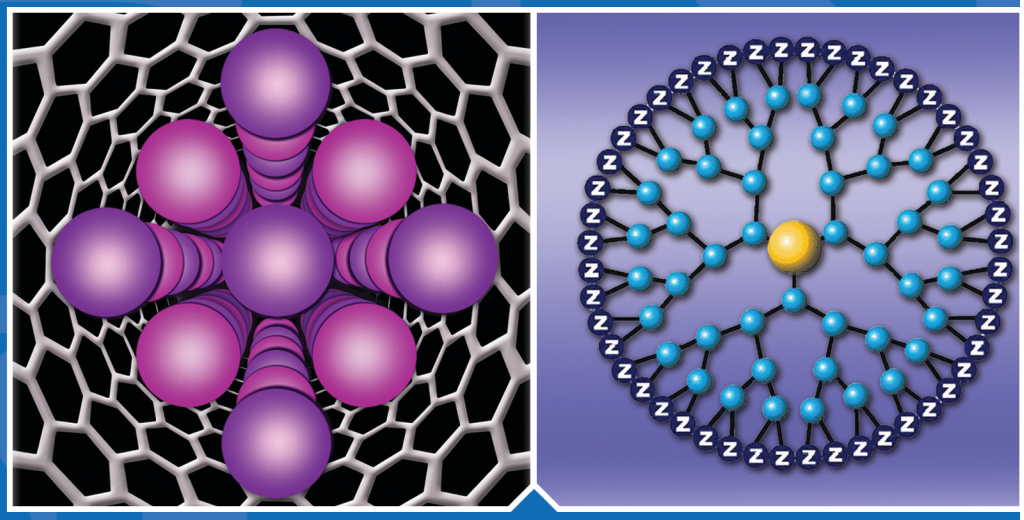
United States
Environmental Protection
Agency

U.S. EPA NANOTECHNOLOGY GRANTEES MEETING
—REPORT—

IN CONJUNCTION WITH
SETAC NORTH AMERICA 31ST ANNUAL MEETING
BRIDGING SCIENCE WITH COMMUNITIES

NOVEMBER 8 - 9, 2010

OREGON CONVENTION CENTER
ROOMS D135 AND D136 ON LEVEL 1
PORTLAND, OREGON



National Center for Environmental Research
Science To Achieve Results (STAR) Research Program

Table of Contents

PM Session 2: Environmental Effects on Nanoparticles

Day 2, Tuesday, November 9, 2010

AM Session 1: Effects on Cells

AM Session 2: Effects at Sub-Cellular Level

PM Session 1: Effects on Fish and Oysters

PM Session 2: Nanoparticles and Waste Treatment

Handouts on Centers for Environmental Implications of Nanotechnology (CEIN)

U.S. EPA NANOTECHNOLOGY GRANTEES MEETING

In Conjunction with the SETAC North America 31st Annual Meeting

Bridging Science with Communities

November 8 - 9, 2010 • Oregon Convention Center • Portland, OR

(Session and presentation times in this agenda are the same as in the SETAC agenda)

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DAY 1, Monday, November 8, 2010

7:30 – 7:45 a.m. **Registration (Rooms D135 and D136)**

7:45 – 8:00 a.m. **Welcome and Ground Rules**

8:00 – 9:35 a.m. **AM Session 1: Systems Approaches**

8:00 – 8:20 a.m. **An Integrated Approach Toward Understanding the Impact of Aggregation and Dissolution of Metal and Metal Oxide Nanoparticles**
Vicki Grassian, University of Iowa

8:25 – 8:45 a.m. **Life Cycle Analysis and Nanostructured Materials**
Thomas Theis, University of Illinois at Chicago

8:50 – 9:10 a.m. **Platinum-Containing Nanomaterials: Sources, Speciation, and Transformation in the Environment**
Martin Shafer, University of Wisconsin-Madison

9:15 – 9:35 a.m. **Role of NLRP3 Inflammasome and Nickel in Multi-Walled Carbon Nanotube-Induced Lung Injury**
Andrij Holian, The University of Montana

9:35 – 10:15 a.m. ***BREAK***

10:15 – 11:50 a.m. **AM Session 2: Effects of Nanoparticle Surface Properties**

10:15 – 10:35 a.m. **Microbial Bioavailability of Polyethylene Oxide Grafted to Engineered Nanomaterials**
Gregory Lowry, Carnegie Mellon University

10:40 – 11:00 a.m. **Surface Oxides: Their Influence on Multi-Walled Nanotubes Colloidal, Sorption, and Transport Properties**
Howard Fairbrother, Johns Hopkins University

11:05 – 11:25 a.m. **Development of Hyphenated and "Particle Counting" ICP-MS Methods Exposure Assessment of Inorganic Nanoparticles**
James Ranville, Colorado School of Mines

11:30 – 11:50 a.m. **Controlled Release of Biologically Active Silver From Nanosilver Surfaces**
Jingyu Liu, Brown University

Note: *Additional Presentation that could not be presented at the meeting:*

Effects of Polyethyleneimine Surface Modifications of Multi-Walled Carbon Nanotubes: Their Toxicity, Sorption Behaviors, and Ecological Uptake by Earthworms and *Daphnia Magna*
Roger Pinto, University of Michigan, Ann Arbor

11:50 a.m. – 1:45 p.m. ***LUNCH***

1:45 – 3:30 p.m. **PM Session 1: Characterization Methods**

1:45 – 2:15 p.m. **A Biological Surface Adsorption Index for Characterizing Nanomaterials in Aquatic Environments and Their Correlation With Skin Absorption of Nanomaterials**
Xin-Rui Xia, North Carolina State University

2:20 – 2:40 p.m. **Flexible Nanostructured Conducting Poly(amic) Acid Membrane Captures, Isolates, and Simultaneously Detects Engineered Nanoparticles**
Wunmi Sadik, State University of New York at Binghamton

2:45 – 3:05 p.m. **Fate and Effects of Nanosized Metal Particles Examined Along a Simulated Terrestrial Food Chain Using Genomic and Microspectroscopic Techniques**
Jason Unrine, University of Kentucky

3:10 – 3:30 p.m. **Determination of Manufactured Nanoparticle Toxicity Using Novel Rapid Screening Methods**
John Rowe, University of Dayton

3:30 – 4:10 p.m. ***BREAK***

4:10 – 5:45 p.m. **PM Session 2: Environmental Effects on Nanoparticles**

4:10 – 4:30 p.m. **Influence of Natural Organic Matter on the Behavior and Bioavailability of Carbon Nanoparticles in Aquatic Ecosystems**
Stephen Klaine, Clemson University

4:35 – 4:55 p.m. **Environmental Photochemical Reactions of nC_{60} and Functionalized Single-Walled Carbon Nanotubes in Aqueous Suspensions**
Chad Jafvert, Purdue University

5:00 – 5:20 p.m. **Impact of Photochemical Oxidation on the Stability of nC_{60} and Multi-Walled Carbon Nanotubes in Aqueous Solutions**
Qilin Li, Rice University

5:25 – 5:45 p.m. **The Environmental Behaviors of Multi-Walled Carbon Nanotubes in Aquatic Systems**
Quingguo Huang, University of Georgia

5:45 – 6:30 p.m. **Open Discussion**

6:30 p.m. **Adjournment**

DAY 2, Tuesday, November 9, 2010

7:30 – 7:45 a.m. **Registration (Rooms D135 and D136)**

7:45 – 8:00 a.m. **Review of Monday and Plans/Ground Rules for Today**

8:00 – 9:35 a.m. **AM Session 1: Effects on Cells**

8:00 – 8:20 a.m. **Functional Effects of Nanoparticle Exposure on Airway Epithelial Cells**
Amiraj Banga, Indiana University-Purdue University at Indianapolis

8:25 – 8:45 a.m. **Toxicity Assessment of Nanomaterials in Alveolar Epithelial Cells at the Air-Liquid Interface**
Galya Orr, Pacific Northwest National Laboratory

8:50 – 9:10 a.m. **Interactions of Nanomaterials With Model Cell Membranes**
Jonathan Posner, Arizona State University

9:15 – 9:35 a.m. **Development of an *In Vitro* Test and a Prototype Model To Predict Cellular Penetration of Nanoparticles**
Yongsheng Chen, Georgia Institute of Technology

9:35 – 10:15 a.m. ***BREAK***

10:15 – 11:50 a.m. **AM Session 2: Effects at Sub-Cellular Level**

10:15 – 10:35 a.m. **Impacts of Quantum Dots on Gene Expression in *Pseudomonas aeruginosa***
Shaily Mahendra, University of California, Los Angeles

10:40 – 11:00 a.m. **Thiol Redox-Dependent Toxicity and Inflammation Caused by TOPO-PMAT Modified Quantum Dots**
Terrence Kavanagh, University of Washington

11:05 – 11:25 a.m. **Bioavailability and Fates of CdSe and TiO_2 Nanoparticles in Eukaryotes and Bacteria**
Patricia Holden, University of California, Santa Barbara

11:30 – 11:50 a.m. **Using Zebrafish Embryos To Test Phototoxicity of TiO_2 Nanoparticles**
Warren Heideman, University of Wisconsin-Madison

11:50 a.m. – 1:45 p.m. ***LUNCH***

1:45 – 3:30 p.m. **PM Session 1: Effects on Fish and Oysters**

1:45 – 2:15 p.m. **Effects of Subchronic Exposure to Nanoparticulate Silver in Zebrafish**
David Barber, University of Florida

2:20 – 2:40 p.m. **Refinements to the Use of Zebrafish for Nanomaterial-Biological Interaction Assessments**
Robert Tanguay, Oregon State University

2:45 – 3:05 p.m. **Impacts of Functionalization of Fullerenes and Carbon Nanotubes on the Immune Response of Rainbow Trout**
Devrah Arndt, University of Wisconsin-Milwaukee

3:10 – 3:30 p.m. **Characterization of the Potential Toxicity of Metal Nanoparticles in Marine Ecosystems Using Oysters – Silver Nanoparticle Studies With Adults and Embryos**
Amy Ringwood, University of North Carolina-Charlotte

3:30 – 4:10 p.m. ***BREAK***

4:10 – 5:45 p.m. **PM Session 2: Nanoparticles and Waste Treatment**

4:10 – 4:30 p.m. **Bioavailability of Metallic Nanoparticles and Heavy Metals in Landfills**
Zhiqiang Hu, University of Missouri

4:35 – 4:55 p.m. **Biological Fate and Electron Microscopy Detection of Nanoparticles During Wastewater Treatment**
Paul Westerhoff, Arizona State University

5:00 – 5:20 p.m. **Analysis and Fate of Single-Walled Carbon Nanotubes and Their Manufacturing Byproducts in Estuarine Sediments and Benthic Organisms**
P. Lee Ferguson, Duke University

5:25 – 5:45 p.m. **Safety/Toxicity Assessment of Ceria (A Model Engineered NP) to the Brain**
Robert Yokel, University of Kentucky

5:45 – 6:30 p.m. **Open Discussion**

6:30 p.m. **Adjournment**

U.S. EPA Nanotechnology Grantees Meeting

Oregon Convention Center
Rooms D135 and D136
777 NE Martin Luther King Jr. Boulevard
Portland, OR

November 8 – 9, 2010

MEETING SUMMARY

The U.S. Environmental Protection Agency held this meeting in conjunction with the Society of Environmental Toxicology and Chemistry's (SETAC) North America 31st Annual Meeting: *Bridging Science with Communities*.

NOVEMBER 8, 2010

OVERVIEW

The U.S. Environmental Protection Agency (EPA) currently funds research that focuses on what happens to nanoparticles, and what impacts on aquatic organisms the particles have, when they enter water environments. EPA holds an annual meeting at which its nanotechnology grantees present their research. There may also be presentations by researchers who have been funded by other Federal agencies with which EPA co-sponsored a Request for Applications. The purpose of the 2010 meeting was to provide a forum for the researchers to share their findings, problems, solutions, and project plans, and to address issues of common concern.

The meeting was held in conjunction with the Society for Environmental Toxicology and Chemistry (SETAC) North America 31st Annual Meeting: Bridging Science with Communities so that EPA researchers could attend the SETAC meeting and people attending the SETAC meeting could attend the EPA meeting. The meetings were coordinated so that the EPA meeting was held at the beginning of the week and the SETAC nanotechnology sessions were held later in the week. As a result, there were 117 attendees from academia, industry, and government at the EPA meeting. For information concerning the SETAC meeting go to: **http://portland.setac.org/**

The meeting was organized by Paul Shapiro of the EPA Office of Research and Development (ORD) National Center for Environmental Research (NCER). The leader of the NCER nanotechnology research program is Nora Savage. Mitch Lasat and Michael McKittrick are also members of the NCER nanotechnology team.

Welcome
Paul Shapiro, EPA

Mr. Shapiro called the meeting to order at 7:45 am and welcomed the participants. He introduced Nora Savage, Mitch Lasat, and the contractor support staff. He explained the logistics of the meeting. He emphasized the need to stick to the schedule because it matched the SETAC schedule, which set the length of each presentation at 20 minutes and the time between each presentation at 5 minutes.

Mr. Shapiro said that in the past attendees have requested an opportunity to have an open discussion of issues that come up during the presentations. He said that the schedule for this meeting includes an open discussion session at the end of each day. There was an easel at the front of the room to serve as a "parking lot" for attendees to write down topics they would like to discuss during these open sessions.

Meeting participants were asked to complete evaluation forms of the sessions each day and to submit them to the meeting staff at the registration table. Mr. Shapiro noted that those presentations for which the presenters give permission will be published on the Web site following the meeting.

Dr. Savage explained that the National Nanotechnology Initiative (NNI) is in the process of finalizing its 2010 Strategic Plan; public comment currently is being accepted. She announced that a Gordon Research Conference focused on environmental nanotechnology will be held at the Waterville Valley Resort in New Hampshire from May 29 to June 3, 2011. The conference steering committee is accepting abstract submissions. Every accepted oral presentation also will be required to have an accompanying poster presented during the conference. EPA also is working with the Organisation for Economic Co-operation and Development (OECD) on a research strategy to understand fate and transport of nanomaterials to ultimately understand toxicity. The next Nanotechnology Grantees Meeting will be held at Duke University in May 2011 in conjunction with a meeting sponsored by the Duke University Center for the Environmental

Implications of NanoTechnology (CEINT) and the University of California, Los Angeles Center for the Environmental Implications of Nanotechnology (commonly known as CEIN). Dr. Savage asked participants who have ideas for future meetings to submit them to her or Mr. Shapiro.

MORNING SESSION 1: SYSTEMS APPROACHES

An Integrated Approach Toward Understanding the Impact of Aggregation and Dissolution of Metal and Metal Oxide Nanoparticles

Vicki Grassian, University of Iowa

This project aims at understanding the environmental and health implications of nanotechnology from the perspectives of air, water, and soil. The researchers are interested in the toxicity of nanomaterials and have partnered with other researchers to examine inhalation exposure to nanomaterials. Also of interest are particles with size-dependent properties and quantifying their effects as they relate to toxicity in water, air, or *in vivo* conditions. Particle dissolution impacts particle size and can impact aggregation by causing deaggregation as the particles within the aggregate dissolve. Particle aggregation impacts size, shape, density, available surface area, and surface chemistry. The researchers have chosen an experimental approach that integrates macroscopic and microscopic measurements and methods to better understand the implications of nanomaterials and are performing toxicity and biological interaction studies. The researchers synthesize or purchase commercial nanomaterial powders and perform bulk and surface characterization of these nanomaterials to determine their fate and transformation in water and aerosol and inhalation toxicity.

Titanium dioxide (TiO_2) nanoparticles from nanostructured and amorphous materials are some of the smallest commercially manufactured oxide nanoparticles, and although they are sold at a primary size of 5 nm, characterization shows them to be 4 nm in size. The researchers determined that these nanoparticles aggregate but do not dissolve in water at a temperature of 293 K. Aggregation and sedimentation in aqueous suspensions will depend on nanoparticle-to-nanoparticle interactions. Research also indicates that there is a switch in stability of TiO_2 nanoparticle suspensions in the presence of citric acid. Derjaguin, Landau, Verwey, and Overbeek (DLVO) calculations along with zeta potential measurements of the surface charge show that TiO_2 nanoparticle suspensions are stable at low pH in the absence of citric acid and at near neutral pH in the presence of citric acid. Surface speciation suggests that pK_a values are lower for surface adsorbed citric acid; less adsorption at higher pH is a result of the surface charge becoming more negative with increasing pH. Thus, mobility in the environment of nanoscale TiO_2 will depend on surface coatings, coverage, and charge and pH in a complex manner.

The researchers compared the dissolution of nanorods to microrods and found that nanorods showed increased surface density of hydroxyl groups compared to microrods. Nanorods can extensively aggregate under certain conditions and form tight bundles. Nanorods have enhanced dissolution but aggregate more readily than microrods in some conditions, but different chemical behavior is seen in different conditions. Enhanced dissolution on the nanoscale is quenched in the aggregated state; therefore, dissolution depends on aggregation and the aggregation state, and nanoparticle aggregation and dissolution are connected in ways that are not fully understood. When researchers compared the inflammatory response of mice to various metal and metal oxide nanomaterial aggregates, the greatest inflammatory response was found for copper-based nanoparticles, and copper nanoparticles showed a higher propensity for dissolution in simulated biological media. Differences between iron and copper nanoparticles are a result of different chemical reactivity in biological media. Lung tissues show no evidence of copper nanoparticles, suggesting that the nanoparticles dissolve, which may increase the inflammatory response.

The results of environmental fate and transport studies indicate that metals and metal oxides show unique reactivity and physicochemical behavior on the nanoscale, and this behavior will be impacted by aggregation. Surface area and chemistry impact aggregation, and aggregation impacts surface reactivity

(e.g., dissolution). Some ongoing environmental fate and transport studies in the laboratory include those of size-dependent dissolution of zinc oxide (ZnO) nanoparticles and nanorods as well as aggregation and dissolution of copper nanoparticles in aqueous media as a function of pH and in the presence of citrate aggregation and dissolution. Inhalation toxicity studies indicate that chemical composition, size, and the ability to undergo dissolution and translocation are important to toxicity in ways that have not been discerned previously. Additional studies on silver, ZnO, and copper nanoparticles currently are underway.

Discussion

Warren Heideman (University of Wisconsin–Madison) asked, in terms of metal toxicity, particularly in the cases of silver and copper, whether the effects of the nanoparticles can be distinguished from those of the carrier ion. Dr. Grassian responded that her laboratory currently is exploring this with follow-up studies.

Bonnie Blazer-Yost (Indiana University–Purdue University Indianapolis) asked how reagents react with mucin in the airway. Dr. Grassian replied that these experiments had not been performed.

Boris Jovanovic (Iowa State University) asked from which company the laboratory ordered the 4 nm TiO_2 nanoparticles. Dr. Grassian responded that they had been supplied from Nanostructured and Amorphous Materials, Inc., but laboratories using them should be sure to characterize them because some were up to 10 nm in size.

Qilin Li (Rice University) asked whether the pH adjustment and nanoparticle contact with citric acid were simultaneous and about the reversibility of absorption. Dr. Grassian responded that the researchers set the pH with citric acid and then added the nanoparticles. Then, pH was measured, and pH changes were not seen. In terms of reversibility, this is a good question, and these studies were not performed.

Mr. Shapiro asked what types of products use copper nanoparticles. Dr. Grassian replied that they are used as catalysts in electronics, and they also are beginning to be used for agricultural applications.

Life Cycle Analysis and Nanostructured Materials
Thomas Theis, University of Illinois at Chicago

Many of the topics discussed during this presentation were addressed at the National Science Foundation (NSF)/EPA Life Cycle Aspects of Nanoproducts, Nanostructured Materials, and Nanomanufacturing: Problem Definitions, Data Gaps, and Research Needs Workshop, which 60 individuals attended. Life cycle assessment (LCA) is a systems methodology for compiling information on the flow of materials and energy throughout a product chain. LCA evolved from industry needs to understand manufacturing and market behavior and make choices among competing designs, processes, and products. It defines four general sections of the product chain: (1) materials acquisition, (2) manufacturing/fabrication, (3) product use, and (4) downstream disposition of the product. LCA is standardized by ISO 14040 and 14044 in a framework whose four steps (goal and scope definition, inventory analysis, impact assessment, and interpretation) can be described as "improvement analysis" and whose outcomes are expressed in common units to allow a comparative systems tool.

EPA's LCA includes potentials for exposure to workers and consumers and disposal exposures. The Agency imposes a risk assessment paradigm on LCA, which is difficult to accomplish. This adaptation of LCA is a method by which to gather information on waste production, energy demand, and the potential for risk to exposed populations. It works best when risks are nonlocal and the population is nonspecific. It is not a substitute for regulatory risk assessment. The nanomaterial health/materials paradox was discussed at the above-mentioned workshop. Those attributes of nanomaterials that are prized for commercial development and application are the same ones that cause toxic reactions.

EPA's nanotechnology research is a two-pronged approach that focuses on environmental applications and implications. This is a worthy approach for environmental regulation but does not apply to LCA. Elements of an LCA-inspired interdisciplinary research program for nanotechnology include use of less toxic and more available components, focus on structures that are less bioavailable, lowering the life cycle energy of manufacturing, design for recovery of nanocomponents at end- of-life, understanding the social contexts in which nano-based products are used and disposed, and application of LCA methodology to the entire product chain. The topic of nanotechnology LCA is not well published and data are lagging. Additionally, manufacturing of nanomaterials causes a variety of impacts including low process yields, significant energy requirements, use of toxic and organic solvents, and high water consumption.

There also is an energy paradox to nanomaterials: Although nanomaterials are some of the most energy-intensive materials known, they currently represent less than 1 percent of manufacturing costs. The costs are low at this point because these materials are not yet commodities, but energy costs may not remain low as they become commodities. Current estimates of world production of various nanomaterials appear to be close to actual amounts, but carbon nanotubes and quantum dots are difficult to mass produce with their current energy requirements. The potential U.S. energy savings from eight nanotechnology applications is approximately 15 percent, but the stability of nanomaterials in the environment is a challenge that must be overcome. Another challenge is that composite materials are not recycled.

In summary, engineered nanomaterials and products are already in use, not widely understood by consumers, often energy intensive and materially inefficient to make, and often difficult to recover once placed in commerce. They have increasingly complex functionalities and provide high added value, although they often are composed of toxic and/or scarce chemicals or use such chemicals in processing. The comparative benefits and impacts of nanoproducts are unknown, and LCA research and applications for nanomaterials are lagging.

Discussion

Gregory Lowry (Carnegie Mellon University) noted that an issue in regard to risk assessment is developing reasonable and reliable numbers for inputs and sources of nanomaterials into the environment. He asked how the annual production figures for nanomaterials are determined and whether they are reliable. Also, can information on potential product types and their release be distributed? Dr. Theis responded that the figures are an estimate based on patents and the open literature. It is too speculative to release information by potential product types, and potential demand is too difficult to predict.

Dr. Grassian noted that many consumer products state that they are "nano" when they actually are "micro." Dr. Theis agreed and stated that the accepted definition of nanomaterials is those smaller than 100 nm in size. When reviewing the literature, only those products identified as smaller than 100 nm were included.

Platinum-Containing Nanomaterials: Sources, Speciation, and Transformation in the Environment
Martin Shafer, University of Wisconsin–Madison

The work on platinum was motivated by several factors, including the increase in platinum levels in many environmental receptors during the past 40 years as a result of platinum use in automobile exhaust catalysts and industrial catalysts, the toxicity of certain platinum species, and the ability of platinum to transform in environmental matrices. Platinum is likely to continue to be used because of a lack of other suitable substances. The toxicological responses of many metals, including platinum, are determined by the specific chemical and physical speciation in the primary source or environmental receptor. Extant modern methodologies, however, provide little relevant speciation information, and traditional techniques that are speciation capable lack the required sensitivity. The specific objectives of the study are to refine analytical tools for measurement and chemical speciation of platinum in environmentally relevant sources and

receptors and integrate source and environmental sampling with advances in platinum analytical speciation tools.

The study examines automobiles, diesel engines, roadside dust and soils, and ambient aerosol from urban centers. Roadway and tunnel dust, which is an excellent integrated receptor from emissions and mobile sources, from Milwaukee, Los Angeles, Atlanta, and Denver was studied. Roadside soils and catalyst materials also were studied, and air sampling was performed adjacent to heavily trafficked roads. The diesel engine dynamometer studies focused on platinum-cerium amended fuel, and the researchers completed a good deal of roadside and ambient aerosol sampling and characterization. Extraction-based and solid-phase speciation and electronic microscopy were used to characterize particulate matter (PM). Physiologically relevant fluids were used for the extraction-based characterization.

The researchers measured the levels of platinum in road dusts and determined that it was from an anthropogenic source. A small fraction of platinum in road dust from the Los Angeles site was found to be soluble, and it is much more soluble in macrophages. Sampling at the Milwaukee site indicated that there is a significant difference in platinum and palladium aerosol mass-size distributions from week to week. The Milwaukee road dust also showed increased solubility in the macrophage of platinum in roadside aerosol; the levels approached the critical range established by EPA. Researchers also noted a potential dilution effect with cerium. The extractable fraction of speciated water-soluble platinum in diesel PM was approximately 3 percent. Studies showed that, in terms of gasoline vehicle catalyst, the modeled fraction of oxidized platinum is significant. Significant contributions from oxidized platinum species are evident in the spectrum in primary vehicle emissions. Early data suggest that oxide and metal are the two dominant platinum species. Additionally, the laboratory is targeting two documented toxic/allergenic chloroplatinate compounds and their hydrolysis products because only very limited information on the concentrations of chloroplatinates in potential environmental sources and receptors is available and environmental fate and transport data are lacking. The laboratory is developing an isocratic and gradient method to examine the toxic form of platinum and will continue this work; once complete, it will apply the methods to engine PM, road dusts, and airborne PM samples. Researchers also will study various environments to examine the transformation state in different environments.

Discussion

Dr. Lowry asked where the chloroplatinate was found in the samples. Dr. Shafer responded that it has the potential to form during the combustion process, so it sits in road dusts and attaches to surfaces. It is more soluble than oxide species. Dr. Lowry asked whether it was possible to distinguish between adsorbed species and others. Dr. Shafer explained that this was not possible with the tools that the laboratory uses.

Dr. Grassian asked whether different regions had specific chloroplatination profiles. Dr. Shafer replied that the method had not been developed to the point that it could be quantitatively applied to field samples.

Quingguo (Jack) Huang (University of Georgia) asked what "SF" stood for in one of the mentioned methods. Dr. Shafer explained that it meant "sector field." Dr. Huang asked whether using solids would return original speciation to the particles. Dr. Shafer answered that the laboratory is collecting a large volume of presize-fractionated aerosols so that species can be associated.

Role of NLRP3 *Inflammasome and Nickel in Multiwalled Carbon Nanotube-Induced Lung Injury*
Andrij Holian, The University of Montana

The researchers have focused on determining the central mechanism to explain how engineered nanomaterials cause pathology and developing a high throughput *in vitro* screening tool to separate bioactive from nonbioactive nanomaterials. The alveolar macrophage was chosen as a vehicle for study because it is the front-line defense against inhaled particles and plays a major role in both the innate and adaptive

immune responses. The alveolar macrophage is responsible for particle clearance from the lung and contributes to the regulation of the inflammatory response. The research focuses on the *NLRP3* inflammasome, which is present in alveolar macrophages and plays an important role in mediating the inflammatory response to various danger signals, including crystalline particles. The inflammasome is activated by cathepsin B, which signals assembly of the NLRP3 inflammasome and results in active caspase 1, which in turn activates gene transcription of pro-inflammatory cytokines (e.g., interleukin [IL]-1β, IL-18). The laboratory tested 24 different multiwalled carbon nanotubes and evaluated cytotoxicity and the inflammasome in THP-1 cells and alveolar macrophages in mice.

Results indicated that the type of metal, diameter, purity, and length were not important following histopathological analysis 7 days postexposure by two blind scorers. Pathology only correlated with nickel content. At 56 days postexposure, multiwalled carbon nanotubes still were present as were granuloma formations. The 7-day and 56-day pathology data are well-correlated; therefore, the 7-day data can be used to predict the 56-day outcomes. There is significant correlation between nickel and various inflammatory response markers (e.g., IL-1β, IL-18, percent viable cells). The increased correlation with *in vivo* cell viability compared to *in vitro* was probably a result of the heterogeneity of the alveolar macrophages versus the cell line. The work has not answered the question of whether there is a relationship between the effect on cell viability and inflammasome activation, which are occurring by separate mechanisms. Additionally, *in vitro* assays are predictive of pathology. There was an excellent correlation between IL-1β production and percent viable cells with prediction of pathology, indicating that measurements of the inflammasome can be used to predict pathological outcomes. Inflammasome production of IL-1β is critical to the inflammatory response.

In summary, the *NLRP3* inflammasome is important in the bioactivity of engineered nanomaterials, and IL-1β is central to initiating inflammation. Nickel on multiwalled carbon nanotubes appears to be a good predictor of *NLRP3* inflammasome activation, and activation of the *NLRP3* inflammasome provides a good explanation of *in vitro* and *in vivo* observations for both multiwalled carbon nanotubes and TiO_2 nanowires. Also, activation of the *NLRP3* inflammasome, which can utilize alveolar macrophages or THP-1 cells, is a good predictor of nanoparticle bioactivity. Disruption of lysosomes, which can be caused by bioactive but not nonbioactive engineered nanomaterials, is required for *NLRP3* inflammasome activation.

Discussion

In response to a question by Dr. Blazer-Yost, Dr. Holian explained that the test materials were selected because there was a clear difference among them in nickel content but not in size, which minimized the variables; therefore, the main variable tested was nickel content. Dr. Blazer-Yost asked whether the nickel was being taken up with the nanotubes, to which Dr. Holian replied that this was definitely the case. Dr. Blazer-Yost asked about the concentration of nanomaterials in the lungs. Dr. Holian responded that each mouse received 100 μg. Agglomeration, suspension, and singlets are critical determinants in the process, and this is what the next phase of the research will study.

Dr. Jovanovic noted that this is an important field of immunotoxicology that has not been explored enough in the past. He asked whether the researchers had considered additional work with neutrophils, especially considering the recent *Nature* article indicating that nanoparticles are important inducers of neutrophil interactions at environmentally relevant concentrations. Dr. Holian agreed that neutrophils are first responders and contribute to cleanup, but he did not think that they contribute to chronic inflammation and injury.

Wen Zhang (Georgia Institute of Technology) asked why the researchers chose a 7-day timeframe to observe pathology. Dr. Holian responded that the time was chosen for practical considerations (e.g., expense), and many publications have indicated that multiwalled carbon nanotubes are able to cause

distinct pathology within 7 days. Because it would be advantageous to perform shorter experiments, the researchers then determined whether this timeframe was a valid predictor.

Howard Fairbrother (Johns Hopkins University) asked whether the correlation with nickel could have been predicted *a priori*. Dr. Holian replied that nickel is more reactive and a better catalyst in redox reactions than iron. A 2009 paper indicated that nickel was capable of activating the inflammasome. Dr. Holian's theory is that nickel is being released by the multiwalled carbon nanotubes, or it has unique bioactive properties and/or catalytic activities. Therefore, the results possibly could have been predicted, but the study provides a deeper understanding. Dr. Fairbrother asked whether toxicological effects of nanotubes are a result of nickel rather than the nanotubes themselves. Dr. Holian thought that contaminants would be an important predictor, and pure nanotubes have less bioactivity. The idea is that nanotubes can interact with lysosomal proteins and cause lysosomal permeability. Dr. Fairbrother noted that the data that Dr. Holian showed indicated that there were nickel subsets that did not correlate with pathology. Dr. Holian responded that the correlation occurs with those multiwalled carbon nanotubes that are composed of at least 2 percent nickel.

MORNING SESSION 2: EFFECTS OF NANOPARTICLE SURFACE PROPERTIES

Microbial Bioavailability of Polyethylene Oxide Grafted to Engineered Nanomaterials
Gregory Lowry, Carnegie Mellon University

The goal of the research was to determine the effect of surface coatings on the environmental and microbial fate of nano-iron and iron oxide (FeO) nanoparticles. The specific objectives were to determine the: (1) fate of nanoscale zero valent iron (nZVI) in the environment, (2) effects of nZVI and its coatings on biogeochemistry, and (3) fate of the coatings. To understand nanoparticle fate and transport, it is necessary to understand coating fate; coatings affect aggregation, deposition, and biological interactions. Therefore, the researchers asked whether nanomaterial coatings are bioavailable. Because nanomaterials must be 5 nm or smaller to enter bacteria, the researchers focused on this size.

The researchers placed polystyrene covalently bound with polyethylene glycol (PEG) in water to determine whether microbes could remove the coating in an aqueous environment and demonstrated that PEGs are nontoxic, provide a permanent coating, and do not hydrolyze in water. Next, water from an urban river with PEG degraders was run through enrichment culture to select for these PEG degraders, and species of *Novosphingobium*, *Pseudomonas*, and *Hydrogenophaga* were found. These bacterial species were provided with PEG, and their growth correlates with the addition of PEG. The same analysis was performed with copolymers, and the same growth was seen, which is evidence that bacteria are able to remove PEG from copolymers. Additionally, the researchers determined that microbes induced PEG copolymer aggregation via a change in surface properties.

The researchers concluded that covalently bound PEG on nanoparticles is bioavailable, and microorganisms can change nanoparticle stability, which in turn changes environmental fate and transport. Bioavailability depends on coating attachment and degradability. The next step is to determine what happens to coatings in the environment. The researchers faced several challenges, including the difficulty of tracking coating fate in real environmental samples, recovering engineered nanomaterials from environmental samples, and measuring the process and effects at realistic nanomaterial concentrations.

Discussion

Robert Yokel (University of Kentucky) asked whether similar results were received with citrate coatings. Dr. Lowry replied that the researchers have not performed extensive studies regarding the bioavailability or biodegradation of citrate. Free citrate would expected to be readily biodegradable, but if it is bound, then Dr. Lowry was unsure of its ability to biodegrade.

Dr. Heideman noted that the opportunity is present to measure molar amounts of carbon with the microsystem and asked how much of the coating is removed. Dr. Lowry responded that the mass balance on the particles indicated that percent levels are converted to carbon dioxide (CO_2), and it clearly is changing the character of the particles. This information has been included in a paper that will be submitted to *Nano Letters* shortly.

Wunmi Sadik (State University of New York at Binghamton) asked whether the researchers performed structural characterization. Dr. Lowry answered that this was difficult for these particular particles. They are uncharged, so measuring zeta potential does not make sense. Dr. Lowry was unaware of any analytical tools available to answer this question, so to indirectly address this, the laboratory examined the nature of the particles postexposure. The mechanism by which the bacteria are removing the coating is interesting but not fully known at this point. Dr. Sadik suggested that one method might be to look at the nuclear magnetic resonance or mass spectrometry of the solution. Dr. Lowry responded that the laboratory would have to restructure its approach to use these methods because of the concentrations involved.

Dr. Grassian asked about the quantitative aspects of surface chemistry and absorption. Dr. Lowry said that the researchers had performed static light scattering on the particles, and this analysis showed that some of the material was removed and converted to CO_2.

Qilin Li (Rice University) asked, because the particles were not taken up by the bacteria, whether enzymes in the extracellular matrix are responsible. Dr. Lowry replied that the next step is to determine the process by which the bacteria are removing the coating.

Elijah Petersen (National Institute of Standards and Technology [NIST]) suggested the use of thermal gravimetric analysis to examine carbon amounts that are released as the nanoparticles are released. Dr. Lowry replied that this method could not be used because of the polystyrene core.

Surface Oxides: Their Influence on Multiwalled Nanotubes' Colloidal, Sorption, and Transport Properties
Howard Fairbrother, Johns Hopkins University

This study focuses on the role that oxygen functional groups play in regulating the properties of multiwalled carbon nanotubes. The laboratory performs physicochemical characterization to develop the functional relationships related to material properties to create models to predict environmentally relevant behavior. Surface analysis is a key component of the research; x-ray photoelectron spectroscopy (XPS) is used to determine surface oxygen concentration because it is the most reliable and convenient method to control the amount of oxygen grafted to the sidewalls. Aggregation properties are examined in a laboratory setting. Surface oxygen may be a predictive metric as stabilization correlates with the amount of surface oxygen. Other properties that the researchers measured were poor metrics for colloidal stability for carbon nanotubes.

The researchers also are interested in studying turbidity, organisms, and natural organic matter to determine the environmental aggregation behavior. To understand complex environmental behaviors, the researchers study colloidal stability and correlate it with adsorption properties to ultimately determine whether surface chemistry of the underlying particle plays a role after natural organic matter adsorption. Surface concentration reduces the adsorption of natural organic matter onto the multiwalled carbon nanotubes' surface. Results clearly indicate inversion of properties in environmental conditions and that surface chemistry plays a significant role in how the multiwalled carbon nanotubes interact in the environment. The researchers designed a column transport experiment to determine how surface oxygen affects the ability to transport in the environment. Results indicated that as the amount of salt increases, multiwalled carbon nanotubes show decreased transport ability. The researchers used a standard calculation method to determine behavior and also found that pH plays a fairly important role in transport; an increase in pH

causes an increased ability of the multiwalled carbon nanotubes to transport. The researchers also determined the optimal conditions under which to obtain reliable and reproducible results.

Future work in the laboratory will focus on the effect of different oxidation on the deposition of surface-oxidized multiwalled carbon nanotubes, the effect of particle sizes on deposition of surface-oxidized MWCNTs, and facilitated transport.

Discussion

Dr. Grassian asked about the morphology of the multiwalled carbon nanotubes in water. Dr. Fairbrother stated that they could be described as floppy rods.

Dr. Li asked Dr. Fairbrother to explain the fact that pulse results were larger than the researchers observed. Dr. Fairbrother said that the confusion might be a result of the order in which he presented his slides, as some of the results were obtained prior to the researchers determining how to consistently reproduce the results. The plan is to return to these experiments now that this is known. Dr. Li asked about the shape of the ethyl concentration profile, which was not typical, and whether it could have been caused because the average was measured. Dr. Fairbrother agreed that this was possible.

A participant asked whether the researchers examined other nanotube-to-natural organic matter ratios besides 10:1. Dr. Fairbrother responded that they studied ratios from zero to 30, and there is a systematic evolution of the particle stability as a function of the amount of natural organic matter.

Hyphenated and "Particle Counting" ICP-MS Methods for the Detection and Characterization of Metal and Metal Oxide Nanoparticles
James Ranville, Colorado School of Mines

The research focuses on risk assessment of nanotechnology. There are many factors that can be identified, and the researchers initially focused on effects (e.g., uptake, toxicity). To understand exposure, it is necessary to understand stability, for which aggregation and dissolution are important. Additionally, to study exposure better metrology (e.g., quantitation, detection, characterization) must be developed. The researchers observed the optical properties over time, which may indicate that reactivity may be changing. Questions to be addressed regarding detection and characterization are: How much sensitivity and selectivity are needed? How can methods be applied to complex matrices? What is exposure? Are researchers studying what they think that they are studying?

With respect to nanosilver, material flow indicates that surface waters and sewage treatment plants should be studied, and environmentally relevant concentrations must be assessed at the parts per trillion (ppt) level although toxic effects are seen at the parts per billion and parts per million levels in the laboratory. The standard hypothesis is that inductively coupled plasma (ICP) mass spectrometry (MS) can be used to detect, count, and size individual silver nanoparticles. The approach is to use element-specific "pulse" counting (e.g., real-time single-particle [RTSP]-ICP-MS; time-resolved ICP-MS; single-particle ICP-MS). The researchers chose to examine health food supplements, but these are polydispersed in size, so the laboratory used nanoComposix, which is monodispersed.

Results indicated that silver nanoparticles up to 100 nm in size could be quantitatively detected by ICP-MS. If the particle counting approach is valid, then the number of pulses will increase with increasing silver nanoparticle concentration, the number of pulses will be reduced by filtration or acidification, and the intensity of the pulse will be related to nanoparticle size. The results correlated with this. The time data can be used to determine the difference between dissolved and particulate materials. Disk centrifuge is another method to analyze particle size, and these data are in agreement with the ICP-MS particle counting method.

The researchers than performed a proof-of-concept study to quantify silver in wastewater, and the results were comparable to estimates from a previously completed materials flow analysis.

Another focus of the project was to determine whether nanotechnology researchers in general are studying what they expect. The researchers examined the biovailability of the cadmium selenide (CdSe) quantum dot core and whether it is toxic to *Daphnia magna* to help answer this question. Field-flow fractionation (FFF)-ICP-MS can be used to sort the nanoparticles by size to allow further analysis of the nanoparticles. Tests indicated that the cadmium-to-selenium ratio was not 1:1. The cadmium was associated with the quantum dot but not with the core, possibly because cadmium associated with the polymer coating as a result of poor washing during synthesis. The tests appeared to study the cadmium on the surface rather than in the core, highlighting the fact that good characterization techniques are needed to ensure that researchers indeed are studying what they expect.

In summary, RTSP-ICP-MS: (1) can be used to detect silver nanoparticles at environmentally relevant concentrations (i.e., ppt levels) with high specificity; (2) can distinguish between dissolved and particle silver, which provides the potential for the method's application in stability and exposure/toxicity laboratory studies; and (3) has limitations in that there is a 40 nm size limit, and it cannot identify nanoparticle type. FFF-ICP-MS can be used to more fully characterize complex nanoparticles and provide information to interpret the results of experiments in which mixtures are used, manufacturing impurities are present, and/or transformation/degradation products are present.

Discussion

Patricia Holden (University of California, Santa Barbara) asked how the method will enable researchers to track mobile particle association. Dr. Ranville answered that the researchers plan to perform experiments to simulate the processes occurring in wastewater at each step. Coupling FFF with particle counting may lead the researchers forward.

Kim Rogers (EPA) stated that crystallography experiments were being performed to determine the association of silver chloride with silver nanoparticles. Dr. Ranville acknowledged the limitations of the current methods and noted that complementary techniques will be performed to obtain more information.

Dr. Huang asked how applicable the method is to other materials. Dr. Ranville replied that it could be used element-by-element to build correlations between silver and other elements.

Controlled Release of Biologically Active Silver From Nanosilver Surfaces
Jingyu Liu, Brown University

Silver is a broad-spectrum antibiotic that has relatively low toxicity in humans and is being manufactured in large quantities and incorporated into consumer and medical products. Is it a risk to the environment and human health? It is known to be more toxic to aquatic organisms than any other metal except mercury. It bioaccumulates quickly, and some organisms have a low toxicity threshold to nanosilver. Silver has potential toxic effects on beneficial soil bacteria. An important research question is whether nanosilver interacting in biological and environmental systems is the particle or the ion. Metal ions may coexist in metal-containing nanoparticle suspensions. Silver ion is a known toxicant that binds to thiol groups in enzymes, such as NADH dehydrogenase, which disrupts the bacterial respiratory chain and generates reactive oxygen species (ROS) that can lead to oxidative stress and cell damage. Nanosilver particles themselves may also contribute by binding to or passing through cell membranes and generating ROS through surface reactions. There is some controversy about the role of particle-based mechanisms, but there is broad agreement that silver ion is an important toxicant. Previous work regarding ion release kinetics and particle persistence in aqueous nanosilver clouds indicate that the reaction produces active peroxide

intermediates, is inhibited by natural organic matter, and leads to complete particle dissolution in aerobic environments.

The researchers are interested in controlled-release nanosilver and application of the drug delivery paradigm. Two questions that are being considered are: Can ion release rate be systematically increased or decreased? Can nanosilver materials be engineered for optimal ion release? Specific benefits of controlled release nanosilver formulations might include: (1) dose control to achieve desired bactericidal or bacteriostatic effects; (2) dose limitation to avoid eukaryotic toxicity; (3) control of product lifetime, before dissolution and diffusion end antibacterial activity; (4) minimization of environmental release through excess ion production beyond that necessary for product performance; or (5) optimization of release profile for targeted delivery to specific tissue or intracellular targets. The researchers use ultrafiltration and atomic absorption to study particle-ion partitioning in aqueous nanosilver colloids. The results indicate that bulk silver oxidatively resolves but much more slowly than nanosilver. Visual MINTEQ software was used to determine the effects of chloride and thiol. The results showed that biological thiol can drive silver equilibrium in a biological system. Nanosilver causes the gradual release of ionic silver because of its affinity to thiol.

Functionalized nanosilver in the presence of citrate, sodium sulfide, or mercaptoundecanoic acid was studied, and all three methods were found to inhibit ion release from silver nanoparticles. Pre-oxidation shows a distinct two-stage release (i.e., fast then slow). The first stage is a result of the rapid dissolution, and the second is because the remaining metal reacts with dissolved oxygen. Other results indicated that antioxidants can inhibit silver ion release. Different surface treatment methods induce different release rates. The primary release mechanism appears to be oxidative dissolution, which can be inhibited through ROS. Other mechanisms are reversible surface binding, inhibition by insoluble silver sulfide, surface passivation, and pre-oxidation. Future work will focus on the biological and environmental implications of ion release kinetics and control.

Discussion

John Rowe (University of Dayton) asked whether this was tested *in vitro* or in tissue culture; he asked because ion effects should be differential, with different toxic effects on prokaryotic and eukaryotic cells. Ms. Liu responded that the researchers plan to perform this type of work in the future, but the current focus is on the basic chemistry of ion release. Dr. Rowe commented that this type of work would be important to perform because there may be two different toxic methods depending on whether the organism is prokaryotic or eukaryotic.

AFTERNOON SESSION 1: CHARACTERIZATION METHODS

A Biological Surface Adsorption Index for Characterizing Nanomaterials in Aquatic Environments and Their Correlation With Skin Adsorption of Nanomaterials

Xin-Rui Xia, North Carolina State University

Currently, most methods to characterize nanomaterials in aqueous environments measure physical parameters. Surface chemistry and core material compositions are the only measurable chemical information on nanomaterials, but these cannot be used directly for quantitative analyses. The octanol-water partition coefficient has been used widely for predictive model development for small molecules, but it is difficult to use for nanomaterials because most nanomaterials form stable suspensions in water or oil but not both. Efforts have been made to understand the chemical interactions between nanoparticles and biological or environmental components. Researchers have demonstrated that lipophilicity is a significant factor in the nanoparticle adsorption of small chemicals. To date, there is no generally applicable approach to quantitatively measure the molecular interactions of nanoparticles with biological or environmental components, which is crucial information needed to develop a quantitative structure-activity relationship

for nanomedicine research and risk assessment and safety evaluation of nanomaterials in occupational and environmental exposures. Many researchers have focused on nanocharacterization of pure nanomaterials in industrial applications, nanoprotein coronas in biological systems, and the nanohumic acid complex in the environment.

The researchers have identified that the adsorption property at the solid-liquid interface is key to understanding the behavior of nanoparticles in aqueous environments. The researchers also have developed a biological surface adsorption index (BSAI) approach to characterize the molecular interaction strengths of nanoparticles with small molecules and macromolecules in biological and environmental systems. The BSAI approach is based on the molecular interaction similarity between nano–small molecule interactions and nano–macromolecule interactions. Forces that govern the chemical and biological behavior of nanoparticles are the Coulomb force, London dispersion, hydrogen bonding, dipolarity/polarizability, and lone-pair elections. Results indicate that nanodescriptors derived from the BSAI approach provide better prediction. The predictive model was cross-validated and determined to be robust.

The BSAI database is the final product of the approach, and it is composed of the five nano-descriptors for each of the nanomaterials. The nanodescriptors are free energy-related quantities quantitatively describing the molecular interaction potentials of the nanomaterials at the nano–water interface. Biological activities are free energy-related quantities; their logarithmic values can be predicted directly via the similar predictive model shown for multiwalled carbon nanotubes. The development of the BSAI approach could open a quantitative avenue toward predictive nanomedicine development, particularly for developing integrated physiologically based pharmacokinetic models and for quantitative risk assessment and safety evaluation of nanomaterials.

The researchers also studied the impact of physicochemical properties on skin absorption of manufactured nanomaterials. Pristine fullerene (C_{60}) in different solvents is used in many industrial and pharmaceutical manufacturing processes; therefore, human exposure to C_{60} could occur in various solvents. Currently, the impact of solvents on its skin penetration is unknown. The laboratory studied four types of representative industrial solvents. The laboratory developed a novel method to prepare nC_{60} nanoparticles with a narrow size distribution. nC_{60} and most of the unprotected nanomaterials have a very narrow window in their colloidal stability, and biological electrolytes will cause their aggregation. The researchers determined that once the nanoparticles aggregate, they cannot get through the skin. Aqueous colloidal nanomaterials with coatings did not penetrate intact skin regardless of particle size. Ion-pairing agents did not promote skin penetration. Skin penetration of C_{60} was observed in different industrial solvents. Significant solvent effects were observed; toluene and chloroform promote skin penetration of C_{60}, whereas mineral oil does not promote skin penetration. The same results were found when the researchers examined deeper skin layers as well.

The laboratory performed short-term studies, but long-term studies also are needed. Skin absorption into aquatic animals should be studied because of their different skin structure (e.g., amphibian skin is very permeable to small molecules). Additionally, more work is needed to make the BSAI approach a generally useful tool for quantitative correlation and risk assessment of various nanomaterials.

Discussion

Mr. Shapiro asked whether the results could be used to design nanoparticles to have specific impacts on the skin. Dr. Xia answered that tailor-made nanoparticles may be possible in the future.

Dr. Lowry expressed concern about applying an equilibrium system to a system so far from equilibrium. Dr. Xia replied that this is a general question for the field. For example, quantitative structure-activity relationship can be used as a driver, but then the kinetics of the actual model are used. Dr. Lowry still had concerns about applying kinetics in this situation. Dr. Xia said that the approach was to correlate

equilibrium parameters. Dr. Lowry asked whether the approach had been applied to macromolecules. Dr. Xia replied that much more work was needed at the current level before moving into macromolecules.

Flexible Nanostructured Conducting Poly(amic) Acid Membrane Captures, Isolates, and Simultaneously Detects Engineered Nanoparticles
Wunmi Sadik, State University of New York at Binghamton

Two types of sensors have been defined by an EPA white paper. Category 1 includes sensors that are nanoscale or have nanoscale materials or components, and category 2 includes sensors that are used to measure nanoscale properties. The overall project objective is to develop novel category 2 nanosensors for application in complex environmental matrices. Nanoparticles must be isolated from complex matrices, and there are several current characterization techniques. Environmental matrices require ultrafiltration of free-engineered nanoparticles. The researchers used functional groups on poly(amic) acid (PAA) to isolate nanomaterials. The researchers have studied nanoparticle crosslinking with PAA for years, as well as the chemistry of the materials used for crosslinking. Additionally, ultrafiltration often is used for the separation of suspended solids, colloids, bacteria, and viruses. If the porosity of the membrane is controlled, then the ions and particles that pass through the membrane can be controlled. The researchers used the phase-inverted membrane method to create several types of flexible PAA membranes. Phase-inverted membranes allow control of pore size and are stable to most organic solvents, conductive, and flexible.

The researchers successfully filtered quantum dots directly from aqueous solution with 99 percent efficiency and were able to control porosity. Next, the researchers analyzed commercially available products, including food supplements and beverages. Nanosilver in food supplements can cause permanent bluish-gray discoloration of the skin and eyes; nanosilver can be toxic at a dose of as low as 15 ppm and is 50 percent more toxic than asbestos. PAA coordinates different nanomaterial functionalities and separates nanosilver, TiO_2 nanoparticles, and quantum dots. The researchers compared the developed membranes to commercially available membranes and found that the membranes developed by the laboratory show superior performance.

In summary, the laboratory has developed a new class of polymeric materials that exhibit spatio-selection via three-dimensional binding interaction with engineered nanomaterials, control porosity, provide accessibility to the underlying transducer, and enable the removal of major interferences. PAA membranes can be regenerated by exposure to fresh solvents or acid washing, and the laboratory successfully filtered nanosilver and quantum dots directly from commercial products with greater than 99 percent efficiency. Future work will focus on improving the fabrication process and testing other nanoparticle combinations to correct defects of the PAA membrane and functionalize the surface of the PAA membrane to improve selectivity.

Discussion

Dr. Huang asked whether the researchers had differentiated between silver ions and other nanoparticles. Dr. Sadik responded that this had not been examined yet.

Dr. Li asked whether the main method of interaction between nanoparticles and the membrane was size or chemical interactions. Dr. Sadik replied that both size exclusion and selective chemistry were occurring. Dr. Li asked what the advantages of the membrane developed by Dr. Sadik's laboratory were compared to commercial membranes. Dr. Sadik answered that the ability to control functional groups on the surface of the membrane allowed for selectivity. Commercial membranes only offer physical selectivity. Dr. Li noted that it would be beneficial to create a membrane that allowed for separation of particles of different sizes. Dr. Sadik agreed and stated that the laboratory currently was working on this.

In response to a question from Mr. Shapiro, Dr. Sadik explained that the researchers had not considered commercializing the membrane that had been developed.

Fate and Effects of Nanosized Metal Particles Examined Along a Simulated Terrestrial Food Chain Using Genomic and Microspectroscopic Techniques
Jason Unrine, University of Kentucky

The researchers are examining the fate, transport, and effects of manufactured nanoparticles in the environment by focusing on uptake of nanoparticles by soil invertebrates, microbes, and plants and their subsequent transfer to higher trophic levels. The worm *Eisenia fetida* is a semimodel organism that is important to the toxicity testing model; the test medium is natural sandy loam, and gold nanoparticles are used as a probe for particle uptake. Nanoparticles up to 50 nm in size can be absorbed by earthworms. The researchers examined the effect of source on bioavailability and determined that primary particle size alone does not determine uptake in complex media, such as soil. The researchers next hypothesized that nanoparticles are more bioavailable through trophic rather than direct exposure and added frogs to their experimental procedures. Transformation appears to occur during the first few weeks of exposure that affect uptake; therefore, future studies should examine this. Results indicated that there was slow elimination of the gold by the earthworms with no significant decrease of gold particles. Frogs that were exposed to gold via ingestion of earthworms showed much higher levels of gold accumulation than those that were directly exposed through gavage. Therefore, the hypothesis is correct, and persistence has significant implications for the food chain. Although there was no difference in frog growth between the two experimental groups, frogs exposed via earthworms showed greater gold concentrations in kidney, liver, and muscle tissues compared to those directly exposed. One alternative hypothesis is that once particles enter earthworm tissues, they acquire a protein corona and become more bioavailable, and another alternative is that earthworms absorb only the most bioavailable particles from the total population of particles, thus enriching the transferable fraction.

Next, the researchers tested various silver nanoparticles with different coatings in two different media; the sandy loam increased oxidation compared to artificial soil media, and the percentages correlate well with the toxicity seen. Results also indicated transient changes in gene expression, so studies should be performed in a time-result manner to observe changes while the organism is adapting. Studies involving protein carbonyl showed an increased amount of protein carbonyl, which correlates with downregulation of catalase gene expression. Catalase transcription is complex and context dependent. There is a cascade of effects leading to the downregulation of catalase, and what most likely is being observed is accumulation of peroxide, which can accelerate the dissolution of particles; therefore, this could be a self-feeding cycle. Following molecular exploration, the researchers examined integrated orgamismal response to nano-particles. Initial avoidance was seen in soil, but there are intact particles. It may be that dissolution is occurring close to the biological surfaces, but the researchers did rule out that it was the result of changes in microbial community composition.

The researchers concluded that nanoparticles are bioavailable from soil and can be transferred to higher trophic levels, and particle size and redox properties are important for uptake and toxicity. Silver particles cause a variety of adverse effects in earthworms translating from the molecular level to the population level, some at concentrations similar to those expected in sewage sludge. Environmental variables are probably more important than particle variables for silver toxicity.

Discussion

Christian Andersen (EPA) asked whether the differences seen between the two experimental frog groups exposed directly or trophically were an experimental artifact from gavage. Dr. Unrine responded that this was not the case; the doses and their confidence levels are known. Dr. Andersen asked whether the waste

products were collected. Dr. Unrine explained that this was not possible because the frogs live in water, and the waste products disperse.

Dr. Heideman asked Dr. Unrine to explain why the removal phase occurred more rapidly than the outflow phase. Dr. Unrine replied that the mass of the worm at different time points needs to be examined, and this study has not been completed yet. Worms can detach part of their body, which could be one possibility, but it is puzzling.

Maria Victoria Peeler (Washington State Department of Ecology) asked whether the researchers had examined sediments. Dr. Unrine answered that this type of work had not been completed, but there are plans to collaborate with laboratories that work with sediments.

In response to a comment from Dr. Grassian, Dr. Unrine explained that the redox potentials listed in one of his slides were for illustration purposes only.

Determination of Manufactured Nanoparticle Toxicity Using Novel Rapid Screening Methods
John Rowe, University of Dayton

The focus of this project is to devise biological systems to rapidly assess the potential toxic effects of nanoparticles and correlate *in vitro* results with *in vivo* outcomes. The approach is multidomain, using viruses, plants, bacterial assays, mammalian *in vitro* cells, and *Drosophila melanogaster* as an *in vivo* model, and examines the biogeochemical cycle and its effects on plants. *D. melanogaster,* which has a fast life cycle, is used to study acute toxicity, and studies have moved to examine chronic toxicity. The overall objective of the project is to establish *D. melanogaster* as an *in vivo* model system for rapid assessment of nanoparticle toxicity. The current project objective is to study the effects of nanoparticle ingestion on *D. melanogaster* growth and development.

Nanoparticle behavior is function of size, shape, and surface reactivity, and the researchers compare the effects of different sizes and coating of nanoparticles on *D. melanogaster* development and reproduction. Polysaccharide-coated silver nanoparticles were used in the experiments that were characterized by transmission electron microscopy (TEM) and dynamic light scattering. Food was treated with uncoated silver 10 nm in size, resulting in a linear effect on survivorship up to 30 μg/ml of silver. Survivors showed a significant increase in pupation time and had a phenotype significantly different than untreated *D. melanogaster*. The same toxic effects, although less, were seen with coated silver nanoparticles and silver nanoparticles that were 60 nm in size. Nanoparticles have been shown to increase ROS, which may result in oxidative stress, inflammation, and consequent damage to proteins, membranes, and DNA. The researchers tested whether oxidative stress occurs *in vivo* using the model system and determined the effect of treatment with ascorbic acid, which is a protector against oxidative stress through the antioxidant defense mechanism, a pathway that provides protection against the harmful effects of ROS. Silver nanoparticles induced superoxide dismutase activity, which is part of the antioxidant defense mechanism. Results also indicated that ascorbic acid has protective effects. Additionally, results showed that silver nanoparticles induced oxidative stress, which may be a mechanism of silver nanoparticle toxicity.

In summary, the researchers established an *in vivo D. melanogaster* model for studying nanoparticle toxicity and demonstrated induction of oxidative stress by silver nanoparticles and the protective effect of ascorbic acid treatment. Future directions will include elucidation of the pathway of oxidative stress involved in the process and evaluation of the efficacy of an array of antioxidants.

Discussion

Paul Westerhoff (Arizona State University) asked whether the researchers had examined the first generation offspring for the presence of nanoparticles. Dr. Rowe responded that other laboratories have

demonstrated this, and researchers are studying an inhalation model to determine whether nanoparticles enter the system completely via respiration.

Dr. Rogers asked whether the researchers knew how ROS production occurs in response to silver nanoparticles. Dr. Rowe replied that the activation appears to be direct because it can be reversed with an antioxidant.

Dr. Blazer-Yost asked whether the researchers tested lower concentrations of nanoparticles. Dr. Rowe explained that this would be one of the next steps of the laboratory. The researchers used high concentrations to ensure that an effect was seen before moving to lower concentrations. This is an important question because the ultimate fate of nanoparticles is unknown.

Dr. Lowry asked whether the researchers experimented with silver nitrate in food. Dr. Rower answered that they had not, but it may be worthwhile to do so.

AFTERNOON SESSION 2: ENVIRONMENTAL EFFECTS ON NANOPARTICLES

Influence of Natural Organic Matter on the Behavior and Bioavailability of Carbon Nanoparticles in Aquatic Systems

Stephen Klaine, Clemson University

The researchers are examining how water quality parameters (e.g., natural organic material) influence the bioavailability of carbon nanoparticles, and a major goal of the research is to examine food chain uptake. A standard *D. magna* bioassay was used to measure the toxicity of a variety of carbon nanomaterials. Multiwalled carbon nanotube toxicity did not change as a function of natural organic material. Natural organic material-stabilized C_{60}, C_{70}, and single-walled nanotubes were nontoxic.

The researchers explored whether carbon nanomaterials (multiwalled carbon nanotubes, carbon dots, single-walled carbon nanotubes) are absorbed from the intestinal tract. Results indicated that there was no movement of nanotubes in between the microvilli, and they do not appear to be biochemically toxic but are physically toxic because they clog the intestinal tract. Acidified single-walled carbon nanotubes, however, are found in between and in the microvilli, indicating that they have moved into the organism. Aggregation inside the tissue also was observed. Raman spectroscopy was used to determine where the single-walled carbon nanotubes were located within biological tissues, and results showed that they were within the intestinal tract. Movement outside of the intestinal tract also was seen, and acidified single-walled carbon nanotubes stabilized by natural organic material move farther outside of the tract than other single-walled carbon nanotubes.

Carbon dots are useful for examining where nanomaterials travel after digestion by *D. magna*. The carbon dots used by the researchers possess a carbon core with a PEG coating and have the same fluorescence as quantum dots. Confocal microscopy was used to observe movement outside of the intestinal tract and showed that there was a buildup around various organs and organ systems outside of the tract.

The researchers observed that multiwalled carbon nanotubes are acutely toxic to *D. magna*, and this is not a function of natural organic material but appears to be a result of interference with orgamismal food processing. It took 29 hours for *D. magna* to clear multiwalled carbon nanotubes from the intestinal tract, compared to 30 minutes for clearance of clay. Multiwalled carbon nanotubes are not taken up from the intestinal tract. Carbon dots migrate from the intestinal tract and appear to be associated with organelles. Hydroxyl-functionalized single-walled carbon nanotubes may migrate from the intestinal tract, whereas PEG-coated single-walled carbon nanotubes do not. The next steps are to continue to examine uptake from the intestinal tract using fluorescent-labeled single-walled carbon nanotubes and employing other surface

modifications. Food chain studies will use labeled carbon nanoparticles that are known to be bioavailable to determine their movement through the aquatic food chain.

Discussion

Dr. Petersen asked about any impurities present in the original single-walled carbon nanotubes. Dr. Klaine replied that they were pure by the time that they were treated with acid. Dr. Petersen suggested that the researchers use electron energy loss spectroscopy to gain more definitive results.

Dr. Fairbrother asked why the acidified single-walled carbon nanotubes were more likely to move through the organisms. Dr. Klaine answered that he was unsure, but possibly it was because items that are more hydrophilic better associate with the microvilli. Dr. Fairbrother asked if similar results were seen with multiwalled carbon nanotubes. Dr. Klaine answered that multiwalled carbon nanotubes are very stable.

Galya Orr (Pacific Northwest National Laboratory) asked about the zeta potential. Dr. Klaine was unsure whether the laboratory had obtained these data. Dr. Petersen added that disbursement of nanotubes could be the result of bundling and stronger interactions among the single-walled carbon nanotubes, which probably are easier to disperse following acid treatment. Dr. Klaine agreed that this correlated with the data.

Environmental Photochemical Reactions of nC_{60} and Functionalized Single-Walled Carbon Nanotubes in Aqueous Suspensions
Chad Jafvert, Purdue University

Dr. Jafvert described published results of the grant, which is coming to an end. A paper focusing on the photochemical transformation of aqueous C_{60} clusters in sunlight was the first paper to report on C_{60} photochemical decay, measured by high-performance liquid chromatography (HPLC), in aqueous media under sunlight. Results indicated that smaller clusters result in faster loss of C_{60}, and the photo-transformation rate is not pH dependent. There is a negligible rate change with humic acids present, and molecular oxygen is required for the process. A paper reporting on the photochemistry of aqueous C_{60} clusters highlighted that singlet oxygen forms during solar irradiation of nC_{60}, consistent with known reaction mechanisms involving singlet oxygen. The photo-transformation of nC_{60} is mediated by singlet oxygen, and the rate of singlet oxygen production is auto-catalyzed by nC_{60} water-soluble products formed during irradiation. The singlet oxygen production rate increases with decreases in the size of nC_{60}. The concentration of singlet oxygen induced by nC_{60} in sunlight is four- to 65-fold higher than the average concentration typically found in sunlit natural surface waters.

A paper focusing on wavelength dependency and product characterization in terms of the photochemistry of aqueous C_{60} clusters showed that several laboratory methods indicate that oxidation of C_{60} occurs in aqueous suspensions of nC_{60} under sunlight, and C_{60} photo-transformation and singlet oxygen production occur in visible light. Another paper focused on the photoreactivity of carboxylated single-walled carbon nanotubes in sunlight and ROS production in water. In oxic aqueous solutions under sunlight, carboxylated single-walled carbon nanotube dispersions generate singlet oxygen, superoxide anion, and hydroxyl radicals. Reactions with probe molecules were corroborated, and photo-induced aggregation occurred at a low pH. Another paper highlighted projects focusing on solar light-induced ROS production by single-walled carbon nanotubes in water and the role of surface functionalization. Results indicated that oxic aqueous colloidal dispersions of both types of functionalized nanotubes generated ROS in sunlight, and Type I and Type II photochemical pathways occur by the functionalized single-walled carbon nanotubes in sunlight. It appears that the functionalized single-walled carbon nanotubes can act as the electron donor directly, resulting in a change in their properties, or can shuttle electrons from other electron donors to form ROS.

Discussion

Richard Zepp (EPA) asked whether the researchers attempted to generate ROS by nonphotochemical methods to observe how they react with substrate. Dr. Jafvert answered that others have performed this in organic solvents, and it would be worthwhile to attempt.

Dr. Jovanovic asked whether the researchers exposed the C_{60} species from the Materials and Electrochemical Research Corporation to sunlight or ultraviolet (UV) light to determine if it generated ROS. Dr. Jafvert said that the compounds being generated could not be quantified. C_{60} was not used as the starting material in any of the experiments.

Dr. Petersen asked whether multiwalled carbon nanotubes had been tested. Dr. Jafvert replied that this is planned for the future.

Impact of Photochemical Oxidation on the Stability of nC_{60} and Multiwalled Carbon Nanotubes in Aqueous Solution
Qilin Li, Rice University

The main objectives of the study are to understand the changes in physicochemical properties of carbon-based nanomaterials, specifically C_{60} and carbon nanotubes, in natural aquatic systems as a result of interactions with NOM and sunlight and determine the subsequent changes in their aggregation and deposition behaviors. To simulate the particle structures that may form when C_{60} and multiwalled carbon nanotubes are released into the natural aqueous environment, the researchers prepared the nanoparticle suspensions using a direct sonication method without using any organic solvent. Carboxylated multiwalled carbon nanotubes were used for easier dispersal; C_{60} and the carboxylated multiwalled carbon nanotubes were well dispersed in water. Sunlight irradiation was simulated with a photoreactor equipped with UV lamps, and samples taken at various times of irradiation were characterized for their physicochemical properties.

Results indicated that the outer surface layer of nC_{60} particles was oxidized following 7 days of irradiation. When the aggregation of these surface-oxidized nC_{60} particles was examined, it was found that they were significantly more stable than the pristine nC_{60} particles, as demonstrated by the reduced aggregation rate. Comparison of stability curves shows that the surface oxidation caused by irradiation increased the critical coagulation concentration by more than fivefold. Additionally, irradiated nC_{60} responds to humic acid differently from the pristine nC_{60}, showing no change in particle stability, and shows differences in calcium chloride ($CaCl_2$) as well. The steric hindrance effect of humic acid in $CaCl_2$, however, did not seem to be affected by UVA irradiation. An adsorption experiment confirmed that this was a result of significant humic acid adsorption on the irradiated nC_{60} surface, aided by calcium.

A similar study used carboxylated multiwalled carbon nanotubes. Contrary to the nC_{60} results, irradiation reduced multiwalled carbon nanotube stability, and the surface negative charge decreased after irradiation, suggesting changes in surface chemistry. Carboxylated multiwalled carbon nanotubes also appear to lose surface hydroxyl and/or carboxyl groups following irradiation. In $CaCl_2$ solutions, however, the stability before and after irradiation was very similar. Multiwalled carbon nanotubes are unstable in the presence of calcium, so it is important to remember that these nanotubes most likely will aggregate and settle quickly in most natural aquatic systems.

In conclusion, sunlight irradiation and humic acid sorption mediate nC_{60} and carboxylated multiwalled carbon nanotubes aggregation, and specific and nonspecific interactions are involved. Nanocarbon surface chemistry plays a key role in its environmental fate and transport. Ongoing and future work in the laboratory focuses on the impact of irradiation and natural organic material on sorption/deposition and

transport in a subsurface porous medium as well as on the impact of irradiation and natural organic material on bioavailability and bioaccumulation of nanoparticles.

Discussion

P. Lee Ferguson (Duke University) asked whether the researchers had plans to examine truly pristine nanotubes. Dr. Li responded that the laboratory is interested in this, but original attempts with pristine nanotubes did not allow for high enough concentrations.

Dr. Fairbrother noted that the techniques used were meant for solids rather than powders, and the method may need to be modified. Dr. Li acknowledged the limitations of the technique and explained that to counteract this the laboratory analyzed the XPS spectrum. Dr. Fairbrother cautioned that there still might be issues, and the two researchers agreed to discuss the specifics later.

The Environmental Behaviors of Multi-Walled Carbon Nanotubes in Aquatic Systems
Quingguo Huang, University of Georgia

The objective of the research project is to examine solubilized carbon nanotubes currently under development for a variety of applications. Their mobility and exposure also are being examined. The project focuses on sorption, transformation, bioaccumulation, and trophic transfer. Because sediments affect dispersed carbon nanotubes and dissolved organic matter, it is necessary to design experiments to better understand each of these situations. The three treatments applied were control, peat with dissolved organic matter, and solid peat. Results indicated that in peat, sodium is necessary for sorption in a dose-dependent manner; in shale, there is strong sorption.

Dr. Huang noted that an inner nanotube core may slide, almost without friction, within its outer nanotube shell, thus creating an atomically perfect linear or rotational bearing. Additionally, studies show that C_{60} can be degraded by microbes via an enzyme. The researchers examined whether white rot fungus, used in bioremediation, could degrade multiwalled carbon nanotubes and found that it could not. Bacterial degradation was evidenced by multiwalled carbon nanotube mineralization, so the researchers attempted to determine the method of degradation using DNA extraction, propagation, isolation, sequencing, and comparison. Three bacteria (*Burkholderia*, *Delftia*, and *Stenotrophomonas*) were identified, all of which are Gram-negative aerobes involved in the degradation of organic contaminants. These field bacteria probably work in concert to degrade. Bacterial degradation has implications for nanotube behavior and sequestration.

The researchers also examined chronic exposures using *Ceriodaphnia dubia* with the goal of evaluating reproductive toxicity and accumulation of multiwalled carbon nanotubes by adult and neonate *C. dubia* under two different solubilization protocols. Results indicated that sonication increased toxicity, whereas natural organic material stabilized the nanotubes. Sonicated multiwalled carbon nanotubes adhered to adult organisms and prevented molting and release of neonates. Natural organic material was protective against reproductive toxicity, with no observed adherence to adults. There was significant accumulation of natural organic material-solubilized multiwalled carbon nanotubes in neonates. The next step is a feeding study that will determine whether there is trophic transfer from *C. dubia* exposed to multiwalled carbon nanotubes following ingestion by *Artemia* and fathead minnows. Also, full life cycle exposures of multiwalled carbon nanotubes will be evaluated in fathead minnows.

Discussion

Dr. Zepp asked about the strategy used to locate the bacteria. Dr. Huang replied that the bacteria were found attached to samples that were being examined for the white rot fungus. Dr. Zepp asked how the researchers synthesized the labeled materials. Dr. Huang stated that he used a common method that has been described in many papers.

A participant asked about the purity of the carbon-labeled material. Dr. Huang responded that amorphous carbon is not seen because it has been removed. The participant asked about the size distribution. Dr. Huang answered that a variety of sizes had been examined. Dr. Petersen added that these sizes were 100 to 420 nm. The participant asked whether the researchers examined distribution following aggregation. Dr. Huang explained that this would be difficult to accomplish using scanning electron microscopy with the mixed system; the researchers will be examining the chemistry, however.

OPEN DISCUSSION

Mr. Shapiro explained that in the past, participants have asked for an open discussion session to be included in the meeting to discuss issues that are introduced throughout the day's presentations. Four issues were introduced during the Day 1 discussions: (1) How can more be done toward LCA? (2) Is there a substitute for platinum as a catalyst? (3) Should research focus on plumes or far lower concentrations? (4) EPA Administrator Lisa Jackson is interested in examining methods by which to treat groups of drinking water contaminants, including nanoparticles.

Mr. Shapiro asked Dr. Grassian to discuss her concern, which is item 3 above. Dr. Grassian wondered what is the "right" concentration to study. Sometimes, research is conducted at high concentrations that may be relevant to plumes. Dr. Westerhoff agreed that this is an important consideration and noted that it does not make sense to study concentrations that are orders of magnitude lower than those at which an effect is seen. Concentrations studied should be environmentally relevant. Dr. Lowry commented that lower doses may be relevant to chronic toxicity. Dr. Orr agreed that bioaccumulation is important. Dr. Holden added that what constitutes a dose also is an important question. Mr. Shapiro asked Dr. Holden's opinion on what constitutes a dose, and she responded that it depends on the mechanism. Dr. Xia said that it was hard to generalize, and the approach to determining this should include dose response, exposure, and screening. Dr. Petersen noted that there could be a wide range of doses (e.g., plume vs. environmental concentrations). It is necessary to be cautious when interpreting toxicity results, which must be placed in context and compared to other compounds in the environment.

Dr. Savage asked the best method for writing a solicitation that would address chronic toxicity of nanomaterials, which requires a significant amount of time and money. Dr. Lowry stated that this issue cannot be confined to nanotechnology. There is an EPA model that addresses similar issues; therefore, the uncertainty already has been dealt with. Dr. Grassian noted that the National Institutes of Health fund long-term studies (greater than 20 years). Dr. Savage asked whether a reasonable approach would be to focus on fate and transport and then examine chronic toxicity after the fate and transport studies have yielded results. A participant noted that extrapolating from acute toxicity is difficult. Terrence Kavanagh (University of Washington) agreed that this extrapolation was difficult because often the targets of acute and chronic toxicity are completely unrelated (e.g., organophosphate [OP] acute toxicity results in cholinesterase inhibition, whereas OP chronic toxicity results in neurotoxicity).

A participant asked whether carbon nanomaterials, reactive metal nanomaterials, and so forth could each be grouped together for study. Dr. Savage explained that the Request for Applications (RFA) should focus on mixtures because the compound-by-compound approach is not working. Dr. Orr suggested that libraries of data be created that focus on an array of compound modifications to develop the whole picture. Dr. Petersen commented that some trends may be emerging (e.g., carbon nanotubes and physical effects). Classification could be based on how nanoparticles cause toxicity generally to organisms. A participant stated that a good deal of research has focused on the toxicology of chemicals and pharmacology of toxicity. From a pharmacological point of view, receptors are important. Some nanotubes may have properties and interact in ways for which researchers have no foundation; this is an infant science. Dr. Lowry noted that even though this is an infant science, there are 20 years of toxicological PM research, and particle science is not new.

Dr. Rogers suggested considering nanotechnology from a purpose or functional standpoint. Nanomaterial designers do not think about toxicity; they focus on making the best material for their purpose. If manufacturers are encouraged or required to consider toxicity, then they will be more receptive to hearing recommendations from the research community. The perception that nanotechnology toxicological research is performed for the benefit of industry so that manufacturers avoid potential disasters should be perpetuated. Dr. Kavanagh agreed that industry would appreciate feedback from nanotechnology researchers.

The participants discussed persistence. A participant noted that even if a compound degrades quickly, chronic exposures are possible if there is continual loading. Dr. Yokel pointed out that ceria nanoparticles can persist for at least 3 months.

In response to a question from Mr. Shapiro, Dr. Lowry explained that there were two different thoughts: to either make nanomaterials safe or minimize exposure. His opinion is that both approaches should be implemented. Dr. Savage noted that it is difficult to design green nanotechnology because often a nanomaterial appears safe, but in the aquatic environment it is not. Dr. Lowry stated that it was necessary to obtain as much information as possible to make informed decisions. A participant noted that some sources can be controlled, but it is difficult for other source streams; there is no one solution.

Dr. Orr commented that positively charged nanoparticles increase toxicity in mammalian cells, so positively charged nanomaterials should be avoided. Research can start building similar "rules."

Mr. Shapiro asked what the participants thought about performing joint research with industry. Dr. Yokel noted the example of the Health Effects Institute, which was cofounded by EPA and the automobile industry. Any nanoparticle research that relates to combustion could have a funding source in place. Dr. Savage added that the National Nanotechnology Initiative aims to increase private-public partnerships (e.g., CEINT). Dr. Lowry stated that BASF Corporation and IBM Corporation would like to engage the nanotechnology research community, although they are not interested in providing funding. He suggested that making research relevant to the needs of industry may increase private funding. Industry has some answers, but they are not publicized because of the nature of their confidential business materials. Industry will release research and development materials. A participant cautioned that patents must be considered when dealing with industry.

Mr. Shapiro asked whether it would be helpful if EPA emphasized, assisted, or encouraged the commercialization of research products; this is another manner in which to partner with industry. Dr. Lowry noted that NSF has programs that require grantees to have industry partners. Also, there are Small Business Innovation Research grants available from various federal agencies.

Mr. Shapiro thanked the participants for attending and recessed the meeting at 6:22 p.m.

NOVEMBER 9, 2010

Review of Day 1 and Plans/Ground Rules for Day 2
Paul Shapiro, U.S. EPA

Mr. Shapiro called the meeting to order at 7:54 a.m. and welcomed the participants to the second day of the meeting. He reiterated the ground rules and expectations for the meeting and discussed logistical issues. He reminded the participants that the schedule is based on the SETAC schedule, and it was important to adhere to this schedule. He pointed out the "parking lot" for the open discussion session at the end of the day.

During breaks between presentations, Mitch Lasat explained that EPA has partnered with other agencies and international organizations to facilitate the flow of ideas and increase innovation. The resulting program will support innovative nanotechnology research co-funded by EPA and a partner in the United Kingdom. Each project is funded for 4 years at $1 million per year. Dr. Savage provided information about the nanotechnology RFA that closed in February 2010. There were more than 100 submissions, and five were funded by EPA, five by NSF, and four by the U.S. Department of Agriculture. The two research categories are: (1) environmental matrices and (2) biological matrices with a food focus. A new $4 million center will attempt to understand environmental matrices.

MORNING SESSION 1: EFFECTS ON CELLS

Functional Effects of Nanoparticle Exposure on Airway Epithelial Cells
Amiraj Banga, Indiana University–Purdue University at Indianapolis

Nanoparticles are being scrutinized as a health hazard, and humans are exposed to nanoparticles in various ways. Workers handle nanoparticle materials in many industrial jobs, and nanoparticles can enter the body via inhalation, ingestion, and penetration through the skin. Complete information about health effects of nanoparticles is lacking. The research used three different unpurified and as-manufactured carbon nanoparticles: multiwalled carbon nanotubes, single-walled carbon nanotubes, and C_{60}. The hypothesis is that manufactured, nonfunctionalized carbon nanoparticles, when exposed to barrier epithelia, exert a biological effect on the cell membrane and may alter the cell function. Dr. Banga explained the laboratory approach and noted that all concentrations are expressed in micrograms per square centimeter.

Results indicated that both nanotubes significantly decreased the resistances of cells over a wide range of concentrations, but C_{60} did not. It is interesting to note that the effects of these low concentrations have not been reported in the literature; the laboratory hypothesizes that these concentrations are physiologically more relevant. Additional experiments highlighted the fact that chloride moves in a secretory direction, causing water to follow and leading to hydration of the passageway. Exposure to different types and concentrations of carbon nanoparticles showed a variable response, but the effect of nanoparticles still is observed at the lowest concentration (0.004 $\mu g/cm^2$). Because the initial increase in chloride secretion is mediated predominantly by an increase in intracellular cyclic adenosine monophosphate (cAMP), resulting in activation of protein kinase A and consequently phosphorylation and activation of *CFTR*, the researchers examined cAMP in the treated and control cells. After epinephrine stimulation, the rise in cAMP was found to be the same in nanoparticle-exposed and control monolayers. These results suggest that the ion transport element affected by the nanoparticles lies beyond the basolateral membrane epinephrine receptor and intracellular cAMP production.

In summary, low-dose nanotube exposures decrease the barrier function of airway epithelial cells. Low-dose nanotube exposures affect the ability of the airway epithelial cells to secrete chloride. These data suggest that the levels of nanotubes found in the workplace, particularly during chronic exposures, are likely to have physiological effects that can cause or exacerbate respiratory problems.

Discussion

Dr. Holian asked if the researchers had examined nanotubes of varying metal content or considered that the nanotubes might be conducting. He also asked about the purity of the materials. Dr. Banga responded that the purity was 99 percent, and the nanotubes contain certain metals (nickel, cobalt, iron), but as 1-hour experiments showed no significant effects, the nanotubes were not conducting. Dr. Holian noted that nanotubes must enter the cells to conduct. He asked whether the researchers had tried carboxylation. Dr. Banga responded that they had not yet done so.

Toxicity Assessment of Nanomaterials in Alveolar Epithelial Cells at the Air-Liquid Interface
Galya Orr, Pacific Northwest National Laboratory

The rationale of the project is that airborne nanomaterials that enter the respiratory tract are likely to be deposited in the alveolar region, where alveolar epithelial cells are found at the interface with ambient air. To date, the majority of *in vitro* studies characterizing the interactions and impact of engineered nanomaterials in these cells have been carried out in cells submersed under growth media. To more closely mimic *in vivo* exposures, the researchers have established the growth of alveolar type II epithelial cells at the air-liquid interface, enabling realistic exposures to aerosolized nanoparticles. Type II cells play critical roles in the function of the alveoli by secreting pulmonary surfactants, and by differentiating into type I epithelial cells when these are damaged. Importantly, type II cells participate in the immune response to certain particles and pathogens by releasing chemokines. By collecting the particles on millimeter-size grids placed randomly over the cells and visualizing them using electron microscopy, it is possible to accurately quantify the number of particles delivered per square centimeter or per cell. This approach also enables physical and chemical characterizations of the collected nanoparticles, providing properties that are relevant to airborne nanoparticles and the actual exposure at the air-liquid interface.

The project studies manufactured amorphous silica nanoparticles, which are used extensively in a wide range of industrial applications. The results did not show decreased membrane integrity or proliferation of alveolar type II epithelial cells following exposure to 50 nm bare amorphous silica nanoparticles. The researchers estimated equivalent doses in submersed and air-liquid interface conditions using the computational *In Vitro* Sedimentation, Diffusion, and Dosimetry (ISDD) Model. The ISDD model integrates the influence of particle properties and cell culture conditions to calculate the actual deposited cellular dose (particles per cell). Using estimates from the particokinetics model, cells were exposed to submersed conditions, and no membrane compromise, toxicity, or decrease in proliferation was observed.

Next, the researchers focused on ZnO nanoparticles, which can be highly toxic, an effect that might originate from the dissolved molecules. Large aggregates were created in two different solutions. Following exposure to aggregates, toxicity in cells emerged at a concentration of 9–10 μg/ml (300 aggregates per cell). Under submersed conditions, ZnO toxicity was observed at a concentration of 25 μg/ml. Therefore, ZnO toxicity can be induced by intact particles or dissolution of the molecule in a local area, which provides insight into ZnO toxicity. Testing the same outcome but in a different manner still showed ZnO toxicity at a concentration of 25 μg/ml.

In conclusion, exposures of alveolar type II epithelial cells to 50 nm bare amorphous silica nanoparticles at the air-liquid interface elicit no significant cytotoxic response at concentrations ranging from 10 to 1,000 particles per cell. These observations agree with the response of submersed cells exposed to equivalent doses as estimated by a computational particokinetics model. Dose-response evaluations of 300 nm ZnO aggregates (25 nm primary particle size) in alveolar type II epithelial cells exposed at the air-liquid interface show a toxic response starting at approximately 300 aggregates per cell (10 μg/ml) 24 hours following exposure. Toxicity evaluation of these aggregates in submersed cells elicits a toxic response at approximately 25 μg/ml, indicating that they might be slightly more toxic at the air-liquid interface. These

findings support the idea that ZnO aggregate toxicity can originate from the intact nanoparticles or from molecules dissolved locally at the cell membrane or inside the cell.

Discussion

Dr. Yokel asked whether surfactants had any effect on the nanomaterials. Dr. Orr replied that the laboratory is attempting to obtain artificial surfactants prescribed to premature infants to study next.

Dr. Sadik asked how background was accounted for by the researchers and whether the researchers were aware that flow cytometry often introduces artifacts into results. Dr. Orr responded that controls always are included to manage these types of issues. In terms of the flow cytometry, filters are applied or oxidative trace studies are performed.

Interactions of Nanomaterials With Model Cell Membranes
Jonathan Posner, Arizona State University

This project attempts to measure particle properties and perform toxicity assays to develop global descriptors that predict bioaccumulation for use in models. The main global descriptor is the octanol-water partition coefficient, which is a ratio of concentration of solute in between two immiscible phases, generally octanol and water. It is used in water quality models to predict fate, accumulation, and aquatic toxicity of organic pollutants in the environment. It is not defined for particles, however. To examine octanol-water partitioning of engineered nanomaterials, the researchers studied a variety of materials and determined that surface charge is important, but it is difficult to identify trends. The researchers attempted to quantify partitioning at various interfaces and conditions. Partitioning occurs because of the minimization in Helmholtz free energy. Although zeta potential correlates with pH, it cannot be used to predict partitioning.

Challenges with determining octanol-water partitioning of engineered nanomaterials include importation into EPA models and treatment of mass at the interface. Additionally, partitioning provides no information on the state of engineered nanomaterials (e.g., aggregation, dissolution), is path dependent, does not correlate with bioaccumulation, and is dependent on the poorly defined interfacial area. Therefore, researchers have taken an analogous approach using lipids. The lipid bilayer is an important interface between life and its environment and a potential exposure route for engineered nanomaterials. The lipid bilayer-water distribution has been shown to be a more appropriate indicator than octanol-water partitioning for bioaccumulation of ionizable organic molecular and surface active compounds, with which engineered nanomaterials share some properties. Lipid bilayer-water distribution is being used increasingly in environmental research regarding molecular pollutants. Lipid bilayers are the primary constituent of many biological cellular membranes and often are used to model passive transport into cells.

The researchers used commercially available lipid bilayers noncovalently bound to silica in their experiments. The engineered nanomaterials used were aqueous C_{60} aggregates, fullerol, and gold nanoparticles. The concentration of nC_{60} was determined by HPLC, fullerol concentration by UV-visible absorption spectroscopy, gold nanoparticle concentration by ICP-optical emission spectroscopy, lipid concentration by malachite green dye assay, and the sizes and zeta potential of the liposomes and engineered nanoparticles by dynamic light scattering. Fullerols and nC_{60} were found to have similar size distributions and charge. The researchers quantified all of the mass in the system and determined that there was no loss to the glass walls at pH 7.4 and that distribution of nC_{60} and fullerol in lipid-water is pH dependent. The next goal was to compare isotherms in environmentally relevant situations, and the laboratory found qualitative agreement with other studies that suggest higher bioaccumulation and toxicity of nC_{60} compared to fullerol. Lipid-water distribution isotherms of gold nanoparticles suggest that number of particles appears to be a reasonable metric.

In summary, the lipid bilayer-water distribution of the selected engineered nanomaterials is a pseudo-equilibrium process that can be described by isotherm behaviors. The distribution behavior and accumulation to lipid bilayers are pH dependent. Size dependency studies show that 20 nm gold nanoparticles exhibit the highest propensity to accumulate in lipid bilayers. Comparisons with bioaccumulation and toxicity studies using organisms suggest that the lipid bilayer-water distribution is promising for assessing the bioaccumulation and toxicity potentials of engineered nanomaterials. Bioaccumulation data (i.e., bioconcentration factor) data are needed for a variety of engineered nanomaterials to verify whether lipid-water distribution can be used to predict the fate of engineered nanomaterials.

Discussion

Mr. Shapiro asked about the next steps. Dr. Posner responded that the laboratory will examine a variety of particles and collect additional bioconcentration factor data to determine trends.

In response to a question from a participant, Dr. Posner explained that when nanoparticles and electrolytes are mixed, zeta potential is modified slightly in the final solution. When asked if this would occur with all nanoparticles, Dr. Posner replied that it depended on the nanoparticle. For example, the researchers did not observe gold absorption to glass; therefore, he did not want to generalize.

In response to a question from Dr. Huang, Dr. Posner explained that particles have ionizable surfaces; therefore, the rationale is that the surface chemistry is similar.

Development of an In Vitro *Test and a Prototype Model To Predict Cellular Penetration of Nanoparticles*

Yongsheng Chen, Georgia Institute of Technology

Surface interactions are the first step for nanomaterials to act in a beneficial or detrimental manner. Governing parameters that contribute to interactions include nanoparticle properties, cell properties, and the environment; these parameters lead to biological consequences (e.g., interfacial forces, sorption processes, cellular damages). The researchers addressed the question of how particle size impacts biological interactions and focused on hematite as a reference material because it is relatively stable and displays uniform size distribution in culture media. *Escherichia coli* is used because it is a common model for toxicity tests and ubiquitous in the environment. A model epithelium cell line for human intestinal cells was used as well. The researchers evaluated surface property changes of *E. coli*, adsorption kinetics, size effects on the adsorption kinetics, and DNA binding with ultrafine nanoparticles.

Results indicated that hematite accumulates on the surface of *E. coli*, causing deformity, death, and flagella damage. Surface disruption can disrupt cellular respiration without nanoparticle entry into the cell. Adsorption kinetics of hematite nanoparticles on *E. coli* cells also show the dependency on particle size, with adsorption rates being faster for small nanoparticles compared to large ones. The contradiction in the trend of size effects on adsorption kinetics caused by concentration expressions can be interpreted via the Interaction Force Boundary Layer (IFBL) Theory. IFBL and DLVO are combined to interpret the size effect on the adsorption kinetic, and the model agrees with the experimental observations.

Results of DNA binding experiments following *E. coli* exposure to quantum dots indicate that ultrafine quantum dots can permeate into *E. coli* cells and unintentionally bind with DNA. Results of human cell line experiments indicate that bio-nano interactions cause microvillus disruption, including structural damage and decreased cellular integrity and nutrient absorption, and adhesion junction disruption. Cells lose their integrity and eventually die. Adsorption kinetics on the human cell line show similar features to hematite nanoparticle adsorption in *E. coli*. Large particles adsorbed faster by mass-based concentrations, and small particles adsorbed faster in number-based concentrations. In terms of the size effects on the disruption of

the adhesion junction and cell penetration, small nanoparticles penetrated cell lines faster and led to more severe junctional disruption.

The researchers concluded that hematite nanoparticles are ideal for use as a reference nanomaterial because of their high stability, uniform size distribution, and low aggregation. Adsorption kinetics are size dependent, which can be interpreted by IFBL Theory. The exposure to hematite nanoparticles induced reorganization and distortion of surface structure damages and cell penetration. Challenges include determination of the role of interfacial forces and diffusion in the transport of nanoparticles toward biological systems and DLVO theory versus mass transport mechanisms. During the next year, the laboratory will continue to extend its developed methodologies (e.g., models) to evaluate other types of nanoparticles regarding their environmental and biological behaviors. The researchers plan to develop sophisticated imaging and quantifying techniques for the surface characterization of nanoparticles and their interactions with the biological system at the nanoscale. The laboratory has published 15 papers in peer-reviewed journals, submitted six manuscripts, and presented 20 invited talks at national and international conferences.

Discussion

Mr. Shapiro thought that the presentation related to the previous day's discussion regarding providing industry with recommendations and information.

Dr. Holian asked how the researchers took agglomeration into account. Dr. Chen replied that the researchers verified that the small particles still were stable in the cell culture media.

Dr. Petersen asked how the researchers differentiated between adsorption and absorption because both could be occurring; this is important to consider in a model based primarily on surface interactions. Dr. Chen agreed that this was a good point.

Dr. Heideman asked how the researchers distinguished between live and dead *E. coli* with atomic force microscopy. Dr. Chen replied that they mobilized *E. coli* cells on a silicone chip and verified whether they were alive or dead via colony numbers. Dr. Heideman asked how the researchers could tell whether the particles bound DNA *in vivo*. Dr. Chen dispersed quantum dots into the suspension for a 1-hour exposure, extracted the DNA, and observed changes in the DNA, some of which may not be conformational. He agreed, in response to a comment by Dr. Heideman, that this could have occurred following the opening of the cells.

Dr. Unrine noted that receptor-mediated endocytosis occurs in eukaryotes. He asked whether the researchers considered the strength of the interactions with cell surface receptors in the model. Dr. Chen answered that this was difficult, and the laboratory is providing compelling evidence that penetration cannot be controlled.

MORNING SESSION 2: EFFECTS AT THE SUBCELLULAR LEVEL

Impacts of Quantum Dots on Gene Expression in Pseudomonas aeruginosa

Shaily Mahendra, University of California, Los Angeles

Quantum dots are semiconducting nanocrystals that have biomedical and electronics applications. Biocompatible quantum dots have a hydrophobic core, often containing toxic metals, surrounded by an inorganic shell. Because of the hydrophobic core, these quantum dots can be stabilized in water by derivatizing the surface with amphiphilic organic coatings. In terms of quantum dot weathering, the laboratory's hypothesis is that the toxicity of quantum dots primarily is a result of free metal, and environmental weathering of the coating will increase their toxicity to cells. They are safe for intended

uses; therefore, decreasing exposure and/or degradation will eliminate, to the extent possible, quantum dot toxicity. Degraded quantum dot cores increase bioavailability to cells and microbes.

Laboratory results indicate that coated quantum dots retard cell growth, and weathered quantum dots kill bacteria. Additionally, cadmium and selenite are toxic to cells. *Pseudomonas aeruginosa* tolerated high concentrations of cadmium from quantum dots; the bacterial species apparently has mechanisms by which to expel quantum dots. Therefore, this may be a good candidate at the molecular (genetic) level to understand the impact of sublethal doses, which would allow proactive predictions of risk. Dr. Mahendra outlined several mechanisms of bacterial toxicity, including protein oxidation by nC_{60}, disruption of cell membranes by single-walled carbon nanotubes, generation of ROS by TiO_2, DNA damage by multiwalled carbon nanotubes, and release of toxic ions by ZnO and nanosilver.

The researchers use microarrays to provide a snapshot of genome expression following exposure and analyze global gene responses to quantum dot exposure. The researchers analyzed differences in gene expression, functional genes and pathways affected by coated quantum dots, and functional genes and pathways affected by weathered quantum dots. Results indicated that metal resistance genes were induced by weathered quantum dots but not coated quantum dots.

In summary, coated and weathered quantum dots affected gene expression in *P. aeruginosa*, and the functional categories of amino acid metabolism, energy production, and carbohydrate metabolism were primarily regulated. Metal-resistance genes were upregulated following weathered quantum dot exposure. Results also indicated that there is an apparent change from ammonium-assimilating aerobic metabolism toward anaerobic, denitrifying metabolism in response to stress.

Discussion

Dr. Rowe stated that *P. aeruginosa* tends to switch to anaerobic respiration after sitting, so this may be the cause of that observation. He also recommended that the researchers examine the proteosome to determine whether there is translation, which is more relevant, in addition to transcription.

Dr. Petersen asked whether the researchers examined amounts of cadmium and selenium in the cells following exposure to weathered and coated quantum dots. Dr. Mahendra replied that the laboratory used TEM to image cells exposed to quantum dots and identified cadmium ions and zero-valent CdSe, which were associated mostly with the surface membrane. Dr. Petersen asked whether the researchers performed ICP-MS. Dr. Mahendra answered that they had, and the data are in a manuscript under review.

Dr. Lowry asked whether the researchers had examined different kinds of particles (e.g., FeO, silica) that do not injure the bacteria to ensure that the bacteria are responding to the nanoparticle. Dr. Mahendra responded that these types of experiments had been performed; bacterial and fungal responses were compared, and bacteria responded only to the cadmium in the nanoparticles. The ion appears to be more important than the nanoparticle.

Thiol Redox-Dependent Toxicity and Inflammation Caused by TOPO-PMAT Modified Quantum Dots
Terrence Kavanagh, University of Washington

Dr. Kavanagh explained that there was a recent review in *Science* regarding activities of nanoparticles in the environment and how important surface chemistry is to induce the various forms of oxidative stress. The hierarchical model of oxidative stress induced by exposure to nanoparticles consists of tiers: (1) antioxidant defense mechanisms, (2) inflammation, and (3) cytotoxicity. These increase as oxidative stress increases. The researchers used quantum dots, which have multiple uses, including gene and drug delivery. Because uncoated quantum dots often have poor solubility and are unstable in biological systems, the researchers chose manufactured quantum dots that are exceptionally stable in aqueous solution and display

red-orange fluorescence for use as *in vivo* tracers. The project examined the interactions of cell types with macrophages, and results indicated that the effects (changes in NADPH, thiols, and viability) were relatively minor after 24 hours, but colony forming efficiency decreased after 7 days. Researchers also observed an increase in hemoxygenase and glutamate-cysteine ligase (GCLM), which is involved in glutathione production. Hemoxygenase induction and necrosis are highly correlated with quantum dot uptake, and a number of proinflammatory cytokines are upregulated as well.

Quantum dots can release heavy metals, causing oxidative stress and toxicity in biological systems. Glutathione is important in preventing oxidative damage to cellular macromolecules and has been shown to be an important modulator of the immune response. Therefore, glutathione could be an important determinant of quantum dot-induced toxicity and inflammation. Glutathione, a heterodimer, is important in scavenging free radicals, and its levels are controlled by cysteine availability, synthesis, and utilization, and organ import and export. The researchers found that glutathione depletion does not increase the toxicity of the quantum dots to the mouse macrophage cell line, and, unexpectedly, glutathione depletion suppresses cytokine responses in this cell line. To more thoroughly investigate this phenomenon, researchers used a *Gclm*-null mouse as an *in vivo* model of glutathione depletion. Humans are known to have polymorphisms in GCLM, which predispose them to heart disease, lung diseases, schizophrenia, and heavy metal body burden.

The researchers tested the susceptibility of mice with varying amounts of GCLM production to nanoparticle-induced lung injury by exposing them to quantum dots. *Gclm*-null mice have low GCLM activity and low levels of glutathione in most tissues. The researchers exposed the mice to quantum dots via nasal instillation. There is correlation between neutrophil influx and protein in bronchoalveolar lavage fluid 8 hours postexposure. Surprisingly, nasal instillation of quantum dots increases neutrophils in the airways of wild-type and *Gclm*-heterozygous mice but not *Gclm*-null mice, and quantum dots increase inflammatory cytokine levels in the bronchoalveolar lavage fluid of wild-type and *Gclm*-heterozygous mice but not *Gclm*-null mice. Possible reasons for the lack of inflammation in *Gclm*-null mice could be the failure of their macrophages to take up the quantum dots, produce and/or secrete chemotactic peptides and cytokines, or produce ROS. Alternatively, perhaps the lack of glutathione has resulted in an adaptive response (e.g., upregulation of protective genes), which acts to squelch oxidative stress or the immune response. Researchers also found that *Gclm*-null mice have attenuated myeloperoxidase activity but not matrix metalloproteinase activity in their lungs after quantum dot exposure, and quantum dot-induced cytokine responses are attenuated in cultured peritoneal macrophages from *Gclm*-null mice. Glutathione depletion enhances nuclear factor-kappa B translocation induced by quantum dots in the mouse macrophage cell line.

Ongoing studies focus on the mechanisms of quantum dot uptake by macrophages, markers of oxidative stress in lung tissue and bronchoalveolar lavage cells and fluid, chronic effects of exposure to quantum dots, DNA microarray analysis of gene expression for additional biomarkers of lung injury, systemic inflammation/markers of lung injury, translocation of quantum dots and cadmium to other organs, and effects on the olfactory epithelium and brain.

Discussion

Dr. Rowe asked if the phenotype of the knockout mouse was generally healthy. Dr. Kavanagh responded that they are relatively healthy but have compromised fertility, and one research group saw behavior similar to schizophrenia. Dr. Rowe asked whether the researchers examined weathered dots. Dr. Kavanagh replied that various coatings and stability were examined.

David Barber (University of Florida) stated that a theme in the literature is that dramatic toxicity is not seen until mitochondrial glutathione is depleted. He asked whether the researchers had examined the difference between cytosolic and mitochondrial glutathione in the knockout mice. Dr. Kavanagh answered that they

had; the knockout mice have depleted glutathione in the mitochondria (30% of normal) but not to the extent of cytosolic glutathione depletion (10–15% of normal).

Bioavailability and Fates of CdSe and TiO_2 Nanoparticles in Eukaryotes and Bacteria
Patricia Holden, University of California, Santa Barbara

Through manufacturing and use, nanoparticles will enter the environment, particularly via waste streams, where they will be taken up by individual cells, especially bacteria. The bioavailability continuum (agglomeration, adhesion, entry, accumulation) is simplistic. Four questions to consider are: (1) When do nanoparticles enter cells? (2) Do the particles stay intact? (3) What are the cellular effects? (4) What are the variables? The hypothetical framework of the interactions of nanomaterials and cells has become more complex as more research is completed.

The researchers used laboratory-synthesized CdSe/zinc sulfide (ZnS) quantum dots and laboratory- and industrial-synthesized TiO_2 nanoparticles and varied light and dark conditions. Certain laboratory methods were selected to determine whether electron transfer is occurring between nanoparticles and cells that could contribute to cell oxidation and generation of free radicals that ultimately could allow nanoparticles to enter cells. Other methods were used to characterize and quantify the nanomaterials and measure exposure to and effects on cells. Previous studies have shown that CdSe quantum dots enter planktonic cells in light conditions. Quantum dot fluorescence lifetime is examined to study energy transfer to quantum dots to understand how energy transfer may ultimately be linked to the generation of free radicals that could affect cells with which quantum dots are associated. These quantum dot lifetimes vary with different cores, caps, and conjugates.

Results indicated that CdSe/ZnS quantum dots photosynthesized with dopamine increased in superoxide dismutase, intracellular ROS, and reactivity and decreased in metabolism in the cells. Bare CdSe quantum dots enter and are toxic to *Pseudomonas* in dark conditions, and cadmium telluride quantum dots differentially bind and transfer electrons to bacterial strains. As a result of electron transfer, Gram-positive bacterial membranes are depolarized, but bacterial growth is not slowed. The researchers concluded that quantum dots can enter cells with ROS-mediated membrane damage, but the ROS form varies. Quantum dots can enter cells intact, but caps slow dissolution. Cells show consequences of uptake of quantum dots (e.g., slow growth rate and lower yield), but membrane depolarization does not appear to be fully toxic. When the laboratory examined the consequences for the next trophic level, it showed that CdSe quantum dots can be trophically transferred from *Pseudomonas* to *Tetrahymena*, a protozoan. Furthermore, *Pseudomonas* binds and disagglomerates TiO_2.

In summary, quantum dots can damage and enter cells and activate electron transfer. TiO_2 binds to cells but does not enter. Variables include light versus dark conditions, strain, specific nanoparticle, cap, conjugate, and oxygen. The next steps of the laboratory are to perform high throughput studies on membrane effects and quantify cell loading and bioprocessing.

Discussion

Dr. Rowe asked about the size distribution used when uptake was observed. Dr. Holden responded that it was 5 nm. Dr. Rowe asked if the researchers used a size curve, to which Dr. Holden replied that they did not. Dr. Rowe thought that bacterial surfactants might be involved, but Dr. Holden explained that the researchers had proven that they were not by measuring surface tension; dispersion in citrate also did not occur. Dr. Rowe asked whether the size and shape of the bacteria were taken into account in terms of surface binding. Dr. Holden answered that for the quantum dot experiments, the researchers quantified the amount of cadmium that was associated with the cell using cadmium as a tracer and used microscopy to observe orientation. Therefore, the size and shape were not taken directly into account.

Using Zebrafish Embryos To Test Phototoxicity of TiO_2 Nanoparticles
Warren Heideman, University of Wisconsin–Madison

The laboratory is examining the theory that light causes ROS production *in vivo*, and the question is whether this matters *in vivo*. Zebrafish show cardiotoxicity following exposure to nanoparticles. Testing TiO_2 nanoparticles *in vivo* requires TiO_2 nanoparticles, zebrafish embryos, and light. Because coral is photodependent, the aquarium hobby industry has developed aquarium lights that mimic sunlight; the researchers used these lights for their experiments. Results indicated that zebrafish embryos exposed to TiO_2 nanoparticles and illumination do not survive. When zebrafish embryo survival at various times and TiO_2 nanoparticle concentration were examined, the researchers found that decreased nanoparticle concentrations increased survival time. There are several phenotypic defects associated with TiO_2 nanoparticle exposure and illumination, including malformed head and tail, stinting, edema, and extended yolk.

Because the researchers realized that it was possible that toxicity unrelated to the nanoparticles might have been caused by a new reactive species of chemical created as a result of the plastic well in which the experiments were conducted, they illuminated the TiO_2 nanoparticles prior to exposing embryos, which did not cause toxicity. Embryos pre-exposed to TiO_2 nanoparticles, washed, and then illuminated showed toxicity. TiO_2 nanoparticles have a pronounced tendency to aggregate, and TiO_2 nanoparticle exposure adds measurable titanium to the fish. TiO_2 nanoparticles are found throughout the zebrafish embryo. TEM determined that the egg chorion shields the embryos from toxicity. Dehydroergosterol fluorescence was used to detect superoxide production, and the yolk showed autofluorescence under all experimental conditions. Additionally, DNA adducts are formed when TiO_2 exposure is combined with illumination. Fish have a clear defense mechanism to protect cells from oxidative stress; it involves transcription factors that bind to a canonical sequence called "ARE" that drives production of enzymes that protect the organism from oxidative stress. Using green fluorescent protein as an ARE reporter shows activation by TiO_2 nanoparticles combined with illumination; therefore, this is the normal response to oxidative stress. Preloading embryos with N-acetyl cysteine (NAC) can prevent some of the effects of TiO_2 nanoparticle exposure.

The photochemistry of TiO_2 nanoparticles predicted that the nanoparticles might cause phototoxicity as a result of ROS production. The uncertainty was whether this occurs *in vivo*. Using zebrafish embryos, the researchers showed that TiO_2 nanoparticles cause light-dependent toxicity associated with uptake and ROS production. The findings in zebrafish may be relevant to humans because many biological systems are strongly conserved, and mechanisms that work in zebrafish often are found in humans.

Discussion

Dr. Savage asked whether oxidative stress was seen in all of the same cells. Dr. Heideman responded that he was unsure which cells are being affected, but the pattern is the same.

Dr. Jovanovic was concerned about the environmental relevance of the study because of the artificial nature of the lights designed for coral, despite the *in vivo* construct. Many studies show that the amount of energy needed to cause photoactivation is much higher than the particles can receive from sunlight. Dr. Heideman replied that the response tends to be seen with high concentrations of nanoparticles. It is difficult to determine whether this is environmentally relevant because zebrafish are relatively hearty. The artificial light was developed by scientists in a very scientific manner. The illumination used likely is lower than sunlight received on a sunny day, but it is necessary to remember that sunlight changes throughout the day, so it is difficult to equate the two types of illumination.

Xinyu Yang (Duke University) noted that NAC is a chelator and wondered whether it was possible that NAC is chelating the metals in TiO2. Dr. Heideman said that he had not considered this, and another set of controls would need to be added to test this.

AFTERNOON SESSION 1: EFFECTS ON FISH AND OYSTERS

Effects of Subchronic Exposure to Nanoparticulate Silver in Zebrafish
David Barber, University of Florida

Nanosilver is not as toxic as other nanomaterials in terms of gill proliferation in zebrafish, but accumulation of nanosilver could cause chronic toxicity. The experimental design incorporates zebrafish exposed to various concentrations of 25 nm nanosilver. Results indicated that accumulation was approximately 50 percent of nominal, and levels dropped to zero 4 days following the removal of nanosilver exposure. Silver levels on day 3 following exposure were similar despite the exposure concentrations being different by orders of magnitude. This may be because the researchers are not measuring soluble silver or because the system is being saturated with particulate silver. Carcass tissue burden was found to be dose and time dependent, indicating absorption and accumulation of particulate and dissolved silver in tissues outside of the intestinal tract. After nanosilver exposure was discontinued, tissue silver concentrations remained stable. Gill silver concentrations were greater than tissue concentrations, which is expected because gills have increased accumulation compared to other organs. This concentration, however, decreased after 2 weeks, possibly indicating an adaptive response.

The bioconcentration factor decreased as concentration increased, indicating that the bioconcentration factor is concentration dependent. There also is significant correlation between carcass/gill burden and nanosilver concentration but a lack of correlation with soluble silver. Gill morphology (i.e., cell proliferation in the interlamellar space) appears unchanged following the 28-day exposure. Although accumulation of silver is seen in the skin and nasal epithelium, there is no evidence of morphological injury; the same is true for several other tissues and organs (e.g., liver, heart). The researchers examined transcriptional effects on the gill following 28 days of exposure, and a cluster analysis found three distinct clusters: control, solubility, and high concentration. Although hundreds of genes were up- or downregulated in response to the various concentrations, only 55 genes were common to all concentrations. Increases in nanosilver concentrations increase the number of genes, but the genes differ by treatment. A pathway analysis indicated that ribosomal and organ development effects were significant pathways.

The researchers concluded that zebrafish accumulate significant silver tissue burdens, gill levels are 10 times greater than carcass levels, and nanosilver remains for up to 4 days in the absence of additional nanosilver exposure. There is a significant correlation between nanosilver concentration and tissue burden, and soluble silver is not significant. There is no observable effect on epithelial morphology. Microarray data indicate significant alterations in gene expression patterns and that there is a dose response pattern for the number of genes affected. Pathway analysis indicates two pathways: organ development and ribosome biogenesis.

Discussion

Dr. Westerhoff asked about the experiments in which bioconcentration decreased as a function of concentration and whether on a nanogram per gram basis of tissue the results were similar. Dr. Barber responded that tissue concentrations at later times were similar between the nominal and high doses.

Dr. Heideman suggested that the researchers examine a subset of genes affected by nanosilver versus soluble silver. Dr. Barber answered that these types of studies have been performed in the past, and there definitely is such a subset, but it is not annotated very well, so further work is needed to characterize it.

Dr. Kavanagh asked whether the researchers looked specifically for widely recognized genes that might be responsive (e.g., metallothionein). Dr. Barber answered that the researchers examined metallothionein, but there was very little success in obtaining reproducibility with silver. Past studies indicate that there is induction of a number of metal transport factors, heat shock proteins, and ROS.

Dr. Petersen asked about the laboratory's definition of nanosilver. Dr. Barber replied that finding soluble silver was a challenge, and centrifugation yields more reproducible results compared to filtration at this size. The researchers are aware, however, that complete dissolution probably is not being measured.

Refinements to the Use of Zebrafish for Nanomaterial–Biological Interaction Assessments
Lisa Truong, Oregon State University

Physicochemical properties influence nanoparticle behavior. Nanoparticle exposure to air, water, and ground result in a variety of responses, including agglomeration, accumulation, aggregation, dissolution, and so forth. Interaction of nanoparticles with environmental and biological systems remains largely unknown. The research community is missing toxicological data to understand biocompatibility and needs to identify the risk associated with nanoparticle exposure. The goal of the research is to determine what influence each nanoparticle parameter has on biological activity. The hypothesis is that more than one parameter (size, surface charge, functional group) activates different biological responses. Researchers used a zebrafish model to test the hypothesis because zebrafish embryos develop within 120 hours. Zebrafish are continuously exposed to various nanoparticles from 6 to 120 hours postfertilization. The researchers assessed more than 200 nanoparticles via high throughput screening and found that a large portion did not induce a biological response. Whether there are false negatives has not been established.

Nanoparticle properties change depending on the aqueous environment and conditions, aggregation can occur in high-ionic-strength media, biological response can be altered, and it is necessary to characterize aggregation in test media and throughout the exposure period. Therefore, the laboratory assessed nanoparticle aggregation in aqueous media using gold nanoparticles. The three research questions were: Does ionic strength play a role in aggregation? Can zebrafish develop and behave normally in low- or no-ion media? Will suspension of gold nanoparticles in low-ionic-strength media induce biological activity? Results regarding the first question indicate that high-ionic-strength media cause gold nanoparticle aggregation. In terms of the second question, zebrafish morbidity, mortality, and phenotype were similar in all media. Additionally, there was no statistical difference in the biological media following a period of darkness. The researchers concluded that zebrafish develop normally in low-ionic-strength media. In answering the third question, results indicated that decreasing the ionic concentration increased mortality and behavioral effects. The researchers concluded that low-ionic-strength media favor dispersion of gold nanoparticles, which are more toxic when dispersed.

The implications of these results are that every parameter must be taken into consideration when performing nanomaterial–biological interaction studies and that refinement of the current high throughput screening to include avoid false negatives and assess nanoparticles was deemed problematic. Finally, zebrafish are a versatile model.

Discussion

Dr. Jovanovic asked whether the original medium was egg water or embryo water. Ms. Truong replied that it was E2 embryo medium. Dr. Jovanovic asked how the low-ionic-strength medium was derived. Ms. Truong responded that it was E2 embryo medium diluted with reverse osmosis water.

Dr. Zhang commented that this is an open carbon system that should allow CO_2 to transfer to liquid, which results in carbon speciation that could contribute depending on pH. He asked whether the researchers considered this. Ms. Truong answered that they did.

Dr. Holden stated that the point of using high throughput screening is to survey many different types of nanoparticles and asked how the researchers plan to approach this issue with other nanoparticles. Ms. Truong replied that most collaborations are characterizing media to determine whether nanoparticles are available prior to assessment so that false positives and negatives are eliminated or at least minimized.

Dr. Heideman asked how the researchers buffered the system to prevent the pH from turning acidic. Ms. Truong explained that embryo medium is not made with buffering capacity, and removal of ions further decreases buffering capacity. The pH was measured throughout the experiment to ensure that it remained neutral.

Dr. Xia asked about the ability of various coatings to aggregate depending on ionic strength. Ms. Truong replied that all of the coatings were screened, and none of them aggregated.

Impacts of Functionalization of Fullerenes and Carbon Nanotubes on the Immune Response of Rainbow Trout
Devrah Arndt, University of Wisconsin–Milwaukee

The immune system of all vertebrates is designed to recognize something as foreign, pathogens in particular. The immune system recognizes molecular patterns on the outside of pathogens, which then triggers inflammatory and other biochemical responses. Different pathways are stimulated in the primary immune system depending on the type of pathogen.

The hypothesis of the laboratory is that nanomaterials may instigate the same pathways and some unique responses from the immune system. The laboratory's specific hypotheses are that: (1) nanoparticles should be considered foreign and will stimulate the immune system, (2) core structure will impact the ability to stimulate the immune system, (3) functionalization will impact the ability to stimulate the immune system, and (4) nanomaterials will cause unique gene expression patterns that differ from each other and from traditional stimulants. The researchers chose macrophages to assess the primary immune response to nanomaterials because they are key to the innate primary immune response. The laboratory produces carbon based nanoparticles of different types with various functionalizations, testing the impacts of these particles first on cell viability. Next they chose nontoxic concentrations to evaluate key gene expression; currently, the researchers are evaluating global gene expression in macrophage cells. Particles and their suspensions were characterized using TEM, ICP-MS, and dynamic light scattering. Additionally, the researchers have examined single-walled carbon nanotubes with carboxyl, amide, PEG, and other functional groups.

Results indicated that cell viability does not decline with nanomaterial exposures when not suspended with surfactants. Phagocytosis was initiated following 24-hour exposure to nanomaterials. Macrophage responses to nanomaterials were more similar to that following bacterial exposure rather than viral exposure. The researchers determined that the surfactants that were used stimulated an immune response by themselves, so suspensions were created through sonication or stirring to eliminate surfactant use. The results also indicated that multiwalled carbon nanotubes appear to be slightly more stimulatory than single-walled carbon nanotubes. The researchers found that C_{60} appears to be equally as stimulatory to the immune system as multiwalled carbon nanotubes with anionic functional groups. IL-1β also increased in response to carbon nanotube exposure.

Current work compares nanomaterials in terms of global gene expression profiles. The researchers plan to use the data to determine whether these profiles are similar to those of known pathogens and identify any unique signatures these materials have on the immune system. The goal is to begin to group nanomaterials by their toxicity based on these gene expression patterns. RNA from control and exposed fish were replicated in the arrays and then compared to a database of more than 200 different exposures that have been carried out on this platform. Preliminary results indicate that C_{60} causes a change in the total number

of genes that is similar to a major component of the outer membrane of Gram-negative bacteria. Single-walled nanotubes cause a change in approximately 20 percent of the genes responsive to the C_{60} treatment. There is great similarity between the bacterial membrane component and C_{60} exposures but some difference in the genes expressed and the extent of the fold-change, indicating a potentially different mechanism for dealing with these nanomaterials.

In conclusion, trout macrophages are a sensitive tool to investigate the effects of nanoparticles on the immune system. Nanomaterials stimulate the immune system without complete cell toxicity, and the level of stimulation depends on the core structure and surface chemistry of nanomaterials. Functionalization may increase toxicity, and C_{60} may bind RNA and influence total gene expression in cells. Finally, nanomaterials have unique gene expression signatures.

Discussion

Dr. Petersen asked whether the researchers looked for fullerols inside the cells. Dr. Arndt responded that they had not, but it would be interesting.

Dr. Jovanovic asked whether there is proof that fullerols bind to everything. Dr. Arndt replied that this is a hypothesis that the laboratory will investigate. Dr. Jovanovic added that a design for Parkinson's disease is to bind the second messenger to stop second messenger pathways.

Characterization of the Potential Toxicity of Metal Nanoparticles in Marine Ecosystems Using Oysters—Silver Nanoparticle Studies With Adults and Embryos
Amy Ringwood, UNC Charlotte

Oysters are coastal estuary organisms. Filter-feeding bivalves are good models because they are highly effective at removing particles, have high filtration rates, and sample the water column and surface and resuspended sediments. Additionally, there is extensive information regarding their toxic responses to metals and organic contaminants. Oyster nanoparticle studies in adults indicate that lysosomal destabilization, lipid peroxidation, antioxidant responses, and tissue and cellular accumulation occur. Embryo exposure to nanoparticles results in antioxidant responses and normal development. The researchers used lysosomal destabilization assays extensively and determined that lysosomal endpoints have biological and ecological relevance. The researchers worked with a variety of nanoparticle types and shapes.

Results showed a dose-dependent lysosomal destabilization response to fairly low concentrations of nanosilver "seeds" in a citrate-based preparation. The researchers attempted to work with environmentally relevant concentrations. Lipid peroxidation is significant in the hepatopancreas at higher doses of nanosilver seeds, but no significant effect is seen in gills, suggesting that oyster gill responses differ from those of fish gills. Glutathione was not significantly upregulated, as was expected. There was a threshold response in terms of embryo development at the highest concentration. Data indicated an increase in metallothionein gene expression, particularly in embryos.

Similar experiments were carried out with polyvinylpyrrolidone (PVP)-coated "spheres" and "prisms." Spheres significantly increased lysosomal destabilization compared to control, and prisms significantly increased lysosomal destabilization compared to control and other treatments. There was no significant increase in lipid peroxidation as a result of sphere or prism exposure. Prisms appear to increase toxicity, showing a shape-based effect. Exposed oyster embryos showed a threshold-based response, and prisms were toxic at lower concentrations compared to other shapes. Seeds, prisms, and "plates" significantly increased embryo ROS production compared to control, and prisms significantly increased embryo ROS production compared to other treatments.

In summary, nanosilver prisms were found to be more toxic than spheres and plates in adult and embryo oyster studies. Mechanisms of toxicity were associated with lysosomal dysfunction and oxidative stress. PVP-coated particles may be slightly less toxic than citrate-based preparations. Oysters and other filter feeding bivalves are valuable model organisms for characterizing potential nanoparticle toxicity.

Discussion

In response to a question from Dr. Lowry, Dr. Ringwood explained that oyster hepatopancreas cells have a pH of 7.3. Dr. Lowry asked if this is related to differences in dissolution and amounts of available ion. Dr. Ringwood responded that this is a good question, and she was unsure of the answer, but there is evidence of a shape-based effect. Dr. Lowry asked whether the researchers had used prism forms of other nanoparticles. Dr. Ringwood answered that they have completed some studies with titanium, and they will continue to explore this.

Dr. Barber asked about the strength of the seawater, to which Dr. Ringwood responded that it is about 25 parts per thousand, which is not full strength. Dr. Barber asked about the solubility and effects of silver toxicity at various concentrations of chloride. Dr. Ringwood answered that the researchers have not examined the range of salinities, but they have worked with dynamic light scattering and TEM analysis, which suggest increased aggregation in the distilled water and lowest salinity preparations.

Dr. Xia thought that the shape-based effect was interesting and asked whether the different geometries were prepared by the same chemical process. Dr. Ringwood explained that seeds are a precursor for prisms, which in turn are precursors for plates. The effect was geometric and not chemical.

Dr. Chen was surprised that PVP was found to be less toxic, and Dr. Ringwood agreed.

AFTERNOON SESSION 2: NANOPARTICLES AND WASTE TREATMENT

Bioavailability of Metallic Nanoparticles and Heavy Metals in Landfills
Zhiqiang Hu, University of Missouri

Silver ions and nanoparticles are commonly used in consumer products, and predicted silver concentrations in sludge in wastewater treatment plants range from 7 to 39 mg/kg. In North America, approximately 2,200 mg of silver per year are wasted through landfill, accounting for approximately one-half the total wasted silver. Nanosilver flows from products to the environment with potentially high exposure, and a significant amount ultimately goes to landfills. Silver ion has been found to affect bacterial growth, interact with thiol groups, deactivate vital enzymes, and inhibit DNA replication. Silver nanoparticles inhibit autotrophic bacterial growth and are highly toxic to zebrafish, daphnids, and algal species. Silver nanoparticles less than 10 nm in size may enter cells directly to release silver ions. There are two types of sanitary landfills. Conventional landfills are based on the storage/containment concept and offer slow and natural degradation with no recirculation. Bioreactor landfills offer leachate recirculation, increased degradation rates, improvement of the setting ability of solids, and recovery of landfill space; they also enhance methane generation in the leachate. Major biological processes in bioreactor landfills are hydrolysis, acidogenesis/acetogenesis, and methanogenesis. Methanogens are important microorganisms for final biogas production and good indicators of functional anaerobic bioreactor landfills.

The experimental design utilized municipal solid waste from a bioreactor landfill site in Columbia, Missouri. Results indicated that there was a significant difference in gas volume between the control and each of the reactors treated with the low and high concentrations of nanosilver. Solids treated with a low concentration of nanosilver showed no inhibition of anaerobic process, whereas those treated at the higher concentration affected biogas generation rate and volume. The pH drop resulting from volatile fatty acid accumulation and the changes of leachate chemical oxygen demand in the bioreactor treated with the higher

nanosilver concentration confirmed the inhibitory effect of nanosilver on anaerobic biodegradation of solid waste. The dynamic changes of volatile fatty acids and acetic acid from the reactor containing the higher nanosilver concentration confirmed the accumulation of volatile fatty acids and acetic acid, resulting in a consistently low pH in the leachate; these results are consistent with the biogas production profile.

During the early stage of anaerobic decomposition, Methanobacteriales was the dominant organism (greater than 90%) in the control and low-nanosilver-concentration bioreactors. By comparison, *Methanosaeta* accounted for 40 percent of the bacterial species present in the high-nanosilver-concentration bioreactor. Additionally, the methanogenic bacterial population continues to evolve in bench-scale bioreactor landfills. Results of experiments focusing on total silver in leachate indicate that silver could be precipitated or absorbed in landfill solid waste.

In summary, there was no significant difference in the cumulative gas production between the low-nanosilver-concentration bioreactor and the control, whereas the high-nanosilver-concentration bioreactor resulted in reduced biogas production, volatile fatty acid accumulation, and lower pH in the leachate. Other results demonstrated a dominant population shift from acetoclastic methanogens to hydrogenotrophic methanogens at the early stage of anaerobic solid degradation. These results could be useful to regulatory agencies and landfill operators for decision-making and remedial actions.

Discussion

In response to a question by Dr. Holden, Dr. Hu replied that methane was not measured initially because of CO_2. At the early stages, there is no methane present.

Dr. Zhang asked how the researchers inoculated the methanogens and whether they used organic sludge. Dr. Hu answered that the source of the methanogens was the municipal solid waste landfill, so they already were inoculated. The food source was the organic waste from the landfill.

Dr. Mahendra asked Dr. Hu to clarify whether he thought that the acetoclastic methanogens were more sensitive to silver than the hydrogenotrophic methanogens and whether this was the reason for the metabolism shift. Dr. Hu replied that examining the substrate at the earliest stages was beneficial to attempt to answer this question. The predominant reactions at early stages of the process favor the hydrogenotrophic methanogens. Dr. Mahendra commented that metals comprised 1 percent of the waste at municipal solid waste landfills and asked whether the researchers had characterized what metals are present, as methanogens are susceptible to copper. Dr. Hu responded that silver was the only metal measured, but the controls helped to determine that copper susceptibility is not the cause of the results.

Biological Fate and Electron Microscopy Detection of Nanoparticles During Wastewater Treatment
Paul Westerhoff, Arizona State University

The goal of the project is to quantify interactions between manufactured nanoparticles and wastewater biosolids. The laboratory hypothesizes that dense bacterial populations at wastewater treatment plants should effectively remove nanoparticles from sewage, concentrate nanoparticles into biosolids, and/or possibly biotransform nanoparticles. The relatively low nanoparticle concentrations in sewage should have negligible impact on the wastewater treatment plant biological activity or performance. The researchers aim to develop mechanistic models for nanoparticle removal in wastewater treatment plants. Dr. Westerhoff highlighted three papers that examine the release of nanosilver in consumer products and noted that his laboratory submitted a paper that examines detection of fullerenes in cosmetic products. The dominant removal mechanisms at wastewater treatment plants are settling and biosorption; therefore, the research evaluated batch sorption to biomass, continuous loading bioreactors, and occurrence at full-scale treatment plants.

Previous research indicated that surface properties were important to biosorption of nanoparticles on heterotrophic wastewater biomass. The researchers confirmed that the EPA sorption method is not valid for nanosilver, and it is likely that it is not valid for other nanomaterials as well. Data also indicate that the primary mechanism of the removal of nanomaterials is the interaction of the nanomaterials with wastewater biomass. Results also indicated that freeze-dried biomass has different morphology. Next, the researchers performed a continuous nanomaterial loading study. The removal of functionalized silver is a function of the amount of biosolids present in the system. The same pattern is seen with titanium (i.e., biosolids concentration decreases).

In terms of occurrence at full-scale wastewater treatment plants, nanoscale, microscale, and mixed element titanium already are found in biosolids at these plants. The researchers evaluated the presence of TiO_2 at several wastewater treatment plants, as well as membrane technologies to characterize or remove nanomaterials. Data indicate that titanium is well removed at wastewater treatment plants in Arizona. Other experiments showed that nanomaterial surface properties were more important than membrane material properties. Tighter ultrafiltration rejection was high, but recovery indicates significant absorption.

In summary, nanomaterials will accumulate in biosolids. Approximately 60 percent of wastewater treatment plant biosolids are land applied, 22 percent are incinerated, and 17 percent are sent to landfills. Better tools are needed to differentiate engineered from "other" nanoparticles in wastewaters, and pollutant removal models for wastewater treatment plants currently are not suitable for predicting the fate of nanoparticles. Better relationships between surface charge and core composition versus biosorption are needed. Finally, the fate of nanomaterials in biosolids is poorly understood.

Discussion

Mr. Shapiro asked what the most cost-effective treatment would be for wastewater treatment plants. Dr. Westerhoff responded that the best goal would be to design wastewater treatment plants to stop all pollutants via a membrane bioreactor and tighter membranes.

A participant asked whether the biosolids were returned to the anaerobic bioreactor directly from sludge. Dr. Westerhoff answered that all activated sludge is returned from the aeration basin; there are plans to perform anaerobic digester sampling.

Dr. Holden asked whether settling characteristics are affected by the affinity to biomass. Dr. Westerhoff replied that it was much more difficult with nanosilver compared to C_{60} to control the sequencing batch reactors.

Dr. Huang asked how the researchers determined TiO_2 plus and minus. Dr. Westerhoff explained that the researchers did not determine these factors as the TiO_2 was acquired from a commercial source.

Analysis and Fate of Single-Walled Carbon Nanotubes and Their Manufacturing Byproducts in Estuarine Sediments and Benthic Organisms
P. Lee Ferguson, Duke University

Single-walled carbon nanotube composites have made their way into the marketplace, and numerous companies now supply single-walled carbon nanotubes on a kilogram scale. Annual worldwide production of single-walled carbon nanotubes is estimated to be greater than 1,000 tons by 2011. Currently, there are no reliable methods to detect single-walled carbon nanotubes in complex mixtures at low concentrations. The laboratory takes advantage of unique structural properties of single-walled carbon nanotubes that create unique electronic properties. The overall research objective is to implement and apply near-infrared fluorescence (NIRF) spectroscopy for qualitative and quantitative analysis of single-walled carbon nanotubes in complex environmental media. The specific objectives are to: (1) develop sample preparation

methods for isolating single-walled carbon nanotubes from sediment and tissue prior to NIRF spectroscopy, (2) explore asymmetric flow field flow fractionation coupled with NIRF spectroscopy for separating single-walled carbon nanotubes and reducing interferences, and (3) apply asymmetric field flow fractionation (AFFF)-NIRF spectroscopy to analysis of single-walled carbon nanotube uptake and accumulation in sediment-dwelling organisms. Researchers use a multilaser near-infrared spectrofluorometer to excite the samples and measure emissions at defined excitation. The laboratory method is quantitative with little matrix effect and is reproducible.

Results indicated that single-walled carbon nanotubes are detectible in complex sediment extracts using AFFF-NIRF spectroscopy, and single-walled carbon nanotubes do not degrade in sediments during a 1-month timescale. Single-walled carbon nanotubes were undetectable in sediment-exposed amphipods and mysid shrimp. When the researchers measured single-walled carbon nanotube body burden in sediment- and/or food-exposed organisms, they found that the nanotubes are present in nondepurated amphipods. Accumulation of single-walled carbon nanotubes in benthic macroinvertebrates and single-walled carbon nanotube bioaccumulation and trophic transfer using worm and clam species also were examined. No internal filter artifacts were present in the NIRF analysis of clam extracts. Additional microcosm-based experiments will track the uptake of single-walled carbon nanotube manufacturing byproducts in sediment-dwelling organisms as well as degradation in sediments, investigate chirality and diameter-dependence of single-walled carbon nanotube interaction with sediment and organisms, and survey environmental media for contamination with single-walled carbon nanotubes.

The researchers concluded that a novel and highly sensitive method based on NIRF spectroscopy for analysis of single-walled carbon nanotubes in sediments has been developed. NIRF spectral features of single-walled carbon nanotubes were retained after extraction from sediment, allowing diameter and chirality characterization for dilute solutions. AFFF can be used as a clean-up tool prior to NIRF analysis. Single-walled carbon nanotubes do not appear to be highly bioaccumulative in estuarine invertebrates exposed via sediment or dietary routes.

Discussion

Dr. Zhang asked whether the extraction procedure was sensitive to the sample matrices and whether there was a positive control to show nanotubes in biomass. Dr. Ferguson responded that calibration curves are used to compare sediment extract spiked with nanotubes at different concentrations and clean surfactant solution at the same concentration to ensure that there is no matrix effect. In terms of the second question, positive control experiments always are performed to ensure that they can matrix spike and recover.

Dr. Zepp asked whether there was a faster, less expensive method to clean up the samples other than AFFF. Dr. Ferguson replied that the nanotubes are "sticky." The researchers tried ultrafiltration, which did not work. XAD is a possibility that the laboratory could try.

Dr. Holden asked whether the developed method could be used to identify rare earth nanomaterials. Dr. Ferguson answered that it was possible if the excitation and emission pairs could be matched, but time-resolved fluorescence might be more appropriate for rare earths.

A participant from EPA asked about the recovery with the AFFF and noted that there appeared to be bimodal distribution in the sediment extract based on the results that were presented. Dr. Ferguson agreed that there was bimodal distribution. The sediment matrix type makes a difference in peak shapes of the AFFF. Natural organic material sorption to nanotubes is important. There is significant recovery of nanotubes on membranes.

Safety/Toxicity Assessment of Ceria (A Model Engineered Nanoparticle) to the Brain
Robert Yokel, University of Kentucky

The objective of this research is to characterize the physicochemical properties of a model engineered nanomaterial that influence its biodistribution and effects, including distribution across the blood-brain barrier, effects on oxidative stress endpoints in the brain, uptake into selected peripheral organs, and persistence over time. The researchers studied ceria (also known as cerium dioxide or cerium oxide) because it is an insoluble metal oxide that can be readily observed and quantified in tissue. Also, ceria has current commercial applications and has been reported to be cytotoxic as well as neuroprotective, representing the controversy about nanoscale materials. The laboratory prepared and characterized citrate-coated ceria of five different sizes.

When the researchers assessed the influence of size on engineered nanomaterial distribution, persistence, translocation, and toxicity, highest concentrations were found in the spleen and liver. Cerium found in the brain did not necessarily cross the blood-brain barrier. Liver and spleen showed little decrease in cerium concentration during a 30-day time period. The researchers concluded that brain cortex cerium always was less than 1 percent of the dose, and ceria was seen only in brain vasculature. Spleen cerium concentration was greater than liver cerium concentration, although liver had the greatest mass amount of the ceria dose. There was little decrease in liver and spleen cerium up to 30 days.

Oxidative stress markers and antioxidant enzyme levels and activities were determined following exposure to 5, 30, and 65 nm ceria, and significant changes were seen. *In vivo* exposure to ceria indicated that cerium concentrations in the blood decreased over time. The 15 and 30 nm ceria predominantly associated with blood cells, whereas the 5 and 65 nm ceria were generally evenly distributed between the two compartments. The greatest association of the 30 nm citrate-coated ceria with blood cells in the clot fraction is consistent with reports showing that this size is optimal for protein wrapping of engineered nanomaterials. A 90-day survival study to assess longer term distribution, persistence, and effects revealed that exposure resulted in modestly decreased body weight gain, and ceria was retained primarily in reticulo-endothelial tissues. No significant decrease of the mass amount of ceria in liver and spleen was seen during the 90-day period. Liver pathology was examined 30 and 90 days postexposure for 5 and 30 nm ceria, and results 30 days after exposure to 5 nm ceria showed nonuniform granuloma formations that contained ceria-loaded Kupffer cells and mononucleated cell infiltration among the hepatic parenchyma and at perivascular sites. Mononucleated cells appeared to encircle Kupffer cells, and there was no evidence of fibrosis or abscess formation. Live pathology 90 days after exposure to 30 nm ceria showed granulomatous formations.

Ultimately, the researchers concluded that citrate-coated 5 to 65 nm ceria do not enter the brain to any significant extent, and ceria primarily is cleared by reticuloendothelial organs and sequestered in intracellular agglomerates. The cerium valence does not change *in situ* during the first 30 days. There is little clearance of 5 to 65 nm ceria from reticuloendothelial organs. The smaller the ceria, the longer it remains in blood before being cleared. Maximal distribution into blood cells was seen with 30 nm ceria, and granulomatous formations were seen. Ceria and the cerium ion are very slowly eliminated, and ceria does not always behave in a manner similar to the cerium ion in its distribution in blood or tissues. These results further support the concern about the long-term fate and adverse effects of inert nanoscale metal oxides that reach systemic circulation, from which they can distribute throughout the body, resulting in persistent retention and potential adverse effects in multiple organs.

Future plans are to complete the histopathology, agglomeration extent and localization, cerium valence, and oxidative stress marker analyses as a function of time following 30 nm ceria infusion; assess the biodistribution and effects of a noncubic/nonpolyhedral ceria in the rat; and perform more direct assessment of the physicochemical properties of ceria that influence brain uptake and blood-brain barrier effects.

Discussion

Vishal Shah (Dowling College) noted that most of the results appeared to be primarily dependent on the stimulation and asked whether this might have more to do with stabilization than the nanoparticles. Dr. Yokel replied that surface properties probably are the most important variables.

OPEN DISCUSSION

Mr. Shapiro opened the discussion regarding all issues that were introduced during the day. Dr. Xia thought that the lipid bilayer-water partitioning approach was a better technique than octanol-water partitioning, but he was concerned about the size and thickness of the lipid bilayer. Dr. Posner stated that the thickness of the lipid bilayer is 4 nm, so it is small relative to the particle. One of the models that the researchers use is a biologically relevant surface, another model that examines passage is extruded, and the third measures conductance. Dr. Xia asked whether the effective surface was used when the partition coefficient was measured. Dr. Posner responded that examination of particle numbers showed a nice trend. The surface area does not deform in any way, so the surface area properties of the biological surface are well known. Dr. Xia asked whether this would be published, and Dr. Posner replied that it would be.

Dr. Huang thanked EPA for a very informative meeting and asked the EPA staff members to share their view of future directions. Dr. Savage replied that the next RFA will be released by February 2011. The specific details are unknown, but the general theme will pertain to the lifecycle of nanomaterials. There was a suggestion for EPA to request preproposals, and the EPA team still is working on the details.

Mr. Shapiro asked the participants what they thought should be the focus of the solicitation. A participant noted that the literature does not allow all results to be compared. Another participant noted that no study had demonstrated a nanoparticle that exerts acute toxicity at environmentally relevant levels. More research is needed on understanding mechanisms of factors other than acute toxicity. Dr. Yokel stated that it is acute versus chronic in terms of compensatory changes to repeated or prolonged exposure; therefore, regulation should be function of exposure. There could be a significant difference between acute and chronic toxicity. Mr. Shapiro asked if the typical 3- to 4-year grant cycle would be enough time to study chronic toxicity. Dr. Yokel replied that it depends on the model.

A participant thought that, in terms of susceptibility factors, acute toxicity beyond cell death and gene expression changes should be examined. How can researchers interpret an adaptive response? These types of data should be incorporated into risk assessment.

Dr. Heideman commented that there is a significant diversity regarding what people see as a potential risk and the best methods by which to examine these risks. He cautioned not to "put all of the eggs in one basket" so that this diversity is not missed. Dr. Savage noted that the strategy of EPA's Office of Research and Development identified classes of materials, which were included in the last solicitation; examining susceptible populations may go beyond populations currently known to be susceptible (e.g., children, elderly) when the genomic databases are established.

Dr. Heideman thought that preselecting a theme might prevent the submission of proposals that are too broad. Dr. Savage replied that the RFA must have a research topic. The current thought is that the RFA will focus on understanding nanomaterials throughout their lifecycles. Possibly, materials could be tiered. The compound-by-compound approach is not working, so a better method is needed to examine outcomes.

Dr. Heideman thought that each proposal should demonstrate a clear and present danger via preliminary results so that it is plausible that the research is addressing a truly hazardous situation. Dr. Hu commented that the purpose of EPA-funded research is to help the Agency with its regulatory needs, and decision-makers need to know the critical numbers to allow them to make informed decisions. Dr. Ringwood had

concerns about focusing on acute toxicity. Sublethal effects are different than chronic effects and can be important. She did not think that the analytical tools currently are available to definitively declare something a clear and present danger. It is more important to examine different kinds of potential receptors to identify those that increase susceptibility. Researchers must continue to examine diverse systems because different species may have different responses. She thought that there still was too much uncertainty to eliminate any systems because important risk issues may be missed.

Dr. Barber said that a missing element was how to apply the 5 years' worth of data that have been collected and begin to synthesize them into a product to help with risk assessment, which would help identify knowledge gaps. A participant noted that the National Institute of Environmental Health Sciences released a solicitation with a risk assessment core and agreed that the ability to apply data would be useful.

Dr. Savage explained that EPA is under more scrutiny than any other federal regulatory agency. Solicitations must be released for the sake of science, although program offices do supply feedback. All of the NNI agencies are realizing that data must be assimilated, but the question is which agency will maintain the resulting database. There has been some discussion that OECD will maintain it, but this is not confirmed. The Woodrow Wilson International Center for Scholars no longer is maintaining its Nanotechnology Consumer Products Inventory. These are issues with which federal agencies are struggling as budgets decrease.

Dr. Huang commented that a good use of the data would be to be able to generalize key issues in an intercorrelated manner. The various nanotechnology research groups could determine relationships.

Dr. Yokel asked the EPA staff members how useful data that have not been extensively characterized are in terms of risk assessment. Dr. Savage responded that characterization of data is very important to risk assessment. The new environmental health and safety strategy emphasizes characterization as a key issue. Unfortunately, many manufacturers incorrectly characterize their nanomaterials or cannot divulge characteristics because of their confidential business practices.

Dr. Petersen asked whether the EPA staff members had any recommended reading regarding what EPA has done in the past regarding uncertainty of pH and metals; this possibly could be applied to this field. He noted that other fields have had 30 years to work through these issues, and nanotechnology research is expected to have answers after only 5 years. Dr. Savage responded that past approaches are not working, so they should not be repeated. Nanomaterials are novel, but it may be possible to glean generalities that allow use of traditional chemical knowledge. Dr. Petersen thought that the new approach involving tiering was helpful in providing decision-makers with the best possible data to make informed decisions. Dr. Savage said that it also would be helpful for NIST to provide characterization. Dr. Petersen said that there are some options, but they require funding.

Dr. Heideman suggested the idea of identifying rules for nanomaterial groups so that it is not necessary to investigate each new one as it is developed. This is the only reasonable manner by which to approach this problem. His initial remark about determining a clear and present danger was intended to communicate the fact that research is not ready to develop these rules. Information on characterization and compound concentration are needed to compare results because there are so many potential hazards. Dr. Savage asked, if the research is not ready, how to get it ready. Dr. Heideman said that chemical companies constantly develop new products and could be a model for how to proceed in terms of nanotechnology.

Dr. Xia stated that Dr. Savage and the EPA team did an excellent job in developing the last RFA; the diversity of researchers and the amount of results and data presented during the meeting were impressive. He thought that it was beneficial to have many researchers to increase the diversity of the research.

Dr. Orr thought that the research community is "drowning" in too much data. The science is good, but researchers need to focus the data to develop practical guidelines and predictions. There needs to be a compromise so that the focus is not too narrow or broad. She suggested that EPA choose a common nanomaterial and have all of the researchers focus on it.

Dr. Yokel asked whether OECD was assembling the data to synthesize U.S. collaborations. Dr. Savage explained that currently OECD is gathering its own data. Dr. Yokel asked whether there were enough data to start a data-gathering effort. Dr. Savage stated that there are enough data but no one to maintain a database. Dr. Yokel thought that regulatory agencies would have a stake in this and, therefore, would be interested in funding an effort. Dr. Savage agreed and said that the Consumer Product Safety Commission, Occupational Safety and Health Administration, Food and Drug Administration (FDA), and EPA are the agencies with the most interest, but it is falling to EPA, the agency with the smallest budget.

Dr. Ringwood asked whether industry could be pressured to provide funding. Dr. Savage explained that public-private partnerships were being explored; this is in the strategic plan, but the data would not be available to the general scientific public. Dr. Kavanagh noted that in addition to the example of the Health Effects Institute, the Superfund Basic Research Program and a training effort funded via oil taxes also serve as examples. The latter recognize that many problems were the result of synthetic manufacturing based on petroleum. Although taxation should not be used to the extent that it stifles innovation, it is one possibility. Another example is that pharmacological companies pay FDA for each new investigative drug. Dr. Savage agreed that taxation was not favorable in the current political and economic climate, and Dr. Xia suggested that industry be charged a "registration fee." Eric Grulke (University of Kentucky) added that many of the U.S. companies that manufacture nanomaterials are small businesses and would not be viable if taxes were leveraged against them. Much of the value added for nanomaterials is how they are used in various media; therefore, functionalization is critical. This is an important clash that needs to be addressed. Dr. Savage explained that many small businesses came to EPA and were very proactive regarding potential problems.

Dr. Holden thought that larger federal agencies that benefit from the research (e.g., Department of Defense) should be lobbied to increase funding for nanomaterial research. Environmental toxicologists could partner with these agencies' researchers. Dr. Savage agreed that some agencies have dedicated more funding since 2003, and they could increase funding significantly, but it is not their mission. Even the Department of Energy should be more interested because it is in their best interest and the interest of the United States.

A participant said that the training of young scientists should be a priority. Dr. Savage explained that EPA's People, Prosperity, and the Planet (commonly known as P^3) Program accomplishes this.

Mr. Shapiro thanked the participants for attending on behalf of himself and Drs. Savage and Lasat and adjourned the meeting at 6:44 p.m.

Abstracts and Presentations

Day 1, Monday, November 8, 2010

AM Session 1: Systems Approaches

Vicki H. Grassian

An Integrated Approach Toward Understanding the Impact of Aggregation and Dissolution of Metal and Metal Oxide Nanoparticles

Vicki H. Grassian
Department of Chemistry and Nanoscience and Nanotechnology Institute, University of Iowa, Iowa City, IA

Nanoparticles, the primary building blocks of many nanomaterials, may become suspended in air or get into water systems (e.g., drinking water systems, ground water systems, estuaries and lakes, etc.). Therefore, manufactured nanoparticles can become a component of the air we breathe or the water we drink. One important issue in understanding the environmental fate, transport, toxicity, and occupational health hazards of nanoparticles is in characterizing the nature and state of nanoparticles in air, water, or *in vivo*. For the nanoparticles of interest in these studies, metals and metal oxides, it can be asked: (1) will metal oxide and metal nanoparticles be present in air or water as isolated particles or in the form of aggregates?; (2) will metal oxide and metal nanoparticles dissolve in aqueous solution or *in vivo*?; and (3) under what conditions will metal oxide and metal nanoparticles aggregate or dissolve? As the size regime will be very different depending on the state of the nanoparticles, as dissolved ions, isolated nanoparticles, or nanoparticle aggregates, these questions are important to address as it impacts the size regime that needs to be considered or modeled in, for example, environmental transport or lung deposition models. Furthermore, the effect on biological systems including nanoparticle-biological interactions and toxicity will depend on the state of nanoparticles. In the studies discussed here, macroscopic and molecular-based probes that include quantitative solution phase adsorption measurements, ATR-FTIR spectroscopy, dynamic light scattering techniques and zeta-potential measurements are used to investigate the physicochemical properties including nanoparticle interactions as a function of important environmental variables such as pH, presence of organic ligands, surface chemistry, nanoparticle concentration, and solar irradiation. We have focused on several different metal and metal oxide nanoparticles in aqueous environments, including those that contain Fe, Ag, Zn, Cu, Ce, and Ti. Results for these different metal-containing nanomaterials will be presented with a focus on aggregation and dissolution in the presence of citrate, a common organic ligand found in the environment. This research is beneficial as it significantly contributes to the growing database as to the potential environmental and health implications of nanoscience and nanotechnology and how nanomaterials will behave in the environment and impact human health.

EPA Grant Number: R833891

An Integrated Approach Toward Understanding the Impact of Aggregation and Dissolution on the Fate, Behavior and Toxicity of Metal and Metal Oxide Nanoparticles

Vicki H. Grassian
Departments of Chemistry, Chemical and Biochemical Engineering and Occupational and Environmental Health
and Nanoscience and Nanotechnology Institute at the
University of Iowa

EPA Grantees Meeting - 2010
Portland, Oregon

Motivation

Interest in understanding the environmental and health implications of natural and engineered nanomaterials

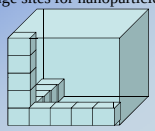
- Environmental Fate and Transport of Nanoparticles in Air and in Water Systems
  - -Nanoparticle Fe oxides as a reactive constituent in air, water and soil.
  - -Manufactured nanoparticles may become suspended in air during production, distribution, use and disposal(*i.e. any time during its life cycle*) or get into water systems (lakes, estuaries, ground water). Therefore, manufactured nanoparticles can become a component of the the air we breath or the water that we drink.
- Occupational Hazards and Toxicity of Airborne Nanoparticles
  - -Majority of reports indicate that exposure by inhalation is the greatest hazard faced by w-orkers in the nanotechnology industry. Furthermore, it is well known that ultrafine particles are associated with health problems. Therefore, in occupational settings, there may be associated risks with the production of nanomaterials.

Nanomaterials composed of metals and metal oxides are a large percentage of the commercially developed nanomaterials on the market and a focus of these studies.

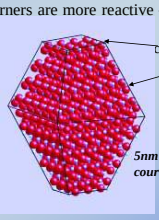
Nanoparticles Less Than ca. 20 nm Are of Particular Interest

Quantum Size Effects and Other Size-Dependent Properties Become Increasingly Important
Reactive Edge and Corner Site Density Increase

Most inorganic nanoparticles are not spherical in shape but in fact more cubic or octahedral in nature.
Defect sites which include edges and corners are more reactive – many corner and edge sites for nanoparticles.



1-5 cm x 5 cm cube has length of edges = 60 cm
125-1cm x 1cm cubes have length of edges = 1500 cm (25 times greater).
Edge length increases by 1/(factor of size change)^2
Number of corners increases by 1/(factor of size change)^3



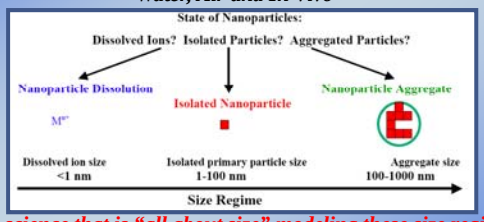
5nm TiO_2 particle courtesy of MJ Shultz

Literature Studies Suggest Unique Surface Reactivity for Smaller Metal and Metal Oxide Nanoparticles

Differences in reactivity due increase number of edge and corner sites? Does this make smaller nanoparticles more or less toxic, or behave differently in the environment compared to larger particles?

One Issue is Related to the *State* of Metal and Metal Oxide Nanoparticles in Different Environments and Under Different Conditions?

Water, Air and In Vivo

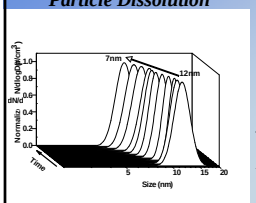


For a science that is "all about size" modeling these size regimes in e.g. transport and lung deposition models will be very different

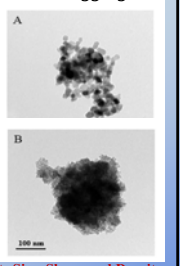
Size Issues Beyond Primary Particle Size

Particle Dissolution and Aggregation From the Particle Perspective

Particle Dissolution



Particle Aggregation



Effect Each Other

-Impacts Particle Size

-Specifically, With the Formation of Metal Ions and There is Also the Formation of Smaller Nanoparticles

-Dissolution Can Impact Aggregation By Causing Deaggregation as the Particles Within the Aggregate Dissolve

-Impacts Size, Shape and Density

-Impacts Available Surface Area

-Impacts Surface Chemistry Including Nanoparticle Dissolution

An Experimental Approach That Integrates Macroscopic and Microscopic Measurements and Methods Taken From *Surface Science, Surface Chemistry, Solid State and Materials Chemistry, Colloid Science and Aerosol Science* to Better Understand the Implications of Nanomaterials

X-Ray Diffraction and Microscopy
SEM, TEM and AFM

Surface Area
BET

Particle Sizing
SMPS
DLS

Metal and Metal Oxide Nanomaterials in Gas and Liquid Phase Environments

Surface Spectroscopy
ATR FTIR
Transmission FTIR
X-Ray Photoelectron Spectroscopy

Quantitative Reactor Studies
Adsorption Measurements
Reactivity Studies
Dissolution Measurements

along with studies of toxicity and biological interactions

Combined Integrated Approach to Better Understand Implications of Nanomaterials

Synthesize or Purchase Commercial Nanomaterial Powders

Bulk and Surface Characterization of Nanomaterials

Fate and Transformation in Water

Molecular, Particle and Batch Reactor Studies of Surface Adsorption and Dissolution in the Aqueous Phase

pH, ionic strength, organic ligands

Air/Aerosol and Inhalation Toxicity

Inhalation Toxicity with Aerosol Characterization, Inflammatory Response and Studies of Dissolution in Simulated Biological Fluids

Environmental Fate and Transport

Examples of the Physicochemical Properties and Conditions that Influence the Aggregation and Dissolution of Nanomaterials in Aqueous Environments

TiO_2 Nanoparticles and α-FeOOH Nanorods

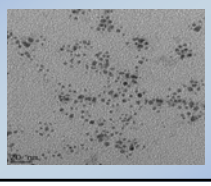
Titanium Dioxide Nanoparticles - ca. 4 nm

TiO_2nanoparticles from Nanostructured and Amorphous Materials are some of the smallest commercially manufactured oxide nanoparticles and is sold as having a primary particle size of 5 nm.

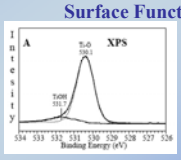
Characterization of Bulk and Surface Properties

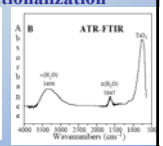
XRD anatase phase

TEM of isolated particles
3.5 ± 1.0 nm
(sonicated in methanol before deposition on to the TEM grid)



Surface Spectroscopy - Surface Functionalization





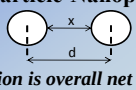
Surface Area - BET measured
219 ± 3 m^2 g^{-1}

Will they dissolve in water, aggregate or remain as isolated particles?

-No dissolution observed at 293 K

-Aggregation is observed at 293 K

Aggregation and Sedimentation in Aqueous Suspensions Will Depend on Nanoparticle-Nanoparticle Interactions



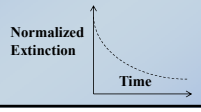
and whether that interaction is overall net repulsive or attractive ($V_{tot} = V_{rep} + V_{att}$) DLVO (Derjaguin, Landau, Verwey and Overbeek) theory can be used to calculate V_{tot}

Attractive Interactions ($V_{tot} < 0$)

DLS Measurements

Large Particle Size (Aggregate)

Light Scattering Measurements of Sedimentation **- Changes in light scattering as a function of time as the aggregates settle out**

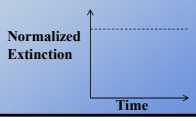


Repulsive Interactions ($V_{tot} > 0$)

DLS Measurements

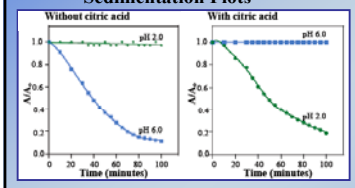
Small particle size

Light Scattering Measurements of Sedimentation **- Stable signal as particles remain suspended**



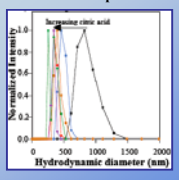
4 nm TiO_2 Particles in the Presence and Absence of Citric Acid, A Common Organic Acid Found in the Environment, at pH 2 and pH 6

Sedimentation Plots



Switch in stability of TiO_2 nanoparticle suspensions in the presence of citric acid.

DLS measurements with increasing addition of citric acid at pH 6



DLVO Calculations Along with Zeta Potential Measurements of the Surface Charge Show that TiO_2 Nanoparticle Suspensions Are Stable at Low pH in the Absence of Citric Acid and at Near Neutral pH in the Presence of Citric Acid

DLVO Calculations

$V_{tot} = V_{elec} + V_{vdW}$

Repulsive

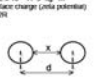
$V_{elec} = 4\pi\varepsilon R\Psi^2 \left[\frac{k_B T}{e}\right]^2 \frac{\exp(-\kappa x)}{x + 2R}$

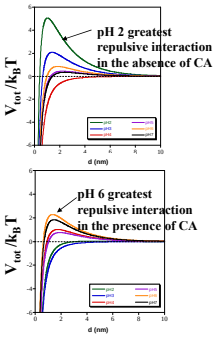
$\kappa = \left[\frac{1000 e^2 N_A (2I)}{\varepsilon k_B T}\right]^{1/2}$

$Y = \frac{8 \tanh(e\Psi_d/4k_B T)}{1 + \left[1 - \frac{(2\kappa R + 1)}{(\kappa R + 1)^2} \tanh^2(e\Psi_d/4k_B T)\right]^{1/2}}$

Attractive

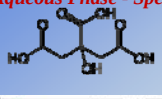
$V_{vdw} = -\frac{A_{12}}{6}\left[\frac{2R^2}{d^2 - 4R^2} + \frac{2R^2}{d^2} + \ln\frac{d^2 - 4R^2}{d^2}\right]$



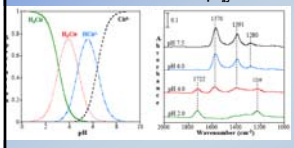


Surface Speciation and Surface Coverage of Adsorbed Citric Acid as f(pH)

Aqueous Phase - Speciation and pKa values



Citric Acid
pK_{a1} = 3.13
pK_{a2} = 4.76
pK_{a3} = 6.40



Surface Coverage as f(pH)

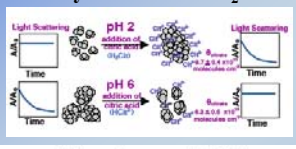
pH	Maximum Surface Coverage (molecules cm^{-2})
2.0	$9.7 \pm 0.4 \times 10^{13}$
4.0	$7.5 \pm 0.3 \times 10^{13}$
6.0	$6.3 \pm 0.5 \times 10^{13}$
7.5	$3.1 \pm 0.4 \times 10^{13}$

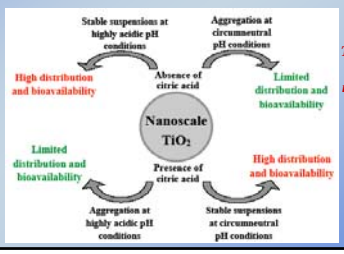
Surface Speciation

QuickTime™ and a decompressor are needed to see this picture.

Surface speciation suggests pKa values are lower for surface adsorbed citric acid. Less adsorption at higher pH a result of surface charge becoming more negative with increasing pH.

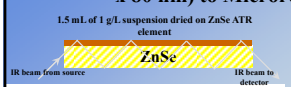
Summary of the Behavior of Some of the Smallest Commercially Available TiO_2 Nanoparticles

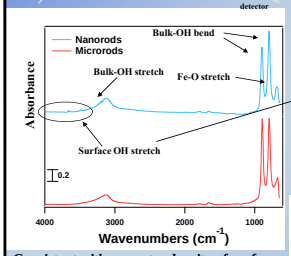


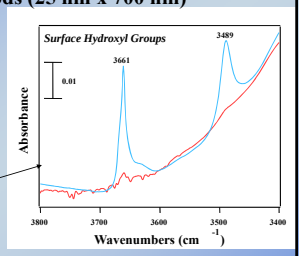


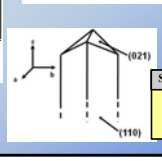
Thus mobility in the environment of nanoscale TiO_2 will depend on surface coatings, surface coverage, surface charge and pH in relatively complex ways.

Comparison of the Dissolution of of α-FeOOH Nanorods (7 nm x 80 nm) to Microrods (25 nm x 700 nm)







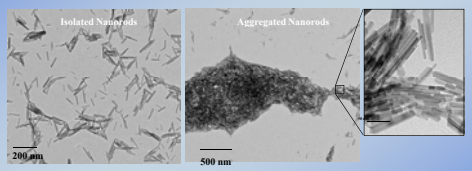


Surface Hydroxyl Groups	
(021)	8.2 groups/nm^2
(110)	3.0 groups/nm^2

Consistent with a greater density of surface hydroxyl groups found for nanorods. But even greater than surface area considerations

Barron & Torrent, JCIS, 1996

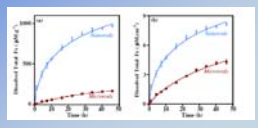
Nanorods Can Extensively Aggregate Under Certain Conditions



Enhanced Dissolution on the Nanoscale Is Quenched in the Aggregated State

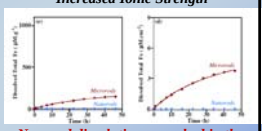
α-FeOOH Acid Assisted Dissolution at pH 2 Nanorods vs Microrods

Isolated Rods



Enhanced dissolution on the nanoscale

Aggregated Rods
Increased Ionic Strength



Nanorod dissolution quenched in the aggregated state

Rubansinghege, Lentz, Scherer and Grassian, Langmuir 2010

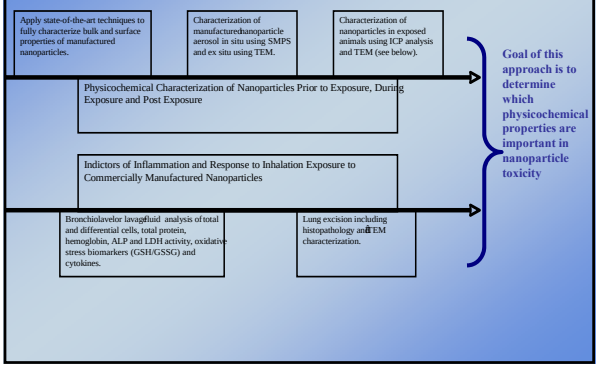
Therefore, dissolution will depend on aggregation and the aggregation state. Thus nanoparticle aggregation and dissolution (nanoparticle reactivity in general?) are connected in ways that are not yet fully understood.

Inhalation Toxicity

Size and Composition

Metal and Metal Oxides

Integrated Approach To Nanoparticle Inhalation Toxicity: Research Design



Comparison of Inflammatory Response of Mice to Different Metal and Metal Oxide Nanomaterial Aggregates on the order of 100 - 200 nm

Greatest Inflammatory Response Found for Cu-Based Nanoparticles as Determined by Elevated Cell Count in BAL Fluid and Greater Percentage of Neutrophils and Lymphocytes. Copper Nanoparticles Showed a Higher Propensity for Dissolution in Simulated Biological Media (Which Contain Citric Acid).

Adamcakova-Dodd, A.; Thorne, P.S.; Grassian, V.H. "In Vivo Toxicity Studies of Metal and Metal Oxide Nanoparticles" Handbook of Systems Toxicology, John Wiley and Sons 2010 (in press).

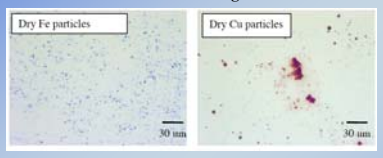
Fe and Cu Nanoparticle and Aerosol Characterization

	Iron nanoparticles	Copper nanoparticles
Primary Particle Size	25 ± 2 nm	12 ± 1 nm
Crystalline or Amorphous Material	Crystalline	Crystalline
Crystalline Phases	Fe, Fe_3O_4, γ-Fe_2O_3	Cu, Cu_2O, CuO
Surface Phase	γ-Fe_2O_3	CuO
Surface Functionality	O, O-H and H_2O	O, O-H and H_2O
Surface Area		
BET	17 ± 1 $m^2 g^{-1}$	12 ± 0.2 $m^2 g^{-1}$
Aerosol Size Distribution*		
Acute Exposure	187.0 (1.3)	187.9 (1.3)
Sub-acute Exposure	199.9 (1.3)	190.1 (1.3)

*GM, nm, (GSD) in Exposure Chamber

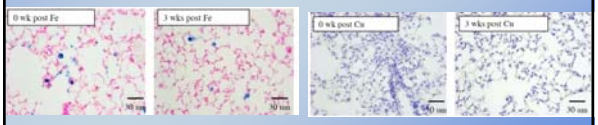
Lung Tissues Show No Evidence for Cu particles

Controls - Staining Alone



Fe (blue stain) present in macrophages

Cu (red stain) *not* present in macrophages



Suggesting Dissolution and/or Translocation of Cu particles

Conclusions and Acknowledgements

Environmental Fate and Transport: Metal and metal oxides show unique reactivity and physicochemical behavior on the nanoscale and this behavior will be impacted by aggregation. Surface chemistry and surface impacts aggregation and aggregation impacts surface reactivity (e.g. dissolution). Some ongoing studies include: size-dependent dissolution of ZnO nanoparticles and nanorods; aggregation and dissolution of copper nanoparticles in aqueous media as a f(pH) and presence of citrate aggregation and dissolution

EPA
Imali Mudunkotuwa, Thillini Rupasighege, Gayan Rubasinghege, Dr. Shaowei Bian

Inhalation Toxicity: Chemical composition, size and ability to undergo dissolution and translocation are important in the toxicity in ways that have not been discerned previously through inhalation toxicity studies. Additional studies on Ag, ZnO and Cu nanoparticles are currently underway

NIOSH
Professors Peter Thorne and Patrick O'Shaughnessy, Drs. John Pettibone, Andrea Adamacova-Dodd and Larissa Stebonouva

Thomas Theis

Life Cycle Analysis and Nanostructured Materials

Thomas Theis*[1], Bhavik Bakshi[2], Delcie Durham[3], Vasilis Fthenakis[4], Timothy Gutowski[5], Jackie Isaacs[6], and Thomas Seager[7]
[1]University of Illinois at Chicago, Chicago, IL; [2]Ohio State University, Columbus, OH;
[3]University of South Florida, Tampa, FL; [4]Columbia University, New York, NY;
[5]Massachusetts Institute of Technology, Cambridge, MA; [6]Northeastern University,
Boston, MA; [7]Arizona State University, Tempe, AZ

The term nanotechnology is now widely employed to describe the unique properties and applications of materials in the nm size range, typically taken to be 1-100 nm. Advances in our understanding of molecular events at the atomic or near-atomic level, coupled with new methods of measurement and observation, have led to the development of new products and manufacturing processes that comprise the domain of nanotechnology. Nanoproducts are defined as small structures of controlled shape, size, composition, and function (e.g., nanoparticles, carbon nanotubes, nanowires, nanofilms, quantum dots). Examples of industries or sectors where nanoproducts or nanomanufacturing methods are being used today include ceramics, membranes, coatings, composites, skin care products, biotechnology, semiconductors, and thin films. However, this area is growing rapidly, thus new applications and products will undoubtedly be developed in the near term.

Present environmental research on nanotechnology appears to be proceeding along two separate pathways; one as a receptive view recognizing nanotechnology as an enabling force providing benefits such as innovative remediation alternatives, improved catalysts and membranes, and better sensors for detection of contaminants, and the other as a precautionary view seeking to identify fate and transport, potential toxicity, risk, and health effects of nanostructured materials and resultant products. Significant research efforts on human health impacts are underway; however, there are comparatively few studies that have focused on the application of life cycle concepts.

This presentation will review the findings from a U.S. Environmental Protection Agency/National Science Foundation-sponsored workshop on life cycle analysis and nanostructured materials and products. It will examine the function and composition of nanostructured materials, their manufacture, and explore ways in which a life cycle approach can be used to guide research on their environmental and health properties, manufacturing methods, and end-of-life disposition.

EPA Grant Number: R831521

Life Cycle Analysis and Nanostructured Materials

Thomas L. Theis
Institute for Environmental Science and Policy
University of Illinois at Chicago

Nano Grantees Meeting
8 November 2010

NSF/EPA Workshop

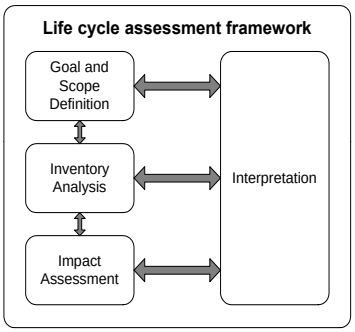
Life Cycle Aspects of Nanoproducts, Nanostructured Materials, and Nanomanufacturing:

Problem Definitions, Data Gaps, and Research Needs

Life Cycle Assessment

- A systems methodology for compiling information on the flow of materials and energy throughout a product chain
- LCA evolved from industry needs to understand manufacturing, and market behavior, and make choices among competing designs, processes, and products
- Defines four general sections of the product chain:
  - materials acquisition
  - manufacturing/fabrication
  - product use
  - downstream disposition of the product

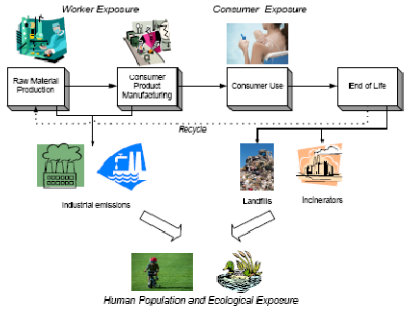
ISO 14040 & 14044



Major Impact Categories

HH (cancer)	kg benzene eq/unit
HH (non cancer)	kg toluene eq/unit
Global Warming	kg CO_2 eq/unit
Eutrophication	kg N eq/unit
Ecotoxicity	kg 2,4 D eq/unit
Acidification	eq H^+/unit
Smog Formation	kg NO_x eq/unit
Ozone Depletion	kg CFC eq/unit
Land Use	(in progress)

Life Cycle Assessment Stages (USEPA)



LCA and Environmental Regulation

•Adaption of LCA as a way to gather information on waste production, energy demand, and the potential for risk to exposed populations

•Works best when risks are non-local, and the population is non-specific

•Not a substitute for regulatory risk assessment

The Health/Materials Paradox

Why might nanostructured materials be toxic?

size
shape
composition
photoactivity
redox activity
solubility
environmental instability
potential for exposure

Those attributes of NSM's that are prized for commercial development and application, are the same ones that cause toxic reactions

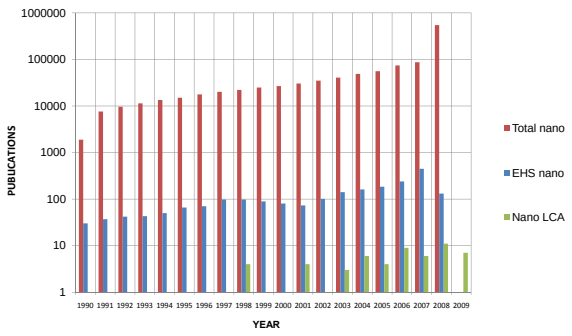
EPA Nanotech Research Focus

- Environmental Applications
  - Membranes, remediation, etc.
- Environmental Implications
  - End-of-pipe
  - Toxicity
  - Fate, transport, transformation
  - Focus on NPs already in commercial production
    - CNTs
    - Ag^0
    - Fe^0
    - TiO_2
    - CeO_2

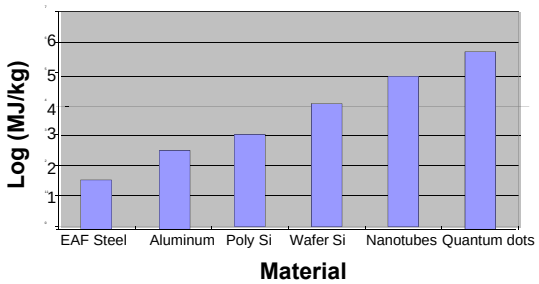
Elements of a LCA-Inspired Interdisciplinary Research Program for Nanotechnology

- Use of less toxic, more available components (eg. Cd, Pb-free, AlP)
- Focus on structures that are less bioavailable (e.g. coatings, solubility, stability, kinetics)
- Lowering of life cycle energy of manufacturing
- Design for recovery of nano-components at end-of-life
- Understanding the social contexts in which nano-based products are used and disposed of
- Application of LCA methodology to the entire product chain

Nanotechnology Publication Trends



Embodied Energy (Cradle-to-Gate)



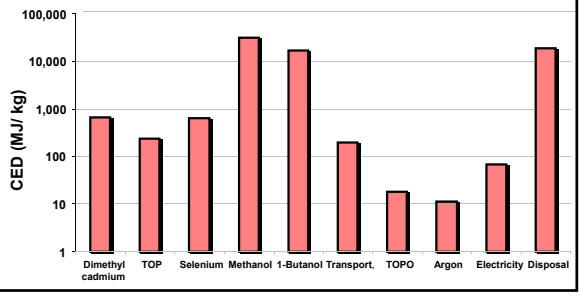
Adapted from Gutowski et al. 2007, and Sengul and Theis 2008.

Sources of Impacts During Manufacturing of NSMs

- Strict purity requirements and less tolerance for contamination during processing (up to "nine nines")
- Low process yields
- Significant energy requirements
- Batch processing (post-processing, reprocessing), or very low-yield continuous processing
- Use of toxic/basic/acidic chemicals and organic solvents
- High (or low) temperatures, pressures
- High water consumption

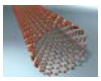
Sengul and Theis JIE, 2008.

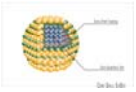
Cumulative energy demand (embodied energy) CdSe q-dots



The Energy Paradox

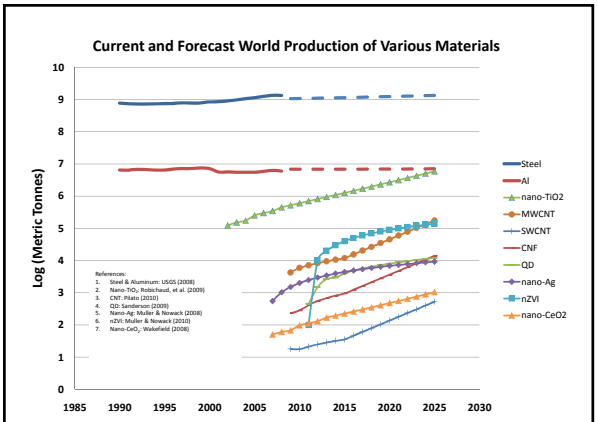
- Some of the most energy intensive materials known to humankind

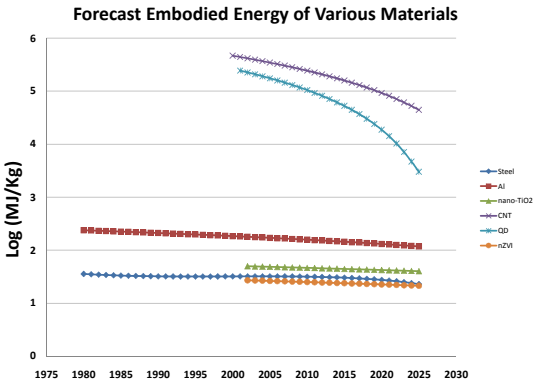




- Less than 1% (currently) of the mfg cost

(Healy, Isaacs, 2008)





Nano-based Energy Savings

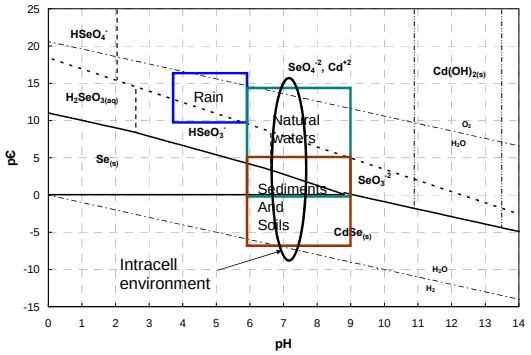
Table 3. Potential U.S. Energy Savings from Eight Nanotechnology Applications (Adapted from Brown, 2005 a)

Nanotechnology Application	Estimated Percent Reduction in Total Annual U.S. Energy Consumption**
Strong, lightweight materials in transportation	6.2 *
Solid state lighting (such as white light LED's)	3.5
Self-optimizing motor systems (smart sensors)	2.1
Smart roofs (temperature-dependent reflectivity)	1.2
Novel energy-efficient separation membranes	0.8
Energy efficient distillation through supercomputing	0.5
Molecular-level control of industrial catalysis	0.2
Transmission line conductance	0.2
Total	14.5

*Assuming a 5.15 Million BTU/ Barrel conversion (corresponding to reformulated gasoline – from EIA monthly energy review, October 2005, Appendix A)

**Based on U.S. annual energy consumption from 2004 (99.74 Quadrillion Btu/year) from the Energy Information Administration Annual Energy Review 2004

CdSe in aquatic environments



Composite materials are not recycled

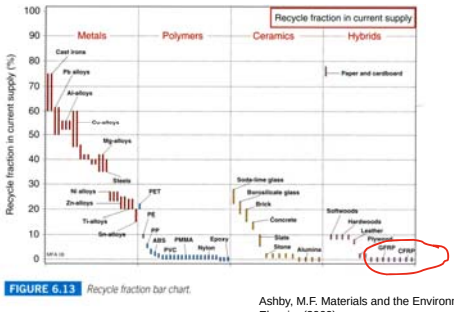


FIGURE 6.13 *Recycle fraction bar chart.*

Ashby, M.F. Materials and the Environment, Elsevier (2009)

To summarize…

Engineered nano-materials and products

- are already in use (how are they actually used?)
- are not widely understood by consumers
- are often energy intensive and materially inefficient to make
- have increasingly complex functionalities
- have very high "value added"
- often use or are composed of toxic and/or "scarce" chemicals in processing (availability?)
- are often difficult to recover once placed in commerce (recyclability of bulk matrices)?
- comparative benefits and impacts of nanoproducts?
- LCA research and applications for NSMs are lagging

Martin Shafer

Platinum-Containing Nanomaterials: Sources, Speciation, and Transformation in the Environment

***Martin Shafer*[1], *James Schauer*[1], *and Brandy Toner*[2]**
[1]University of Wisconsin-Madison, Madison WI; [2]University of Minnesota-Twin Cities, Minneapolis MN

Platinum is the archetypal element where chemical and physical speciation is essential for valid toxicology assessments, yet critical basic information on environmental pools, speciation, and reactivity is lacking. Anthropogenic platinum emissions to the environment have dramatically risen over the past 2-3 decades and consumptive use, particularly in nano-catalytic applications, is projected to increase. Nano-particulate species of platinum represent a major fraction of total platinum in most primary emissions, though it was thought to be present in relatively benign elemental species. Recent evidence, however, indicates that primary emissions may contain a significant oxidized platinum component and some studies suggest that the speciation of nano-platinum can change rapidly after release into the environment—a factor that must be considered in fate/transport and toxicology modeling. Information on environmental levels of the recognized toxic species of platinum (chloroplatinates) is essentially absent.

Our research addresses three major questions: (1) what are the primary sources and environmental receptors of platinum and nano-platinum? (2) what are the chemical forms of platinum introduced into the environment from current and potential major sources? and (3) how does the speciation of platinum change within specific environmental reservoirs after release? Our focus is on aerosol-mediated emissions, transport, and exposure in non-occupational settings. Emissions from vehicles (exhaust catalysts [e.g., Three-Way-Catalysts, TWC] are a major source of environmental platinum) are being addressed using roadside aerosol sampling and a synoptic program of roadway dust sampling. Engine dynamometer experiments are being conducted to evaluate platinum emissions from platinum-cerium based fuel-borne catalysts (FBC). High-volume air samplers are used to collect ambient aerosols in several urban environments. Concentrations and chemical speciation of platinum in particulate and "soluble" phases of these samples is being determined with a suite of analytical tools. Synchrotron XAS (sXAS) is applied to solid phases. "Soluble" species, as defined with physiologically relevant fluid extractions, including Gamble's Saline and Alveolar Macrophage Vacuole Fluid, are characterized for particle size (Ultrafiltration and STEM), and charge (Ion Chromatography). The presence of the particularly toxic chloroplatinate species is being probed using an HPLC-IC-ICPMS method. Platinum species transformation will be evaluated in controlled laboratory experiments with both environmental and model samples.

Road dust collections from multiple sites in cities across the country (including Atlanta, Denver, Los Angeles, Milwaukee) exhibit elevated levels of total platinum (200–800 ng/g). Significant (8-23% of total) soluble pools of platinum, with measureable anionic character, were measured in these vehicle emission receptor samples. Our sXAS studies (ANL-APS, 20-BM) of aerosol emissions (PM) from diesel engines burning a Pt/Ce-based FBC reveal a large fraction of oxidized platinum. Spectral fitting suggests that a platinum(IV)oxide-hydrate is the dominant oxidized platinum species in the engine PM. Similarly, a substantial component of the platinum pool in used TWCs was found to be oxidized. The majority of the primary emissions of platinum from diesel engines burning a Pt-FBC was present in fine and ultra-fine particle-size fractions. We have advanced the HPLC-IC-SFICPMS analytical methodology for separation and detection of hexa- and tetra-chloroplatinate to achieve quantification limits of lower than 10 ng/L—an order-of-magnitude better than reported in the literature—and we are working to further improve these limits.

Through our multidisciplinary approach, we expect to substantially advance our understanding of the sources, speciation, transformation, and potential human exposures to nano-platinum materials in the

environment. We expect to provide some of the first measurements of the recognized toxic species of platinum in environmental media. Vital information on the concentrations and chemical species of platinum in mobile source emissions and important environmental receptors will be provided. Fundamental data on rates of species transformation will be acquired. The chemical speciation and exposure data will enable enhanced assessments of the toxicological relevance of environmental nano-platinum species.

EPA Grant Number: R833892

Platinum-Containing Nanomaterials: Sources, Speciation and Transformation in the Environment

Martin Shafer[1], James Schauer[1], Brandy Toner[2]

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Environmental Chemistry & Technology
Program and State Laboratory of Hygiene
[2]University of Minnesota-Twin Cities

U.S. EPA Nanotechnology Grantees Meeting
November 08-09, 2010
Portland, OR

This research is funded by U.S.EPA - Science To Achieve Results (STAR) Program
Grant # R833892

General Motivation

- Platinum is an archetypal element where speciation is essential for valid toxicological assessments, yet relevant information on environmental pools, speciation and reactivity is lacking.
- Certain platinum species (most notably the chloroplatinates) are toxic (allergenic).
- Platinum levels in many environmental receptors has increased over the past 40 years due to platinum use in automobile exhaust catalysts and industrial catalysts.
- Platinum-based catalysts are likely to remain a key strategy for reduction of regulated pollutants from mobile sources.
- Though platinum in most primary emission sources was thought to be present in relatively benign elemental [Pt^0] species, evidence is mounting that the speciation of platinum (particularly nano-sized platinum) can change rapidly after release.

Motivation (Mobile Sources)

- **Controlling emissions from mobile sources are critical for continued reduction in health impacts of air pollution, and for addressing regional and global climate impacts.**
- **Most current and proposed emission control strategies for diesel and gasoline engines employ metal catalysts to reduce tailpipe emissions of regulated species.**
  - ***Gasoline Three-Way-Catalysts (Pt, Pd, Rh)***
  - ***Diesel Fuel-Borne Catalysts (Pt-FBC)***
  - ***Diesel Particulate Filters (Pt-Catalyzed)***
  - ***Diesel Selective Catalytic Reactors (V-SCR)***
- **The use of these metals raises concerns about environmental dissemination.**

MOTIVATION – Chemical Speciation

- **The toxicological responses of many metals (e.g. Cr, Cu, Mn, Pt, V) are determined by the specific chemical & physical speciation in the primary source or environmental receptor.**
- **Extant modern methodologies provide little relevant speciation information.**
- **Traditional techniques that are speciation capable lack the required sensitivity, particularly in the context of (a) ambient aerosols, and (b) lower emissions from vehicles equipped with modern control devices.**

Oxidized, halogenated (e.g. chloroplatinic acids) species (H, NH4, K, Na) are very soluble and are 500-fold more toxic than metallic species.

- **OSHA-PEL / ACGIH-TLV**
  - **Soluble salts – 0.002 mg/m³ (Pt)**
  - **Metal – 1 mg/m³**
- **EPA – Toxicological Review (2009)**
  - **$NOAEL_{ADJ}$ $1x10^{-6}$ mg/m³**
  - **RfC (halogenated Pt salts) $1x10^{-9}$ mg/m³**

Specific Objectives of Study

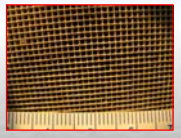
- Refine analytical tools for measurement and chemical speciation of platinum in environmentally relevant sources and receptors.
- Integrate source and environmental sampling with advances in platinum analytical speciation tools.
  - **Determine the physical and chemical forms of platinum introduced into the environment from selected current and potential major sources.**
  - **Evaluate changes in the speciation of platinum within specific reservoirs after release to the environment.**

- ✓**Aerosol Sources**
- ✓**Soluble Species**
- ✓**Toxic Species**
- ✓**Nano-sized Species**

Soluble Species	"Insoluble" Species
Halogenated Pt Salts (chloroplatinates)	Pt metal
Cisplatin, Carboplatin	Pt oxides (PtO, PtO_2)
$PtCl_4$	$PtCl_2$
$Pt(SO_4)_2$	Pt sulfides (PtS_2)
$Pt(NH_3)_4Cl_2$	Pt(cyclo-octadiene)
$PtBr_4$	$PtO_2 \cdot H_2O$

Primary Platinum Sources and Receptors Under Study

- ❑ **Automobiles: Three-Way-Catalysts (TWC).**
- ❑ **Diesel Engines: Platinum-Amended Diesel Fuel (FBC) and Platinum-Catalyzed Particulate Filters (DPF)**
- ❑ **Roadside Dust / Soils**
- ❑ **Ambient Aerosol from Urban Centers**



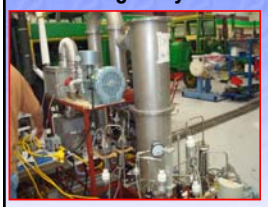
Gasoline Engine Catalytic Converter

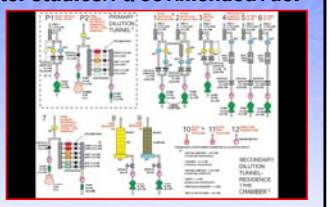
Mobile Source Emissions: Pt-Catalyst Equipped Vehicles

- **Roadway and Tunnel Dust**
  - ❖Several Urban Centers (Milwaukee, Los Angeles, Atlanta, Denver)
  - ❖Excellent integrated receptor for emissions from mobile sources
  - ❖Sieved and resuspended → PM_{10} and $PM_{2.5}$
  - ❖PGE concentrations 50-500x background
- **Roadside Soils**
- **Air Sampling adjacent to heavily trafficked roads.**
  - ❖**Size-fractionated** recently emitted PM.
- **Catalyst Materials** New and Used.

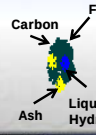
Lough, G.C., J.J. Schauer, J-S. Park, M.M. Shafer, J.T. Deminter, and J.P. Weinstein. 2005. Emissions of metals associated with motor vehicle roadways. Environ. Sci. Technol., 39:826-836.

Diesel Engine Dynamometer Studies: Pt/Ce Amended Fuel





Our sampling train installed at MATC Engine Research Laboratory. Platinum speciation was examined as a function of engine load, hot/cold start, particle size, and [Pt/Ce].



- **Catalyst dosed directly into diesel fuel**
  - Pt / Ce fuel-soluble bimetallic catalyst
  - delivered *in situ*
- **Active in high temperature combustion zone**
  - higher efficiency of fuel HC combustion
- **FBC intimate contact with PM**
  - more complete combustion of solid C, HC
- **Delivers Catalyst to DOC / DPF**

Atmospheric Aerosol Sampling

- **Roadside Aerosol Sampling & Characterization**
- **Ambient Aerosol Sampling & Characterization**

Size-resolving impactor (PCIS) sampler

25 mm PCIS Substrate

Majestic B.J., J.J. Schauer, M.M. Shafer, P.M. Fine, M. Singh, and C. Sioutas. 2008. Trace metal analysis of atmospheric particulate matter: A comparison of personal and ambient samplers. J. Environ. Eng. & Sci. 7(4):289-298.

Ntziachristos L., Z. Ning, M. D. Geller, R. J. Sheesley, J. J. Schauer, and C. Sioutas. 2007. Fine, Ultrafine and Nanoparticle Trace Element Composition Near A Major Freeway With Heavy Duty Diesel Traffic. Atmospheric Environment 41(27): 5684-5696.

Particulate Matter Characterization

- Total Elemental and Isotopic: SF-ICPMS
- Extraction-Based Speciation
- Solid-Phase Speciation: XAS (XANES & EXAFS)
- Electron Microscopy: STEM

Applied to each of our target source and receptor samples:

a) PM from diesel engines burning Pt-amended fuels
b) Roadway dusts and roadside soils
c) Urban atmosphere aerosols
d) Catalyst materials

Total Pt (+48 additional elements) by SF-ICP-MS after microwave-assisted mixed acid digestion in micro-Teflon bombs.

Microwave digestion system

Extraction-Based Characterization Strategy

Solubility – Biochemically relevant fluids

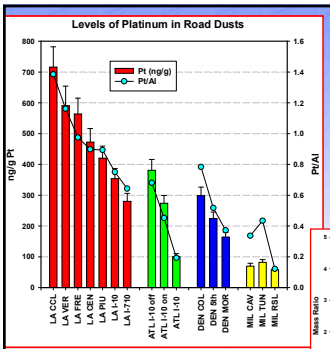
- **I. Gambles Saline (pH=7.4)**
- **II. Macrophage Vacuole Cytoplasm Fluid (pH=4.6)**
- **III. MQ**
- **IV. 1 M HCl**
- **V. Methanol** (access binding sites sequestered in hydrophobic soot matrix)

- Each extract filtered at 0.22 µm
- Time points of 2, 6, 24, and 48 hours (kinetics of release)
- Three solid-solution ratios (200, 500, and 2000 mg L^{-1})
- Room temperature and protected from light
- Soluble ions (nitrate, chloride, sulfate & ammonium) and TOC determined.

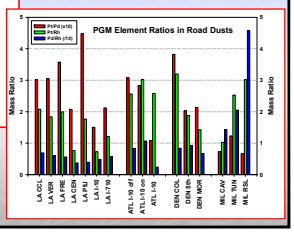
Each filtered extract subjected to the following separations:

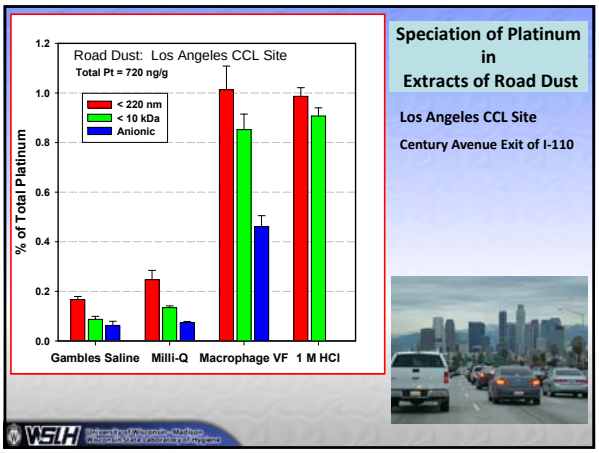
- Colloid/Nano-Particle Charge: *Ion Chromatography (DEAE chromatography). Anionic versus Cationic+Neutral. Fractions → SF-ICP-MS.*
- Colloid/Nano-Particle Size: *Ultrafiltration (10 kDa). Nano-particulate versus "dissolved". Fractions → SF-ICP-MS*

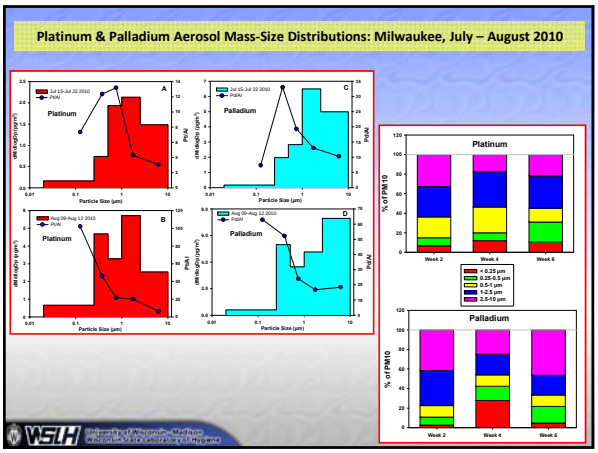
Complementary Total and Extractable Methods

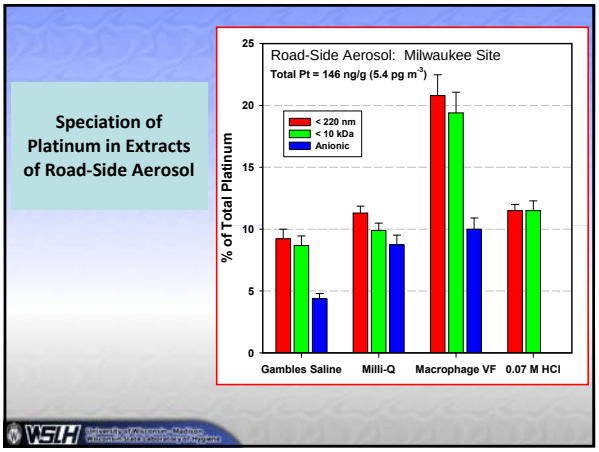


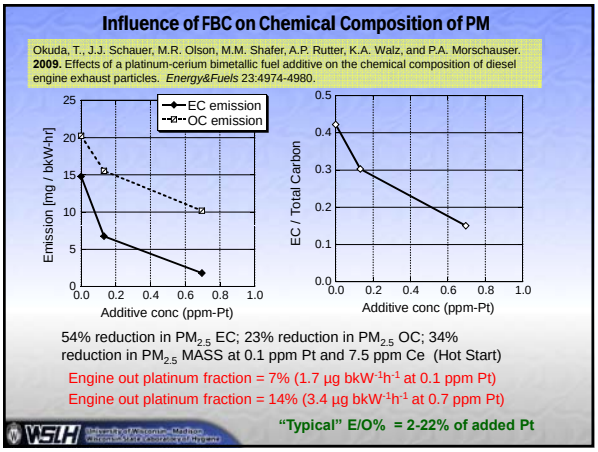
Platinum & PGM Concentrations in Road Dusts

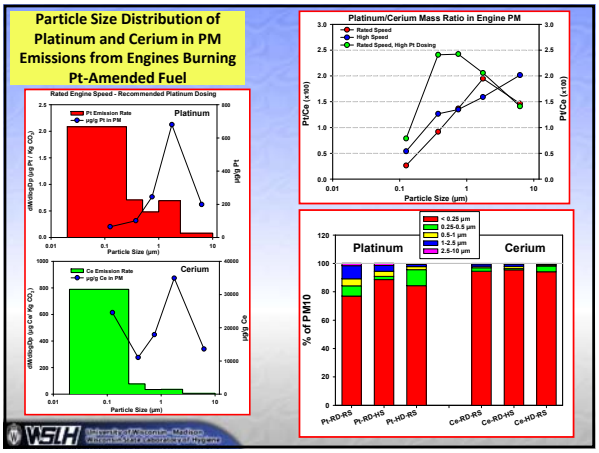


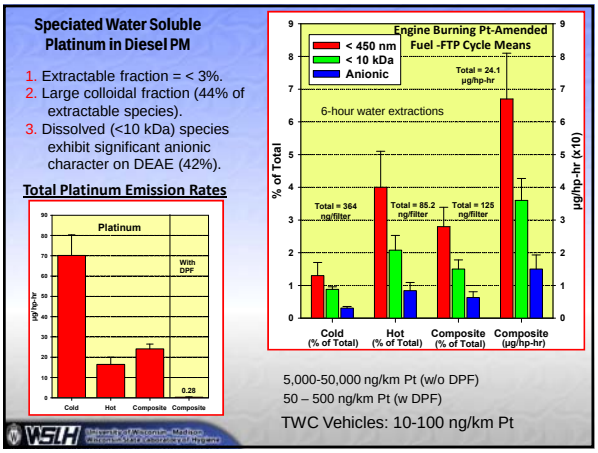




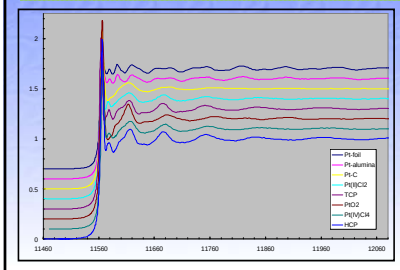








Synchrotron XAS: EXAFS Spectra of Platinum Reference Materials

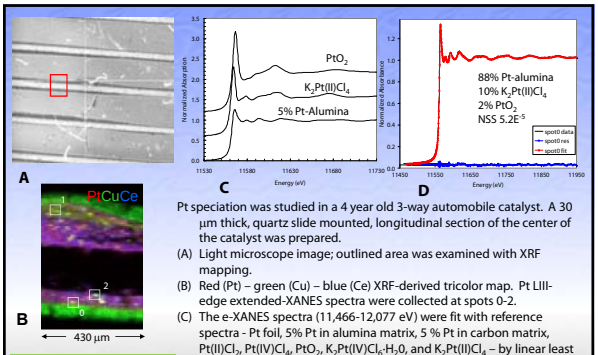


EXAFS Spectra of 18 Platinum Reference Compounds Measured to Date

LBL-Advanced Light Source

Pt-foil = platinum foil; Pt-alumina = 5% platinum on alumina; Pt-C = 5% platinum on graphite; $Pt(II)Cl_2$ = platinum(II) chloride; TCP = potassium platinum(II) tetrachloroplatinate; PtO_2 = platinum(IV) oxide; $Pt(IV)Cl_4$ = platinum(IV) chloride; HCP = potassium platinum(IV) hexachloroplatinate.

Majestic, B.J., J.J. Schauer, M.M. Shafer. 2007. Application of Synchrotron Radiation for Measurement of Iron Red-Ox Speciation in Atmospherically Processed Aerosols. Atmospheric Chemistry and Physics 7:2475-2487.



Gasoline Vehicle Catalyst (TWC).

Pt speciation was studied in a 4 year old 3-way automobile catalyst. A 30 µm thick, quartz slide mounted, longitudinal section of the center of the catalyst was prepared.

(A) Light microscope image; outlined area was examined with XRF mapping.

(B) Red (Pt) – green (Cu) – blue (Ce) XRF-derived tricolor map. Pt LIII-edge extended-XANES spectra were collected at spots 0-2.

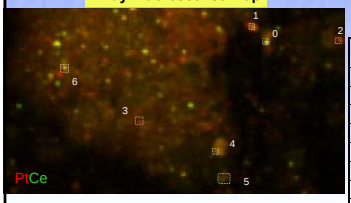
(C) The e-XANES spectra (11,466-12,077 eV) were fit with reference spectra - Pt foil, 5% Pt in alumina matrix, 5 % Pt in carbon matrix, $Pt(II)Cl_2$, $Pt(IV)Cl_4$, PtO_2, $K_2Pt(IV)Cl_6{\cdot}H_20$, and $K_2Pt(II)Cl_4$ – by linear least squares method.

(D) Select reference spectra and an example fit shown in C and D.

Modeled fraction of oxidized Pt is significant.

XRF Map, and Extended-XANES Fitting Results, of Diesel Exhaust Particulates Trapped on a Diesel Particulate Filter (engine running with Pt-FBC)

X-ray Fluorescence Map



Particle – Pt Speciation

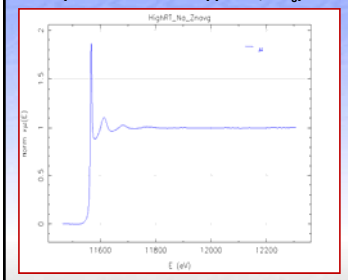
Spot #	Elemental Composition	Linear Least-Squares Fit Results
0	Ce, Zn, S	88% Pt-alumina, 7% Pt-foil, 5% **TCP**
1	Ce	56% Pt-alumina, 21% Pt-C, 24% PtO_2
2	Fe-rich	87% Pt-alumina, 10% PtO2, 3% HCP
3	Pt alone	81% Pt-alumina, 18% PtO2, 2% TCP
4	Ca, S	59% Pt-foil, 41% PtO_2
5	S-rich	86% Pt-C, 11% TCP, 7% Pt-alumina
6	Ce	86% Pt-alumina, 14% PtO_2, 3% HCP
7	Pt-rich	100% Pt-foil
8	Ce, Pt-rich	64% Pt-alumina, 11% PtO_2, 26% Pt-foil
9	Ce-rich	36% Pt-foil, 7% HCP, 59% Pt-C

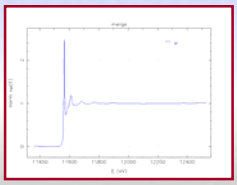
Large heterogeneities in particle composition are observed with many particles exhibiting a significant oxidized platinum component.

Strong evidence for PtO_2 (14-25% in many spots, up to 40% when associated with Ca and S).

Platinum XAS

XAS Spectra of Diesel Engine Exhaust PM (Pt-FBC Fuel at 0.7 ppm Pt; PM_8)

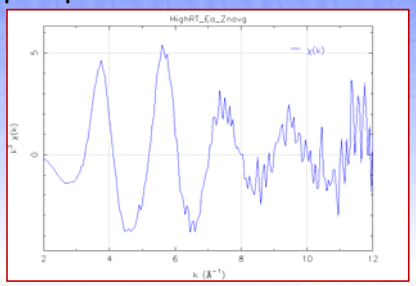




Significant contributions from oxidized platinum species are evident in spectrum.
(in primary vehicle emissions)

•Platinum (IV) oxide-monohydrate

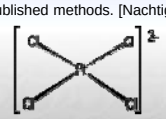
K-space Spectrum of PM from Pt-amended fuel



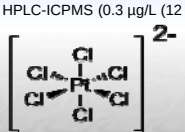
Quality of EXAFS spectrum will support shell-by-shell fitting. Early data suggests that oxide and metal are the two dominant platinum species.

Chloroplatinate Method Development and Application

- We are targeting two documented toxic/allergenic chloroplatinate compounds and their hydrolysis products.
  - hexa-chloroplatinate ($PtCl_6^{-2}$) Pt(IV) (H^+, K^+, Na^+, NH_4^+)
  - tetra-chloroplatinate ($PtCl_4^{-2}$) Pt(II) (H^+, K^+, Na^+, NH_4^+)
- Only very limited information on the concentrations of chloroplatinates in potential environmental sources and receptors is available.
- Environmental fate and transport data is lacking.
- It is unknown whether chloroplatinates may form from environmental processing of other platinum compounds in the environment.
- Very sensitive analytical techniques are required as the levels in environmental samples are expected to be very low (ng g^{-1}, pg ml^{-1}, pg m^{-3}).
- Few published methods. [Nachtigall, Nischwitz : HPLC-ICPMS (0.3 µg/L (12 pg)).

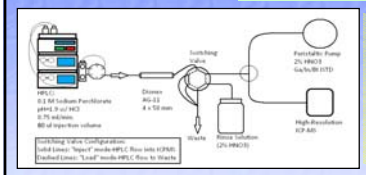


tetra-chloroplatinate



hexa-chloroplatinate

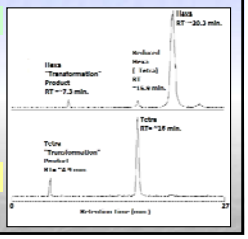
HPLC-SF-ICP-MS Isocratic Method



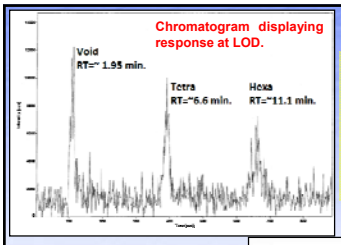
Tetrachloroplatinate ($PtCl_4^{2-}$, "Tetra") and hexachloroplatinate ($PtCl_6^{2-}$, "Hexa") are separated isocratically using a Dionex AG11 guard column containing an alkanol quaternary ammonium stationary phase and a mobile phase consisting of 0.1 M Na-perchlorate/HCl at pH= 1.9 and detected with magnetic sector ICP-MS.

HPLC-SF-ICP-MS Gradient Method

A gradient elution method (total run time = 25 min.) was developed to help elucidate the identity of chloroplatinate transformation products.



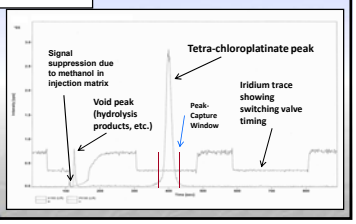
$$PtCl_x^{2-} + H_2O \rightarrow PtCl_{x-1}(H_2O)^- + Cl^-$$



With an 80 µl injection (sample loop) the current limit of detection of the method is approximately 15 parts-per-trillion (ppt) [1 pg] in both standard mixtures and in spiked tunnel dust (CRM 723) extracts (1M HCl). (10-fold improvement over published methods). We are working toward another 10-fold improvement. Our goal is <0.05% of total Pt (<50 pg/g, <0.5 pg/10 mg).

Chromatogram of "tetra" spike, post turbovap blowdown of MeOH/EDTA extract.

With extract pre-concentration and peak-capture we have achieved LODs of 0.2 pg.



Chloroplatinate Method Development Plans

- Continued method development on chloroplatinate speciation
  - Further improvement in already achieved sub-pg detection limits
    - ✓volume reduction (turbo-vap)
    - ✓off-line peak capture and concentration
    - ✓Br-PADAP or trioctylamine ligands (in MIKB) to selectively complex (and preserve) chloroplatinates
  - Further validation of extraction methods (MeOH/HCl and MeOH/EDTA) for target environmental matrices
  - Synthesize stable-isotopically enriched (^{194}Pt and ^{196}Pt) target compounds for isotope dilution and tracer experiments
- Apply methods to engine PM, road dusts and airborne PM samples
  - Determination of ambient chloroplatinate concentrations
  - Investigation of transformation and degradation of chloroplatinates in the environmental matrices

Follow-up with our previous work with tandem mass spectrometry (hydrolysis products).

Environmental Transformation Studies

University of Wisconsin-Madison Biotron

- **Environments**
  - **Aerosol in contact with air**
  - **Soil-sediment system**
  - **Aquatic suspension**
- **Samples**
  - **PM from Pt-FBC-treated diesel exhaust**
  - **Tunnel /road dust and roadside aerosol**
  - **Size-resolved PM from urban air**
- **Variables**
  - **Time**
  - **Humidity**
  - **Light**
  - **Oxidant**

Acknowledgments

This research is funded by U.S. EPA - Science To Achieve Results (STAR) Program
Grant # R833892

Andrij Holian

Role of NLRP3 Inflammasome and Nickel in Multi-Walled Carbon Nanotube-Induced Lung Injury

Andrij Holian, Teri Girtsman, Mary Buford, and Raymond Hamilton, Jr.
Center for Environmental Health Sciences, The University of Montana, Missoula, MN

There is insufficient information on what characteristics of engineered nanomaterials (ENM) result in the greatest health risk. Significant questions regarding chronic inflammation and the subsequent development of fibrosis as observed in animal models need to be addressed. In addition, discrepancies in study outcomes for the same class of materials makes risk assessment difficult. Specifically, carbon nanotubes have been reported by some to have minimal effects while others have reported significant pathological outcomes following exposure. It is likely that variations in the manufacturing methods of these materials are responsible for the inconsistent results in the literature. For example, multiwall carbon nanotubes (MWCNT) are prepared by a variety of methods using different metals as catalysts. This variability in manufacturing method results in tubes that not only vary in size, but also metal content.

The molecular mechanism of action where ENM such as MWCNT causes lung inflammation leading to lung fibrosis has not been elucidated. Studies with other particles such as silica and asbestos indicate that activation of the NLRP3 inflammasome resulting in the release of potent inflammatory cytokines such as IL-1β is important in the resulting pathogenesis. Furthermore, we have reported that long TiO_2 nanobelts activate the NLRP3 inflammasome and generate an inflammatory response *in vivo*. Therefore, the current study utilized the availability of a family of MWCNT that were provided by the National Toxicology Program and characterized by the Research Triangle Institute to test the hypothesis that the inflammatory potential of MWCNT correlated with activation of the NLRP3 inflammasome. These studies were conducted *in vitro* using primary alveolar macrophages (AM) isolated from C57Bl/6 mice and human macrophage like THP-1 cells. *In vivo* studies were conducted to examine the pathology at 7 and 56 days. All MWCNT were suspended in dispersion medium and administered by pharyngeal aspiration.

Pathology varied from little to no evidence of lung injury to significant inflammation and pathology. Correlations were made depending on contaminants. When a subset of MWCNT was evaluated with similar diameters, there was an excellent correlation between pathology and Ni content, but not Fe, Co or Mo. The correlation held for pathology at 7 and 56 days, although there was a tendency towards resolution at 56 days compared to 7 days. Also, there was significant correlation between Ni content and inflammasome stimulation (IL-1β and IL-18 release) in both primary AM and THP-1 cells. Furthermore, inflammasome activation correlated with *in vivo* pathology using both primary AM and THP-1 cells. Activation of the NLRP3 inflammasome required lysosomal rupture and release of cathpesin B. In summary, the bioactivity of a broad range of MWCNT could be predicted from NLRP3 inflammasome activation using either primary AM or THP-1 cells. For MWCNT, Ni content was an excellent predictor of lysosomal rupture, NLRP3 activation and pathology.

EPA Grant Number: R828602

Principal investigator did not authorize publication of the presentation.

AM Session 2: Effects of Nanoparticle Surface Properties

Gregory V. Lowry

Microbial Bioavailability of Polyethylene Oxide Grafted To Engineered Nanomaterials

Teresa Kirschling[1,2], Kelvin Gregory[1], Robert Tilton[2], and Gregory V. Lowry[1]
[1]Department of Civil and Environmental Engineering, [2]Department of Chemical Engineering, Carnegie Mellon University, Pittsburgh, PA

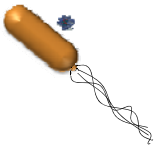
Coatings are an integral part of nanoparticle design, imparting changes in particle reactivity, stability, and toxicity. These coatings frequently consist of polymers adsorbed or grafted to particle surfaces. The fate of these polymeric coatings will affect the long-term fate, partitioning, and ecological impact of engineered nanoparticles in the environment. Depending on the polymer composition and method of surface attachment, some coatings may be removed from particle surfaces by desorption or non-biological hydrolysis processes. Direct microbiological removal or degradation of nanoparticle coatings has not been demonstrated. In this study, we synthesized 70 nm diameter star copolymers consisting of 2000 molecular weight polyethylene oxide (PEO) arms emanating from dense, cross-linked polystyrene-like cores via atom transfer radical polymerization (ATRP). These serve as model engineered nanomaterials with a covalently grafted polymer brush coating for a study of direct coating degradation by microbes. Because the arms are covalently linked to the cores, the possibility of PEO desorption and subsequent microbial degradation of free polymers in solution is eliminated. A consortium of PEO degrading microorganisms was enriched from Monongahela River (Pittsburgh, PA) water. Cultures were grown on either a 2000 molecular weight PEO homopolymer solution or a PEO star polymer solution as the sole carbon source. Cultures grew on both carbon sources indicating that the covalently attached PEO coatings on these nanoparticles are bioavailable. PEO star copolymers aggregated after microbial degradation, demonstrating the loss of colloidal stability caused by PEO arm degradation. Such microbiological processing of nanoparticle coatings would have significant implications for the long-term mobility of engineered nanoparticles in the environment. Furthermore, because a growing body of evidence shows that toxicity of engineered nanoparticles to cells and organisms is decreased by aggregation, microbial processing also may impact the long-term ecotoxicity of engineered nanoparticles.

EPA Grant Number: R833326

Microbial Bioavailability of Polyethylene Oxide Grafted Nanomaterials

Teresa L. Kirschling[1], Patricia L. Golas[2], Kris Matyjaszewski[2], Hye-Jin Kim, Brian C. Reinsch, Edwin G. Minkley Jr., Christopher Kim (Chapman University), Pedro J.J. Alvarez (Rice University), Kelvin B. Gregory[3], Gregory V. Lowry[3], Robert D. Tilton[1,4]

Carnegie Mellon University Departments of [1]Chemical Engineering, [2]Chemistry, [3]Civil and Environmental Engineering and [4]Biomedical Engineering



CEINT Center for the Environmental Implications of Nanotechnology

EPA STAR Grantees Meeting
November 8-9, 2010

Grant # R833326

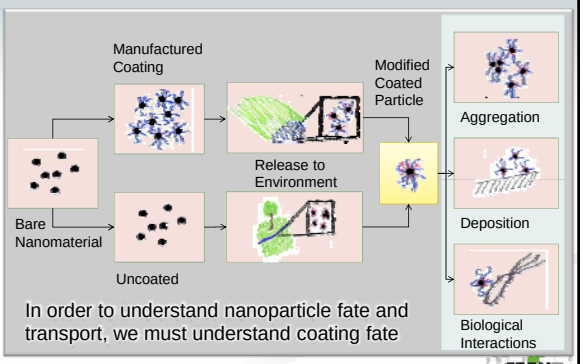
Carnegie Mellon

The Effect of Surface Coatings on the Environmental and Microbial Fate of Nanoiron and Fe-Oxide Nanoparticles

- Objectives
  - Determine the fate of NZVI in the environment
    - EXAFS characterization after aging (Reinsch et al., *EST* 2009)
  - Effects of NZVI and coatings on biogeochemistry
    - Effect of coatings on NZVI toxicity (Li et al. *EST* 2010, Phenrat et al., *EST* 2010
    - Examine shifts in native microbial populations and dehalococcoides spp. upon exposure to NZVI (3 g/L)
    - Kirschling et al., *EST* 2010, Xiu et al., *Biotech Bioproc.*, 2010, *EST* 2011
  - Determine the fate of the coatings
    - Desorption of coatings from NZVI (Kim et al., *EST* 2009)
    - **Biodegradation of covalently bound polymers on ENMs**

Carnegie Mellon

Impacts of Nanoparticle Coatings



In order to understand nanoparticle fate and transport, we must understand coating fate

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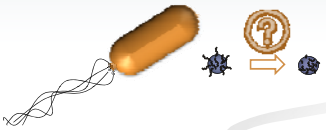
3

5nm cutoff



ARE NANOMATERIAL COATINGS BIOAVAILABLE?

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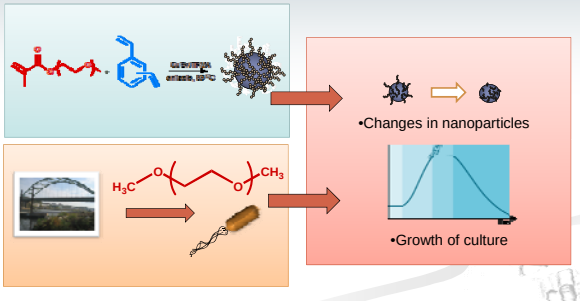
Bioavailability experiments



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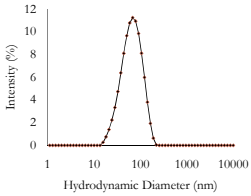
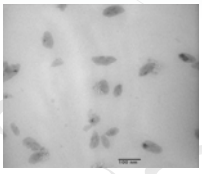
Model nanoparticles: PEO star copolymers

No question about permanency of attachment





- Nontoxic
- Permanent coating
- Does not hydrolyze in water

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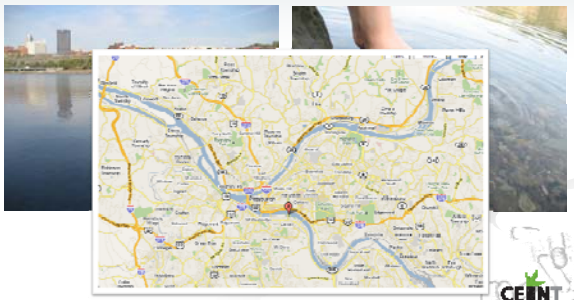
Enrichment culture



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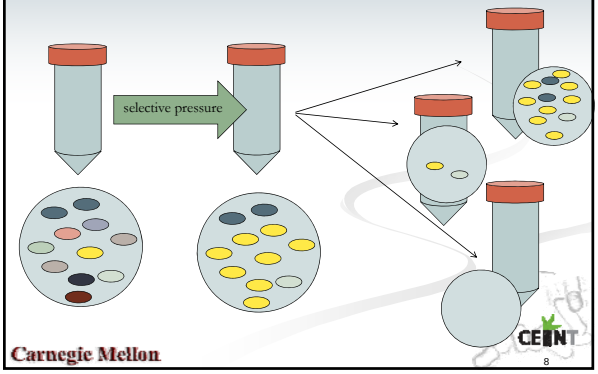
Enrichment culture



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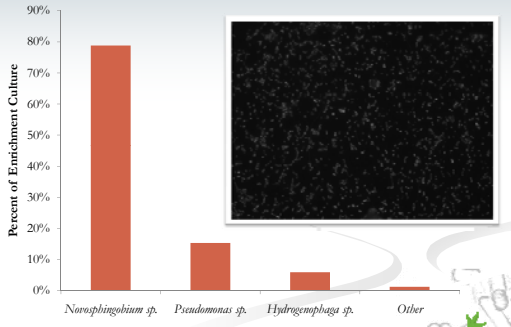
Enrichment Culture



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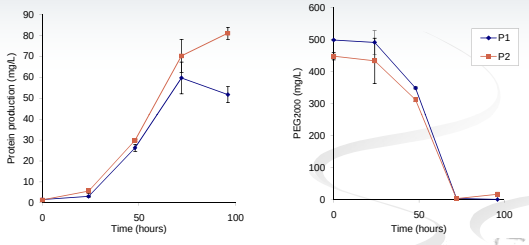
Enrichment culture



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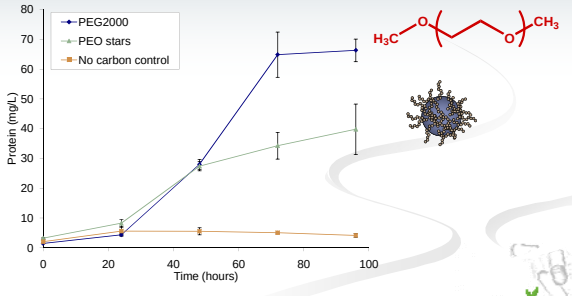
Growth on PEO star copolymers



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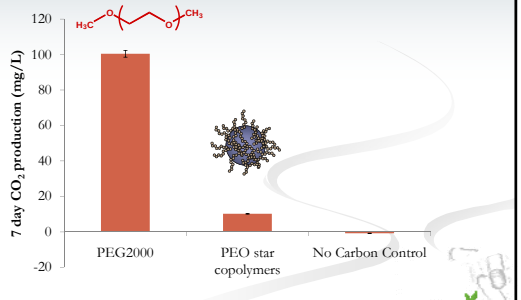
Growth on PEO star copolymers



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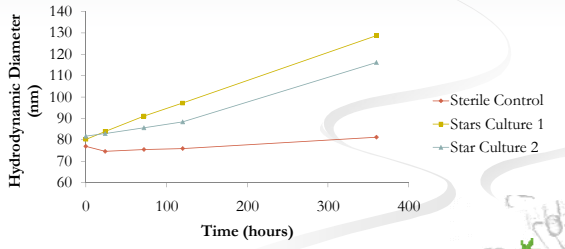
Microbial induced PEO star copolymer aggregation



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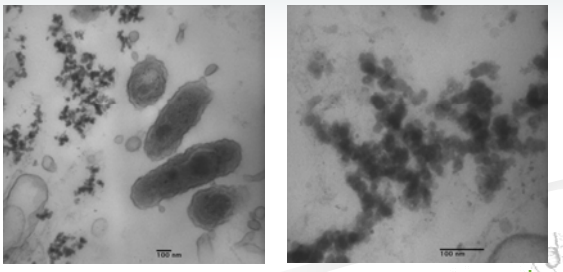
13

Microbial induced PEO star aggregation



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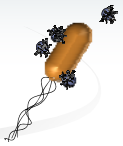
14

Conclusions

- Covalently bound PEO on nanoparticles is bioavailable
- Microorganisms can change nanoparticle stability which will change the fate and transport in the environment.
- Availability will depend on
  - Coating attachment
  - Degradability of coating



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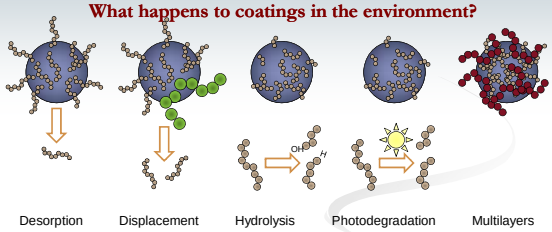
15

Next Steps

What happens to coatings in the environment?



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Problems Encountered

- Difficult to track coating fate in real environmental samples
  - ^{14}C labeled coatings
- Recovering ENMs form real environmental samples
- Measuring processes and effects occurring at realistic concentrations of ENMs

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- Questions?

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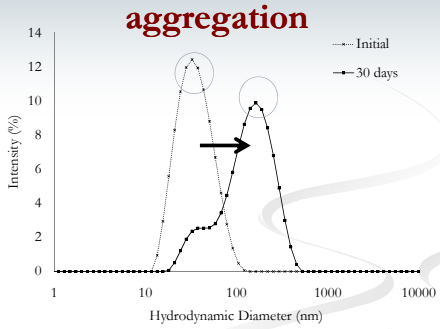
Microbial induced PEO star aggregation



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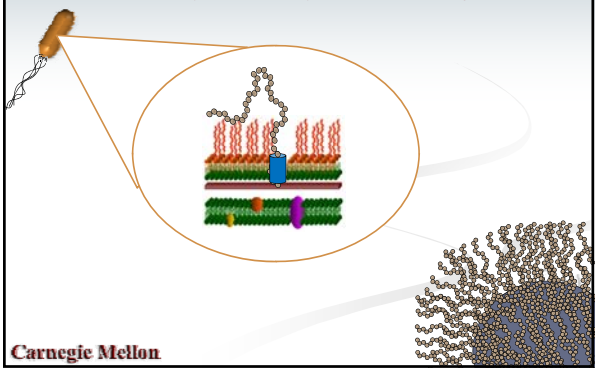
Availability of polymer end groups



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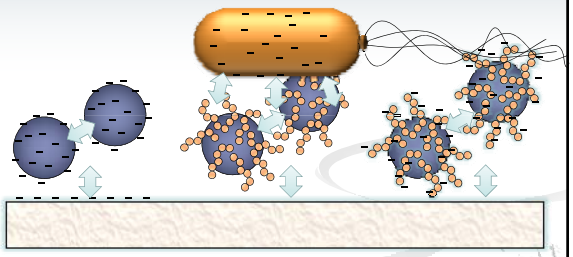
How coatings influence interactions



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21

Howard Fairbrother

Surface Oxides: Their Influence on Multi-Walled Nanotubes Colloidal, Sorption and Transport Properties

Howard Fairbrother*, William Ball, Billy Smith, Jin Jang, Kevin Wepasnick, and Julie Bitter
Johns Hopkins University, Baltimore, MD

Nanomaterials are being produced and integrated into consumer products and specialized applications at an accelerating rate, and concern has increased about their environmental fate and effect. Fueling this apprehension, in part, is the fact that many nanomaterials are being deliberately surface functionalized to enhance their aqueous colloidal stability and biocompatibility. As a consequence, these surface modified nanomaterials are likely to exhibit different behaviors in aquatic environments as compared to the pristine nanomaterials. In our research group, we have focused on understanding how oxygen-containing functional groups (surface oxides) influence the environmental properties (e.g., colloidal stability, transport through porous media, and sorption) of multi-walled carbon nanotubes (MWNT), a prominent class of engineered nanomaterials. In doing so, we hope to provide the information that can be used to predict and rationalize the effect of surface chemistry on the environmental fate of MWNTs.

Our scientific approach has been to develop structure-property relationships between the MWNT's surface oxygen concentration and their colloidal, transport and sorption properties. To accomplish this task, we have used a suite of wet chemical-treatments that allow us to controllably vary the extent of MWNT surface oxidation. Typical oxidants include HNO_3, $KMnO_4$, and mixtures of H_2SO_4-HNO_3. To determine the concentration of surface oxides imparted by these treatments, we have used X-ray photoelectron spectroscopy. Additional characterization of our as-received and oxidized CNTs has been carried out using the techniques listed in **Table 1**.

Analytical Technique	Information Obtained on MWNTs
Transmission Electron Microscopy (TEM)	Structural Integrity
Atomic Force Microscopy (AFM)	Length Distribution - Before/After Oxidation
BET Isotherm	Surface Area
Chemical Derivatization	Surface Concentration Hydroxyls, Carbonyls and Carboxyls
Dynamic Light Scattering (DLS)	Spherically Equivalent Particle Size
Potentiometric Titration	Surface Charge
Electrophoretic Mobility	Sense of Surface Potential

Table 1. Analytical techniques used to characterize oxidized MWCNTs and the information acquired

Colloidal Stability: To examine the aqueous colloidal stability and aggregation properties of oxidized MWNTs, sedimentation and time-resolved dynamic light scattering (TR-DLS) experiments were conducted on single component suspensions prepared by prolonged sonication of MWNTs in Milli-Q water. Over a range of environmentally relevant pH values (4-9) and electrolyte (NaCl, $CaCl_2$) concentrations (0.001-1.000 M), the aggregation and colloidal properties of MWCNTs were found to agree with the basic tenants of DLVO theory, in that (1) more highly oxidized, negatively charged MWNTs remained stable over a wider range of solution conditions than lowly oxidized tubes, (2) oxidized MWNTs adhered to the empirical Schulze-Hardy rule, and

(3) MWNTs exhibited reaction- and diffusion-limited aggregation regimes. To complement investigations conducted under ideal solution conditions, the effect that natural organic matter (NOM) had on the MWNT's colloidal properties also was examined. Due to steric stabilization, the colloidal stability of MWNTs was greatly enhanced in the presence of NOM, as expected. However, bench-top sedimentation and TR-DLS studies indicated that the colloidal stability of less oxidized MWNTs was greater than that of more highly oxidized MWNTs at environmentally relevant NOM concentrations (~3 mg/L). This effect was due to the fact that although the presence of negatively charged surface oxides increases the colloidal stability of MWNTs, they also decrease their sorption capacity towards NOM. Consequently, surface oxidation has the effect of increasing the colloidal stability of MWNTs in the laboratory but decreases the relative colloidal stability of MWNTs in the natural environment.

Transport: Studies examining the transport properties of MWCNT through model columns are underway. Suspensions of MWCNTs are prepared by prolonged sonication in Milli-Q water and model columns have been prepared using spherical glass beads (0.355-0.425 mm diameter). Current results show that the transport of MWCNTs through idealized porous media obeys traditional DLVO and clean bed filtration theory. Specifically, the deposition rate of colloidal MWCNTs increases with increasing ionic strength until reaching a diffusion-limited deposition regime. For a set of highly oxidized MWCNTs, critical deposition was found to increase significantly with pH. Having completed these initial studies, the next step in this investigation is to examine the role that MWCNT surface oxidation plays in transport.

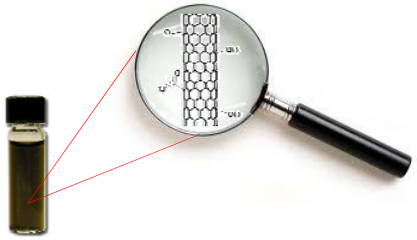
Next Steps: Two new avenues of research are being undertaken to further examine the role that surface oxides play in regulating the environmental fate of CNTs. In one study, solid phase MWCNT powders are being continuously stirred in water to mimic natural currents. The goal of these experiments is to examine the rate of CNT transfer from one phase to another (solid to colloidal) and to determine how releases rates are influenced by particle and aqueous phase conditions. The other avenue of research is to determine the extent to which results from our MWCNT studies apply to single-walled CNTs (SWCNT). While seemingly straightforward, issues associated with purity arise when using pristine and oxidized SWCNTs. According to TEM analysis, purity issues are predominantly associated with amorphous carbon. Methods to purify as-received and oxidized SWCNTs are currently being investigated. One method that has shown some promise is rinsing SWCNTs with strong NaOH.

EPA Grant Number: R828771

Effect of Surface Oxygen on Environmentally Relevant Properties of Carbon Nanotubes (Aggregation, Transport and Sorption)

Howard Fairbrother
Department of Chemistry
Johns Hopkins University

Surface Oxides and Their Effect on MWCNT Properties



Research Questions

PHYSICOCHEMICAL CHARACTERIZATION
Surface Chemistry, Size, Shape

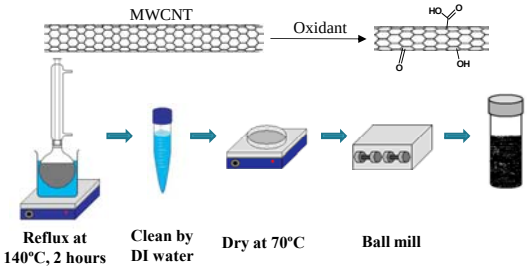
Develop functional relationships related to

MATERIALS PROPERTIES
Surface charge, Colloidal Stability, adsorption ability

Create models used to predict

ENVIRONMENTALLY RELEVANT BEHAVIOR
Aggregation, Deposition, Facilitated transport

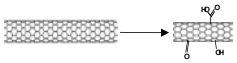
Surface Oxidation of MWCNTs



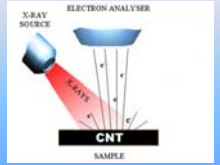
Oxidants:
H_2SO_4/HNO_3, HNO_3, $KMnO_4$, O_3, H_2O_2

Prevalent Oxidative Method

Surface Analysis

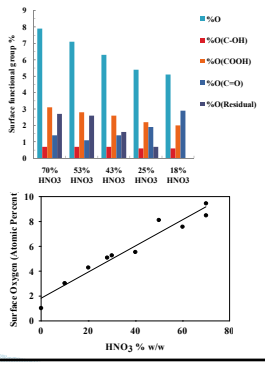


Surface Analysis
X-ray photoelectron Spectroscopy



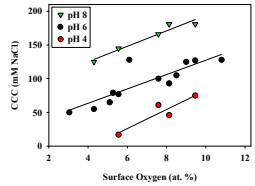
Determine Surface Oxygen Concentration (at. %)

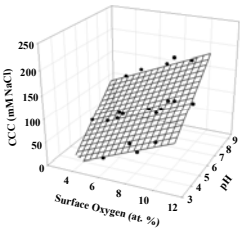
Controllable Oxidation



Aggregation Properties

Surface Oxygen and Colloidal Stability





Surface Oxygen (at. %)
A possible predictive metric

PLANE OF STABILITY:
CCC = −173 +12(%O)+ 30(pH)

Laboratory vs. Environment

Aggregation Behavior

Turbidity

Organisms

NOM

NOM & CNTs - Our Approach

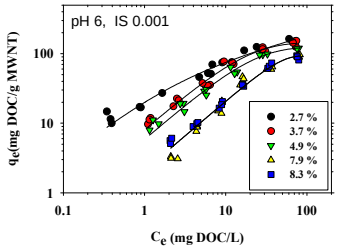
PHYSICOCHEMICAL CHARACTERIZATION
Chemical composition, size, shape

SORPTION PROPERTIES
As a function of background NOM Conc., pH and ionic strength

COLLOIDAL STABILITY
As a function of background NOM Conc., pH and ionic strength

GOAL
To see if the surface chemistry of the underlying particle plays a role after NOM adsorption.

MWCNT Sorption Properties: Influence of Surface Oxygen



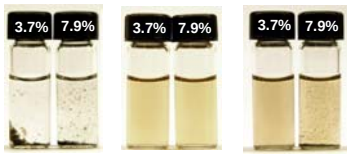
Surface oxide concentration reduces the adsorption of NOM onto the CNT surface

Aggregation in NOM: Observations

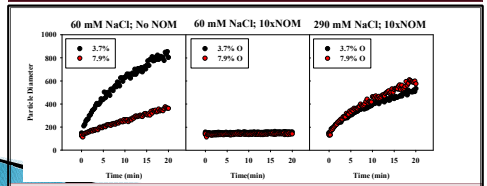
4.2 mg/L MWCNT @ pH 6

- 60 mM NaCl
- No NOM

Low-Ox Tubes aggregate first – as expected



Pictures: 4.2 mg/L MWCNT @ pH 6 after 13 hours

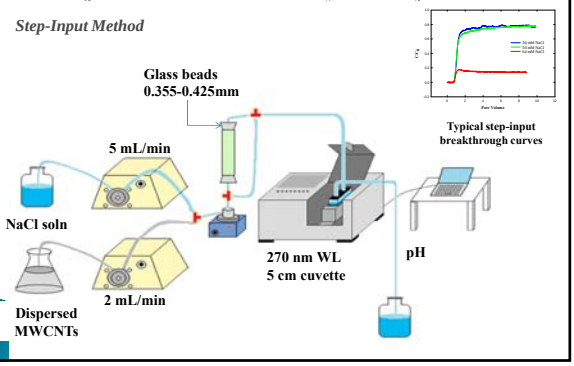


TR-DLS: 0.8 mg/L MWCNT @ pH 6

Transport Properties

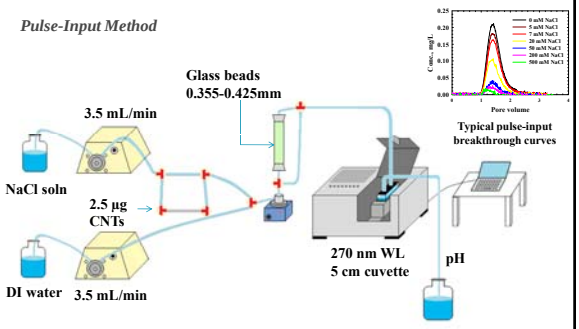
Transport: Column Transport Experiment

Step-Input Method



Transport: Column Transport Experiment (cont'd)

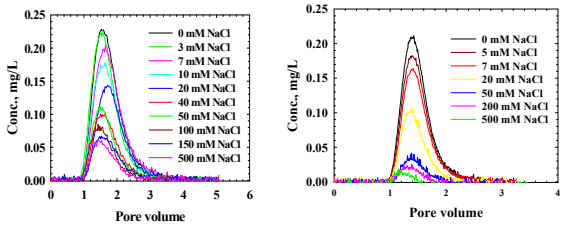
Pulse-Input Method



Transport: Experimental Parameters

Flow parameters		Column parameters	Long column	Short column
Volumetric flow rate	7 ml/min	length	10.2 cm	5.2 cm
Linear velocity	0.0238 cm/s	Intersection area	4.91 cm^2	4.91 cm^2
Superficial velocity	0.0624 cm/s	Porosity	0.38	0.38
		Pore volume	18.67 cm^3	9.73 cm^3

Transport: Breakthrough curves



Breakthrough curves of 53% HNO3 treated MWCNTs at different ionic strengthes at pH 5.6~5.8

Transport:Calculation method

- One-dimensional advection-dispersion equation with a sink

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} - v_p\frac{\partial c}{\partial x} - kc$$

- For step inputs

$$k = -\frac{1}{t_p}\ln\left(\frac{C_f}{C_0}\right)$$

- Colloid deposition rate coefficient *k*

$$k = \lambda_0 v_p = \lambda_0 L/t_p$$

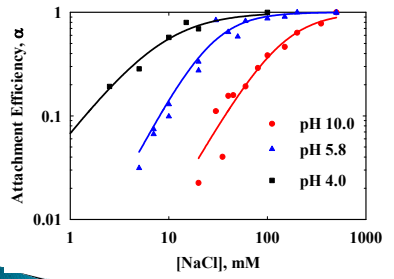
- For short-pulse input

$$k = -\frac{1}{t_p}\ln\left(\frac{q}{N_0}\int_0^{tf} C(t)dt\right)$$

- Collision efficiency

$$\alpha = \frac{k}{k^{fast}}$$

Transport: CDC curves at different pH



$$\alpha = \frac{1}{1+\left(\frac{CDC}{[NaCl]}\right)^{b}}$$

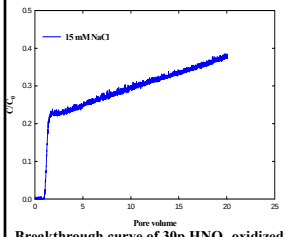
pH 4.0
CDC = 5.2 mM

pH 5.8
CDC = 28.6 mM

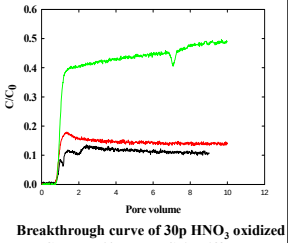
pH 10.0
CDC = 114.8 mM

Transport Issues (& The Solutions)

Increasing plateau and irreproducible results in step-input experiments

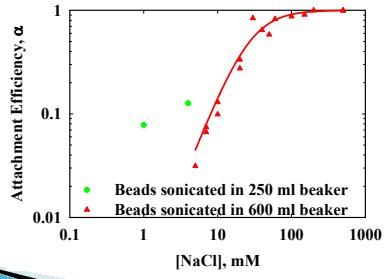


Breakthrough curve of 30p HNO_3 oxidized MWCNTs at 15 mM NaCl

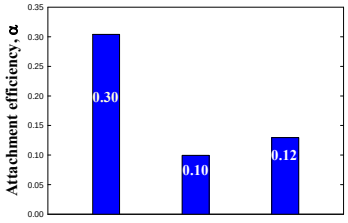


Breakthrough curve of 30p HNO_3 oxidized MWCNTs at 64 mM NaCl in different runs

The Importance of Column Treatment – Sonication



The Influence of Sonication Time in Creating a Reliable Porous Media



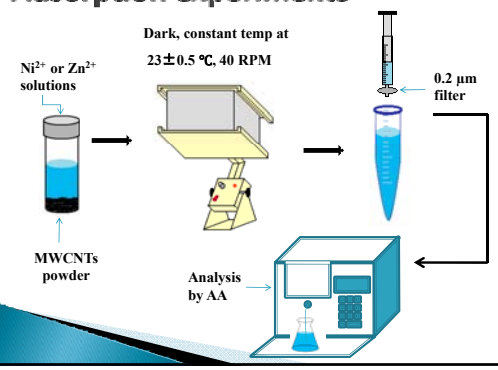
Attachment efficiency of O-MWCNTs through porous media treated for different sonication times. (pH 5.8, 10 mM NaCl)

Preparation of columns for reliable data

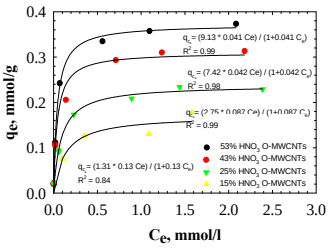
- Acid & base wash glass beads.
- Pack a quartz column with wet-pack method.
- Take the column apart, sonicate beads for 1 h and repack the column.

Sorption Properties

Adsorption experiments

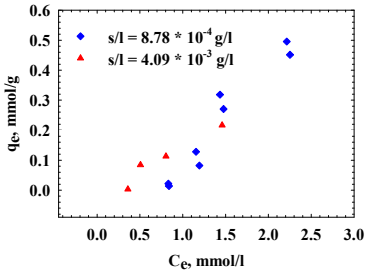


Adsorption: Effect of Surface Oxidation



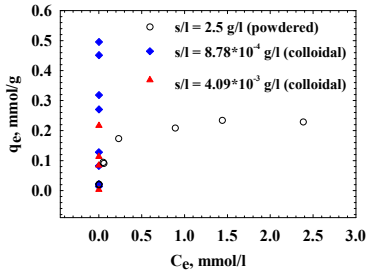
Effect of oxidation degree (oxygen concentration) on the adsorption of Zn (II) onto MWCNTs, total ionic strength is 30 mM, pH = 6.0 was buffered by MES.

Adsorption: Effect of solid-to-liquid ratio



Ni2+ adsorption affinity of colloidal O-MWCNTs at different solid-to-liquid (s/l) ratios; pH = 7.0 buffered by NaHCO3.

Adsorption: colloidal versus powdered phases



Ni2+ adsorption affinities of colloidal (open symbols) versus powdered (solid phase, closed symbols) O-MWCNTs. pH = 7.0 buffered by $NaHCO_3$.

The Future

- Effect of different oxidation on deposition of O-MWCNTs
- Effect of particle sizes on deposition of O-MWCNTs
- Facilitated transport

James Ranville

Development of Hyphenated and "Particle Counting" ICP-MS Methods Exposure Assessment of Inorganic Nanoparticles

***James Ranville*[1] *and Christopher Higgins*[2]**
[1]Department of Chemistry and Geochemistry, [2]Environmental Science and Engineering Division, Colorado School of Mines, Golden, CO

Quantifying environmental loadings and organism exposures is critical for the development of nanoparticle (NP) risk assessment models. Development of detection, characterization, and quantitation methods could lead to direct measures of organism exposure, both in laboratory and field settings. Inductively coupled plasma (ICP) techniques, which are generally capable of achieving ppt detection limits, are well suited to the analysis of metal-containing NPs. The power of ICP for detecting trace amounts of the elemental constituents of the NP must be combined with a means of discriminating between dissolved and NP-associated elements. Furthermore, techniques that provide size distribution information for NPs greatly increase our ability to understand their environmental transformations and implications. Although generally an accepted technique for NP size characterization, serial filtration and ultrafiltration are prone to numerous artifacts. New methods for using ICP-mass spectrometry (ICP-MS) include hyphenated techniques such as field flow fractionation (FFF-ICP-MS) and hydrodynamic chromatography (HDC-ICP-MS). Use of ICP-MS as an element specific single particle counter (SP-ICP-MS) can be achieved by using the ICP-MS in a non-traditional mode of operation. Recent developments in these methods and their potential for use in environmental fate and effects studies will be the central topics of the presentation.

EPA Grant Number: RD-83332401-1

Hyphenated and "Particle Counting" ICP-MS methods for the detection and characterization of metal and metal oxide nanoparticles

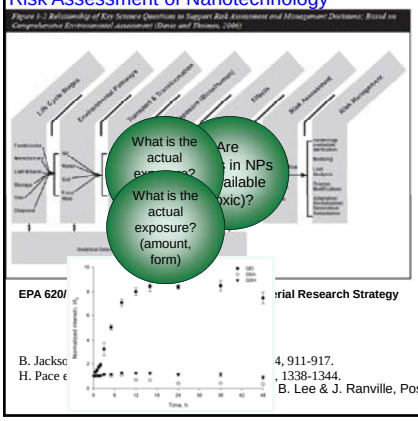
Dr James F Ranville
Department of Chemistry & Geochemistry

Dr. Chris Higgins
Environmental Science & Engineering

Colorado School of Mines
Golden CO

Presented at
EPA PI meeting, Portland, Nov 8th 2010

Risk Assessment of Nanotechnology



Our initial research question was on effects; Uptake and toxicity

For exposure we need to understand stability: aggregation and dissolution

For exposure we need to develop better metrology: Quantitation, detection, characterization

EPA 620/ …rial Research Strategy

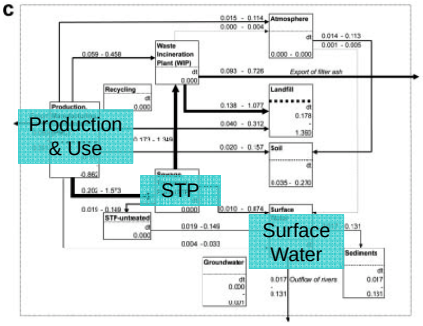
B. Jackso… 4, 911-917.
H. Pace e… , 1338-1344.
B. Lee & J. Ranville, Poster RP059, Thursday

Detection & Characterization

- Questions to be addressed
  - Detection & Quantification (counting methods)
    - **How much sensitivity & selectivity do we need?**
    - How do we apply methods to complex matrices (waters, tissues, sediments)?
    - Example: Nano Ag in wastewater
  - Characterization (hyphenated methods)
    - What is the exposure (form and amount)?
    - **Are we studying what we think we are?**
    - Example: FFF applied to QD toxicity on D. magna

Detection: How much sensitivity & selectivity do we need?

Material Flow Analysis: Ag



Gottschalk et al., ET&C, 29, 1036-1048, 2010

Detection: How much sensitivity & selectivity do we need?

Predicted Environmental Concentrations (PECs)

nano-Ag	Mode	$Q_{0.15}$	—	$Q_{0.85}$	
Air	0.021	0.017	—	0.074	$ng \cdot m^{-3}$
Surface water	0.72	0.56	—	2.63	$ng \cdot L^{-1}$
STP effluent	38.7	29.8	—	127	$ng \cdot L^{-1}$
STP sludge	1.88	1.46	—	6.24	$mg \cdot kg^{-1}$
Sediment	1203	965	—	10184	$\Delta ng \cdot kg^{-1} y^{-1}$
Soil	11.2	8.7	—	41.2	$\Delta ng \cdot kg^{-1} y^{-1}$

Parts per trillion

Gottschalk et al., ET&C, 29, 1036-1048, 2010

- Environmental levels unknown, likely ppt
- From laboratory toxicity testing, effects seen at ppb, ppm

Hypothesis: We can use ICP-MS to:

- detect
- count
- size

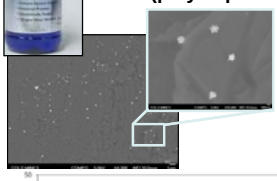
individual Ag nanoparticles

Approach is to use element specific "pulse" counting

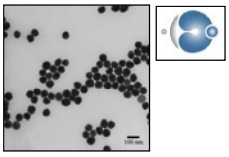
Real time single particle ICP-MS
or
Time resolved ICP-MS
or
Single particle ICP-MS

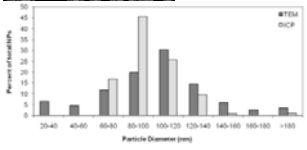
Silver Nanomaterials

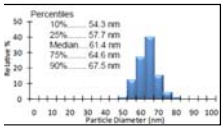
ASAP (polydisperse)



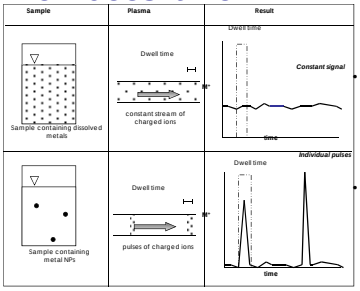
Nanocomposix (monodisperse)





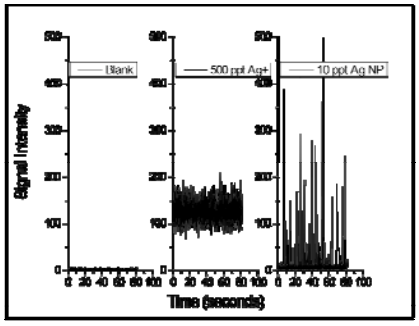


How does it work?

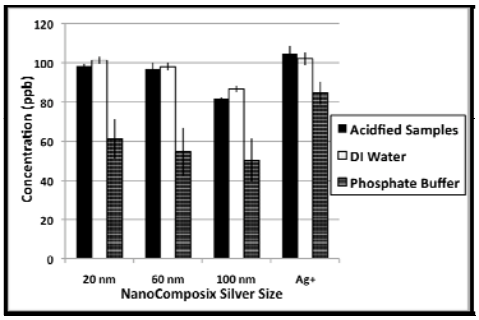


- ICP-MS of dilute solutions
  - Dissolved metals produce relatively constant signal
  - NP appear as a pulse which deviates from the baseline or "dissolved" background
- ICP-MS Parameters
  - Collect individual data points 10 to 20 ms dwell times
  - Optimize parameters (flow rate, neb. Gas flow, etc.) for best Ag detection
- Analyze Signal Intensity
  - Ideally in dilute solns only one NP will be ionized and will appear as a packet of ions
  - Compare pulse height to dissolved standards

What the data look like.



Are Ag Nanoparticles quantitatively detected by ICP-MS ?



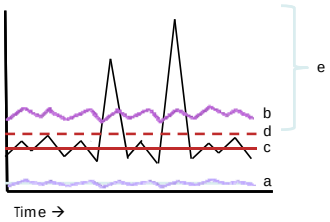
Defining NPs

Run blanks (a) and dissolved standards (b)

Determine background concentration (c)

Find appropriate way to differentiate between background and NP (d)

Determine number concentration and mass of NP (e)

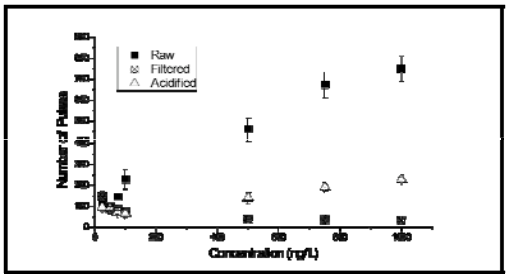


If the particle counting approach is valid:

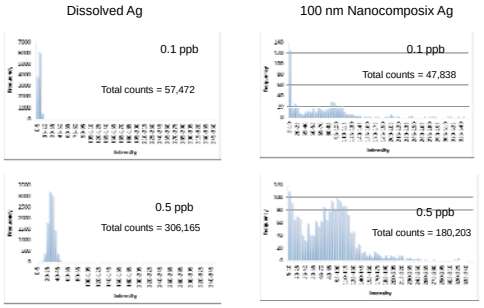
- Number of pulses will increase with increasing nano Ag concentration
- Number of pulses will be reduced by filtration or acidification

- The intensity of the pulse will be related to NP size

ASAP Results



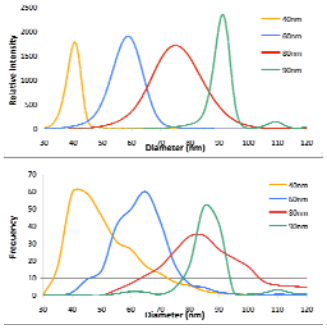
What is the minimum size that can be detected?



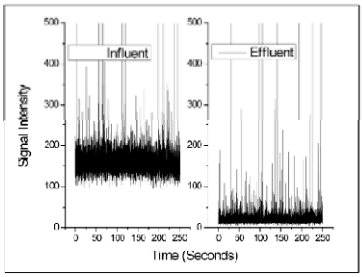
What is the minimum size that can be detected?

Disk Centrifuge

SP-ICP-MS



Quantitation (Estimation) of Ag in wastewater

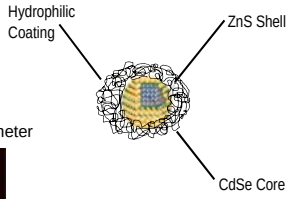


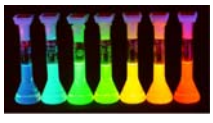
- Summation of baseline signal = "dissolved" Ag
- Summation of pulses = concentration of Ag-NP
- Raw Wastewater influent: Dissolved Ag = 520 ppt Ag-NP = 200 ppt
- Final Effluent: Dissolved Ag = 60 ppt Ag-NP = 100 ppt
- Results comparable to estimates from materials flow analysis (Nowack)
- In what form is the Ag-NP ? Need complimentary analysis (TEM)

Characterization: Are we testing what we think we are?
Question: Is the CsSe core toxic to D. magna

Quantum Dots (QDs)

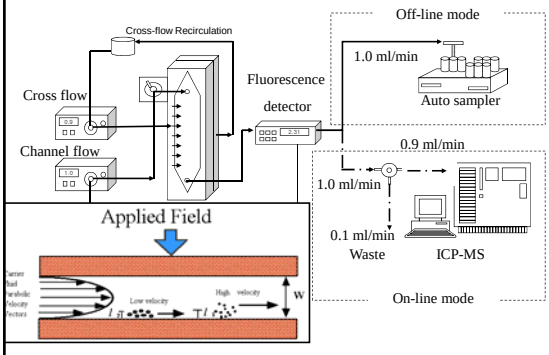
- Basic Structure
  - Metalloid core (1-5 nm in diameter), usually with protective shell
  - Can add coating to make hydrophilic (e.g. PEO or MUA)
- Intense fluorescence
- Fluoresce 490-680nm
  - Determined by CdSe core diameter





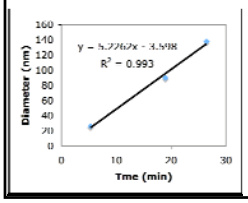
http://lamp.tu-graz.ac.at/~hadley/nanoscience/week2/Nano-CdSe.png

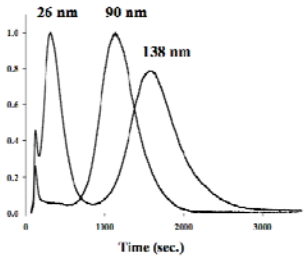
Sizing Hydrodynamic Radius by Fl FFF - ICP - MS



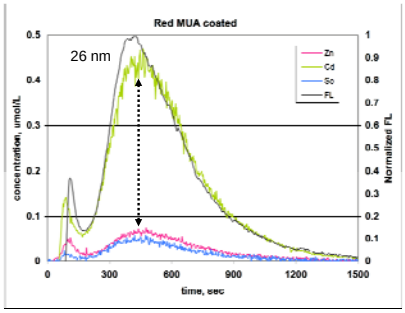
Size analysis by FFF theory or calibration

Field: 0.9 ml/min
Carrier flow:1.0 ml/min
PSS standards (Duke)
20 microliter injection
Fl detector



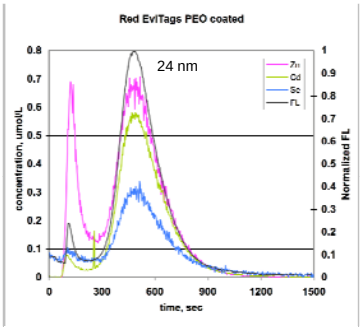


Elemental Size Distribution by Fl FFF-ICP-MS



Non 1:1 Cd: Se ratio
Possible explanation: Cd associated with polymer coating due to poor washing during synthesis
Low Zn indicates thin ZnS shell

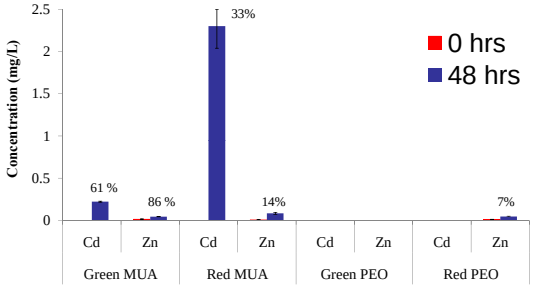
Elemental Size Distribution by Fl FFF-ICP-MS



Thick ZnS shell

Large Zn peak at early time
Possible explanation:Zn associated with free polymer
No dissolved Zn detected in a 3K Dalton filtrate

Dissolved metals (mg/L) and % dissolved metals for a 7.5 nmol/L QD solution



0hrs (mg/L)	BDL	0.014	BDL	0.006	BDL	BDL	BDL	0.013
48hrs (mg/L)	0.222	0.044	2.3	0.083	BDL	BDL	BDL	0.045

MUA NPs release Cd to solution.

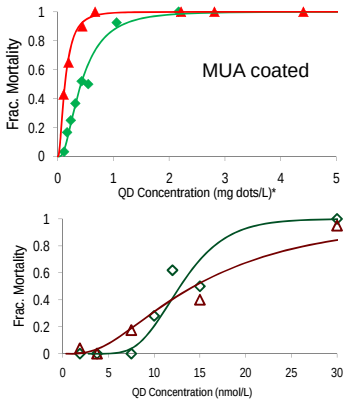
"Excess" Cd source of toxicity?

Are we testing CeSe core bioavailablity or some other form?

PEO NP stable during test

Appears to be a size affect when comparing mass dose

No Size effect when comparing number dose



Summary

RTSP-ICP-MS can be used to:

- Detect NP Ag at environmentally relevant concentrations (ppt levels)
  - •High specificity (contrast to DLS)
- Distinguish between "dissolved" and NP Ag
  - •Potential for application in stability and exposure/toxicity laboratory studies
- Current limitations
  - • About 40 nm size limit
  - •Cannot identify NP type

FFF-ICP-MS can be used to:

- More fully characterize complex NPs
- Provide information to interpret results of experiments where:
  - • Mixtures are used
  - • Manufacturing impurities are present
  - • Transformation/ degredation products are present

Acknowledgments

Collaborators
Dr. Anthony Bednar: USACE
Dr. Nicola Rodgers: CSIRO
Dr. Antonio Nogueira: U. of Aveiro
Dr. Brian Jackson: Dartmouth College

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US Environmental Protection Agency

US Army Corps of Engineers

Students
E. Lesher, D. Mitrano,J. Monserud, H. Pace

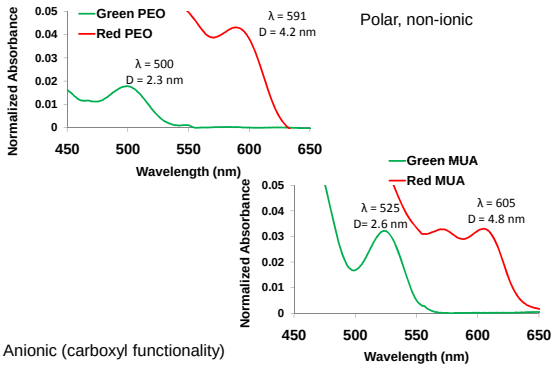
Unexpected metal ratios

Core		Red		Green	
Metal Mole Ratio		Cd/Se	Zn/Cd	Cd/Se	Zn/Cd
PEO	a	2.1	1.4	1.3	5.6
	b	3.2	1.4	ND	5.6
MUA	a	23	0.14	ND	0.23
	b	22	0.14	11	0.23

a. ICP-AES: QD in hard water
b. ICP-AES: QD in DI water

- **Cd:Se ratio not 1:1**
- **Excess Cd in MUA QDs, especially high for red MUA QDs**

Sizing of Core by UV-Vis



Polar, non-ionic

Anionic (carboxyl functionality)

Jingyu Liu

Controlled Release of Biologically Active Silver from Nanosilver Surfaces

***Jingyu Liu*[1], *David Sonshine*[2], *Saira Shervani*[2], *and Robert Hurt*[2,3]**
[1]Department of Chemistry, [2]Division of Engineering, [3]Institute for Molecular and Nanoscale Innovation, Brown University, Providence, RI

Major pathways in the antibacterial activity and eukaryotic toxicity of nano-silver involve the silver cation and its soluble complexes, which are well-established thiol toxicants. Through these pathways, nano-silver behaves in analogy to a drug delivery system, in which the particle contains a concentrated inventory of an active species, the ion, which is transported to and released near biological target sites. Although the importance of silver ion in the biological response to nano-silver is widely recognized, the drug delivery paradigm has not been well developed for this system, and there is significant potential to improve nano-silver technologies through controlled release formulations. This work applies the drug delivery paradigm to nano-silver dissolution and presents a systematic study of chemical concepts for controlled release. After presenting thermodynamic calculations of silver species partitioning in biological media, the rates of oxidative silver dissolution are measured for nanoparticles and macroscopic foils and used to derive unified area-based release kinetics. A variety of competing chemical approaches are demonstrated for controlling the ion release rate over four orders of magnitude. Release can be systematically slowed by thiol and citrate ligand binding, formation of sulfidic coatings, or the scavenging of peroxy-intermediates. Release can be accelerated by pre-oxidation or particle size reduction, while polymer coatings with complexation sites alter the release profile by storing and releasing inventories of surface-bound silver. Finally, the ability to tune biological activity is demonstrated through bacterial inhibition zone assay carried out on selected formulations of controlled release nano-silver.

EPA Grant Number: R833862

Brown University

Institute for Molecular and Nanoscale Innovation

Controlled Release of Biologically Active Silver from Nanosilver Surfaces

Jingyu Liu, David A. Sonshine, Saira Shervani, Robert H. Hurt

Department of Chemistry, Division of Engineering, Institute for Molecular and Nanoscale Innovation

Brown University, Providence, RI

This research is funded by U.S. EPA - Science To Achieve Results (STAR) Program. Grant # R833862

Nanosilver – Two Faces of Janus

A new generation of antimicrobials

Silver is a broad spectrum antibiotic

- has relatively low toxicity in humans;
- is being manufactured in large quantities and incorporated into consumer and medical products.

A risk to the environment and human health?

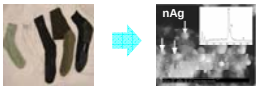
Silver is a known toxicant to aquatic organisms

- is more toxic than any other metal except mercury;
- bioaccumulates quickly;
- nanosilver has toxicity threshold as low as 10 ng/L (zebrafish embryos).

Argyria

Silver has potential toxic effects on beneficial bacteria in soil

Nanoparticle Silver Released into Water from Commercially Available Sock Fabrics



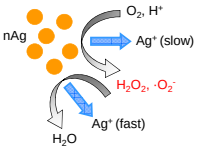
Nanosilver in Biological and Environmental Systems – Is It the Particle or the Ion?

- Metal ions may coexist in metal-containing nanoparticle suspensions.
- Silver ion is a known toxicant that binds to thiol groups in enzymes, such as NADH dehydrogenase, which disrupts the bacterial respiratory chain generating ROS that can lead to oxidative stress and cell damage.
- Nanosilver particles themselves may also contribute by binding to or passing through cell membranes, and generating ROS through surface reactions.
- There is some controversy about the role of particle-based mechanisms, but there is broad agreement that silver ion is an important toxicant.

What determines particle/ion partitioning?

Ion Release Kinetics and Particle Persistence in Aqueous Nano-Silver Colloids

Liu J.; Hurt R.H. *Environ. Sci. Technol.* 2010, 44, 2169–2175



This reaction produces active peroxide intermediates

Is inhibited by natural organic matter

Leads to complete particle dissolution in aerobic environments

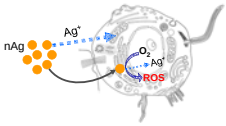
Dissolution kinetic law $-\frac{1}{m}\frac{dm}{dt} = Ae^{-E/RT}\left(\frac{[H^+]}{10^{-7}\,M}\right)^{0.22} e^{-a[NOM]}$

Institute for Molecular and Nanoscale Innovation

"Controlled Release" Nanosilver - application of the drug delivery paradigm

Can we systematically increase or decrease ion release rate?

Can we engineer nanosilver materials for optimal ion release profile?



Specific benefits of controlled release nanosilver formulations might include:

(i) dose control to achieve desired bactericidal or bacteriostatic effects;

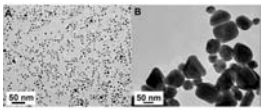
(ii) dose limitation to avoid eukaryotic toxicity that can, for example, slow wound healing in bandage applications;

(iii) control of product lifetime, before dissolution and diffusion end antibacterial activity;

(iv) minimization of environmental release through excess ion production beyond that necessary for product performance;

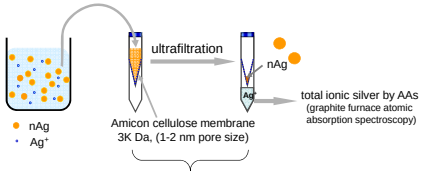
(v) optimization of release profile for targeted delivery to specific tissue or intracellular targets.

Particle-Ion Partitioning in Aqueous nAg Colloids



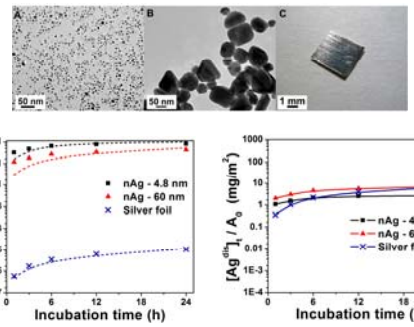
Basic Experiment

Ultrafiltration + Atomic absorption



Incubation → Separation → Quantification

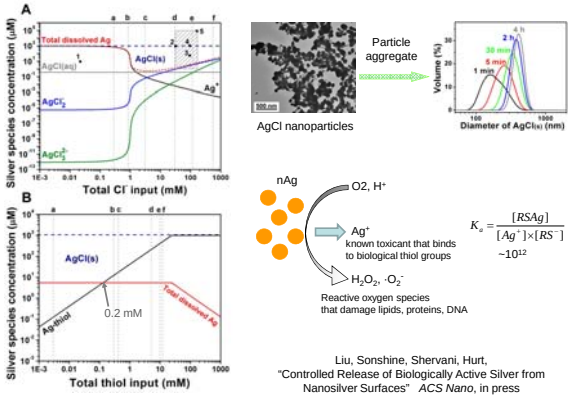
If nano-silver oxidatively dissolves, why doesn't bulk silver?



Answer: it does! (but slowly – about 2 nm/day)

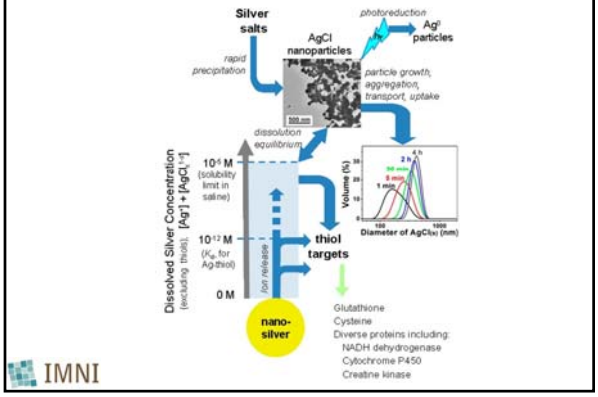
Speciation in Biological Media – Effect of Chloride and Thiol

(By Visual MINTEQ)

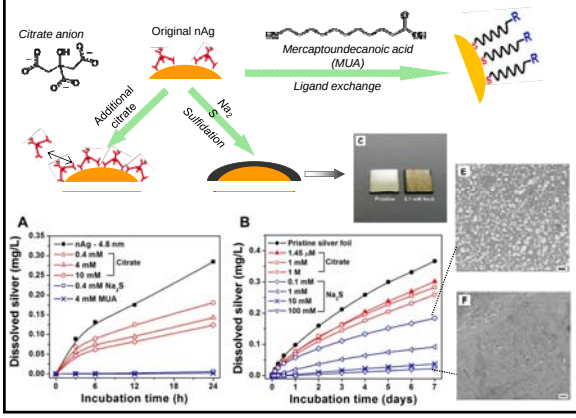


Liu, Sonshine, Shervani, Hurt, "Controlled Release of Biologically Active Silver from Nanosilver Surfaces" *ACS Nano*, in press

Nanosilver Behavior in Biological Environments – Nanosilver vs. Silver Salts

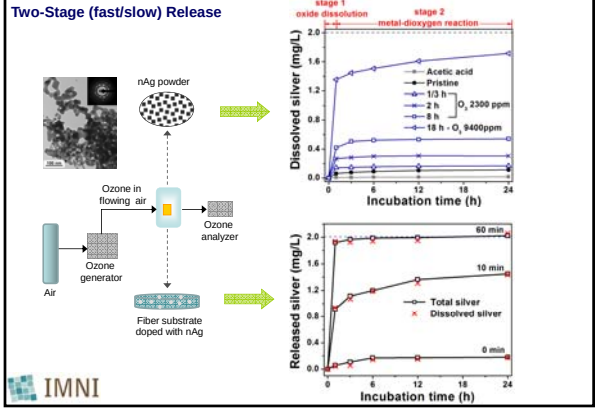


Controlled Release Approaches: Coatings and Ligands

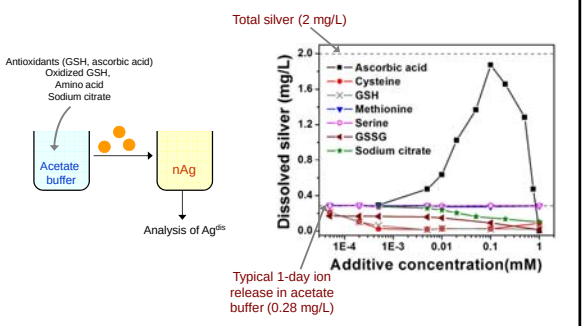


Controlled Release Approaches: Pre-Oxidation

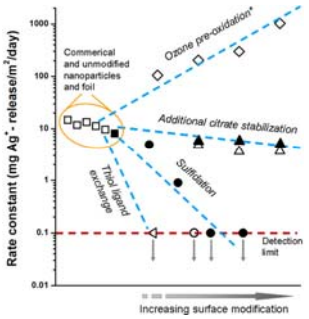
Two-Stage (fast/slow) Release



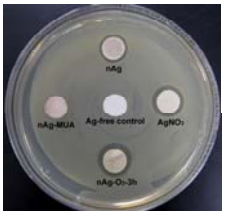
Controlled Release Approaches: Media Composition



Comparison of Surface Treatment Methods for Release Control



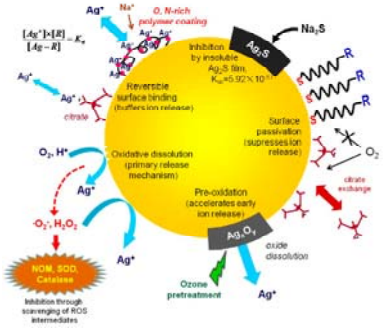
Biological Response to Controlled Release nAg



E. coli, 18 hr incubation
With 10 mm nAg-doped filter papers

Experimental assistance from Dr. Charles Vaslet, Kane laboratory

Approaches for Controlling Biologically Active Silver Release from Nanosilver Surfaces



Liu, Sonshine, Shervani, Hurt
"Controlled Release of Biologically Active Silver from Nanosilver Surfaces"
ACS Nano, in press

Future work – Biological and Environmental Implications of Ion Release Kinetics and Control

Hurt lab – dissolution kinetics and controlled release formulations

How can the transformations of nAg be engineered and controlled?
Role of sulfides and the role of photochemistry

Pennell – coupled reactive dissolution kinetics laws with environmental material flow modeling

What are the ultimate amount, fate, and form of nAg in the environment?

Kane lab – uptake and biodistribution in *Xenopus*

What is the fate and form of nAg in whole biological organisms?

REUSE IN RHODE ISLAND
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Financial support from the US EPA Science to Achieve Results Program and the NIEHS Superfund Research Program grant at Brown is gratefully acknowledged

Grant # R833862

The Laboratory for Environmental and Health Nanoscience At Brown University

Roger A. Pinto

Effects of Polyethyleneimine Surface Modifications of Multi-Walled Carbon Nanotubes: Their Toxicity, Sorption Behaviors, and Ecological Uptake by Earthworms and *Daphnia magna*

***Roger Pinto*[1], *Elijah Petersen*[2], *and Walter Weber, Jr.*[1]**
[1]Department of Chemical Engineering, University of Michigan, Ann Arbor, MI; [2] Chemical Science and Technology Laboratory, National Institute of Standards and Technology, Gaithersburg, MD

In support of the mission of the U.S. Environmental Protection Agency to provide substantive information for ecological risk assessments, this research focuses on investigation of the environmental fate, bioaccumulation potential, and toxicity of surface-modified carbon nanotubes (CNTs) in terrestrial and aquatic ecosystems. Carbon-14 multi-walled carbon nanotubes (MWNTs) were synthesized by a CVD process, grafted with polyethyleneimine (PEI) polymers, and further modified to render them with a range of different surface charges and resultant higher stability in aqueous suspensions. Assessments of the extent to which these modifications influence CNT ecotoxicity, accumulation, and elimination behaviors were performed using the earthworm *Eisenia foetida* and the water flea, *Daphnia magna*.

Liquid scintillation counting of residual ^{14}C *in vivo* provided insights on the uptake and elimination behaviors for the organisms tested. *D. magna* exposed to PEI-coated and acid-modified MWNTs at concentrations of approximately 25 or 250 μg/L indicated that the PEI surface coatings did not appear to substantially impact nanotube accumulation or elimination rates. Microscopy observations revealed substantial aggregation in the guts of *D. magna* similarly to previous studies with acid-treated MWNTs and fullerenes. Algae feeding to *Daphnia* was necessary to achieve almost complete elimination in 48 h, whereas the absence of algal amendments caused minimal CNT elimination. Immobilization studies allowed for the determination of EC_{50} values and indicated that PEI modifications increased MWNT acute toxicities, though this trend corresponded to the overall size of the grafted polymers.

Phase distribution experiments with soils measuring a combined effect of sorption and attachment to particles indicated linear sorption isotherms for the regular MWNTs and non-linear trends for the PEI-modified MWNTs. Differences in uptake behaviors by earthworms were not apparent among the different types of PEI-modified and MWNTs, results that indicated limited interaction of these carbon nanotubes with the organism tissues. In contrast to previous results for unmodified MWNTs, elimination patterns for the grafted PEI-MWNTs were well fit by an exponential decay. To determine whether earthworm exposure to these MWNTs elicits a stress response, two biomarkers of oxidative stress (glutathione-S-transferase (GST), catalase) and two biomarkers of neurological stress (monoamine oxidase, cholinesterase) were measured in whole-body samples. A dose-response relationship was not observed within the concentration range of the exposure treatments (3-1,000 mg MWNT kg^{-1} soil). However, positively (PEI-amino) and neutrally (PEI-acetate) charged nanotubes consistently revealed a toxic response with catalase, monoamine oxidase, and cholinesterase, while negatively charged CNTs (PEI-succinic and acid-treated MWNTs) had no effect.

EPA Grant Number: RD-833321

Principal investigator did not authorize publication of the presentation.

PM Session 1: Characterization Methods

Xin-Rui Xia

A Biological Surface Adsorption Index for Characterizing Nanomaterials in Aquatic Environments and Their Correlation With Skin Absorption of Nanomaterials

Xin-Rui Xia, Nancy Monteiro-Riviere, and Jim Riviere
North Carolina State University, Raleigh, NC

As nanoparticles are increasingly being used in commercial products, it becomes more and more important to understand how they interact with living organisms and the environment. The behavior of nanomaterials in a biological or environmental system is governed by the molecular interactions of their surface species with the biological or environmental components. Quantitative assessment of the adsorption properties of nanomaterials is a crucial step for developing predictive structure-activity relationship in nanomedicine and risk assessment of nanomaterials.

We have developed a biological surface adsorption index (BSAI) approach to characterize the surface activity of nanomaterials in biological systems. A set of small molecules having diverse physicochemical properties was used as probe compounds. The adsorption coefficients (k) of the probe compounds were obtained by measuring the quantities of the probe compounds adsorbed on the surfaces of the nanomaterials and the equilibrium concentrations of the probe compounds in the media. The log (k) values were scaled to a set of solvation molecular descriptors of the probe compounds via multiple linear regressions to provide a set of five nano-descriptors representing the contributions of the five types of molecular interactions (hydrophobicity, hydrogen-bond acidity and basicity, dipolarity/polarizability, and lone pair electrons). The nano-descriptors for multi-walled carbon nanomaterials (MWCNT) with different surface chemistries (unmodified, -OH and –COOH modified) and fullerenes were measured; for example, the regression model obtained for MWCNT (-OH modified) was $\log(k) = 0.77R + 2.55\pi - 0.14\alpha - 2.36\beta + 4.90V$; $n = 30$, $R^2 = 0.89$. The measured nano-descriptors can be used to develop predictive structure-activity relationships in nanomedicine and nanomaterial risk assessments.

We have prepared a series of hydroxylated fullerenes, C_{60}(OH)x. Their hydrophobicity was adjusted by controlling the number of hydroxyl groups (e.g., x = 16, 20, 30, 40). After characterization using conventional techniques such as particle size, zeta-potential and solubility, the nano-descriptors were measured using the BSAI approach for each of the C_{60}(OH)x nanomaterials. Then, the adsorption coefficients of different C_{60}(OH)x into the stratum corneum, the primary barrier of skin, were measured by *in vitro* adsorption experiments. The equilibrium adsorption coefficients were correlated with the physicochemical parameters and the nano-descriptors to establish quantitative correlations.

The following findings will benefit EPA: (1) The BSAI approach measures five molecular descriptors for each of the nanomaterials. The BSA indexes are free energy-related physicochemical parameters that can be used for predictive models developments in EPA guidelines for environmental health of nanomaterials; (2) The hydroxylated fullerenes with different hydrophobicity can be used to study the environmental transport and fate of fullerene nanomaterials; (3) The quantitative approach to correlate the adsorption coefficients of stratum corneum with the BSAI nano-descriptors of the C_{60}(OH)x nanomaterials could be a useful approach for developing predictive models for safety evaluation and risk assessment of nanomaterials.

EPA Grant Number: R833328

NC STATE UNIVERSITY

A Biological Surface Adsorption Index for Characterizing Nanomaterials in Aquatic Environments and Their Correlation with Skin Absorption of Nanomaterials

Xin-Rui Xia (PI), Nancy A. Monteiro-Riviere, Jim E. Riviere

Center for Chemical Toxicology Research & Pharmacokinetics (CCTRP)

North Carolina State University, Raleigh, NC

How to characterize nanomaterials in aqueous environments

- Currently, most of the methods measure the physical parameters, such as, particle size, size distribution, shape, charges, specific surface area, …
- surface chemistry and core material compositions are the only measurable chemical information of nanomaterials, but cannot be directly used for quantitative analyses.
- Octanol water partition coefficient log(Kow) has been widely used for predictive model development for small molecules, but it is difficult to be measured for nanomaterials since most of the nanomaterials form stable suspension in water or oil, but in not both.
- Great efforts have been made to understand the chemical interactions between nanoparticles and biological or environmental components in the scientific communities.
- We have demonstrated that lipophilicity was a significant factor in the nanoparticle adsorption of small chemicals.
- To date, there is no general applicable approach to quantitatively measure the molecular interactions of nanoparticles with biological or environmental components, which is the crucial information needed to develop quantitative structure-activity relationship for nanomedicine researches and for risk assessment and safety evaluation of the nanomaterials in occupational and environmental exposures.

Competitive Adsorption onto NP Surfaces.

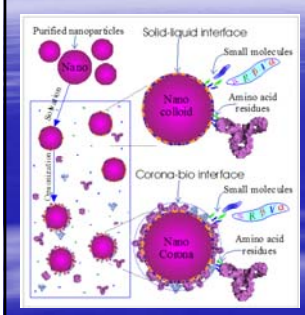


Illustration of the competitive adsorption of small molecules and macromolecules onto NP surfaces

Clean surfaces of pure nanomaterials only exist in high vacuum; having high surface energy, ready to adsorb any compound to reduce their surface energy.

Once contact an aqueous environment, the nanoparticle surface is dramatically altered by solvation to form a solid-liquid interface. This solid-liquid interface is unique feature in aqueous environments.

Small molecules and macromolecules are adsorbed onto the interface to form nano-macromolecule coronas (or complex).

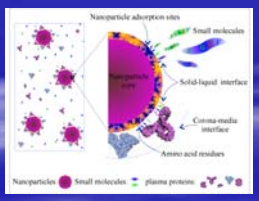
Current Nano-characterization focus:
- Pure nanomaterials in industrial applications
- Nano-protein coronas in biological systems
- Nano-humuic acid complex in environmental

We have identified that the adsorption property at the solid-liquid interface is key to understanding the behavior of nanoparticles in aqueous environments.
- it is not only determined colloidal stability
- but also the affinity and selectivity of environmental components in forming 'nanoparticle coronas'

Molecular interactions in the surface adsorption processes

We have developed a biological surface adsorption index (BSAI) approach to characterize the molecular interaction strengths of nanoparticles with small molecules and macromolecules in biological and environmental systems.

The BSAI approach is based on the molecular interaction similarity between nano-small molecule interactions and nano-macromolecule interactions.



Basic molecular interactions forces:
- Coulombs force (charged nanos) --- measured by zeta potential
- London dispersion (hydrophobicity)
- Hydrogen bonding (acidity/basicity)
- Dipolarity / polarizability
- lone-pair electrons

Obtained by BSAI approach

Nano-descriptors derived by BSAI approach

- It is hypothesized that the adsorption properties of nanomaterials are governed by the contributions of each type of molecular interactions, which can be detected by a set of small molecular probes having diverse physicochemical properties covering the vector spaces of molecular interactions between the nanomaterial and macromolecules.
- The adsorption coefficients (*k*) of the probe compounds on a given nanomaterial were measured using our SPME-GC/MS method.
- Then, the log(*k*) values were scaled to a set of known molecular descriptors of the probe compounds via multiple linear regressions.

$$\log k_i = c + rR_i + p\pi_i + a\alpha_i + b\beta_i + vV_i \qquad i = 1, 2, 3, \ldots, n$$

- Here, the solvation descriptors and a linear free energy relationship (LFER) and multi-walled carbon nanotubes (MWCNT) will be used as examples to demonstrate the BSAI approach.

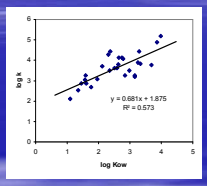
Adsorption coefficients (logk) and solute descriptors of the probe compounds

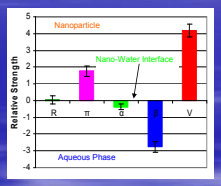
	Probe Compounds*	$\log K_{ow}$	logk	R	π	α	β	V
1	chlorobenzene	2.84	3.25	0.718	0.65	0	0.07	0.839
2	ethylbenzene	3.15	3.19	0.613	0.51	0	0.15	0.998
3	p-xylene	3.15	3.26	0.613	0.52	0	0.16	0.998
4	bromobenzene	2.99	3.50	0.882	0.73	0	0.09	0.891
5	propylbenzene	3.69	3.76	0.604	0.5	0	0.15	1.139
6	4-chlorotoluene	3.33	3.82	0.705	0.67	0	0.07	0.98
7	phenol	1.46	2.57	0.805	0.89	0.6	0.3	0.775
8	benzonitrile	1.56	3.04	0.742	1.11	0	0.33	0.871
9	4-fluorophenol	1.77	2.68	0.497	0.97	0.63	0.23	0.793
10	benzyl alcohol	1.1	2.19	0.803	0.87	0.33	0.56	0.916
11	nitrobenzene	1.85	3.39	1.109	1.11	0	0.28	0.891
12	acetophenone	1.58	3.26	0.818	1.01	0	0.48	1.014
13	3-methylphenol	1.96	3.08	0.822	0.88	0.57	0.34	0.916
14	methyl benzoate	2.12	3.70	0.733	0.85	0	0.46	1.073
15	4-chloroanisole	2.78	4.07	0.836	0.86	0	0.24	1.038
16	phenethyl alcohol	1.36	2.54	0.784	0.83	0.3	0.66	1.057
17	3-methylbenzyl alcohol	1.8	2.85	0.815	0.9	0.33	0.59	1.057
18	4-ethylphenol	2.58	3.62	0.8	0.9	0.55	0.36	1.057
19	3,5-dimethylphenol	2.35	3.49	0.82	0.84	0.57	0.36	1.057
20	ethyl benzoate	2.64	4.14	0.689	0.85	0	0.46	1.214
21	methyl 2-methylbenzoate	2.75	4.12	0.772	0.87	0	0.43	1.214
22	naphthalene	3.3	4.44	1.36	0.92	0	0.2	1.085
23	3-chlorophenol	2.5	3.82	0.909	1.08	0.69	0.15	0.898
24	4-nitrotoluene	2.37	4.44	0.87	1.11	0	0.28	1.032
25	4-chloroacetophenone	2.32	4.28	0.955	1.09	0	0.44	1.136
26	3-bromophenol	2.63	3.79	1.06	1.15	0.7	0.16	0.95
27	1-methylnaphthalene	3.87	4.89	1.344	0.9	0	0.2	1.226
28	biphenyl	3.98	5.18	1.36	0.99	0	0.22	1.324

Predictive Model established for MWCNT

$\log(k) = -1.33 + 0.043R + 1.76\,\pi - 0.37\alpha - 2.76\beta + 4.16V$

n =28, R^2 =0.93 and F =63, Q^2_{LOO}=0.888 and $Q^2_{LMO25\%}$=0.883



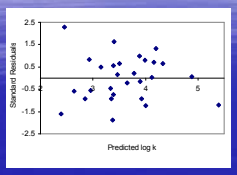


Relative molecular interaction strengths at the nano-water interface.

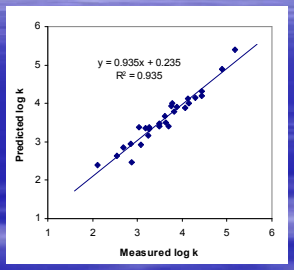
Left: Correlation of log*k* of the probe compounds on MWCNT with their log*K*o/w values, suggesting lipophilicity is significant in the adsorption process (R^2=0.57) but is not the only factor.

Right: The five nano-descriptors [*r, p, a, b, v*] measured by the BSAI approach, representing the five major molecular interactions in nanoparticle adsorption processes: lone-pair electrons, polarity/polarizability, hydrogen-bond donor, hydrogen-bond acceptor, and London dispersion, respectively.

Nano-Descriptors Provide Better Prediction



Residual plot of the linear regression. The random scattering of the data points above and below the zero residual line reveals that the regression model well described the experimental data.

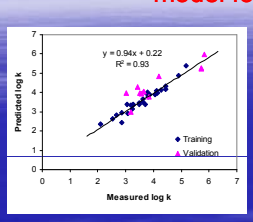


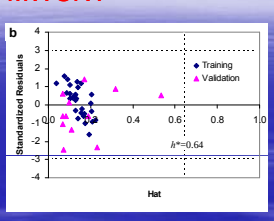
The predicted versus measured log(*k*) values of the probe compounds on MWNCT.

Validation of the predictive model for MWCNT

- The robustness of the model for MWCNT was studied by internal cross-validation using the leave-one-out (LOO) and leave-many-out (LMO25%) techniques with validation coefficients Q^2_{LOO} of 0.888 and $Q^2_{LMO25\%}$ of 0.883, respectively.
- Both of the cross-validation coefficients were greater than 0.7, revealing the robustness of the predictive model.
- External validation was conducted by using 12 different compounds; their log*k* values were measured using same protocols as the probe compounds.
- The external validation coefficient (Q^2_{ext}) of the model was 0.78, suggesting satisfactory predictivity for the external validation compounds.

The applicability domain of the predictive model for MWCNT





Predictive model performance and validation for MWCNT.

Left: The predicted versus measured log*k* values of the training probe and validation compounds. The data points of the validation compounds lying closely along the regression line reveal the robustness of the predictive model.

Right: Williams plot reveals the applicable domain of the predictive model. The dotted lines defined the chemical domain (±3σ and h^*=0.64) by the training probe compounds. The log*k* values for the training probe and validation compounds are all within the chemical domain, suggesting no outliers and the model predictivity is reliable.

BSAI Database and Applications

- BSAI Database is the final product of the BSAI approach, composed of the five nano-descriptors for each of the nanomaterials.

Nano-descriptors [*r, π, α, β, v*]	Physical parameters
r – lone –pair electrons interaction strength	*Particle size*
π – dipolarity/dipolarizability interaction strength	*Size distribution*
α – hydrogen-bond acidity interaction strength	*Particle shape*
β – hydrogen-bond basicity interaction strength	*Specific surface area*
v – hydrophobicity interaction strength	*… …*

- The nano-descriptors are free energy related quantities quantitatively describing the molecular interaction potentials of the nanomaterials at the nano-water interface.
- Biological activities, such as, solubility, partition coefficient, rate constant, binding and inhibition constants are free energy related quantities, their logarithmic values can be directly predicted via similar predictive model shown for MWCNT.
- The development of the BSAI approach could open a quantitative avenue toward predictive nanomedicine development, particularly, for developing integrated physiological based pharmacokinetic models, for quantitative risk assessment and safety evaluation of nanomaterials.

Project Summary

This project is to study the impact of physicochemical properties on skin absorption of manufactured nanomaterials.

Four physicochemical properties were studied;

Particle size (nm): 1 (C_{60}), 20, 50, 80 (silver nanoparticles)

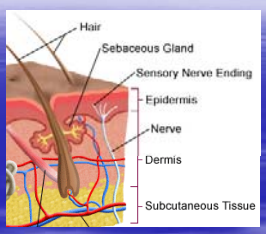
Surface charge: nC60 and coated nC60; the zeta-potential was manipulated by different ion-pairing agents

Hydrophobicity: the difficulty in obtaining log$K_{o/w}$ for nanomaterials forced us to develop the BSAI approach

Solvent effects: 6 industrial solvents (toluene, cyclohexane, chloroform, ethanol, acetone and propylene glycol) using the diffusion, tape-stripping and in vivo methods.

Impact of Physicochemical Properties on Skin Absorption of Nanomaterials

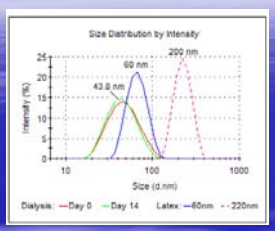
- Pristine C60 in different solvents will be used in many industrial and pharmaceutical manufacturing processes.
- Human exposure to C60 could occur in various solvents.
- Currently, the impact of solvents on its skin penetration is unknown.
- We have studied four types of industrial solvents:
  - Toluene (aromatic)
  - Cyclohexane (aliphatic)
  - Chloroform (chlorinated)
  - Mineral oil (oil)
  - Water (aqueous environmental)



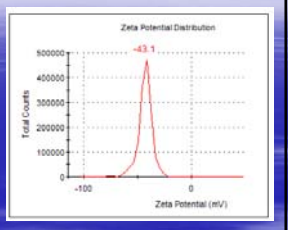
Layered structure of the skin

- Uppermost is the stratum corneum (SC): the primary barrier of skin (~15 μm in thickness),
- C60 distribution in the SC layer was studied by a tape-stripping method.

nC_{60} Characterization



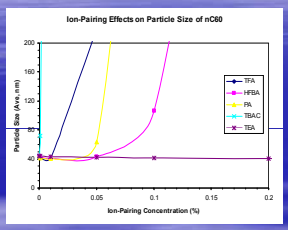
Dynamic size distribution of nC_{60} nanoparticles



Zeta-potential of nC_{60} after 14-day dialysis

We have developed a novel method to prepare nC_{60} nanoparticle with a narrow size distribution. This method does not use THF while provides nC60 concentration in water 100 times higher than the THF method. The nC_{60} nanoparticles are formed in a SDS aqueous solution, then SDS is removed via dialysis. After exhaustive dialysis, the nC60 nanoparticles were stable in water for years.

Impact of nC_{60} Colloidal Stability on Skin Absorption



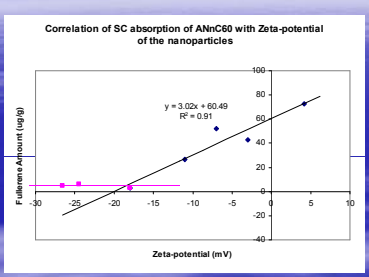
Ion-pairing effects on particle size of nC_{60} in aqueous solutions

nC_{60} and most of the unprotected nanomaterials have a very narrow window in their colloidal stability (even though they are stable in pure water).

- Ion-pairing agents (e.g. > 0.05%TFA) will cause their aggregation.
- Biological electrolytes will cause their aggregation.
- Once the nanoparticles aggregate, they can not get through the skin.

3 anions: TFA (trifluoroacetic acid), HFBA (heptafluorobutyric acid), and PA (phosphoric acid);
2 cations: TBAC (tetrabutylammonium chloride), TEA (triethylamine)

Impact of Zeta-potential on Skin Adsorption



Correlation of SC absorption of ANnC60 with Zeta-potential of the nanoparticles

Aqueous colloidal nanomaterials (nC60, water soluble nAg) did not penetrate intact skin regardless particle sizes.

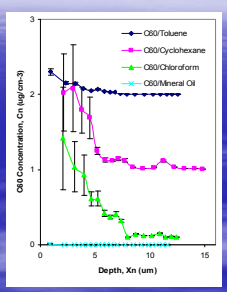
Ion-pairing agent could alter their zeta-potential, even diminish their surface charges, but did not promote their skin penetration (because nano-aggregation occurred as zeta-potential was reduced).

Impact of Solvents on Skin Penetration of C_{60}

Skin penetration of C_{60} was observed in different industrial solvents.

Significant solvent effects were observed; toluene, chloroform promote skin penetration of C60, while mineral oil did not promote skin penetration.

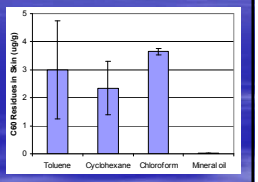
All three solvents have good C_{60} solubility.



Depth distribution profiles of C60 in stratum corneum after exposure in solvents.

Fullerenes Detected in Skin Tissues After 26 Tape-Strips

- **The amounts of fullerenes in the skin tissues (epidermis and dermis) were analyzed using the improved sLLE-HPLC method.**
- **Nanomaterial residues were detected in the skin tissues even after 26 tape-strips.**
- **The amount of fullerene residues was greater when dosed in chloroform than dosed in toluene or cyclohexane.**
- **No C60 was detected in the skin tissues when dosed in mineral oil; this is consistent with the results of the tape-strip analysis.**



Fullerene residues in skin tissues after tape-stripping.

Further Research Needed

All conclusions obtained in this study is human heath oriented using weanling pig skin as a model and short term (< 2 weeks in vivo).

- Long-term studies are needed because the long-term effects on skin could not show in short term studies such as nano contact dermatitis.
- Skin absorption into aquatic animals (fishes, amphibian …) should be studied because they have totally different skin structure (e.g., amphibian skin is very permeable to small molecules).
- Lot works are needed to make the BSAI approach to be a general useful tool for quantitative correlation and risk assessment of different nanomaterials.

Acknowledgements

Supported by the US EPA-Science to Achieve Results (STAR) Program

Wunmi Sadik

Flexible Nanostructured Conducting Poly(amic) Acid Membrane Captures, Isolates, and Simultaneously Detects Engineered Nanoparticles

[*]Wunmi Sadik[1], Nian Du[1], Michael Feurstein[1], Veronica Okello[1], Cheuk Wong[2], and Howard Wang[2]
[1]Center for Advanced Sensors and Environmental Systems (CASE), Department of Chemistry;
[2]Department of Mechanical Engineering and Materials Science Program,
State University of New York-Binghamton, Binghamton, NY

The goal of this project is to develop nanocavity sensor (category II) arrays for the isolation, detection and quantitation of engineered nanoparticles (ENPs) in complex environmental matrices. There is urgent demand for rapid screening methods to isolate, detect, and monitor engineered nanomaterials in the environment. Conventional methods for characterizing nanomaterials such as transmission electron microscopy, scanning electron microscopy, and atomic force microscopy tend to be bulky and inadequate for field and rapid screening of free nanomaterials.[1] At SUNY-Binghamton, we have developed a new class of nanostructured poly(amic acid) –PAA-membranes that are conductive and electroactive by preventing its imidization to polyimide, while retaining its carboxylic acid and amine functionalities.[2-4] We have studied the effect of composition and microstructure on the optical and electrochemical properties of PAA hybrid composites. The uniqueness of PAA lies in its excellent physical and chemical properties: transparency, flexibility, electrical conductivity, and accessibility to forming a large-area device. During the reporting period, our group discovered that this new class of flexible, stand-alone membranes could be successfully used as both sensors and nanofilters. A new nanofilters device based on PAA membranes is hereby introduced. The nanofilters were derived from phase-inverted, copolymers of PAA and other polymers, with the surface and pore sizes systematically controlled by varying the conditions of the synthesis. This presentation will focus on the use of PAA membranes for simultaneous removal and electrochemical detection of silver nanoparticles, quantum dots, and titanium dioxide nanocrystal from food supplements and environmental samples.

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EPA Grant Number: R834091

Principal investigator did not authorize publication of the presentation.

Jason Unrine

Fate and Effects of Nanosized Metal Particles Examined Along a Simulated Terrestrial Food Chain Using Genomic and Microspectroscopic Techniques

***Jason Unrine*, Aaron-Shoults Wilson, Olga Tsyusko, Sarita Hardas, D. Allan Butterfield, and Paul Bertsch**
Department of Plant and Soil Science, University of Kentucky, Lexington, KY

Terrestrial environments are likely to serve as the ultimate sink for a significant fraction of manufactured nanomaterials (MNM) from accidental releases, use in agriculture, and through land application of sewage sludge as biosolids. Risk from exposure to MNM in terrestrial food webs partly depends on their propensity for uptake and retention by detritivorous soil organisms and subsequent trophic transfer to higher trophic levels as well as inherent toxicity. Our research is investigating interactions between chemical composition and particle size in determining bioavailability and adverse effects of Cu, Ag, and Au nanoparticles (NPs). Our results indicate that uptake of nanoparticles from soil by earthworms does not vary systematically with primary particle size on a mass concentration basis; however, on a number concentration basis, smaller particles are much more bioavailable than larger ones. Also, we have found that nature of surface coating (PVP versus oleic acid) has little effect on Ag uptake. It is clear that the redox behavior of metal NPs in soil varies considerably. Although Cu NPs oxidize immediately upon exposure to the air, Au NPs are completely stable and resistant to oxidation. Ag NPs are resistant to oxidation in the air, but they can be readily transformed in natural soil. We have confirmed that either reduced or oxidized metal NPs can be absorbed from soil and taken up into internal tissues using a combination of X-ray microspectroscopy, laser ablation-inductively coupled plasma mass spectrometry, asymmetrical field flow fractionation-multidetection (AF4), and expression of the metal specific gene, metallothionein. We have observed decreased reproductive success associated with exposure to Au and Ag NPs as well as evidence of soil avoidance for Ag NPs. We also have obtained evidence for oxidative damage of proteins as a result of exposure to Ag ions and Ag NPs. In our toxicity tests, Au and Ag NPs were significantly less toxic than their corresponding metal salts; however, NPs, including oxidized NPs, may be bioavailable and cause adverse effects at relatively high concentrations. Behavioral avoidance of Ag NPs is the most sensitive endpoint investigated to date and occurs at concentrations of Ag NPs that are similar to those expected in sewage sludge. The next phase of our research investigated trophic transfer of NPs along a simulated food chain consisting of soil, earthworms, and bullfrogs. We obtained evidence that Au NPs can be transmitted from soil to earthworms to bullfrogs, although the rate of transfer is somewhat limited. It is important for future studies to investigate how aging processes influence the stability and surface chemistry of metal NPs over longer time periods and how this impacts toxicity.

This research is likely to be of great benefit to the U.S. Environmental Protection Agency, which is charged with regulating the land application of biosolids and pesticides. The results will be beneficial for making predictions about how chemical composition and particle size relate to biogeochemical transformations of NPs in soil and NP bioavailability as well as providing information on potential adverse effects on soil invertebrates and by extension ecosystem functions. These predictions will be necessary components of ecological risk assessments for MNMs and for deriving models that predict MNM behavior in the environment based on physicochemical properties.

EPA Grant Number: R833335

Bioavailability and Toxicity of Nanosized Metal Particles Along a Simulated Terrestrial Food Chain

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Postdocs: W. Aaron Shoults-Wilson[1], Simona Hunyadi[1,3]
Undergraduate Students: Alison Willis[4], Oksana Zurbich[5]
Collaborators: Brian Reinsch[6], Greg Lowry[6]

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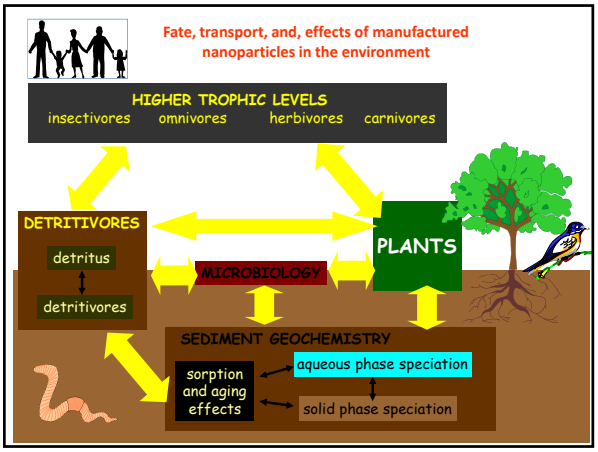
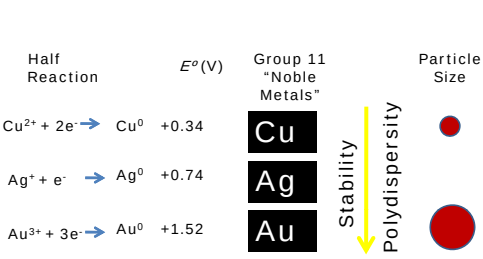
This research is funded by U.S.EPA -Science To Achieve Results (STAR)Program Grant # R833335



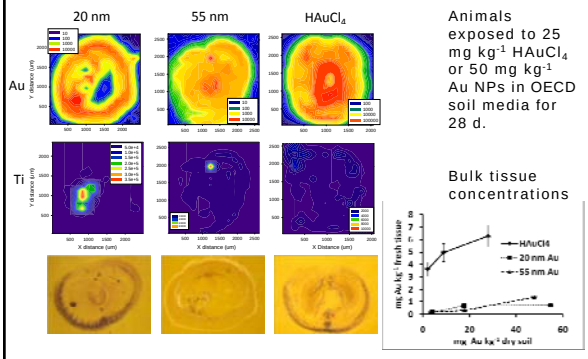


Eisenia fetida
semi-model organism
Important soil toxicity testing model

OECD/EPA test media (70% quartz, 20 % kaolin, 10 % sphagnum peat)

Natural sandy loam

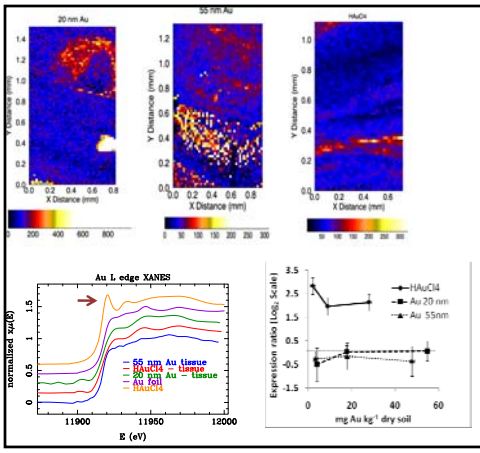
Using Au NPs as a probe for particle uptake –LA-ICPMS



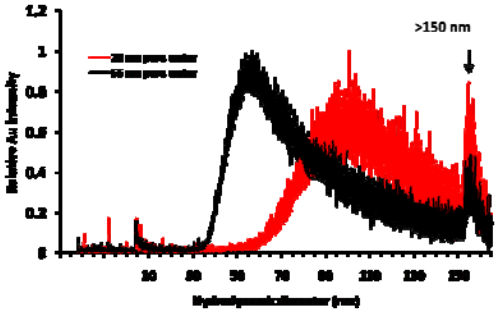
Animals exposed to 25 mg kg^{-1} $HAuCl_4$ or 50 mg kg^{-1} Au NPs in OECD soil media for 28 d.

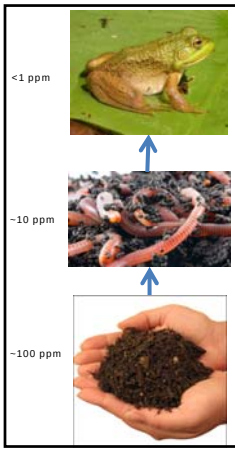
Bulk tissue concentrations

Unrine et al., 2010, ES&T



Unrine et al., in press ES&T





Trophic transfer of Au NPs

Day	Gavage	Worm
1	X	
2		X
3	X	
4		X
5	X	
…		X
14	X	

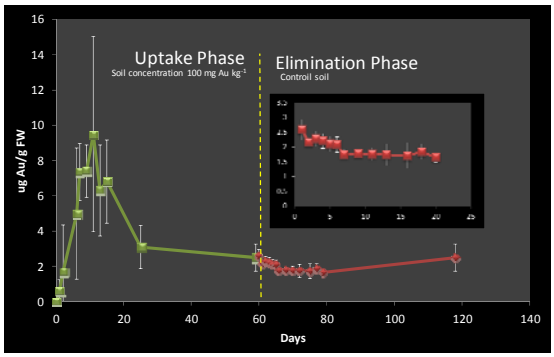
Treatment 1 = Control Gavage, Control worm

Treatment 2 = Au Gavage, Control Worm

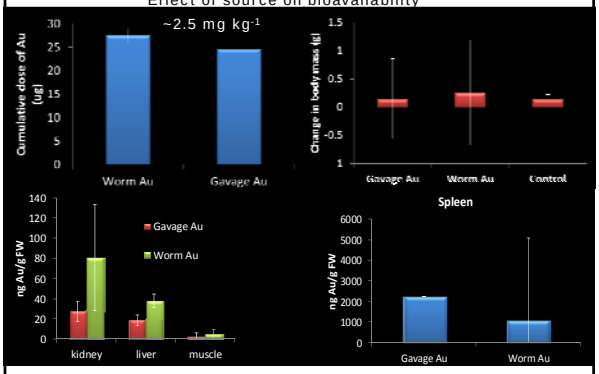
Treatment 3 = Control Gavage, Control Worm

- Gavage dose adjusted each time to maintain similar cumulative dose as earthworm dose

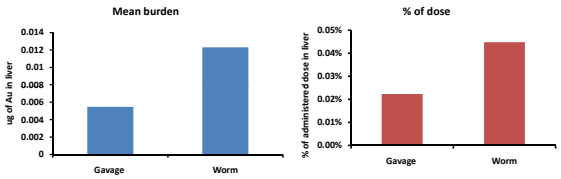
Earthworms



Accumulation of Au NPs in Tissues – Effect of source on bioavailability



Accumulation in liver



Alternative hypotheses

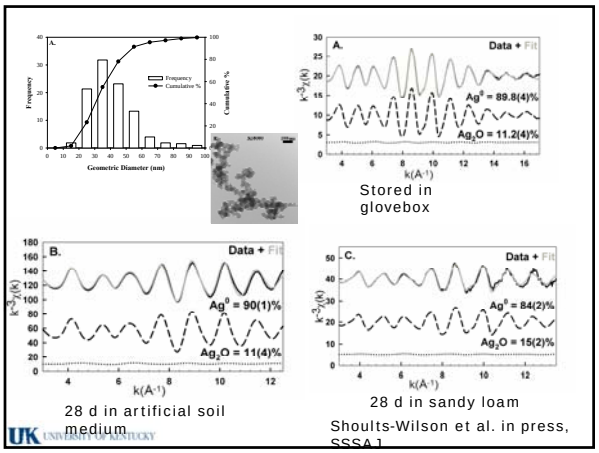
- Once particles enter the earthworm tissues, they acquire a protein corona and thus become more bioavailable
- Earthworms absorb only the most bioavailable particles from the total population of particles, thus enriching the transferable fraction (analogous to trophic enrichment of methylmercury).

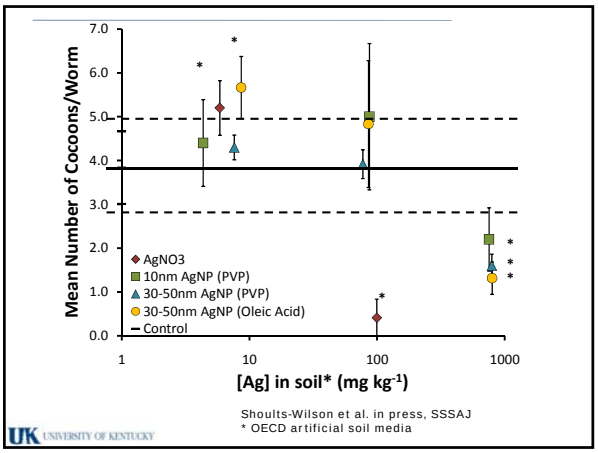
Ag nanoparticles –size and coating

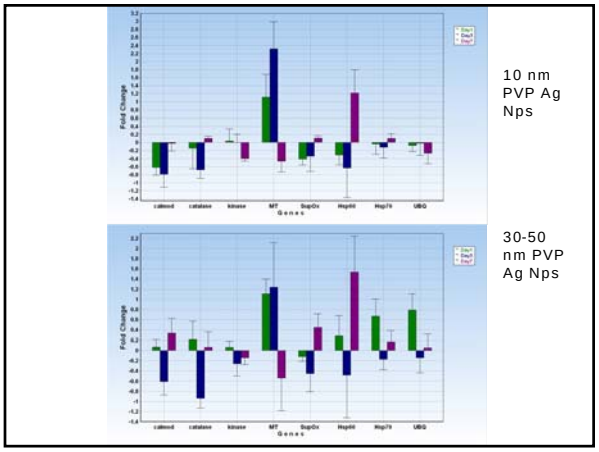
Particle Diameter (nm)	Coating	Properties
35.23 ± 0.81	PVP	Hydrophilic
56.35 ± 1.16	PVP	Hydrophilic
50.60 ± 1.02	Oleic acid	Amphiphilic
27.37 ± 0.36	Citrate	Hydrophilic

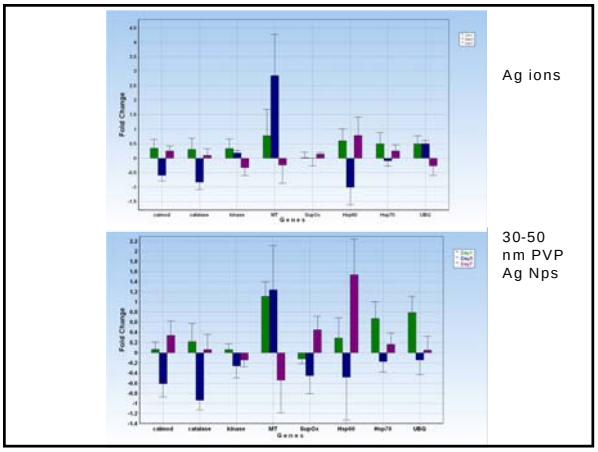
Soils

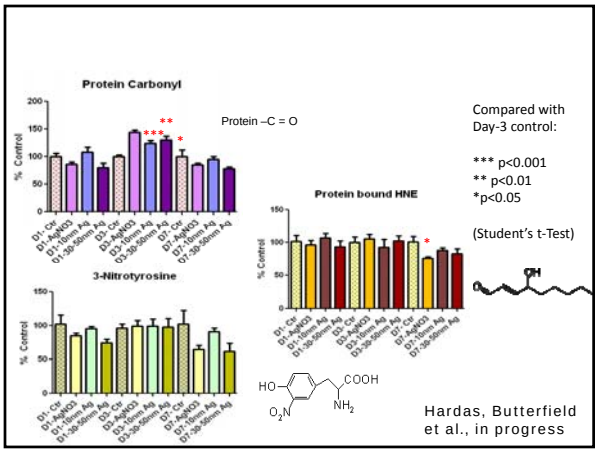
Name	pH_{H2O}	CEC	Sand	Silt	Clay	OM
Yaeger Sandy Loam	5.17	9.18	76.34%	16.53%	7.13%	1.77%
OECD	7.00	14.45	79.12%	6.71%	14.17%	7.65%

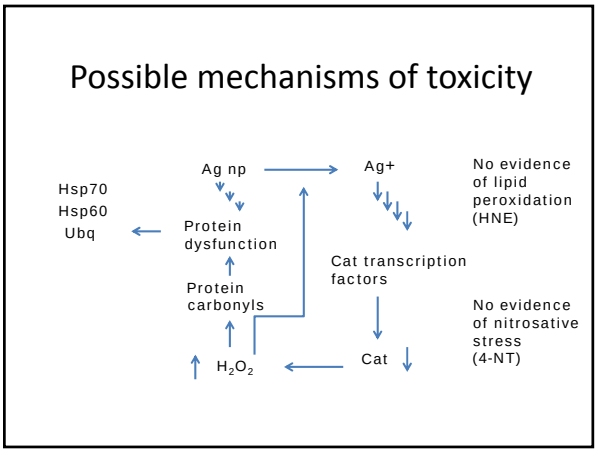






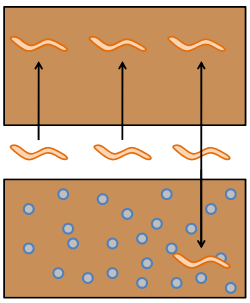






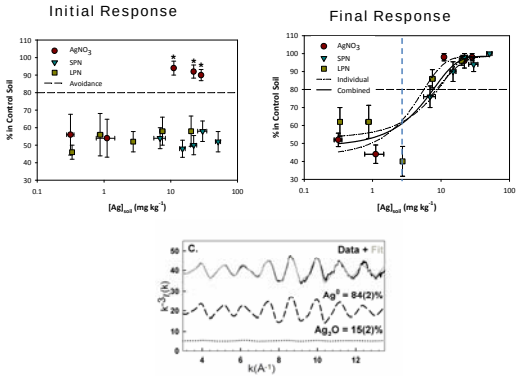
Avoidance of Ag NPs

- Sensitive
  - Initial avoidance
  - Final avoidance
    - 48 h
- High ecological relevance

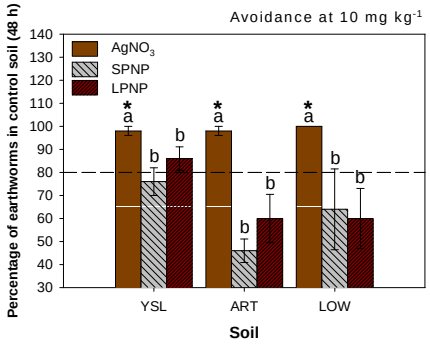


UK UNIVERSITY OF KENTUCKY

Shoults-Wilson et al. in review

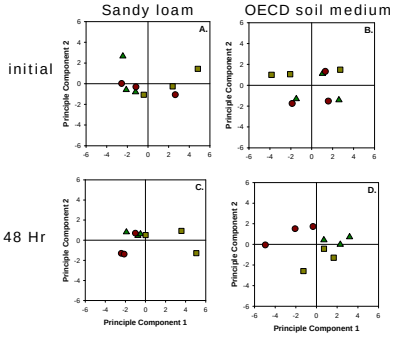


Shoults-Wilson et al. in review



Shoults-Wilson et al. in review

Fatty acid methyl ester analysis (FAME) – Microbial community composition



PC1 – variables associated with bacterial community

PC2- Variables associated with fungal community

Control

Ag NPs

$AgNO_3$

Shoults-Wilson et al. in review

Conclusions

- Nanoparticle are bioavailable from soil and can be transferred to higher trophic levels.
- Particle size and redox properties are important for uptake and toxicity.
- Ag particles cause a variety of adverse effects in earthworms translating from the molecular level up to the population level, some at concentrations similar to those expected in sewage sludge.
- Environmental variables are probably more important than particle variables for Ag toxicity.

Publications

Published

1. W. Aaron. Shoults-Wilson, Brian C. Reinsch, Olga V. Tsyusko, Paul M. Bertsch, Greg V. Lowry, Jason M. Unrine. 2010. Toxicity of silver nanoparticles to the earthworm (*Eisenia fetida*): The role of particle size and soil type. ***Soil Science Society of America Journal***. DOI. doi:10.2136/sssaj2010.0127nps
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3. Jason M. Unrine, Simona E. Hunyadi, Olga V. Tsyusko, W. Aaron Shoults-Wilson, William Rao, Paul M. Bertsch. 2010, Evidence for bioavailability of Au nanoparticles from soil and biodistribution within earthworms (*Eisenia fetida*). ***Environmental Science and Technology*** 44:8308-8313. DOI 10.1021/es101885w
4. Jason Unrine, Paul Bertsch and Simona Hunyadi. 2008. Bioavailability, trophic transfer and toxicity of manufactured metal and metal oxide nanoparticles in terrestrial environments. In: **Nanoscience and Nanotechnology: Environmental and Health Impacts**. Grassian, V.H. Ed., John Wiley and Sons, pp345-360.
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In Review

6. W. Aaron Shoults-Wilson, Oksana I. Zhurbich, David McNear, Olga V. Tsyusko, Paul M. Bertsch, Jason M. Unrine*. *In review,* ***Ecotoxicology***. Earthworms avoid silver nanoparticles.

In Prep

7. J. Unrine, W. Shoults-Wilson, O.Tsyusko. Trophic transfer of Au nanoparticles in a terrestrial food chain. In prep.
8. S. Hardas, A. Butterfield, W. Shoults-Wilson, O. Tsyusko. J Unrine. Oxidative stress and proteomic responses of earthworms to Ag nanoparticles. In prep.
9. O. Tsyusko, W. Shoults-Wilson, J. Unrine. Temporal dynamics of gene expression in earthworms exposed to Ag nanoparticles. In prep.

- Antonio Lanzirotti –U. Chicago/NSLS
- William Rao –UKY/NSLS
- Melissa Lacey- UKY
- Jonathan Judy –UKY
- Greg Joice - UKY
- Diane Addis –Medical College of Georgia
- Ellen Harding – Transylvania University
- The Kim Lab- Chapman University
- Sam Webb, John Bargar, Joe Rogers -SSRL

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John J. Rowe

Determination of Manufactured Nanoparticle Toxicity Using Novel Rapid Screening Methods

***John Rowe*[1], *Saber Hussain*[2], *Rajender Varma*[3], *Ryan Posgai*[1], *Caitlin-Cipolla McCulloch*[1], *Tim Gorey, and Mark Nielsen*[1]**

[1]Department of Biology, University of Dayton, Dayton, OH; [2]Biosciences and Performance Division Human Effectiveness Directorate, Air Force Research Laboratory, Wright-Patterson AFB, Dayton, OH; [3]Clean Processes Branch, Sustainable Technology Division, National Risk Management Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH

We have developed a first tier rapid screening system to determine the toxicity of nanoparticles (NPs) that includes a broad spectrum of organisms, including plants, bacteria, tissue culture, and the fruit fly. The individual organismal (Posgai et al., 2009; Ahamed et al., 2010) and *in vitro (*Ahamed et al., 2008) models have been used to address not only toxicity but also the molecular mechanisms behind toxicity. We will focus in this presentation on Ag NPs and the correlation of surface properties with toxicity. Special emphasis will be placed on the effects of sublethal concentrations on development and reproduction in the fruit fly. The results will be prefaced with a brief review of our earlier molecular findings implicating excess ROS production after exposure to Ag NPs (Ahamed et al., 2008, 2009; Posgai et al., 2009).

Fruit flies provide a powerful model for investigating human health and nanotoxicity. Counterparts of genes responsible for more than 700 different human genetic diseases, including neurological, immunological, cardiovascular, auditory, visual, developmental and metabolic disorders, are found in *Drosophila* (Koh et al. 2006; Wolf et al., 2006; Khurana et al., 2006; Rieter et al., 2001; Sykiotis and Bohmann, 2008). Flies are particularly amenable to investigations of chronic exposure health effects and ecotoxicology, two particularly understudied aspects of NP toxicology. Invertebrates lie at the bottom of food webs and thus are likely to interact with and potentially bioaccumulate environmental NP pollution. Their cost-effectiveness, experimental flexibility, and short generation time permit rapid assessment of the vast number of NPs being produced, including chronic, reproductive, and genotoxic effects less accessible in mammalian systems.

In generating a fly NP toxicity model, we first developed models for different uptake modes (ingestion, Ahamed et al., 2010; inhalation, Posgai et al., 2009). Herein, we report long-term chronic exposure effects. Survival (LD50), developmental rate, reproductive effort, gene expression, and cell physiology (Ahamed et al., 2010) will be assessed, with fully characterized particles generated using different coatings, dispersants and agglomeration states, and particle sizes. Co-exposure with anti-oxidants, phenocopying NP toxicity with known oxidants, and tests in mutant fly backgrounds will be used to experimentally dissect mechanisms of NP toxicity.

The results of our ingestion studies with Ag NPs demonstrate very clear toxic effects on viability, development, and reproduction at levels as low as 10μg/mL. At sublethal concentrations, development was retarded and pupation rate significantly lower than the control. There were also clear differences in phenotype, especially size and coloring at all stages of development. The effects of Ag NPs on development was not reversed by vitamin E but was almost completely reversed by vitamin C.

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Sykiotis GP, Bohmann D. Keap1/Nrf2 signaling re3gulates oxidative stress tolerance and lifespan in *Drosophila*. *Developmental Cell* 2008;14:76-85.

Wolf M, Amrein H, Izatt JA, Choma MA, Reedy MC, Rockman HA. (2006) *Drosophila* as a model for the identification of genes causing adult human heart disease. *Proceedings of the National Academies of Science USA* 2006;103:1394-1399.

EPA Grant Number (vis NSF): CBET-0833953

Model Systems for Rapid Assessment of Long and Short Term Effects of Nanomaterials on Biological Systems

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Department of Biology
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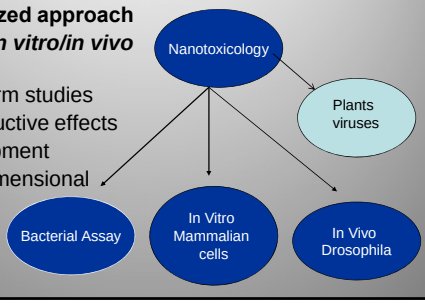
UD/AFRL Nanotoxicity Research Group
John Rowe Ph.D., Jayne Robinson Ph.D., Mark Nielsen Ph.D., Saber Hussain Ph.D., Maqusood Ahamed, Ph.D., Tracy Collins Ph.D., Ryan Posgai, Brittany Demmitt, Caitlin Cipolla-McCulloch, Timothy Gorey, Kyle Murphy

UD/AFRL Nanotoxicity Group

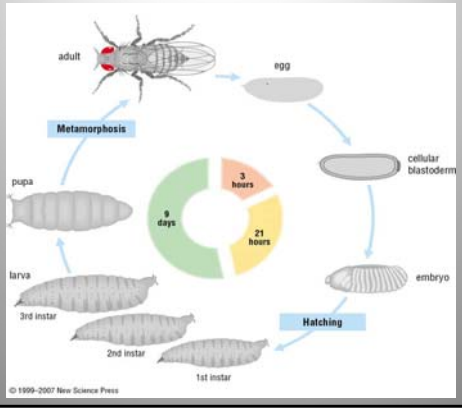
- **Multi-Domain approach**
- **Standardized approach**
- **Coupled *in vitro/in vivo* models**
  - Long term studies
  - Reproductive effects
  - Development
  - Multi-dimensional assays



Life history toxicity effects and vitamin C reversal: a novel *in vivo Drosophila* model for chronic nanoparticle exposure

Department of Biology
University of Dayton
WPAFB/AFRL

Drosophila melanogaster: Life Cycle



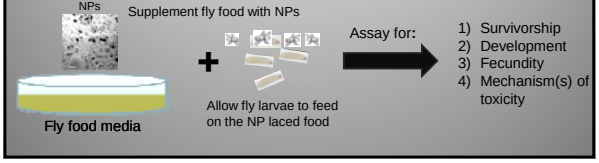
Overall Objective:

- Establish *D. melanogaster* as a model system for rapid assessment of nanoparticle toxicity, *in vivo*

Current Project Objective:

- Study the effects of nanoparticle ingestion on *D. melanogaster* growth and development

Method:



NP Parameters Investigated

NP behavior is function of:

- size
- shape
- surface reactivity

- Compare the effects of different sizes and coating of NPs on *Drosophila* development and reproduction
  - Uncoated or polysaccharide coated

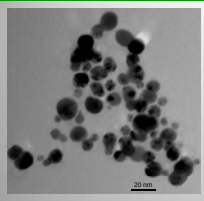
Silver Nanoparticles (Ag NPs)

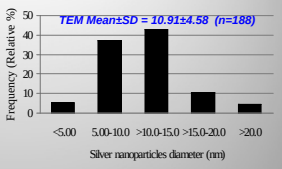
Gift: Dr. Dan Goia, Center for Advanced materials, Clarkson University

Size: 10 nm, 60 nm **Shape:** Almost Spherical

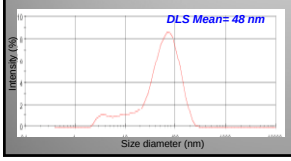
Surface Coating/ Stabilizing Agent: Polysaccharide (Starch) The 10 nm coated Ag NPs were synthesized by the reduction of silver ions in a solution of a polysaccharide (acacia gum), which leads to surface coating.

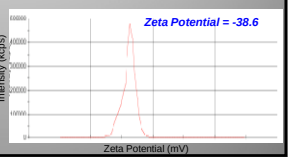
Transmission Electron Microscopy Characterization



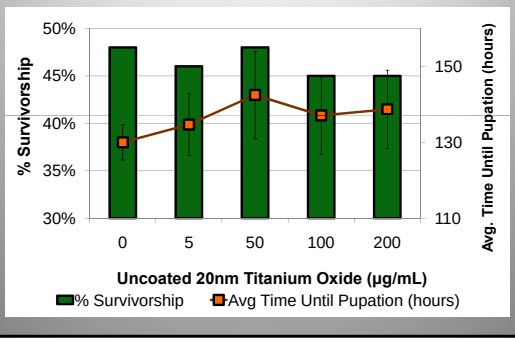


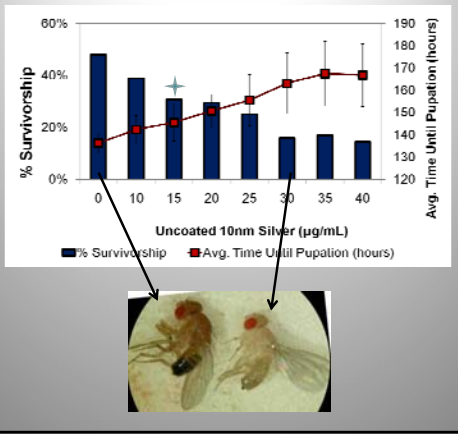
Dynamic Light Scattering Characterization

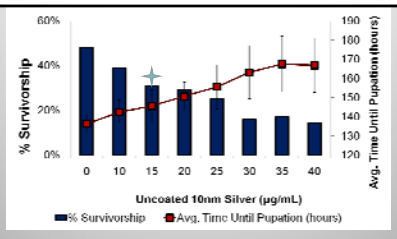


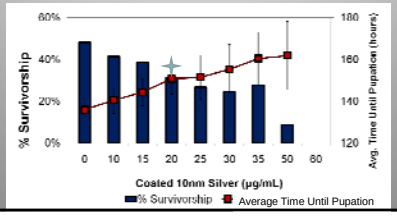


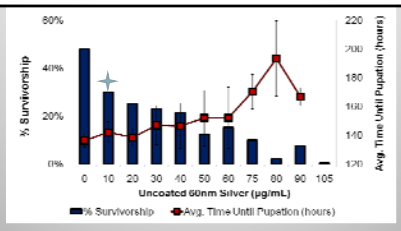
Quantification of NP effect on *Drosophila* larvae

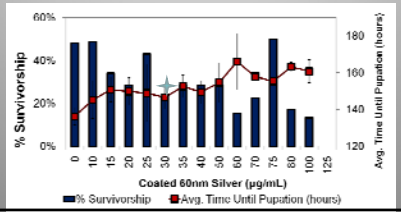












Mechanism of NP Toxicity

- NPs have been shown to increase ROS
  - may result in oxidative stress, inflammation, and consequent damage to proteins, membranes and DNA
- We tested whether oxidative stress occurs *in vivo* using our model system
- Determined effect of treatment with ascorbic acid (Vitamin C)
  - Protector against oxidative stress

Oxidative Stress

•**DEFINITION:**
Oxidative stress occurs when generation of reactive oxygen species (ROS) exceeds the capacity of antioxidant defense mechanisms of cells.

•**LIPID PEROXIDATION (LPO):**
The process whereby ROS "steal" electrons from the lipids in our cell membranes, resulting in cell damage and increased production of ROS

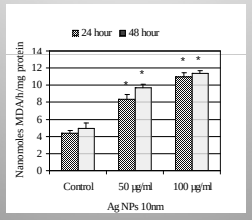
•**REACTIVE OXYGEN SPECIES (ROS):**
Superoxide ion: $O_2^{\bullet}$
Hydroxyl radical: $OH^{\bullet}$
Hydrogen peroxide H_20_2

•**ANTIOXIDANT DEFENSE MECHANISM**
Pathway that provide protection against harmful effects of ROS.

Antioxidant Molecule: e.g. Glutathione (GSH)
Antioxidant Enzymes: e.g. Superoxide dismutase (SOD) and Catalase (CAT)

Ag NPs Enhanced Membrane Lipid Peroxidation

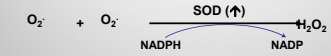
Malondialdehyde (MDA), an end product of lipid peroxidation was quantified to see the extend of membrane lipid peroxidation

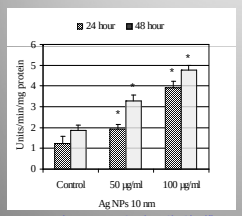


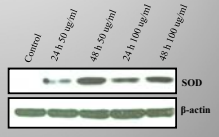
Data represented are mean±SD (n = 3). Significance is ascribed as *p < 0.05 vs. control

Maqsoood Ahamed,[1] Ryan Posgai,[1] Timothy J. Gorey[1], Mark Nielsen,[1] Saber M. Hussain,[2] and John J. Rowe [1]* (2010), Silver nanoparticles induced heat shock protein 70, oxidative stress and apoptosis in Drosophila melanogaster.Toxicology and Applied Pharmacology. 242(3):263-269.

Ag NPs Induced Superoxide Dismutase (SOD) Activity







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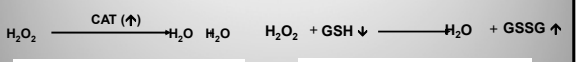
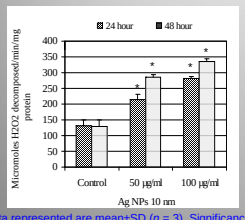
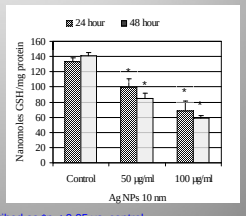
Ag NPs Induced Catalase (CAT) Activity and Depletes Glutathione (GSH) Content







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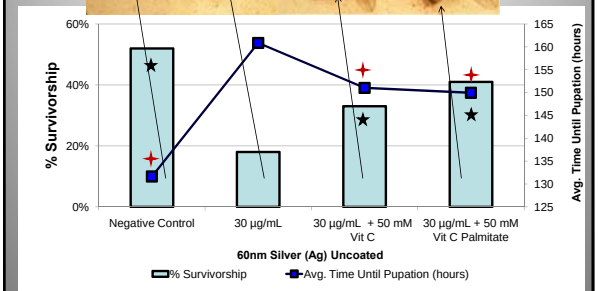
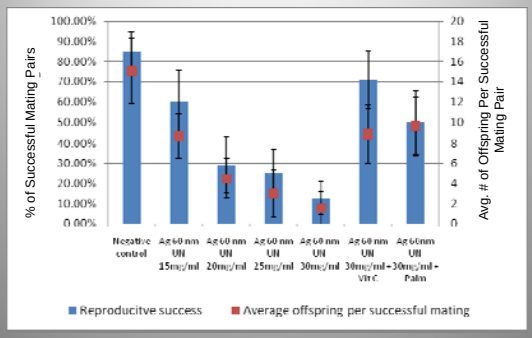
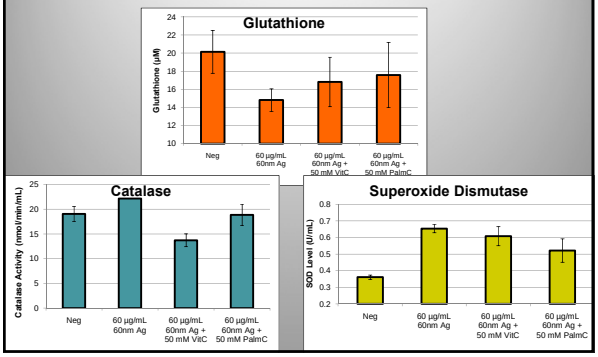


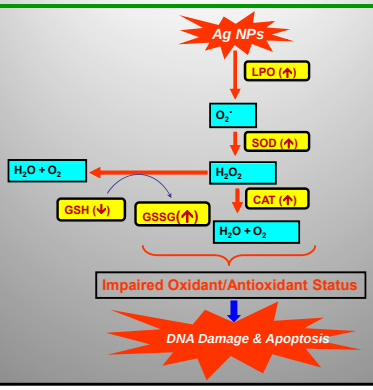
Protection by Vitamin C from Reproductive Effects of Uncoated 60 nm Silver Ingestion



Oxidative Stress as Mechanism of Silver NP Toxicity?



Ag NPs Induced Oxidative Stress



Summary

Conclusions

- Established *in vivo D. melanogaster* model for studying NP toxicity
- Demonstrated
  - Induction of oxidative stress by silver NPs
  - Protective effect of Vitamin C treatment

Future Directions

- Elucidate pathway of oxidative stress involved
- Evaluate efficacy of an array of antioxidants

UD/AFRL
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Ryan Posgai Ph.D. Candidate

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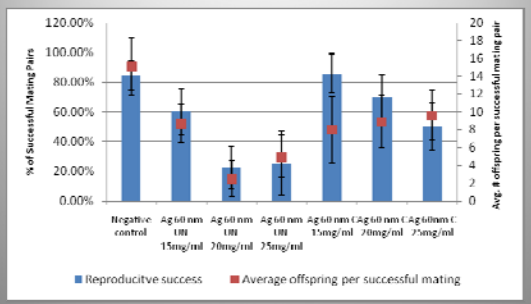
Funding: EPA STAR program via NSF CBET-0833953, Air Force Research Laboratories, Naval Research Laboratories, and Consortium of Universities Research Fellows Program WPAFB/AFRL

Effect of Larval Uncoated and Coated 60 nm Silver NP Ingestion on Reproductive Success



PM Session 2: Environmental Effects on Nanoparticles

Stephen J. Klaine

Influence of Natural Organic Matter on the Behavior and Bioavailability of Carbon Nanoparticles in Aquatic Ecosystems

Stephen Klaine*[1], *Aaron Roberts*[2], *Sharmila Mukhopadhyay*[3], *G. Allen Burton*[4], *Pu-Chun Ke*[1], *and* *E. Michael Perdue*[5]

[1]Clemson University, Clemson, SC; [2]University of North Texas, Denton, TX; [3]Wright State University, Dayton, OH; [4]University of Michigan, Ann Arbor, MI; [5]Georgia Institute of Technology, Atlanta, GA

The overall goal of this research was to characterize the interaction between carbon nanoparticles and natural organic matter (NOM) and the influence this interaction might have on nanoparticle bioavailability. Further, our goal also was to characterize movement of these particles through an aquatic food chain. This research is approximately 18 months into the 36-month project. We have examined the behavior of carbon nanoparticles in solutions of natural organic matter. Suwannee River NOM was obtained from the International Humic Substances Society. This research has utilized transmission electron microscopy, dynamic light scattering, and infrared analysis of tubes before and after NOM adsorption. Stability of multi-walled nanotubes is not influenced by NOM concentrations over 2 mg/L as carbon suggesting that these nanoparticles could be stable in most surface waters. As expected, increased ionic strength decreased the stability of these nanoparticle suspensions. Similar results were obtained with C_{60} and C_{70} fullerenes. However, single-walled carbon nanotubes were not stable in the NOM solution.

Also, we have examined the bioavailability of surface modified carbon nanomaterials. For this research, we conducted static renewal bioassays with the aquatic filter-feeding invertebrate, *Daphnia magna*. Methodology for both rearing organisms and bioassays was as described in the EPA methods. We used transmission electron microscopy to examine the fate of the nanotubes within the organism. Multi-walled carbon nanotube toxicity to *D. magna* was not influenced by the concentration of NOM. The 96 hr LC50 value was 2.2 ± 0.2 for concentrations of NOM ranging from 2 – 20 mg/L carbon. These nanotubes did not appear to aggregate in the gut tract of the organism. Further, these nanotubes appeared to stay within the gut tract and were ultimately eliminated when transferred to clean medium. Toxicity appeared to be due to gut tract clogging and interference with food uptake and processing. This is an energetics effect and similar to that which we described previously for suspended clay particles.

The maximum concentration of fullerenes that we were able to achieve was 15 mg/L in NOM solutions. These suspensions, while stable, did not exhibit sufficient toxicity to generate an LC50 value. However, C_{70}, surface modified with gallic acid (a phenolic acid) was not only stable, but also acutely toxic to *D. magna* with a 96 hr LC50 value 0.4 ± 0.1 mg/L. In a 21-day chronic study, the NOEC was 0.02 mg/L.

Because there was no indication that either multi-walled carbon nanotubes or fullerenes entered the *D. magna* body from the gut tract, we examined small, highly fluorescent carbon dots (4 nm diameter). These particles are very hydrophilic with a polyethylene glycol surface coating. These particles were non-toxic to *D. magna*, and we were able to detect migration out of the gut tract and into the organisms.

Because one of our ultimate goals is to examine the fate of these materials in an aquatic food chain we have begun focusing on which parameters facilitate the movement of nanoparticles accross the *D. magna* gut tract.

EPA Grant Number: R834092

Influence of Natural Organic Matter on the Behavior and Bioavailability of Carbon Nanoparticles in Aquatic Ecosystems.

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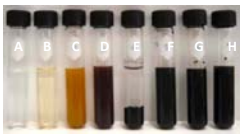
Collaborators

- Aaron Roberts, University of North Texas
- Sharmila Mukhopadhyay, Wright State University
- G. Allen Burton, University of Michigan
- Pu-Chun Ke, Clemson University
- E. Michael Perdue, Georgia Institute of Technology

How do Water Quality Parameters such as NOM Influence the Bioavailability of Carbon Nanoparticles ?

NOM stabilizes <u>most</u> carbon nanoparticle suspensions



***400 mg/L nanoparticles**

- **A: Water**
- **B: 100 mg/L NOM**
- **C: 100 mg/L NOM + C_{60}**
- **D: 100 mg/L NOM + C_{70}**
- **E: 100 mg/L NOM + SWNT**
- **F: 100 mg/L NOM + MWNT**
- **G: 100 mg/L NOM + Nanocoil**
- **H: 100 mg/L NOM + Nanowire**

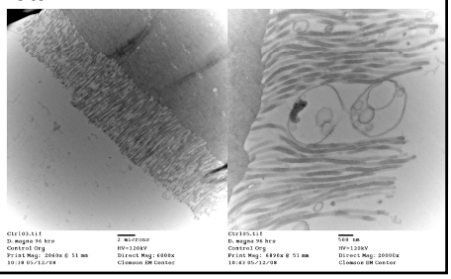
**Sonicated in small quantities for 30 min

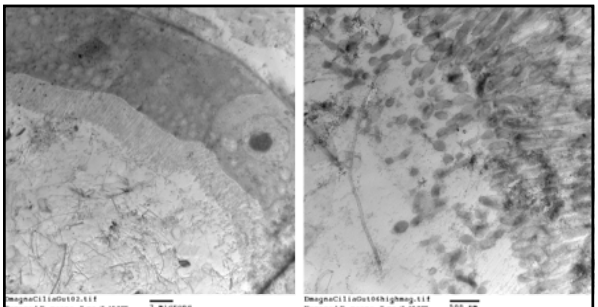
Toxicity of Carbon Nanomaterials (96hrLC50 values)

- MWNT (NOM stabilized) 2.2 mg/L
- C60 (NOM stabilized) >15 mg/L
- C70 (NOM stabilized) >15 mg/L
- C70-Gallic acid 0.4 mg/L
- OH-SWNT (NOM stabilized) no toxicity at 2mg/L
- PEG-SWNT (NOM stabilized) no toxicity at 2mg/L
- Carbon dots >20 mg/L

Are Carbon Nanomaterials Absorbed from the Intestinal Tract?

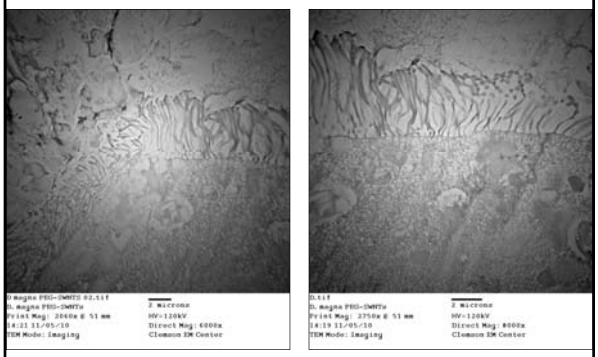
- MWNT
- Carbon Dots
- SWNT
  - OH
  - PEG





Edgington AJ, Roberts AP, Taylor LM, Reppert J, Rao AM, Mao J, Klaine SJ. 2010. The Influence of Natural Organic Matter on the Toxicity of Multiwalled Carbon Nanotubes. Environ. Toxicol. Chem. 29(11):2511-2518.

PEG Functionalized



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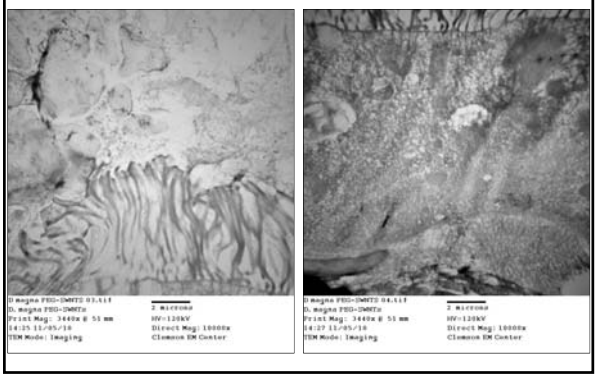
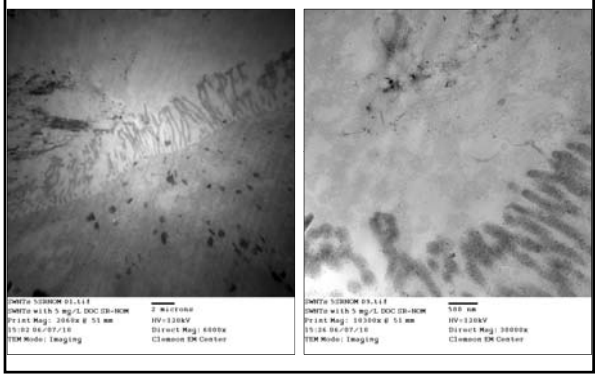
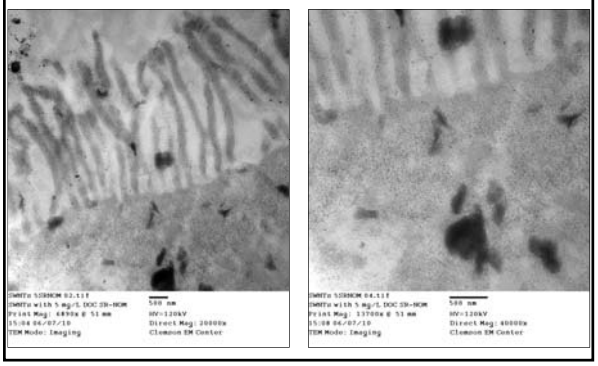


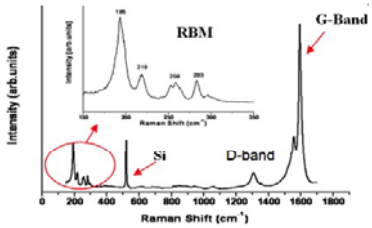
OH-SWNT



OH-SWNT



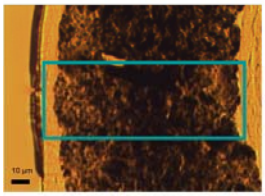
Raman Spectroscopy

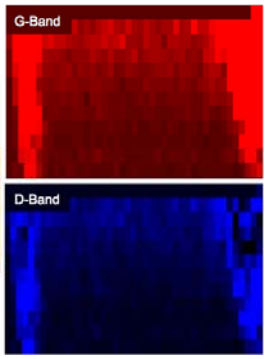


SWNTs posses resonant Raman spectral features. A typical spectra is on the left.

We can track G-band, D-Band for mapping SWNTs in any biological tissue.

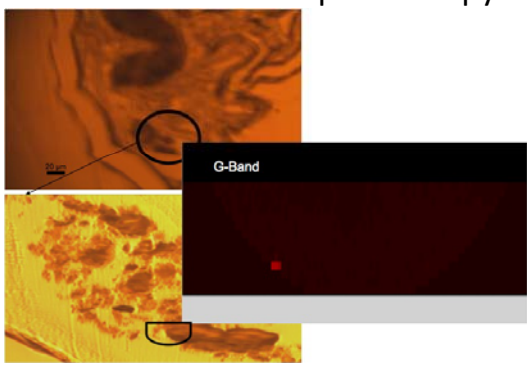
OH-SWNTs Raman Spectroscopy



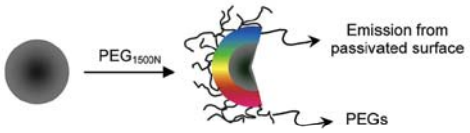


D. magna 96 hr exposure to 2 mg/L OH-SWNTs
8 um section, strong G and D Band signal within gut tract

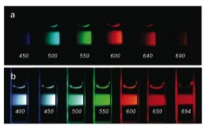
OH-SWNTs Raman Spectroscopy



Carbon Dots



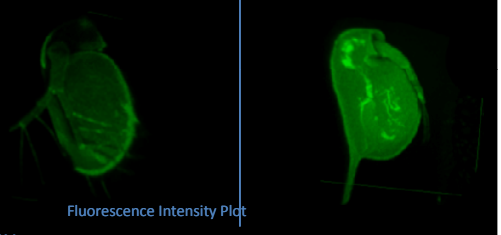
Sun et al.



- Optical effects due to size
- Available in range of sizes
  - 8, 40, 113nm

Confocal Microscopy

Control | Treatment

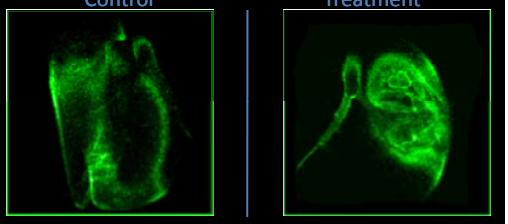


Fluorescence Intensity Plot

10X

Carbon Dot Uptake

Control | Treatment

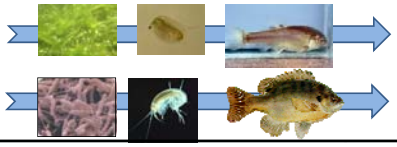


Observations

- MWNTs are acutely toxic to *D. magna*
  - Not a function of NOM
  - Appears to be a result of interfering with food processing.
    - Gut tract clearance: 29 hrs for MWNT; 30 min for clay
- MWNTs are not taken up from the gut tract
- Carbon dots migrate from the gut tract and appear to be associated with organelles
- OH-SWNT may migrate from the gut tract
- PEG-SWNT do not migrate from the gut tract

Next Steps

- Continue to examine uptake from the gut tract
  - Fluorescent labeled SWNT
  - Other surface modifications
- Food chain studies
  - Carbon nanoparticles that are bioavailable will be 13C-enriched and run through our aquatic food chain



Acknowledgments

- Aaron Edgington, Brandon Seda
- U.S. Environmental Protection Agency's STAR program

- Clemson University Public Service Actvities
- Clemson University Office of Research

Any Questions?

Chad T. Jafvert

Environmental Photochemical Reactions of nC_{60} and Functionalized Single-Walled Carbon Nanotubes in Aqueous Suspensions

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School of Civil Engineering and Division of Environmental and Ecological Engineering, Purdue University, West Lafayette, IN

Risk assessment of engineered nanomaterials necessitates the need for information on the reactivity (or conversely, persistence) and transformation pathways of these materials in the natural environment. To this end, we have characterized the reaction rates and products formed when aqueous C_{60} clusters (nC_{60}) are exposed to natural sunlight, and have initiated studies on the photochemical reactivity of functionalized and unfunctionalized single-walled carbon nanotubes (SWCTs). Using furfuryl alcohol (FFA) as a singlet oxygen ($^1O_2$) scavenger, we have shown that aqueous suspensions of nC_{60} clusters produce singlet oxygen ($^1O_2$) upon exposure to sunlight. Mass loss of molecular C_{60} occurs within these suspensions over a period of days in summer sunlight (40° 26' N lat.), whereas mass loss does not occur in dark control samples or in samples containing no O_2. A combination of ^{13}C-NMR analysis of ^{13}C-enriched nC_{60}, X-ray photoelectron spectroscopy, and FTIR analysis indicates that photoproducts have olefinic carbon atoms as well as a variety of oxygen-containing functional groups, including vinyl ether and carbonyl or carboxyl groups, whose presence destroys the native π-electron system of C_{60}. Thus, the photoreactivity of nC_{60} in sunlight leads to the formation of water soluble C_{60} derivatives. Laser desorption ionization time-of-flight (LDI-TOF) mass spectroscopy indicated that most of the photoproducts formed after 947 hours of irradiation in natural sunlight retain a 60-atom carbon structure. Long-wavelength visible light ($\lambda \geq 400$ nm) isolated from sunlight, was shown to be important in both the phototransformation of nC_{60} and in the production of $^1O_2$.

Unlike molecular C_{60} that can be analytically separated and quantified by HPLC methods, the reactivity of carbon nanotubes in sunlight must be studied by examining: (1) formation of indirect photochemical products, (2) changes in spectroscopic properties from which functional group distributions can be deduced, and (3) changes in other bulk physicochemical properties, such as length, colloidal stability, electrophoretic mobility, etc. As a start, we have investigated the production of reactive oxygen species (ROS) (i.e., indirect photochemical product formation) in aqueous suspensions of commercial preparations of carboxylic acid functionalized SWNTs (SWNT-COOH), polyethylene glycol functionalized SWNTs (SWNT-PEG), and unmodified (i.e., pristine or unfunctionalized) SWNTs. Using FFA, a tetrazolium salt, and *p*-chlorobenzoic acid as molecular probes for $^1O_2$, superoxide anion ($O_2^{\cdot-}$), and hydroxyl radial (·OH), respectively, photo-production of all three reactive oxygen species occurred in aqueous suspensions of both types of functionalized tubes, but not to any significant degree over the time period of our experiments in aqueous suspensions of unfunctionalized SWNTs containing sodium dodecylsulfate, used to facilitate disaggregation and dispersion. Defects in the fullerene surface caused by functionalization may facilitate ROS production, as well as differences in amorphous carbon and metal impurity content within the different SWNT preparations. Experiments suggest that the metal impurities may especially contribute to ·OH generation.

These results suggest that functionalization, even with moieties that do not contain sunlight-active chromophores, and/or surface defects strongly influence the environmental photoreactivity of SWCTs, and potentially the environmental persistence of carbon nanotubes in general.

References:

1. Hou Wen-Che, Jafvert Chad T. Photochemical transformation of aqueous C_{60} clusters in sunlight. *Environmental Science and Technology* 2009;43:362-767.

2. Hou Wen-Che, Jafvert Chad T. Photochemistry of aqueous C_{60} clusters: evidence of $^1O_2$ formation and its role in mediating C_{60} phototransformation. *Environmental Science and Technology* 2009;43:5257-5262.
3. Chen Chia-Ying, Jafvert Chad T. Photoreactivity of carboxylated single-walled carbon nanotube in sunlight: reactive oxygen species production in water. *Environmental Science and Technology* 2010;44:6674-6679.
4. Hou Wen-Che, Kong Lingju, Wepasnick Kevin A, Zepp Richard G, Fairbrother D. Howard, Jafvert Chad T. Photochemical transformation of aqueous C_{60} clusters: wavelength dependency and product characterization. *Environmental Science and Technology* (in press, 2010).
5. Chen Chia-Ying and Chad T. Jafvert. Photoinduced reactive oxygen species production by single-walled carbon nanotubes in water: role of surface functionalization. (to be submitted, 2010).

EPA Grant Number: R8333401

Environmental Photochemical Reactions of nC_{60} and Functionalized Single-Walled Carbon Nanotubes in Aqueous Suspensions

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This research is funded by U.S. EPA - Science To Achieve Results (STAR) Program
Grant # 83334001

PURDUE UNIVERSITY School of Civil Engineering

Previous Studies with C_{60}‡

- "Solubility of C_{60} in solvent mixtures"
  (*Env. Sci. Technol.* 42: 845-851, 2008)
- "C_{60}'s K_{ow} and Aqueous Solubility"
  (*Env. Sci. Technol.* 42: 5945-5950, 2008)
- "Sorption of C_{60} to Saturated Soils"
  (Env. Sci. Technol. 43*: 7370-7375, 2009)*

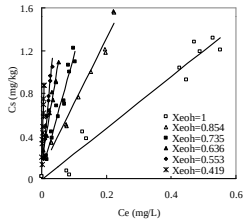
‡Funded by NSF

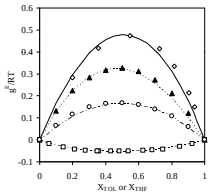
Previous Results

- *Solvated crystals occur*
- $K_{ow} \approx 10^{6.7}$; $K_{oc} \approx 10^{6.2} - 10^{7.1}$
- Aqueous Solubility limit ≈ 8 ng/L

$$RT \ln \gamma_i = \overline{g}_i^E = \left(\frac{\partial n_T g_E}{\partial n_i} \right)_{T,P,n_j}$$

$$\log K_{oc} = -\log S_i - 1.10$$





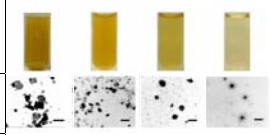
Excess free energy of mixtures (▲TOL-ACN, ○ THF-ACN, □ TOL-THF ◊ TOL-EOH). For the TOL-THF data set, the abscissa is X_{TOL} (not X_{THF}).

Current EPA-funded Study

Project period: May 2007 – April 2009 (currently in extension)

- "Photochemical transformation of aqueous C_{60} clusters (nC_{60}) in sunlight" (*Env. Sci. Technol.* 2009, 43:362-367)
- "Photochemistry of aqueous C_{60} clusters: Evidence of $^1O_2$ formation and its role in mediating C_{60} phototransformation" (*Env. Sci. Technol.* 2009, 43:5257-5262)
- *"Photochemistry of aqueous C_{60} clusters: Wavelength dependency and product characterization" (Env. Sci. Technol. 2010, 8121-8127)*
- *"Photoreactivity of carboxylated single-walled carbon nanotubes in sunlight: Reactive oxygen species production in water" (Env. Sci. Technol.* 2010, 6674-6679*)*
- *"Solar light induced reactive oxygen species production by single-walled carbon nanotubes in water: Role of Surface Functionalization" (under review, Env. Sci. Technol.)*

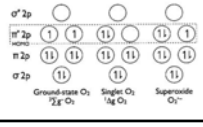
Photochemical transformation of aqueous C_{60} clusters (nC_{60}) in sunlight" (*Env. Sci. Technol.* 2009, 43:362-367)

Irradiation time under lamps (day)	0	10	30	65
$[nC_{60}]$ (mg/L)	65	19.5	2.6	0.47
Color				
TEM image*				
Mean diameter** (nm)	500	350	250	160
After Centrifugation***				

*Scale bars indicate 1000 nm.
**Mean hydrodynamic diameters by DLS.
***Samples after centrifugation (13000xg, 1 h) and filtration (nylon membrane, 0.2-μm pore size

Summary

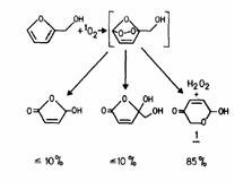
- First paper to report on C_{60} photochemical decay in aqueous media under sunlight.
- C_{60} measured quantitatively by HPLC
- Smaller clusters result in faster loss of C_{60}
- son/nC_{60} and THF/nC_{60} react at similar rates
- Photo-transformation rate is not pH dependent (3-11)
- Negligible rate change with humic acids present
- Molecular Oxygen (O_2) is required.



hydroxyl radical (•OH)

$^1O_2$ measurement

- The production of $^1O_2$ during irradiation of nanomaterials in sunlight and solar-simulated light is monitored by the loss of furfuryl alcohol (FFA) as a trapping indicator.
- [FFA] is analyzed by HPLC with UV detection at 219 nm.



$$-\frac{d[FFA]}{dt} = k_r[^1O_2]_{ss}[FFA]$$

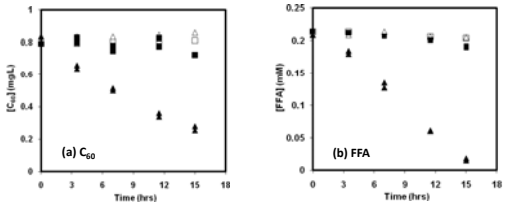
$$-\frac{d[FFA]}{dt} = k_{exp}[FFA]$$

$$[^1O_2]_{ss} = \frac{k_{exp}}{k_r}$$

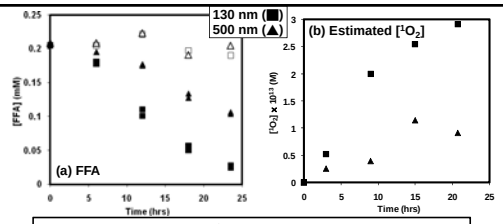
[Haag et al., 1984, 1986]

Photochemistry of aqueous C_{60} clusters: Evidence of $^1O_2$ formation and its role in mediating C_{60} phototransformation" (*Env. Sci. Technol.* 2009, 43:5257-5262)

Irradiated nC_{60},	deoxygenated (■)
Dark control of nC_{60},	deoxygenated (□)
Irradiated nC_{60},	air-equilibrated (▲)
Dark control of nC_{60},	air-equilibrated (Δ)



Irradiation of 0.8 mg/L nC_{60} with 0.2 mM FFA at pH = 7 in lamp light, showing (a) C_{60} and (b) FFA measured in deoxygenated, air-equilibrated, and dark control samples.



$[^1O_2]$ calculation

Steady-state $[^1O_2]_{ss}$ (Haag et al., 1986):

$$-\frac{d[FFA]}{dt} = k_{exp}[FFA]$$

$$-\frac{d[FFA]}{dt} = k_r[^1O_2]_{ss}[FFA]$$

$$[^1O_2]_{ss} = \frac{k_{exp}}{k_r}$$

Non-steady-state $[^1O_2]_t$:

$$[^1O_2]_t = \frac{-\frac{d[FFA]}{dt}_t}{k_r[FFA]_t}$$

Photochemical production of $^1O_2$ by 130-nm (■) and 500-nm (▲) diameter nC_{60} (1 mg/L) under sunlight from July 23 to August 11, 2008 at pH = 7, showing (a) FFA loss, and (b) the calculated $[^1O_2]$ in the irradiated samples, and the recovery of FFA in dark control samples.

Comparison of $[^1O_2]$ measured in this study to values reported for surface waters.

Water source	Sunlight intensity (W/m²)	DOC (mg/L)	$[^1O_2]^0_{ss}$[g] (× 10^{14} M)	$[^1O_2]^0_{60}$/DOC (× 10^{14} M per mg/L)
nC_{60}[a]	525	5	71.1[h,i]	14.2[h]
FA[a]	525	2.6	5.6[i]	2.1
Swiss surface waters[b]	1000[f]	3.2-13	5.9-28[i]	0.8-3.2
Municipal wastewaters[b,c]	1000[f]	8.6-31	11-15[i]	0.3-1.1
US surface and coastal waters[d]	800	4-77	6-71[i]	0.7-2.9
Dutch surface waters[e]	800	8-21	0.4-7.6[i]	0.22

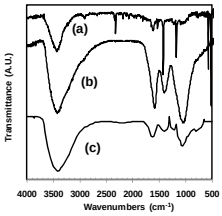
[a]This study. [b]Data from Haag et al. (*18*). [c]Influents and secondary effluents, and the inflow and outflow of a waste stabilization pond in Switzerland. [d]Data from Zepp et al. (*17*). [e]Data from Wolff et al. (*29*). [f]λ = 280-2800 nm in summer-noon sunlight. [g]Corrected to a flat surface water body (*18*). S_λ was calculated at 400 nm for nC_{60}. [h]Value after 10 h of sunlight irradiation. [i]Measured by the FFA method. [j]Measured by 2, 5-dimethylfuran (DMF) method using $k_r = 6.3 \times 10^8$ $M^{-1}s^{-1}$ (*18*).

Summary

- $^1O_2$ forms during solar irradiation of nC_{60}.
- (Loss of FFA (as the probe molecule) in D_2O and in the presence of NaN_3 is consistent with known reaction mechanisms involving $^1O_2$)
- The photo-transformation of nC_{60} is mediated by $^1O_2$.
- The rate of $^1O_2$ production is auto-catalyzed by nC_{60} water-soluble products (formed during irradiation).
- $^1O_2$ production rate is higher when nC_{60} size is smaller.
- $[^1O_2]$ induced by nC_{60} in sunlight is 4-65 fold higher than the average concentration typically found in sunlit natural surface waters.

Photochemistry of aqueous C_{60} clusters: Wavelength dependency and product characterization" *(Env. Sci. Technol. 2010, 8121-8127)*

Wen-Che Hou, Lingjun Kong, Kevin Wepasnick, Richard Zepp, Howard Fairbrother, Chad Jafvert
Purdue University, U.S. EPA, Athens GA, Johns Hopkins U.



(a) Dark control sample
(b) Irradiated sample (780 h)
(c) Fullerenol, $C_{60}(O)_x(OH)_y$, x + y=22

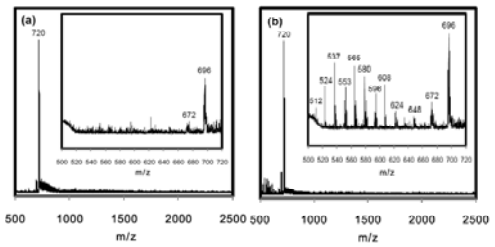
C-O stretch (1060 cm^{-1})

C-O-H in-plane bending or carboxylate asymmetric stretching (1390 cm^{-1})

C=C stretching or carboxylate symmetric stretching (1600 cm^{-1})

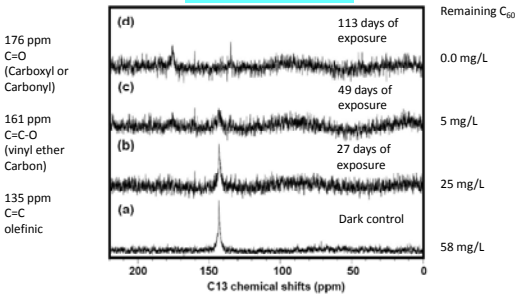
FTIR spectra of AQU/nC_{60} showing (a) the dark control sample, (b) the irradiated sample (780 h), and (c) a commercial fullerenol $[C_{60}(O)_x(OH)_y$, where x + y = 22, (MER Corp.)]. Spectrum (c) is reproduced from Fortner, 2007.

(w/ Carl Wood, Campus-Wide Mass Spectrometry Center)



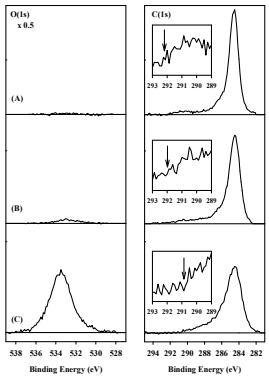
Laser desorption ionization (LDI)-time-of-flight (TOF) mass spectroscopy of AQU/nC_{60}, showing (a) the dark control sample, (b) the irradiated sample (780 h) in sunlight

(w/ Zepp and Kong, EPA)



^{13}C NMR spectra of ^{13}C-enriched (25%) THF/nC_{60} (58 mg/L) exposed to sunlight from February 12 to June 15, 2009. (parent peak occurs at 143 ppm)

(w/ Wepasnick and Fairbrother, JHU)



XP spectral envelopes of the O(1s) and C(1s) regions for (a) as-received C_{60}, (b) dark control AQU/nC_{60}, and (c) AQU/nC_{60} irradiated in sunlight for 947 h. For each sample the integrated area under the C(1s) spectral envelope has been normalized and the O(1s) signal intensity adjusted accordingly. In the C(1s) spectra of (b), the binding energy regions for C−C/C=C and oxidized carbon atoms (i.e., CO_x species) has been shown. The inset in the C(1s) region shows the change in the π-π* shake-up feature centered at 291 eV for each C_{60} sample; in each insert, the vertical arrow indicates the binding energy below which spectral intensity is observed.

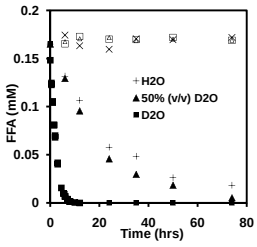
Summary

- NMR
- FTIR
- LDI-TOF-MS
- XPS

- all indicate oxidation of C_{60} occurs in aqueous suspensions of nC_{60} under sunlight (i.e., destruction of π-bonds) (*vinyl ethers, carbonyl and/or carboxyl groups*)

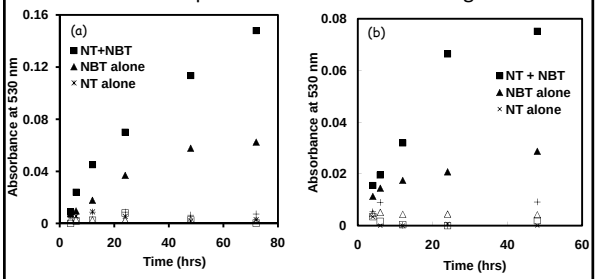
- Experiments with 400 nm cut-off filters and with monochromatic light at λ = 436 nm indicate that C_{60} photo-transformation and $^1O_2$ production occur in visible light (λ > 400 nm).

"Photoreactivity of carboxylated single-walled carbon nanotubes in sunlight: Reactive oxygen species production in water" (*Env. Sci. Technol.* 2010, 6674-6679)



FFA loss at pH 7 under lamp light in 0.01 mg/mL COOH-ARC dispersed in 100 % (v/v) D_2O, 50 % (v/v) D_2O, and H_2O, and FFA recovery in the corresponding dark control samples

Are other Reactive Oxygen Species involve in COOH-ARC phototransformation in sunlight?



Evidence of $O_2^{\cdot-}$ production, via NBT^{2+} (0.2 mM) product formation induced by 10 mg/L COOH-ARC at pH 7 under (a) lamp light and (b) sunlight.

Superoxide anion ($O_2^{\cdot-}$) measurement

• Nitro blue tetrazolium salt (NBT^{2+}) has been one of the most widely used reagent for the detection of $O_2^{\cdot-}$ [Bartosz, 2006]: NBT^{2+} reacts with $O_2^{\cdot-}$ producing products that absorbs light at 530 nm.

• XTT forms a water-soluble reduction product in the presence of $O_2^{\cdot-}$ [Ukeda, 1997; Bartosz, 2006]. The concentration of superoxide was measured by comparing XTT (0.1 mM) reduction with and without superoxide dismutase (40 U/mL)

XTT = 3'-{1-[(phenylamino)-carbonyl]-3,4-tetrazolium}-bis(4-methoxy-6-nitro)-benzenesulfonic acid

•OH Measurement

•*p*-Chlorobenzoic acid (*p*CBA) was used as a reactive ·OH radical scavenger.

•*p*CBA concentrations were measured by HPLC with a UV/Vis detector set at 230 nm.

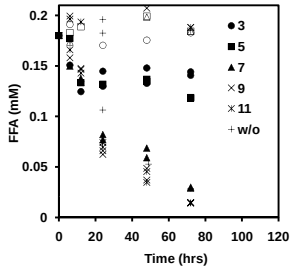
$$-\frac{d[\rho CBA]}{dt} = k_{OH,\rho CBA}[\bullet OH]_{ss}[\rho CBA]$$

$$-\frac{d[\rho CBA]}{dt} = k_{exp}[\rho CBA]$$

$$[\bullet OH]_{ss} = \frac{k_{exp}}{k_{OH,\rho CBA}}$$

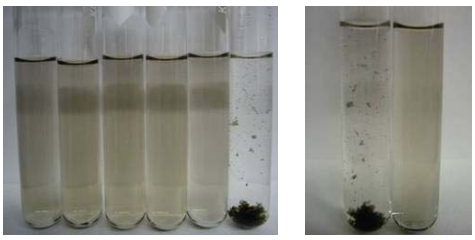
[Elovitz et al., 2000]

pH effect?



FFA loss under lamp light in 0.01 mg/mL COOH-ARC at pH 3, 5, 7, 9, 11, and without buffering , and FFA recovery in the corresponding dark control sample at respective pH

(a) (b)



0.01 mg/mL COOH-ARC in water after 6 hours (λ = 350 ± 50 nm):
(a) without buffer and at pH = 11, 9, 7, 5, 3 (left to right), and
(b) and the same irradiated pH 3 sample (left) and dark control sample (right).

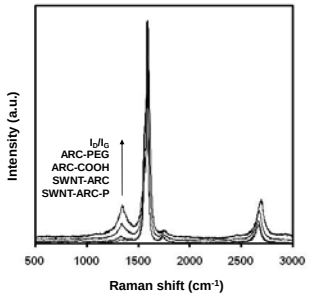
Summary

- In oxic aqueous solutions under sunlight, carboxylated-SWNTs dispersions generate singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\cdot-}$), and hydroxyl radicals (·OH).
- Reactions with probe molecules were corroborated with experiments using D_2O and azide (for $^1O_2$), superoxide dismutase (for $O_2^{\cdot-}$), and tert-butanol (for ·OH).
- Photo-induced aggregation occurred at pH 3.

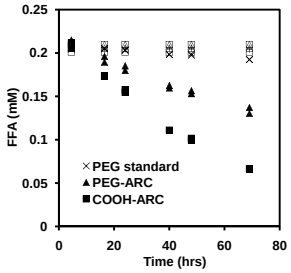
"Solar light induced reactive oxygen species production by single-walled carbon nanotubes in water: Role of Surface Functionalization" *(under review, Env. Sci. Technol.)*

Sample	Synthesis method	Functionalization	Metal residue content*	Carbonaceous purity
SWNT-ARC	Electric arc discharge	No functionalization	30%	~53%
SWNT-ARC-P	Electric arc discharge	No functionalization	5%	>90%
COOH-ARC	Electric arc discharge	Carboxylation	5.9%	>90%
PEG-ARC	Electric arc discharge	PEGylation	5.2%	>90%
SWNT-CVD-P	Chemical vapor deposition	No functionalization	--	>95%

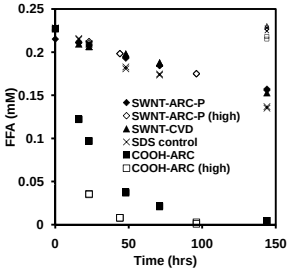
*Vender specification determined by thermal gravimetric analysis (TGA) at 900 ºC in air.
The supplier of "ARC" tubes was Carbon Solutions, Inc.
The supplier of "CVD" tubes was NanoLab, Inc.



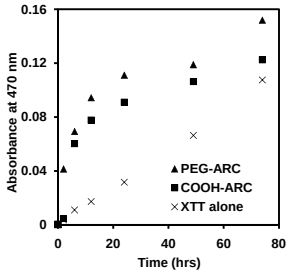
Raman spectra confirms the degree of functionalization: I_D/I_G increases through the following sequence: SWNT-ARC-P ≤ SWNT-ARC << ARC-COOH < ARC-PEG.



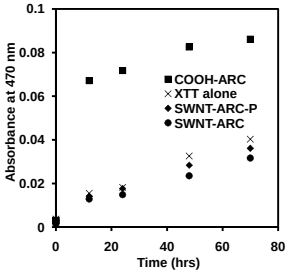
FFA loss indicating $^1O_2$ production at pH 7 in lamp light by aqueous COOH-ARC, PEG-ARC, and 100 mg/mL polyethylene glycol, and the corresponding dark control samples

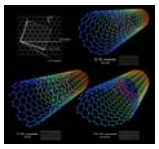


$^1O_2$ detection by FFA loss under lamp light in 1% SDS, and FFA recovery in the corresponding dark control samples at the last sampling time

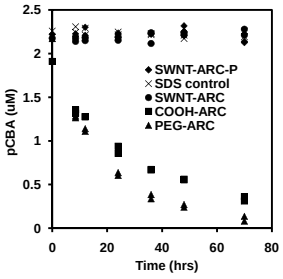


Evidence of $O_2^{\cdot-}$ production via XTT (0.1 mM) product formation, under lamp light in aqueous suspensions

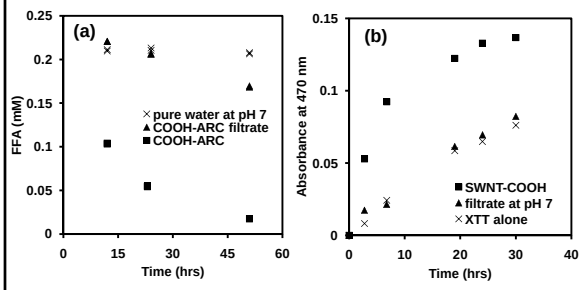




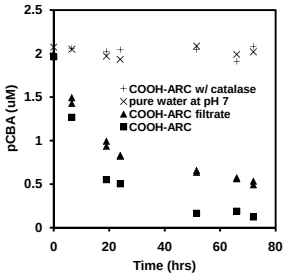
Evidence of $O_2^{\cdot-}$ production via XTT (0.1 mM) product formation under lamp light in 1% SDS suspensions



Detection of ·OH using *p*CBA (2 μM) as the ·OH scavenger at pH 7 under lamp light, in aqueous suspension of COOH-ARC and PEG-ARC, and 1% SDS suspension of SWNT-ARC-P, SWNT-ARC, and SDS alone

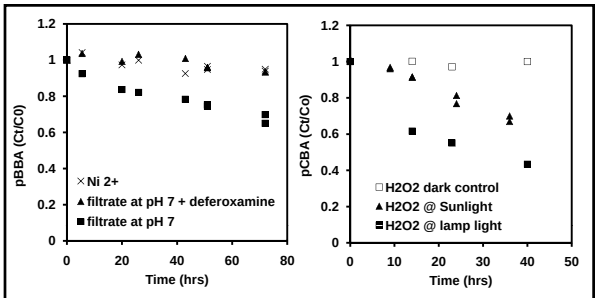


(a) $^1O_2$ and (b) $O_2^{\cdot-}$ production - at pH 7 in lamp light by aqueous COOH-ARC, COOH-ARC filtrate, and pure water.

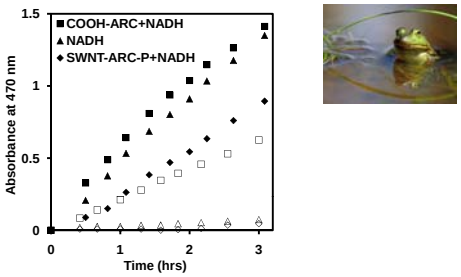


(Catalase reacts with H_2O_2)

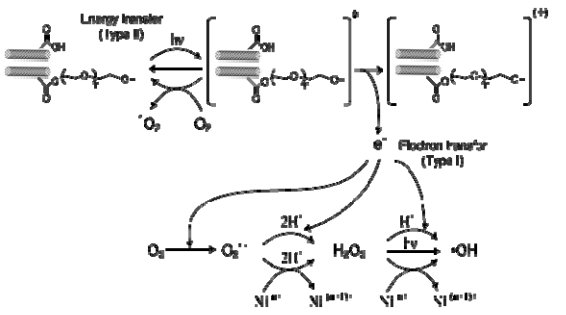
Detection of ·OH using *p*CBA (2 µM) as the ·OH scavenger at pH 7 under lamp light, in aqueous suspension of COOH-ARC, COOH-ARC filtrate, COOH-ARC and catalase (500 U/mL), and pure water control.



Detection of ·OH using *p*CBA (2 µM) as the ·OH scavenger at pH 7 under lamp light and/or sunlight in (a) aqueous suspension of COOH-ARC, COOH-ARC filtrate, COOH-ARC w/deferoxamine, and $NiCl_2$ (2 mg/L), and (b) H_2O_2 100 µM solution, and the corresponding dark control samples



Effects of NADH (0.2 mM) on $O_2^{\cdot-}$ production detected via XTT (0.1 mM) product formation at pH 7 under lamp light and dark controls



Reactions that may occur upon sunlight absorption by functionalized SWNTs

Summary

- Oxic aqueous colloidal dispersions of both types of functionalized nanotubes generated ROS ($^1O_2$, $O_2^{\cdot-}$, and ·OH) in sunlight.
- Both Type I and Type II photochemical pathways occur by the functionalized SWNTs in sunlight.
- It appears that the functionalized SWNTs can act as the electron donor directly (resulting in a change in their properties) or can shuttle electrons from other electron donors to form these reactive oxygen species.

Questions?

PURDUE UNIVERSITY School of Civil Engineering

Qilin Li

Impact of Photochemical Oxidation on the Stability of nC_{60} and Multi-Walled Carbon Nanotubes in Aqueous Solutions

Xiaolei Qu, Yu Sik Hwang, Pedro J.J. Alvarez, and Qilin Li
Department of Civil and Environmental Engineering, Rice University, Houston TX

In natural aquatic systems, various environmental factors including natural organic matter and sunlight can interact with engineered carbon nanomaterials and influence their transport. The main objective of this study was to investigate the impact of natural organic matter and sunlight on the aggregation and deposition behaviors of aqueous C_{60} fullerene nanoparticles (nC_{60}) and multiwalled carbon nanotubes.

Suwannee River humic acid (SRHA) standard (II) and Elliot soil humic acid (ESHA) were used as the model aquatic and soil organic matter, respectively, and the UVA fraction of sunlight was simulated with UV lamps with output wavelength and intensity of 350 ± 50 nm and 1.66 mW/cm^2, respectively. Initial aggregation rates of nC_{60} and carboxylated multiwalled carbon nanotubes (COOH-MWCNTs) before and after irradiation in solutions of different ionic strength, ionic composition, and humic acid concentration were determined from time resolved particle size measurement using dynamic light scattering. Deposition onto SiO_2 surfaces was characterized using a quartz crystal microbalance with dissipation (QCMD) and compared to results from traditional column experiments; the impact of soil organic matter was investigated using ESHA coated SiO_2 crystals or quartz sand.

Our study revealed that UVA irradiation in the presence of dissolved oxygen introduced oxygen-containing function groups on nC_{60} surface, but reduced the oxygen content of the COOH-MWCNTs. Such changes in surface chemistry greatly altered the humic acid adsorption capacity and aggregation and deposition behavior of these carbon nanomaterials. In NaCl solutions, UVA irradiation induced surface oxidation remarkably increased nC_{60} stability by increasing the negative surface charge and reducing surface hydrophobicity. On the contrary, UVA irradiation reduced nC_{60} stability in $CaCl_2$ solutions due to specific interactions of Ca^{2+} with the oxygen-containing functional groups on the UVA-irradiated nC_{60} surface and the consequent charge neutralization. In the absence of Ca^{2+}, the surface photochemical oxidation greatly reduced the adsorption of SRHA on nC_{60} surface, resulting in weak dependence of nC_{60} stability on SRHA; Ca^{2+}, on the other hand, facilitated SRHA adsorption on the UVA-irradiated nC_{60} surface by neutralizing surface charges of both UVA-irradiated nC_{60} and SRHA as well as forming intermolecular bridges, leading to enhanced stability in the presence of SRHA. Deposition of nC_{60} onto silica surface was found to be controlled by electrostatic interactions. The attachment efficiency increased with increasing ionic strength due to surface charge screening. ESHA adsorbed on the quartz crystal and sand surfaces hindered nC_{60} deposition at NaCl concentrations between 5 and 40 mM. However, at lower NaCl concentrations, enhanced deposition was observed on ESHA coated quartz crystal and sand.

UVA irradiation affected stability of the COOH-MWCNTs differently. Unlike nC_{60}, the stability of COOH-MWCNTs in NaCl solutions decreased with increasing UVA irradiation time. Meanwhile, the deposition rate of the COOH-MWCNTs onto silica surface increased by 2.6 times after 1 week of UVA irradiation. Particle electrophoretic mobility measurements suggested that UVA-irradiated COOH-MWCNTs were less negatively charged than the pristine COOH-MWCNTs, consistent with their higher aggregation and deposition rates. Based on the reduced oxygen content observed in XPS analyses, we speculate that the COOH-MWCNT surface underwent decarboxylation during the UVA irradiation, but the underlying mechanism remains unclear and requires further study.

Immediate future study will focus on two tasks to: (1) determine the impact of UVA irradiation on aquatic NOM adsorption to nC_{60} particle surface. NOM sorption will be investigated via batch adsorption and QCMD experiments under various solution conditions and (2) investigate the effect of organic matter content in sediment and soil on nC_{60} deposition/sorption. Batch sorption and column experiments will be conducted.

References:

1. Hwang YS and Li QL. Characterizing photochemical transformation of aqueous nC(60) under environmentally relevant conditions. *Environmental Science and Technology* 2010;44:3008-3013.

2. Qu X, Hwang YS, Alvarez PJJ, Bouchard D, and Li Q. UV irradiation and humic acid mediate aggregation of aqueous fullerene (nC60) nanoparticles. *Environmental Science and Technology* (DOI: 10.1021/es101947f).

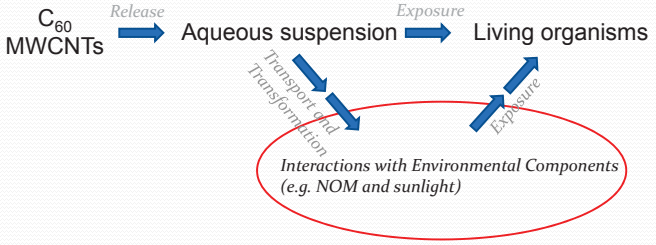
EPA Grant Number: R834093

Impact of Photochemical Oxidation on the Stability of nC_{60} and Carboxylated MWCNTs

Qilin Li

Department of Civil & Environmental Engineering
Rice University

Research Objectives

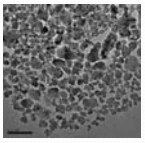


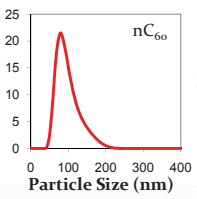
- Changes of physicochemical properties in nature aquatic systems (e.g. interacts with NOM and sunlight)
- Resulting changes of transport pattern (e.g. aggregation and deposition)

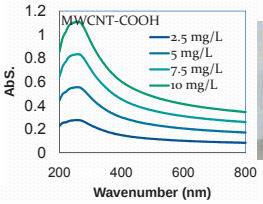
Suspension Preparation by Sonication

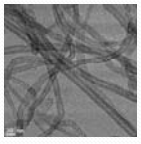
C_{60}
MWCNT-COOH

Sonication/
(2μm Filtration)









Photochemical Transformation

Sonication

- 3.8 – 4.0 mg/L
- pH : 5.5-6.0

UVA-irradiation

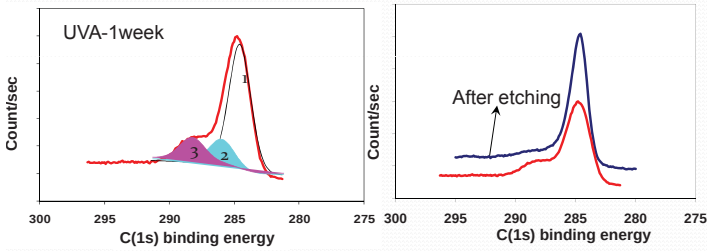
- Luzchem reactor
- Four Hitachi lamps (300-400 nm, 8W)
- 2 mW/cm^2

Characterization

Particle size (DLS)
Zeta potential
C_{60} concentration
UV/Vis
XPS
FTIR

Measurement of aggregation and deposition rate

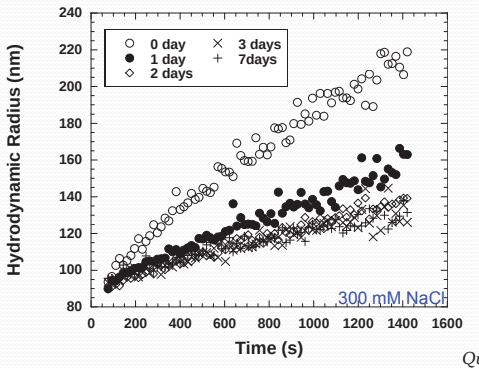
Photochemical Oxidation of nC_{60}



Peak	Position	% C(1s)	Carbon
1	284.3	66%	Underivatized C(C=C)
2	285.6	19%	Monooxygenated C (e.g., C-O)
3	288.0	15%	Di-oxygenated C (e.g., O-C-O and C=O)

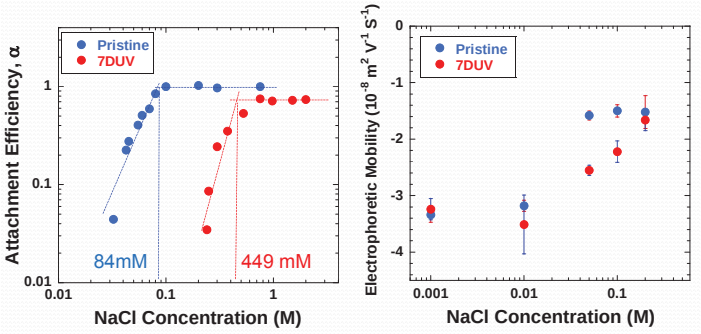
Hwang, Y. S.; Li, Q. L. *ES&T*, **2010**, *44*, 3008-3013.

UVA Irradiation Increases nC_{60} Stability in NaCl



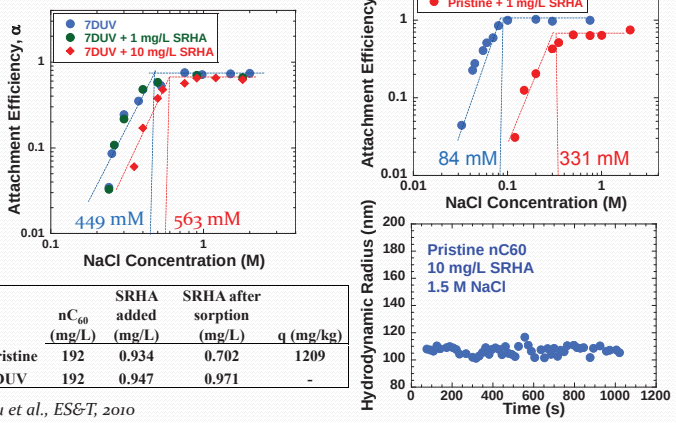
Qu et al., ES&T, 2010

UVA Irradiation Increases nC_{60} Stability in NaCl



Qu et al., ES&T, 2010

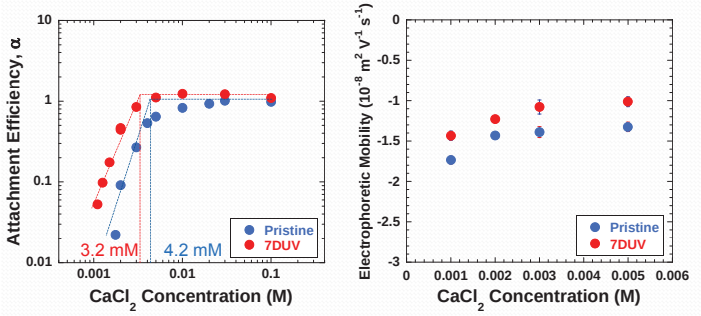
Humic Acid Adsorption Mediated by Surface Oxidation



	nC_{60} (mg/L)	SRHA added (mg/L)	SRHA after sorption (mg/L)	q (mg/kg)
Pristine	192	0.934	0.702	1209
7DUV	192	0.947	0.971	-

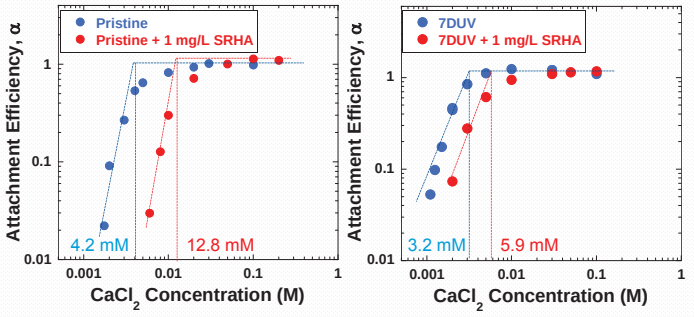
Qu et al., ES&T, 2010

UVA Irradiation Decreases nC_{60} Stability in $CaCl_2$



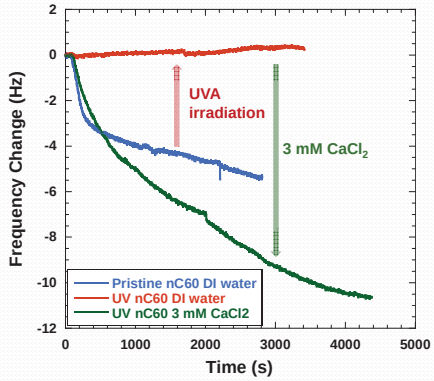
Qu et al., ES&T, 2010

Humic Acid Increases nC_{60} Stability in $CaCl_2$

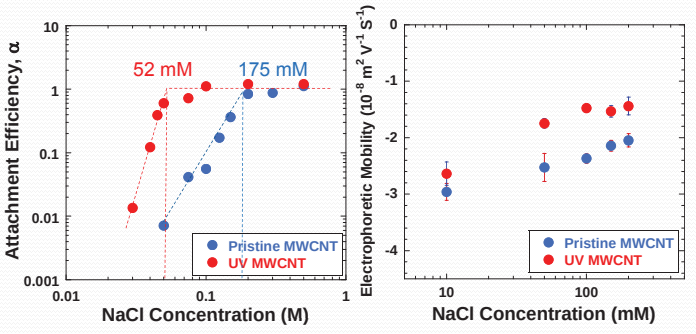


Qu et al., ES&T, 2010

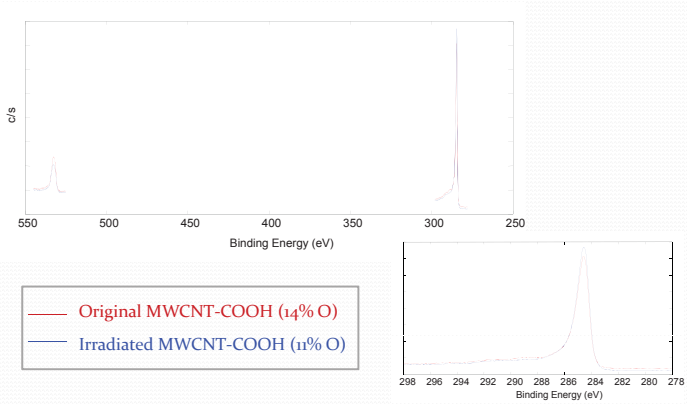
Ca^{2+} Enhances SRHA Adsorption on UVA irradiated nC_{60}



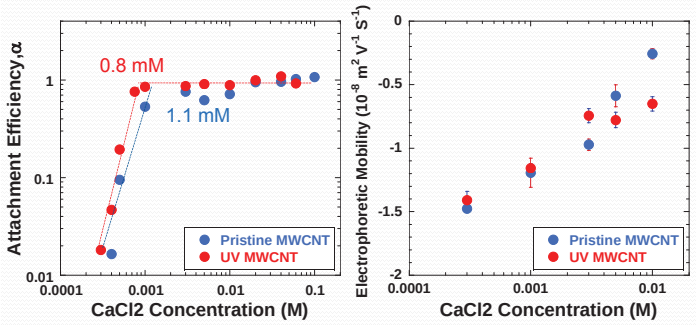
UVA Irradiation Reduces MWCNT Stability in NaCl



Loss of Oxygen Functional Groups



Unchanged Stability in $CaCl_2$



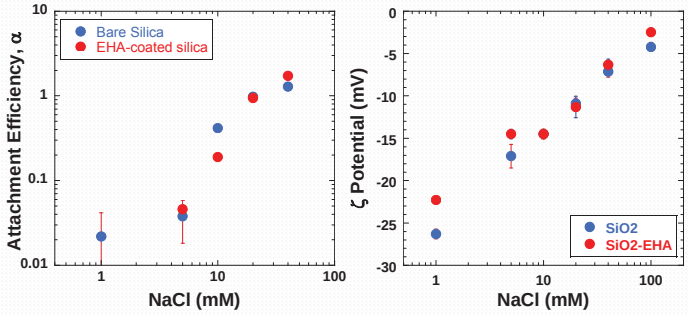
Concluding Remarks

- Sunlight irradiation and humic acid sorption mediate nC_{60} and MWCNT-COOH aggregation
- Both specific and nonspecific (i.e., DLVO) interactions are involved
- nanocarbon surface chemistry plays a key role in its environmental fate and transport.

Ongoing Research and Future Directions

- Impact of UVA irradiation and NOM on sorption/deposition and transport in subsurface porous medium
  - Nature of NOM: aquatic vs. soil
  - Properties of suspended solids/sediment/ aquifer media
- Impact of UVA irradiation and NOM on bioavailability and bioaccumulation

nC60 Deposition on SiO_2



Acknowledgement

- NSF Center for Biological and Environmental Nanotechnology (Award EEC-0647452)
- USEPA STAR program (Grant No. 834093)

Questions and comments

Fullerene cosmetics

Qingguo Huang

The Environmental Behaviors of Multi-Walled Carbon Nanotubes In Aquatic Systems

***Qingguo Huang*[1], *Marsha C. Black*[2], *Liwen Zhang*[1], *Emily R. Roberts*[2], *and Elijah Petersen*[3]**
[1]Department of Crop and Soil Sciences, University of Georgia, Griffin, GA; [2]Department of Environmental Health and Science, University of Georgia, Athens, GA; [3]Chemical Science and Technology Laboratory, National Institute of Standards and Technology, Gaithersburg, MD

Our study was designed to investigate the environmental behavior of water-dispersed carbon nanotubes in natural aquatic systems (i.e., water-sediment phase distribution, possible degradation, ecological exposure and toxicity), thereby providing useful information for environmental risk assessment and potential waste treatment. We have used C14-labeled multi-walled carbon nanotubes (^{14}C-MWNTs) in our experiments to unambiguously identify and quantify carbon nanotubes from various natural materials, including water, sediments and organisms. Our experiments have yielded important information regarding three important behaviors of MWNTs in aquatic systems: water/solid phase distribution, biotic degradation, and possible toxicity and exposure to aquatic organisms. The results on each topic are briefly summarized below, respectively.

We conducted experiments to examine the phase distribution of ^{14}C-MWNTs in aqueous systems containing peat, shale, or clay as model solid phases under a series of varying pH and ionic strength conditions. Our results suggest that solid matter interacts with water-dispersed MWNTs via three interactive processes: (1) dissolved cations tend to promote MWNT aggregation via double layer compression; (2) dissolved organic matters released from the solid phase tend to stabilize MWNT dispersion; and (3) MWNTs sorb to the solid phase, primarily driven by hydrophobic interaction. All processes are variously influenced by aqueous conditions (e.g., pH, electrolytes) and their interplay governs the phase distribution of MWNTs.

Recent studies have discovered biotic degradations of fullerols and single-walled carbon nanotubes (SWNTs), but there has not been a report on microbial degradation of MWNTs. We in our study found an enrichment culture that is capable of mineralizing ^{14}C-MWNTs into $^{14}CO_2$. Our initial study indicates that the mechanism involved in MWNT degradation seems to differ from that of fullerols and SWNTs. The microorganisms responsible for MWNTs degradation may not be fungi, but a consortium of bacteria, and the peroxidases that were found responsible for SWNT and fullerol degradation were absent in the MWNT-degrading culture. Currently, we are conducting a systematic study to identify and characterize the microorganisms that are responsible for degrading MWNTs and the biochemical pathways that are involved in MWNT metabolism.

We studied chronic effects of ^{14}C-MWNTs on *Ceriodaphnia dubia*, an aquatic invertebrate, in 8-day exposures. For chronic exposures, ^{14}C-MWNTs were solubilized in moderately hard water (MHW) by four different methods: bath sonication (Branson) for 2 h; probe sonication for 2 h with 50 sec. pulses (Cole-Parmer 500-Watt Ultrasonic Homogenizer); bath sonication followed by addition of Sewanee River natural organic matter (NOM; final concentration = 4.5 mg/L); and by stirring nanotubes overnight in 4.5 mg/L Sewanee River NOM dissolved in MHW. *Ceriodaphnia* exposed to bath-sonicated MWCNTs had significantly smaller brood numbers and size at the 2.5 mg/L concentration (LOEC), compared with controls. Chronic exposures with probe-sonicated nanotubes showed less reproductive toxicity, with a LOEC of 5 mg/L. No reproductive toxicity was observed for nanotube exposures with added NOM. Reproductive toxicity of the bath-sonicated nanotubes may be related to association of the MWNTs onto the body surfaces of the adults, which likely interfered with molting and prevented neonate release.

EPA Grant Number: R834094

The University of Georgia

This research is funded by U.S. EPA - Science To Achieve Results (STAR) Program. Grant # 834094

The Environmental Behaviors of Solubilized Multi-walled Carbon Nanotubes in Aquatic Systems

Qingguo Huang[1], Marsha C. Black[2], Liwen Zhang[1], Emily R. Roberts[2], Elijah Petersen[3]

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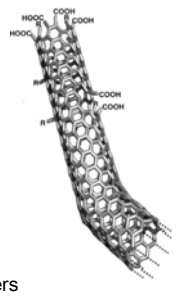
[2]Department of Environmental Health Science, University of Georgia, Athens, GA
[3]Chemical Science and Technology Laboratory, NIST, Gaithersburg, MD

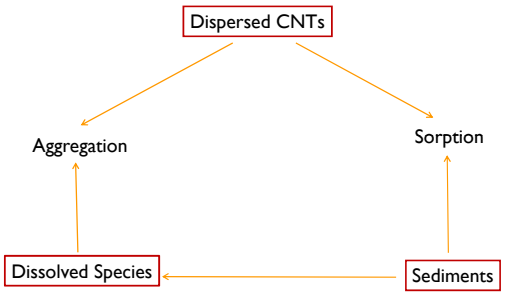
Objective



- **Solubilized CNTs**
  - → Mobility → Exposure
- **Tasks**
  - 1) Sorption
  - 2) Transformation
  - 3)Toxicity, accumulation and transfers

Contents

Phase Distribution



4

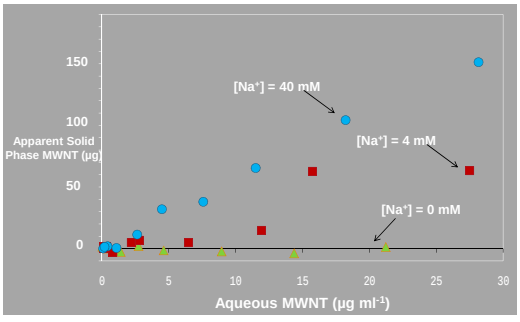
Three Treatments

Treatment 1: H_2O + CNTs

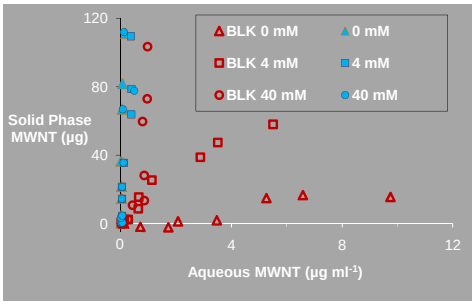
Treatment 2: peat DOM + CNTs

Treatment 3: Peat + CNTs

Effect of Peat

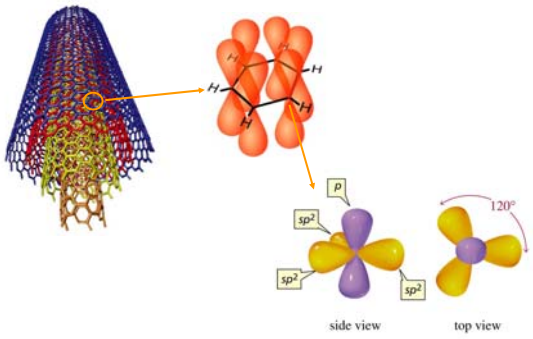


Effect of Shale



Contents

Reactivity



Biotransformation: Fullerene

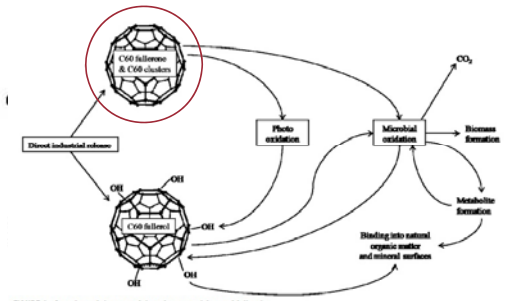
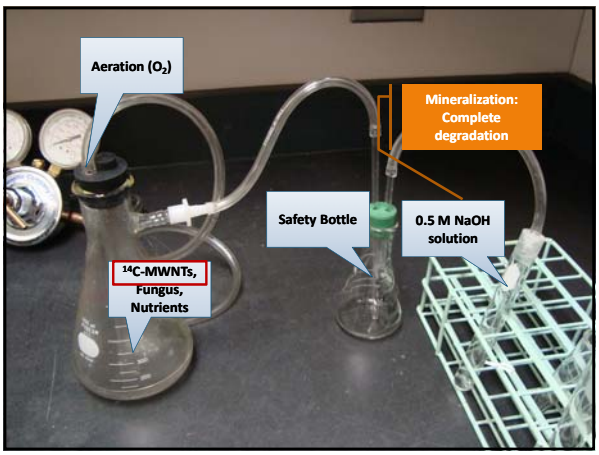


FIGURE 1. Overview of the potential environmental fates of fullerols.

Schreiner, K. M.; et al. *Environ. Sci. Technol.* **2009**, *43* (9), 3162-3168.



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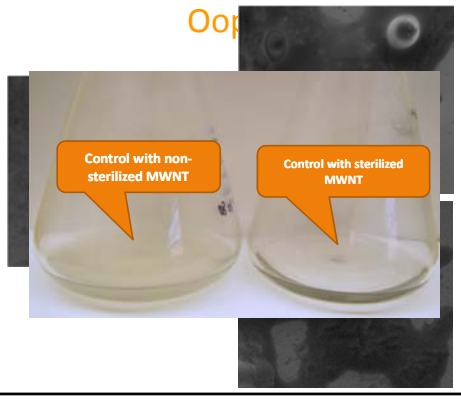
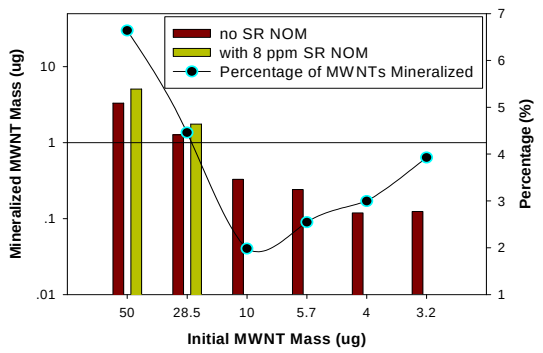
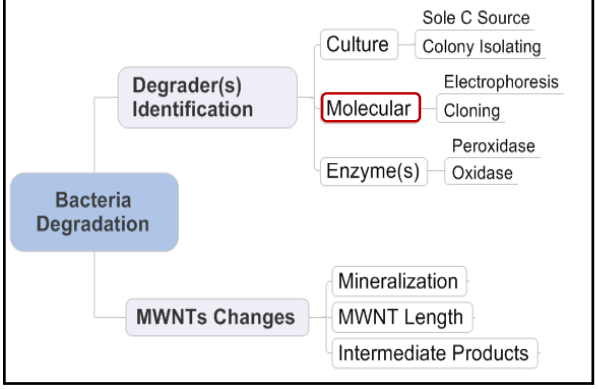
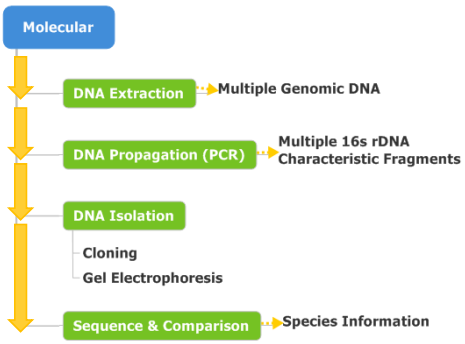


Mineralization

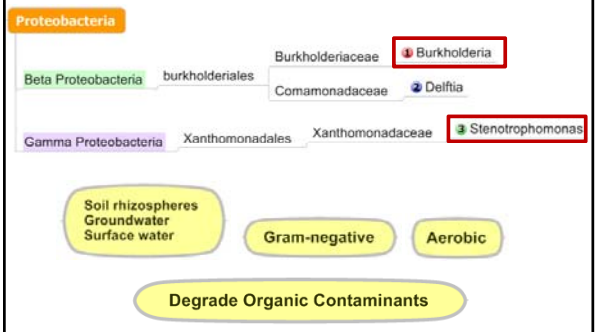




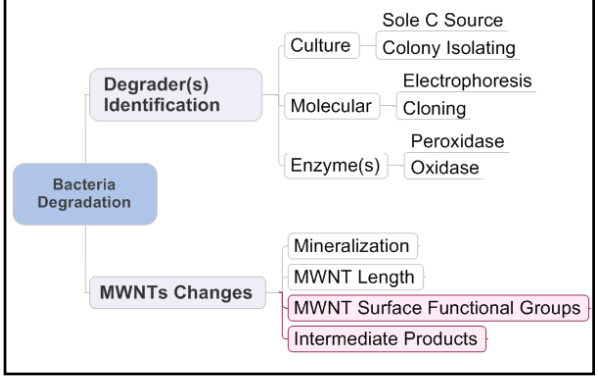
Molecular DNA Identification



Sequence Results



Outline of Research Plan



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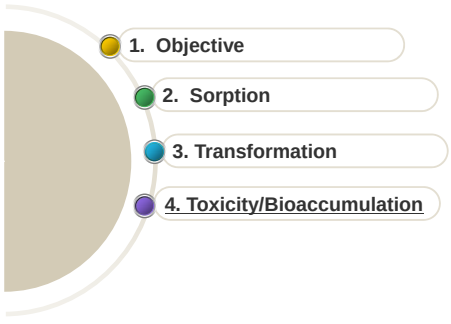


Chronic Exposures with *Ceriodaphnia dubia*

Goal: Evaluate reproductive toxicity and accumulation of MWCNTs by adult and neonate *Ceriodaphnia dubia* in solutions that are prepared by two methods

- Bath sonication
- Sewanee River NOM (4.5 mg/L)

- 7-day chronic test
  - *C. dubia* <24 h old; 3 brood test (US EPA)
  - ^{14}C -MWNT concentrations: 1.25-5 mg/L (in MHW)
  - Solubilization procedures
  - Daily renewal (exposure water + food)
  - Endpoints: # of broods, # of offspring
    - NOEC, LOEC calculated by ToxCalc®
  - Accumulation measured by LSC (^{14}C)

Results

Reproductive Effects of MWNTs in *C. dubia*

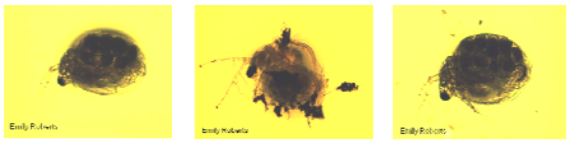
Treatment	NOEC	LOEC
MWNT (sonicated)	1.25 mg/L	2.5 mg/L
MWNT (NOM)	>5 mg/L	>5mg/L

Accumulation of MWNTs in *C. dubia* neonates

Treatment	NOEC	LOEC
MWNT (sonicated)	>5 mg/L	>5 mg/L
MWNTs (NOM)	2.5 mg/L	3.75 mg/L

Discussion and Conclusions

Control (40x) | 2.5 mg/L MWNT (40x) | 2.5 mg/L MWNT+NOM (40x)



- Sonicated MWNTs adhered to adult organisms
  - Prevented molting/release of neonates = fewer broods
- NOM protective against reproductive toxicity
  - No observed adherence to adults
- Significant accumulation of NOM-solubilized MWCNTs in neonates
  - NOM-MWCNTs were consumed (vs. diffusion)?
  - Clumping of sonicated MWCNTs prevented consumption?

What's Next?

- Feeding studies
  - *C. dubia* fed *Artemia* exposed to MWNTs
  - Fathead minnows fed *C. dubia* exposed to MWCNTs
  - Trophic transfer?

- Full lifecycle exposures of MWNTs
  - Fathead minnow
  - Maternal transfer?

Acknowledgements

- Major Participants

Liwen Zhang; Emily Roberts; Dr. Marsha Black; Dr. Zhengwei Pan; Dr. Elijah Petersen; Dr. Mussie Habteselassie

- Major Collaborator

Roger Pinto; Yenjun Zhuang; Vijaya Mantri; Wen Zhang; Dr. Yongsheng Chen; Dr. Aaron Thompson

- EPA STAR support

Day 2, Tuesday, November 9, 2010

AM Session 1: Effects on Cells

Amiraj Banga

Functional Effects of Nanoparticle Exposure on Airway Epithelial Cells

***Amiraj Banga*[1], *Frank A. Witzmann*[2], *and Bonnie L Blazer-Yost*[1,2]**
[1]Department of Biology, Indiana University Purdue University at Indianapolis, Indianapolis, IN;
[2]Department of Cellular and Integrative Physiology, Indiana University School of Medicine, Indianapolis, IN

Nanotechnology, the creation and manipulation of structures and systems at a nanoscale level (< 100 nm), significantly alters fundamental properties from large-scale materials. With nanotechnology being a focused area of exponential scientific and industrial growth in the last few decades, concerns have arisen regarding the potential biological effects of nanoscale materials. These effects remain poorly understood, especially with regard to occupational and environmental hazards. Populations exposed to increasing levels of nanomaterials include not only workers exposed during the production, recycling, and disposal, but also to the general population that uses commercially available nanomaterial-containing products and is exposed to them via environmental contamination.

The unique physico-chemical properties of these nanoscale products cause them to interact with cellular systems in an unknown and undefined manner. Demonstrated effects include oxidative stress, inflammatory cytokine production, DNA mutation membrane damage, and even cell death.[1] Although the nanotechnology industry holds great promise in the future, its darker side has to be explored to obtain the maximum benefits from this industry in a safe manner.

Carbon-based nanoparticles are one group of widely produced nanomaterials both industrially and environmentally. These include fullerenes and nanotubes (Single-wall carbon nanotubes [SWCNT] and Multi-wall carbon nanotubes [MWCNT]). Fullerenes or Buckyballs are the most stable and are composed of 60 carbon atoms with an average diameter of 0.72 nm. Carbon nanotubes are graphite sheets rolled to form seamless tubes or cylinders. Whereas SWCNT consist of a single layer with diameters ranging near 1 nm, MWCNT are larger and consist of many single-walled tubes stacked one inside the other with diameters reaching 100 nm. Because of their nano sizes, fibrous shapes, and carbon base, CNTs are expected to behave differently than the large-sized particles. They are potentially toxic like other small fibers (asbestos and silica) and biopersistent because of their stability.

One primary route of nanoparticle uptake in the body is through inspiration of airborne nanoparticles. Combustion-derived nanoparticles have been shown to cause lung cell injury and inflammation due to oxidative stress[2] that may manifest itself as airway disease, cardiovascular disease, fibrosis, or cancer.[3] Using quartz and carbon nanoparticles at equal mass dose, it was concluded that SWCNT in the lungs were far more toxic than carbon black and even quartz.[4] Following inhalation, ultrafine carbon particles can travel through the circulatory system and invade the brain.[5] Nanoparticles can have prothrombotic effects *in vivo* and demonstrate platelet activation *in vitro,* as has been shown in response to SWCNT exposures.[6] They also demonstrated that MWCNTs can reach the subpleural tissue in mice with a single inhalation dose of 30 mg/m^3 for 6 h. A stable C_{60} suspension has been shown to produce genotoxicity as a result of DNA damage in human lymphocytes.[7]

One of the respiratory cell lines commonly used for tracheobronchial epithelial cell studies is Calu-3. Although it is adenocarcinoma in origin, it is one of the few cell lines that form tight junctions *in vitro* and produces features of a differentiated, functional human airway epithelium. Calu-3 cell line is a human airway epithelial cell line that responds to epinephrine with an increase in Cl^- secretion via Cystic Fibrosis Transmembrane Regulator channel (CFTR). Water follows Cl^- and together with mucous helps to clear the airways of any foreign substances. The cells show a high resistance phenotype after 13 days of growth. The

current studies utilize this well-characterized model to study the effects of unpurified, as manufactured, nanoparticles that are most likely to be found as environmental and occupational pollutants.

We hypothesized that exposure of epithelial cells to unpurified, as manufactured CNPs such as C_{60}, SWNT and MWNT, may alter the function of barrier epithelia. The effect of exposure of each of three different CNPs was studied in air-interface cultured Calu-3 cell model over seven orders of magnitude (4 $\mu g/cm^2$-0.004 ng/cm^2). Electrophysiological techniques were used to study transepithelial ion transport and the barrier function expressed as Trans Epithelial Electrical Resistance (TEER). After 48 h of exposure to CNPs, fullerenes did not show any effect on TEER, whereas the nanotubes significantly decreased TEER over a wide range of concentrations (4 $\mu g/cm^2$-0.004 ng/cm^2). The ion transport response to epinephrine also was significantly decreased by the nanotubes but not by fullerenes. To look at the effect of exposure times, cells were exposed to same concentrations of CNPs for 24 and 1h time periods. Although the 48 h and 24 h time period exposures exhibited same effects, there was no effect seen after 1 h in terms of TEER or hormonal responses. In cells exposed to either of the nanotubes, the TEER was not statistically different from control after treatment with 4 ng/cm^2 concentration, whereas in the case of hormonal responses, the nanotubes, especially multi-walled, still showed significant inhibitory effect. To examine which step of the epinephrine stimulated intracellular pathway is affected by CNPs, cAMP assays were performed. The cAMP levels for the exposed cells vs. the control cells were not different, suggesting an effect manifested after the epinephrine-induced increase in cAMP.

Our results indicate that there are changes in response to physiologically significant nanoparticle concentrations that could, *in vivo*, be manifested as changes in transcellular permeability and hormone responsiveness. Such effects could alter airway function, emphasizing the need of further study on the effect of these nanoparticles.

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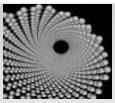
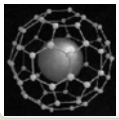
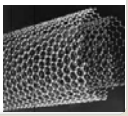
NIH/NIGMS Grant Number: R01GM085218



Functional Effects of Nanoparticle Toxicity on Airway Barrier Epithelial Cell Function

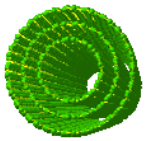
Amy Banga
Blazer-Yost/Witzmann Group
IUPUI, Indianapolis





Health Hazard of Nanoparticles

- Workers handle nanoparticle materials in many industrial jobs to produce consumers' items
- Nanoparticles can enter the body by:
  -Inhalation
  -Swallowing
  -Penetration through the skin
- Complete information about health effects is lacking



MWCNT
(diameter ~2-25 nm;
length: few nm to microns)

SWCNT
(diameter as small as 1 nm;
length: few nm to microns)

C_{60} (fullerene)
(Avg diameter 0.72 nm)

CNP purchased from SES Research, Inc., Houston, TX
(http://www.sesres.com)

Images from: http://www.photon.t.u-tokyo.ac.jp/~maruyama/agallery/agallery.html

Hypothesis

Manufactured, non-functionalized carbon nanoparticles (CNPs), when exposed to barrier epithelia (lung/airway, kidney and intestinal cells), exert a biological effect on the cell membrane and may alter the cell function.

Approach

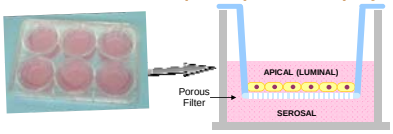
Cell model used – Calu-3 (airway epithelial cells).

Features of cell line

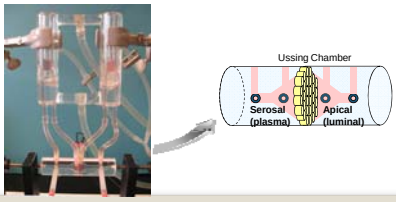
- Forms a monolayer on permeable supports under air-liquid interface culture conditions with Transepithelial Electrical Resistance (TEER) of 800±80 Ω.cm².
- Simulates a barrier epithelium.
- Displays hormonal responsiveness of airway cells *in vivo*.

Technique used - Electrophysiological studies were used to determine the effect of CNPs on Transepithelial Electrical Resistance (TEER) that measures barrier function and Short Circuit Current (SCC) that measures net ion transport.

Short-Circuit Current (SCC) Electrophysiology



Measuring net ion transport across the monolayer



Approach

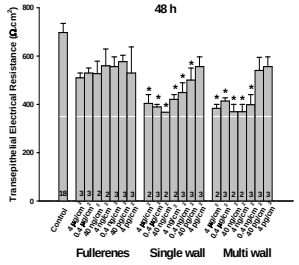
CNP preparation – CNP suspended in Fetal Bovine Serum (FBS), sonicated, autoclaved and added to serum free media. The amount of CNP was regulated so as to obtain a desired final concentration of CNP in media when FBS was added.

CNP exposure – The cells were incubated with CNP-FBS containing media at a normal concentration of 15% for last 1, 24 or 48 h of growth to simulate *in vivo* CNP exposure.

Conversion equation

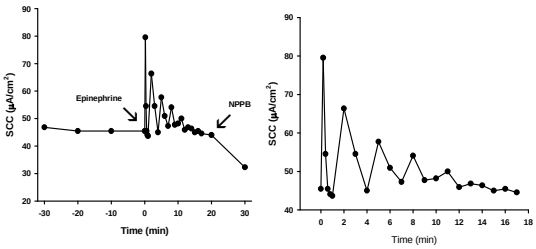
X µg/cm² = 25X µg/ml.

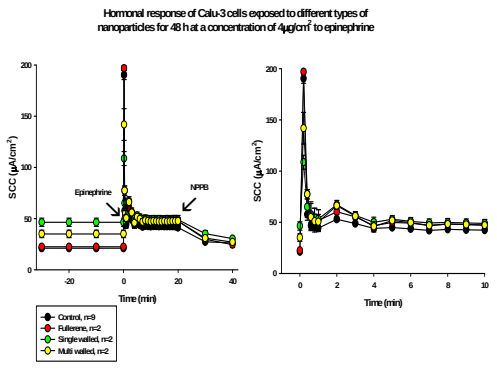
Effect on the TEER of Calu-3 cells by exposure to different types and concentrations of CNPs for 48 h

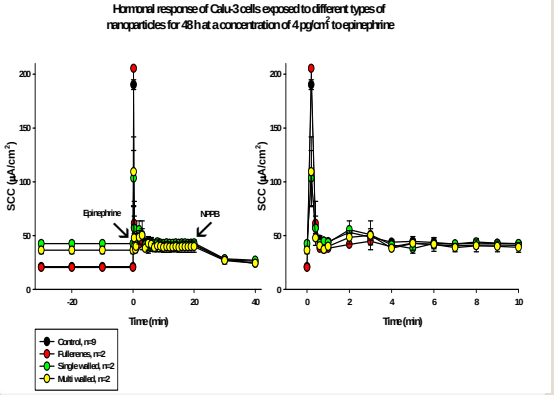


*** indicates that the value was statistically different from the control value (P<0.05) using a Students' t-test.**

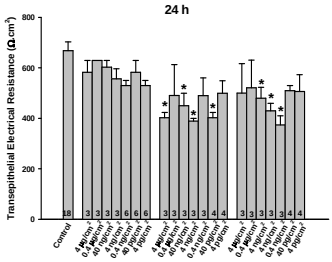
Response of Calu-3 cells to epinephrine





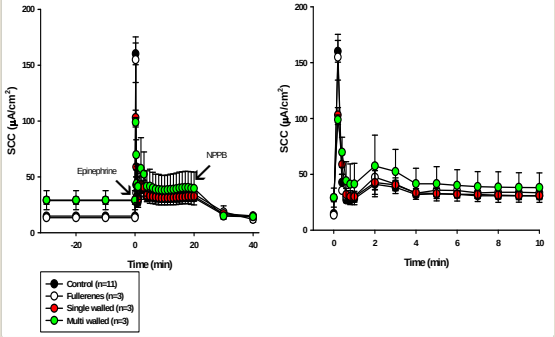


Effect on the TEER of Calu-3 cells by exposure to different types and concentrations of CNPs for 24 h

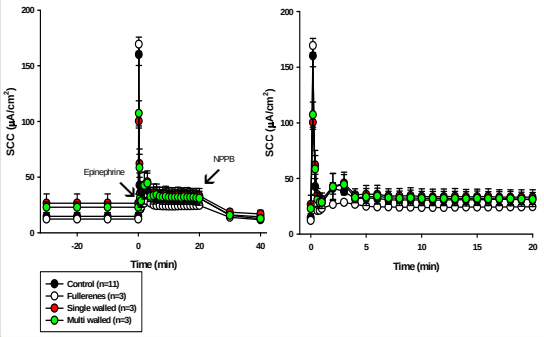


* indicates that the value was statistically different from the control value (P<0.05) using a Students' t-test.

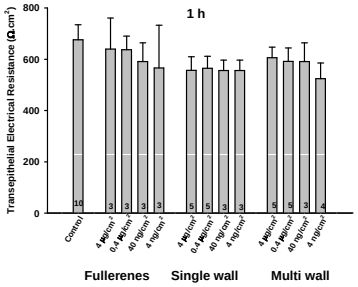
Hormonal response of Calu-3 cells exposed to different types of nanoparticles for 24 h at concentration of 4 μg/cm² to epinephrine



Hormonal response of Calu-3 cells exposed to different types of nanoparticles for 24 h at concentration of 4 pg/cm² to epinephrine

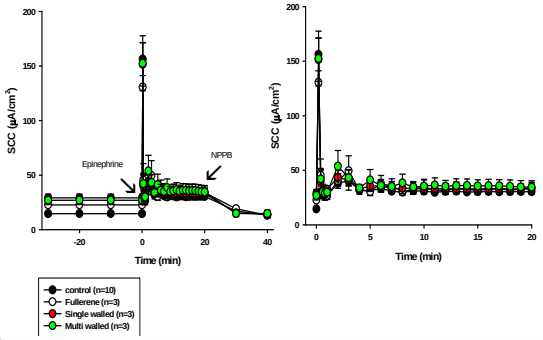


Effect on the TEER of Calu-3 cells by exposure to different types and concentrations of CNPs for an hour

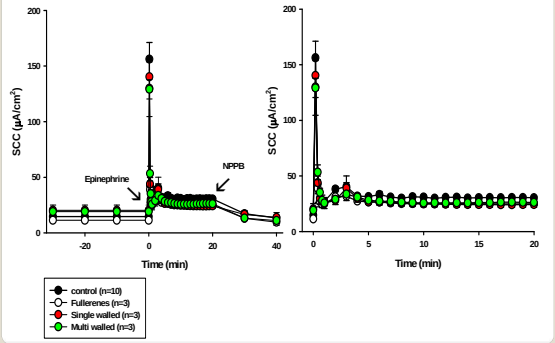


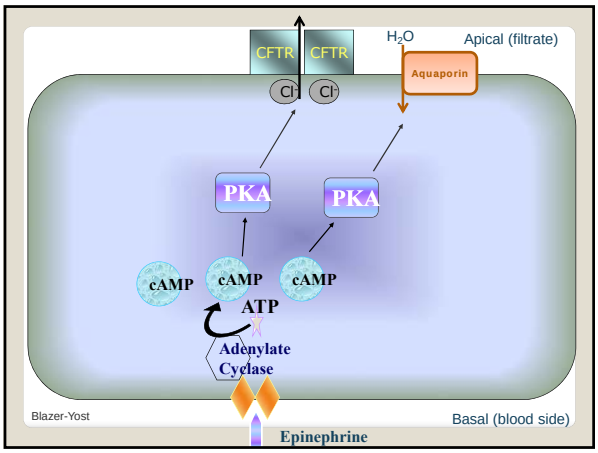
* indicates that the value was statistically different from the control value (P<0.05) using a Students' t-test.

Hormonal response of Calu-3 cells exposed to different types of nanoparticles for 1h at concetration of 4 μg/cm² to epinephrine



Hormonal response of Calu-3 cells exposed to different types of nanoparticles for 1 h at concetration of 4 pg/cm² to epinephrine





Increase in cAMP concentration after epinephrine stimulation

Fold increase in cAMP after epinephrine stimulation

CNP	Concentration of CNP(µg/cm²)	Control	*CNP treated
SWCNT	4	6.5	7.6
MWCNT	4	8.3	6.6
SWCNT	0.004	11.3	11.8
MWCNT	0.004	5.3	7.8

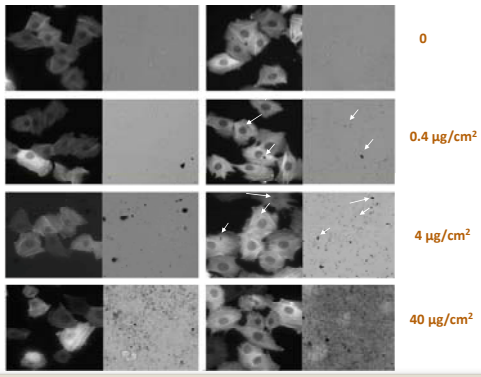
*CNP Treated = 48 hrs Carbon nanoparticle treated cells at concentrations indicated

Summary

- Low dose nanotube exposures decreases the barrier function of airway epithelial cells.
- Low dose nanotube exposures affects the ability of the airway epithelial cells to secrete chloride.
- These data suggest that levels of nanotubes found in the workplace, particularly during chronic exposures, are likely to have physiological effects that can cause or exacerbate respiratory problems.

Thanks

Preliminary Imaging study



Galya Orr

Toxicity Assessment of Nanomaterials in Alveolar Epithelial Cells at the Air-Liquid Interface

***Galya Orr*, Yumei Xie, Nolann Williams, Ana Tolic, Justin Teeguarden, and Alexander Laskin**
Pacific Northwest National Laboratory, Richland, WA

Airborne nanomaterials that enter the respiratory tract are likely to be deposited in the alveolar region, where alveolar epithelial cells are found at the interface with ambient air. These cells provide a vulnerable target for particles that escape the first line of defense by the alveolar macrophages. To date, the majority of *in vitro* studies characterizing the interactions and impact of engineered nanomaterials in these cells have been carried out in cells submersed under growth media. To more closely mimic *in vivo* exposures, we have established the growth of alveolar type II epithelial cells (C10 cell line) at the air-liquid interface (ALI), enabling realistic exposures to aerosolized nanoparticles. This approach supports accurate quantification of the delivered particles per cm^2 (or particles per cell) by collecting the particles on millimeter-size grids or glass cover-slips, placed randomly over the cells and visualized using electron or fluorescence microscopy, respectively. This approach also enables physical and chemical characterizations of the collected nanoparticles, providing properties that are relevant to airborne nanoparticles and the actual exposure at the air-liquid interface. The cells have been cultured on membrane inserts and initially grown under submersed conditions until reached confluence. The apical surface of the cell monolayer was then exposed to ambient conditions for 24 hours, and the integrity of a representative subset of the cells has been monitored using propidium iodide, quantified by fluorescence microscopy. Exposures to aerosolized nanoparticles, generated using a vibrating membrane nebulizer, have been done over 10-minute sessions using an enclosed exposure chamber that ensures uniform particle delivery. The cells have been maintained at the ALI until assayed for lactose dehydrogenase (LDH) release to evaluate cell damage at 6 and 24 hours post exposure, and for proliferation rate (MTS) to evaluate viability at 24 hours post exposure. Using the above conditions, we found that exposures to 50 nm bare amorphous silica nanoparticles, containing embedded fluorescent dye molecules, elicit no significant cytotoxic response at concentrations ranging from 10 to 1000 particles per cell. These observations agree with observations we obtained in submersed cells exposed to equivalent doses, as estimated by a computational particokinetics (sedimentation, diffusion) model. Studies with surface modified aminated 50 nm amorphous silica nanoparticles and other particles that have shown to elicit toxic responses in submersed conditions are currently being pursued at the ALI.

EPA Grant Number: R833338

Principal investigator did not authorize publication of the presentation.

Jonathan D. Posner

Interactions of Nanomaterials With Model Cell Membranes

***Jonathan Posner*[1,2], *Wen-Che Hou*[1,3], *Steve Klein*[1,2], *Babak Moghadam*[1,2], *Charles Corredor*[1,2], *Kiril Hristovski*[4], *and Paul Westerhoff*[4]**

[1]Chemical Engineering, [2]Mechanical Engineering, [3]Environmental Engineering, [4]School of Sustainable Engineering and the Built Environment, Arizona State University, Tempe, AZ

Toxicological studies of engineered nanomaterials (ENMs) have primarily focused on the toxicity and uptake of ENMs by a variety of organisms, including human cell lines, microbes, plants, or aquatic organisms such as fish and *Daphnia magna*. Because the reported results vary depending on the organisms and test conditions, it is difficult to draw a comprehensive conclusion of ENMs' environmental impact based on these empirical studies, especially considering the ecological diversity and wide range of ENMs' properties. The partitioning between the organic solvent phases (typically n-octanol) and water (K_{OW}) has traditionally been used as an empirical approach to evaluate the bioavailability of organic pollutants and is used extensively in current EPA models. For colloids, mechanistic and dynamic fate models in aqueous matrices are more complex than for organic pollutants and require multi-parameter input to describe the colloid transport and interactions with soils and biota. Characterization of ENMs often involves numerous physical measurements of size distribution, surface area, porosity, aqueous zeta potential, surface chemistry, and stability. However, it is challenging to transition from these precise measurements to models suitable to assess fate and bioavailability of ENMs in the environment, especially in complex matrices. Analogous partition type global descriptor methods have not been used extensively for nanomaterials; therefore, there is a need to develop empirical model approaches for predicting bioaccumulation of ENM that account for the collective influence of ENM properties, in a similar way as *Kow* depends on multiple parameters of organic pollutants (molecular weight, conformation, hydration states, ionic charge, etc.).

In this talk, we quantify the lipid-water distribution coefficients for ENMs and use them as a global descriptor that captures the critical interactions between ENM and biological interfaces, which may be used to predict the bioaccumulation potential of ENM. The lipid-water distribution ratio has been shown to be a more appropriate descriptor than *Kow* partitioning for biological uptake and bioaccumulation of hydrophobic ionizable compounds and surfactants, which ENMs share similar properties (e.g. charged, resident at interfaces). Lipid bilayers' mass is nearly all at the interface that eliminates the difficulty encountered in the octanol-water partitioning of surface-active compounds and some types of ENMs that also partition to the interfaces.

We evaluate the lipid-water distribution of aqueous C_{60} clusters (nC_{60}) and polyhydroxylated C_{60} aggregates (i.e., fullerol) using lipid bilayers that mimic biological membranes. The kinetic studies indicate that the distributions of nC_{60} and fullerol between lipid bilayers and aqueous phases reach pseudo-equilibrium after 2 days and 2 h of equilibration, respectively. The pseudo-equilibrium distribution of nC_{60} and fullerol can be described by isotherm-like behaviors that fit reasonably with Langmuir isotherms. Although both nC_{60} and fullerol exhibit pH-dependent distribution behaviors with accumulation in lipid bilayers increasing systematically with the decrease in pH from 8.6 to 3, the distribution coefficients of nC_{60} is up to 1 order of magnitude larger those of fullerol. The pH-dependent distribution trend is consistent with the decrease in the electrostatic repulsion between lipid bilayers and fullerene aggregates, as the zeta potentials of both increased (i.e., became less negative) as pH decreased.

The lipid bilayers that make up cellular membranes are believed to be impenetrable to ions and unfunctionalized macromolecules; however, epidemiological studies have shown that unfunctionalized ENMs can, under some conditions, cross or disrupt the cell membrane through passive, unmediated routes causing

acute cellular toxicity and cell death. The unmediated ENM disruption of cellular membranes is poorly understood. Also, we are developing methods for understanding the mechanisms and conditions under which engineered nanomaterials can cause disruption of, and passive transport through, simplified model cell membranes, namely lipid bilayers. We will show that some ENM disrupt membranes allowing the flux of ions across the membranes.

Distribution of ENM between the aqueous phase and biologically relevant interfaces and disruption of bilayers by ENM may ultimately be used as high-throughput global descriptors for predicting bioaccumulation and toxicity of ENM. Future work includes (1) extending current work to include ENMs of other core compositions, sizes, shapes, and surface functionalities as well as water chemistry parameters such as ionic strength and ion species; (2) correlate with existing or future bioaccumulation studies using aquatic organisms; and (3) translating methods to high-throughput formats.

DOE Grant Number: DE-FG02-08ER64613

Interactions of Nanomaterials with Model Cell Membranes

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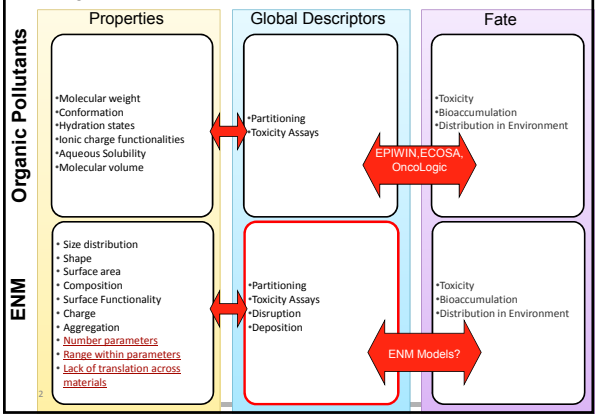
DOE BER: DE-FG02-08ER64613

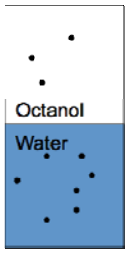
Using Global Descriptors to Predict ENM Fate and Transport



Octanol-Water Partitioning Coefficient

- Ratio of concentration of solute in between two immiscible phases, generally octanol and water.
- Used in water quality models (WASP, QUAL2K, Aquatox, EPD-RIV1) to predict fate, accumulation, aquatic toxicity of organic pollutants in the environment.
- Required for high-volume chemicals
- Methods published in OPPTS
- Methods most appropriate for unionizable chemicals such as many organic chemicals
- More difficult to interpret for ionizable substances
- Not defined for particles

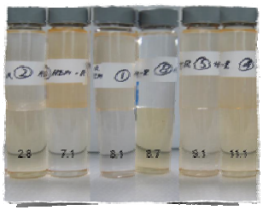
$$K_{ow} = \frac{C_{oct}}{C_w}$$



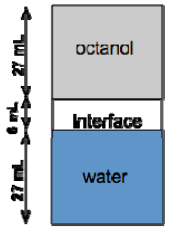
Octanol-Water Partitioning of ENM

- 60 mL Teflon cap glass vials
- 1 mM $NaHCO_3$ buffer (bicarbonate)
- Mixed for 3 days at 30 rpm
- Phases separated
- Vary pH, ionic strength
- high pH > 11 (NaOH)
- low pH < 3 (HNO_3)

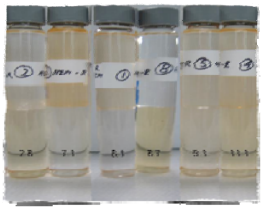
Partitioning of Hematite Fe_2O_3



Octanol-Water Partitioning of ENM

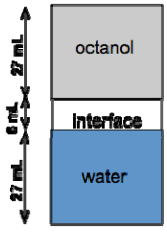


Partitioning of Hematite Fe_2O_3



- Some particles at interface
- Minimization in Helmholtz free energy
- Not quantified or treated in classical partitioning theory

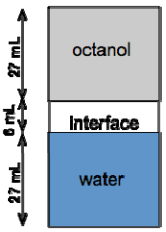
Octanol-Water Partitioning of ENM

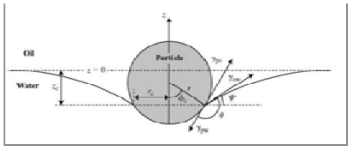


ENM at Octanol-Water Interface

- Some particles at interface
- Minimization in Helmholtz free energy
- Not quantified or treated in classical partitioning theory

Octanol-Water Partitioning of ENM





Minimum in Helmholtz Energy

$$E_0 - E_1 = \Delta E_1 = -\frac{\pi r^2}{\gamma_{ow}}\left[\gamma_{ow} - (\gamma_{po} - \gamma_{pw})\right]^2$$

$$\gamma_{po} - \gamma_{pw} = \gamma_{ow}\cos\theta_{ow}$$

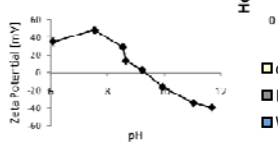
- Some particles at interface
- Minimization in Helmholtz free energy
- Not quantified or treated in classical partitioning theory
- Basis for Pickering emulsions

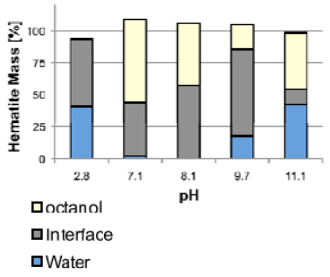
7

Octanol-Water Partitioning

Observe that ENM partitioning experiments result in combination of three primary, path and solution chemistry dependent scenarios:

A – in the aqueous phase
B – In the octanol
C – at interface





8

Challenges with ENM Octanol-Water Partitioning

- Importing into EPA models
- How do we treat mass at interface?
- Partitioning gives no information on state on ENM (aggregation and settled in water, dissolved, suspended, emulsion)
- Partitioning is path dependent
- Does not correlate with bioacumulation*
- Partitioning dependent on poorly defined interfacial area

*Petersen et al. Relevance of Octanol-Water Distribution Measurements to the potential Ecological Uptake of Multi-Walled Carbon Nanotubes, Environmental Toxicology and Chemsitry, 2010

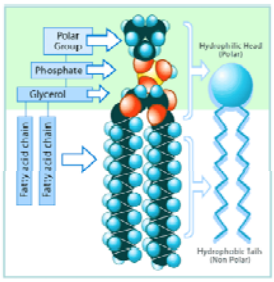
9

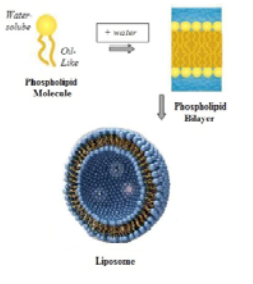
ENM Distributions in Lipid-Water Systems

- Lipid bilayer is an important interfaces between life and its environment and a potential exposure route to ENMs.
- The lipid bilayer-water distribution (K_{lipw}) has been shown to be a more appropriate indicator than (K_{ow}) for bioaccumulation of ionizable organic molecular and surface active compounds, which ENMs share some properties (e.g., charged surface and residence in interface).
- K_{lipw} is increasingly used by the pharmaceutical industry and environmental research for drugs and molecular pollutants.
- All mass is at the surface
- Surface area can be controlled and quantified

10

Lipid Bilayers

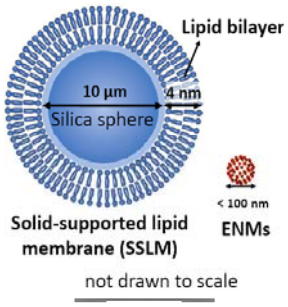


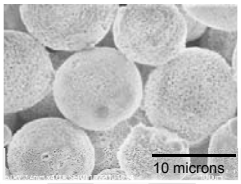


- Primary constituent of many biological cellular membranes.
- Often used to model passive transport into cells.

11

Lipid bilayer-water distribution on Solid Supported Lipid Bilayers

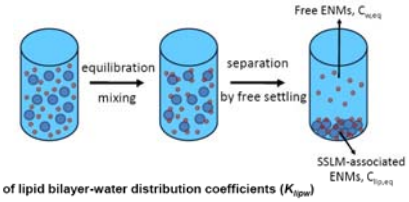




- Lipid Bilayer noncovalent bond to silica
- Bilayer is fluid
- Bilayer robust over wide range electrolyte conditions

12

Lipid Bilayer-Water Distribution of ENMs Method



Calculation of lipid bilayer-water distribution coefficients (K_{lipw})

$$K_{lipw} = \frac{C_{lip,eq}}{C_{w,eq}}(L/kg) \text{ where}$$

$$C_{lip,eq} = \frac{(C_{w,ref} - C_{w,eq})(mg/L)}{m_{lip}(kg/L)}$$

$C_{w,ref}$ = [ENMs] in the control samples

$C_{w,eq}$ = free [ENMs] in supernatants at equilibrium

m_{lip} = lipid concentration

Validated using a reference compound (2,4,6-trichlorophenol).

log K_{lipw} = 3.89 ± 0.03 (L/kg), close to 3.90 reported by Escher et al. (ES&T, 1996)

ENMs and Lipid Sources and Preparations

ENMs:

- **Aqueous C_{60} aggregates (nC_{60})**: dry C_{60} powders (MER, Tucson, AZ) were pulverized and then mixed with DI water for 2 weeks prior to passing through 0.7 and 0.45 µm filters sequentially (Hou et al., 2009).
- **Fullerol ($C_{60}(ONa)_x(OH)_y$, x + y = 24)**: dry fullerol powder (MER, Tucson, AZ) was directly mixed with water and then passed through 0.7, 0.45, and 0.2 µm filters sequentially.
- **Gold nanoparticles (nAu)** were tannic acid coated and well-characterized gold colloids at 5, 10, 20, 50, and 70 nm, purchasing from nanoComposix (San Diego, CA).

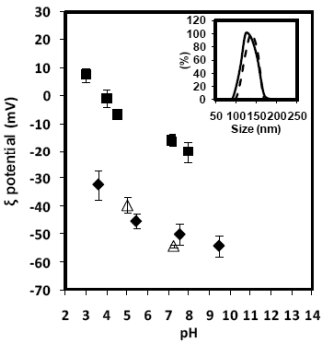
Lipid bilayers:

- SSLMs were purchased from Sovicell (Leipzig, Germany). The lipid composition was 100% phosphatidylcholine from chicken egg.
- Unilamellar lipid bilayer vesicles (i.e., liposomes) were prepared using the same lipid composition as SSLMs by the extrusion method (Hope et al., 1985). Liposomes were used to determined the effective zeta potential of lipid bilayers.

Analytical methods

- **nC_{60} concentration** was determined by high performance liquid chromatography (HPLC) with UV detection at 336 nm. Because HPLC is only applicable to molecules, molecular C_{60} was extracted from the aqueous phase to toluene in the aid of 0.1 M $Mg(ClO_4)_2$. The toluene extract was injected to HPLC (Hou et al., 2009).
- **Fullerol concentration** was determined by UV-visible absorption spectroscopy using UV at 254 nm.
- **nAu concentration** was determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES). Prior to ICP, nAu was dissolved in aqua regia (i.e., 1 part of HNO_3 and 3 parts of HCl).
- **Lipid concentration** was determined by the malachite green dye assay (Petitou et al., 1978). Before assay, lipid was digested, adding concentrated H_2SO_4 and H_2O_2 under heating.
- **The sizes and zeta potential** of liposomes and ENMs were determined by dynamic light scattering (DLS) (NICOMP 380 ZLS, Particle Sizing Systems, Santa Barbara, CA).

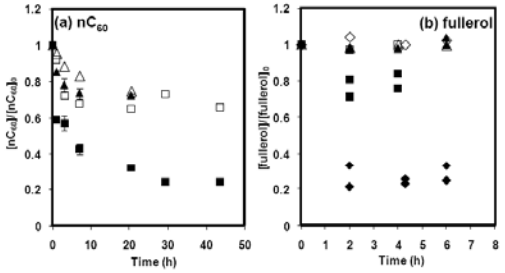
Size and Effective Zeta



Lipid bilayer vesicles (■)
Fullerol 650 mg/L (♦)
nC_{60} 6.5 mg/L (Δ)

nC60 and fullerols have similar size distributions and charge

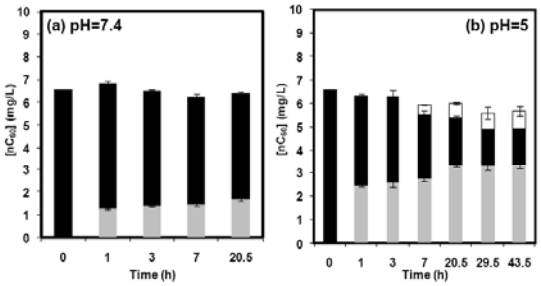
Fullerol and nC60 Lipid Bilayer Interaction Kinetics



pH=3 (♦) and control (◊) pH=5 (■) and control (□) pH=7.4 (▲) and control (Δ)

[lipid] = 0.47 mM --- Controls are vial without SSLM ---- $[nC_{60}]_0$ = 6.5 mg/L at pH = 7.4 and 5. $[fullerol]_0$=8.0 mg/L (pH 7.4); 11.0 mg/L (pH 7.4), and 11.0 mg/L (pH 3)

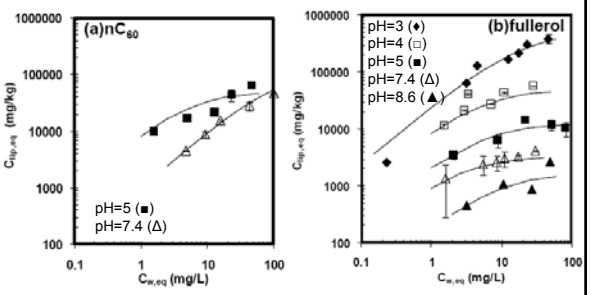
nC60 Mass Balances



Black: free nC_{60} ---- Grey: nC_{60} in SSLMs ---- White: nC_{60} lost to walls

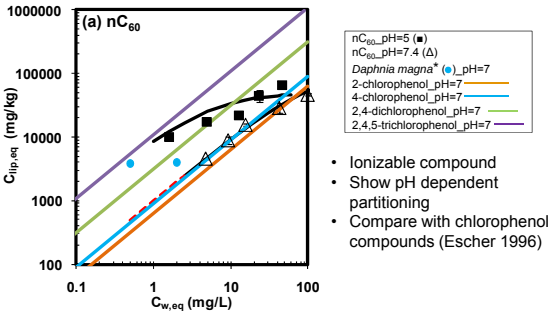
Serial ENM extraction from SSLM by original electrolyte then toluene

Distribution of nC60 and Fullerol in Lipid-Water



- Lines are Langmuir isotherms
- Bars indicate one standard deviation.

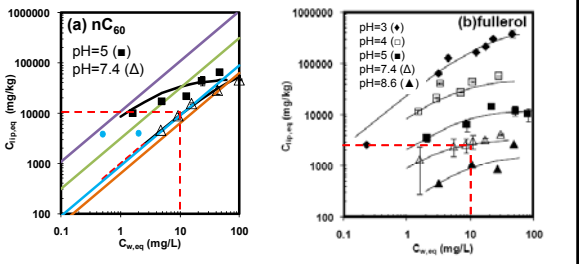
Comparisons with Daphnia Bioaccumulation & Ionizable Organic Compounds



- Ionizable compound
- Show pH dependent partitioning
- Compare with chlorophenol compounds (Escher 1996)

*Bioaccumulation of nC_{60} in aquatic organisms (*Daphnia magna*) (Tervonen et al., 2010)

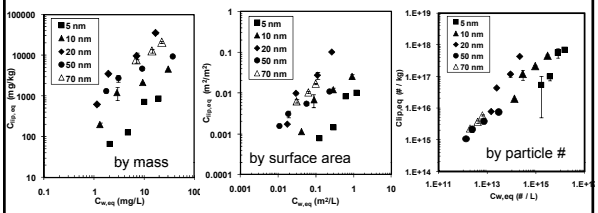
Comparisons with Daphnia Bioaccumulation & Ionizable Organic Compounds



Studies suggest higher bioaccumulation and toxicity of nC_{60} than fullerol:

- Kiser et al., *Water Res.*, 2010. (biosorption using wastewater biomass)
- Sayes et al., *Nano Lett.*, 2004. (toxicity to human cell membranes)
- Zhu et al., *Environ. Toxicol. Chem.*, 2007. (toxicity to fish)
- Fang et al., *Environ. Sci. Technol.*, 2007. (toxicity to bacteria membranes)

Lipid-Water Distribution Isotherms of nAu

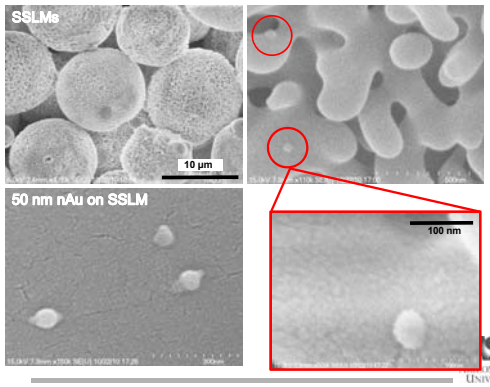


Measure	$[nAu]_0$/[lipid]
mol/mol	0.03 to 0.32
area/area	0.01 to 0.476
particle #/kg	10^{16} to 10^{19}

Also observed for cells. Chithrani et al. Nanoletters 2006

$[nAu]_0$=3-30 mg/L, [lipid]=0.47 mM, and pH = 7.4)

SEM Images of 50 nm nAu Adsorbed on SSLMs



Summary OF Lipid-Water Distributions

- The lipid bilayer-water distribution of the selected ENMs is pseudo-equilibrium process that can be described by isotherm behaviors.
- The distribution behavior is pH dependent
- Accumulation to lipid bilayers increasing as pH dependent (electrostatics)
- Analogous distribution behaviors of ionizable organic pollutants such as chlorinated phenols.
- Size dependency studies show that 20 nm gold nanoparticles exhibit the highest propensity to accumulate in lipid bilayers.
- Comparisons with bioaccumulation and toxicity studies using organisms suggest that the lipid bilayer-water distribution is promising for assessing the bioaccumulation and toxicity potentials of ENMs.
- Need bioaccumulation data (BCF) data on variety of ENM to verify if the lipid-water distribution can be used for predicting ENM fate.

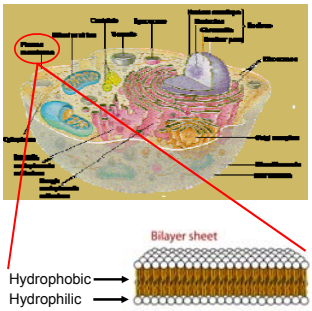
24

backup

25

Lipid bilayers as model cell membranes

- Lipid bilayers are the primary constituents of many biological cellular membranes. Arguably the most important interface between cellular life and its surrounding environment.
- Lipid - amphiphilic molecule that can spontaneously arrange in aqueous solution to have a hydrophobic interior and hydrophilic exterior.



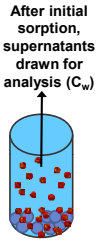
26

Focus

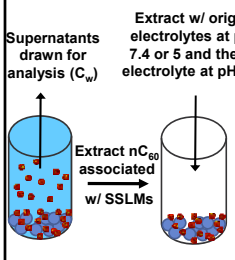
- Assess the affinity of ENPs to model cell membranes by **quantifying ENP distribution between lipid bilayer and water**.
- ENPs: C_{60} and polyhydroxylated C_{60} (i.e., fullerol) aggregates
  - ✓ Applications in cosmetics, energy production, catalysts, etc.
  - ✓ Focus of recent ecotoxicological studies
  - ✓ Fullerol-like materials is potential transformation products of C60 in the environment.
- pH dependency: pH = 3, 4, 5, 7.4, and 8.6 using phosphate electrolytes (5-20 mM), covering the pH range of physiological and environmental conditions,
- Interaction kinetics
- Pseudo-equilibrium isotherms: Langmuir or Freundlich model
- Comparisons with existing aquatic organism bioaccumulation and toxicity studies and with lipophilicity of ionizable organic pollutants.

27

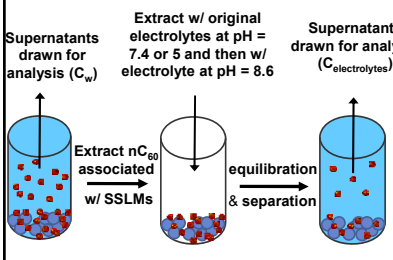
Mass Balance



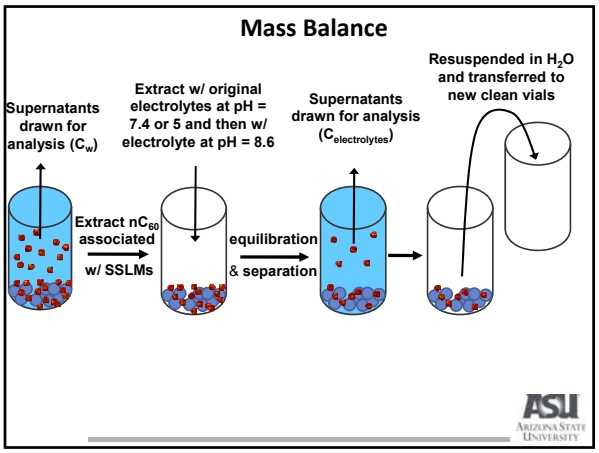
Mass Balance



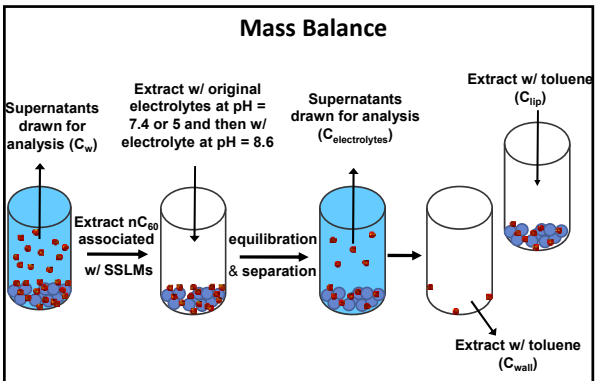
Mass Balance



Mass Balance



Mass Balance

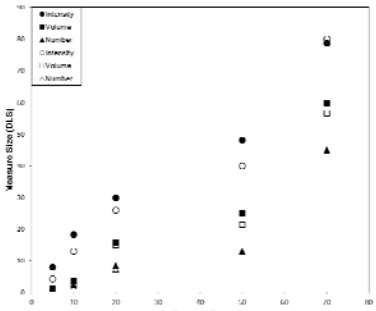


$C_{total} = C_w + C_{electrolytes}$ (combined) + $C_{lip} + C_{wall}$

Table 1. Parameters of the Freundlich and Langmuir models fitted to nC_{60} and fullerol isotherm data.

ENP	pH	Freundlich model: $C_{lip,eq} = K_F \cdot C_{w,eq}^{\ n}$			Langmuir model: $C_{lip,eq} = K_{lip,max} \cdot K_{ads} \cdot C_{w,eq} / (1 + K_{ads} \cdot C_{w,eq})$		
		log K_F (L^n/mg^{n-1}-kg lipid)	n	r^2	log $K_{lip,\ max}$ (mg/kg lipid)	K_{ads} (L/mg)	r^2
nC_{60}	5	3.85	0.55	0.95	4.70	0.2	0.91
	7.4	3.22	0.74	0.98	5.05	0.009	1.00
Fullerol	3	4.21	0.94	0.95	5.70	0.05	0.90
	4	4.02	0.54	0.95	4.70	0.2	0.98
	5	3.52	0.33	0.73	4.10	0.2	0.94
	7.4	3.08	0.37	0.98	3.52	0.38	0.98
	8.6	2.39	0.54	0.76	3.22	0.12	0.85

Gold nanoparticles size measurements in DI water



Yongsheng Chen

Development of an *In Vitro* Test and a Prototype Model To Predict Cellular Penetration of Nanoparticles

***Yongsheng Chen*[1], *David Capco*[2], *and Zhongfang Chen*[3]**
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[2]***School of Life Sciences, Arizona State University, Tempe, AZ;***
[3]***Department of Chemistry, University of Puerto Rico, San Juan, PR***

The aim of this study is to gain fundamental insight into the nanoscale properties of engineered nanomaterials and the relationships with environmental and biological impacts. The overarching questions we are trying to answer are how nanoparticles interact with the environment and cells and how these interactions will impact the environment and biological systems. To answer these questions, we have been focusing on characterizations of NPs in the environment and investigating the nano-bio interactions over the past 1 year.

Characterizations. We are trying to obtain the physicochemical parameters of various nanomaterials of interest by experiments and theoretical computations. One reason is that the environmental behaviors (e.g., fate, transport, and biological impacts) are significantly influenced by their inherent nanoscale properties and characterization of these properties is critical for better understanding their environmental impacts. For experimental research, we focused on Fe_2O_3, CeO_2, and Ag to conduct substantial experiments. Our results indicated that except hematite (α-Fe_2O_3), CeO_2, and Ag NPs had larger hydrodynamic diameters than the diameter measured by transmission electron microscopy (TEM) and atomic force microscopy (AFM). Our AFM images revealed that particle aggregation occurred instantly when they were dispersed, especially at the presence of electrolyte (e.g., KCl) in the solution. The particle size and particle size distribution influenced by the solution chemistry show significant effects on their fate and biological impacts. Thus, we conducted aggregation kinetics experiments, which were studied by time resolved-dynamic light scattering (TR-DLS) technique. Derjaguin-Landau-Verwey-Overbeek (DLVO) theory and the attachment efficiency (or inverse stability ratio) was used to distinguish the aggregation into two regimes, diffusion-limited and reaction limited regimes, which were both observed for the aggregation kinetics of CeO_2 and Ag NPs under ionic strengths between 0.005 and 0.1 M. Based on DLVO theory, we developed a combination of Arrhenius equation, extended DLVO theory, and von Smoluchowski's population balance equation for modeling the aggregation kinetics, which was not only suitable for interpreting the aggregation kinetics in the initial aggregation stages, but also applicable in the transition and post-aggregation stages. In particular, this work lays the groundwork for developing appropriate mathematical descriptions of nanoparticle behaviors and provided insight into aggregation kinetics mechanisms. For example, particle aggregation is dominated by the interplay of van der Waals, electrostatic, and acid-base forces and particle size as well as particle surface charge (indicated by zeta potential) that contribute to the magnitude of energy barrier, which governs the aggregation kinetics rate. In quantum calculation, we obtained theoretical predictions of various nanomaterials (e.g., ZnO) on their structural, electronic, and magnetic properties, and these predictions can not only help our experimental work, but also provide potential physico-chemical properties to develop our prototype model.

The biological interactions. In this aspect, we carried out our studies through two approaches; one is quantifying the cellular exposure to NPs to establish the quantitative description and correlation in the nanotoxicity database, and the other is developing imaging techniques to provide a visualization of the exposure impacts on cells at the nanoscale. For cellular exposure, we conducted experiments with Caco-2 cells, which are a model epithelium for human intestinal cells and *Escherichia coli* (*E. coli*) cells, which are one of the most common microorganisms widely present in the environment and widely used in toxicological tests. Cellular impairment of Caco-2 cells was evaluated by measuring transepithelial electrical resistance (TEER). Cell surface disruption, localization, and translocation of NPs through the cells were further analyzed

with immunocytochemical staining and confocal microscopy. Our results showed that hematite NPs reached adsorption equilibriums after approximately 5 min but adsorption kinetics were size dependent. After the adsorption equilibrium and a longer exposure time (> 3 hr), severe cellular effects were observed from the drop of TEER compared to the control cells. Hematite NPs triggered a dynamic reorganization and detachment of microvilli structures from Caco-2 cell surfaces. Particularly, the confocal microscopy revealed that the exposure to 26 nm disrupted the cellular junctions more severely than larger sizes. Similarly, the adsorption kinetics of hematite NPs upon *E. coli* cells was also found size dependent. The adsorption rates expressed as mg $Fe \cdot L^{-1} \cdot s^{-1}$ decreased in the order of 98 nm > 76 nm > 53 nm > 26 nm. However, adsorption rates expressed as the number of adsorbed hematite NPs per unit cell surface area ($\# \cdot m^{-2} \cdot s^{-1}$) were faster for small NPs than those for large NPs. To interpret the size effects on adsorption kinetics, the extended DLVO theory was combined with interfacial force boundary layer (IFBL) theory. The theories divided the adsorption into two regimes, one is dominated by interfacial forces and the other is dominated by diffusion. Faster kinetics for smaller NPs could be attributed to faster particle mobility and lower energy barriers in the total interaction energy according to the derived adsorption rate from EDLVO-IFBL theories. To visualize the exposure impacts on *E. coli* cells, we developed an AFM-based imaging technique as a novel tool to investigate the NP-cell interactions. Our results demonstrated for the first time AFM's superior performance in resolving the individual hematite NPs interacting with live *E. coli* cells, which provided a striking visualization of the adsorption of hematite NPs onto *E. coli* cells and the subsequent disruption in their extracellular appendages (flagella).

The major challenge we encounter for exposure experiments, also recognized by other groups, is the difficulty in maintaining a stable dispersion of NPs in the biological relevant solutions. In most cases, NPs aggregate rapidly and transit to colloidal particles (amorphous and large size clusters). The biological impacts observed from such toxicity experiments may not be representative of what real NPs exhibit. Another issue we identified through adsorption experiments is the concentration of NPs, which is used to establish the relationship of dose-response in risk evaluation. However, NPs, due to the size distribution, may require a number-based concentration rather than the mass-based concentration alone for establishing dose-response risk assessment.

Next year, we will continue to extend our developed methodologies to other types of NPs (besides, hematite, CeO_2, Ag, and QDs) to study the environmental behaviors such as aggregation and ion release. Furthermore, we will establish AFM-based methods for quantifying the surface characteristics of NPs and their interactions with the biological system.

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EPA Grant Number: R83385601-1

Development of an In Vitro Test and a Prototype Model To Predict Cellular Penetration of Nanoparticles

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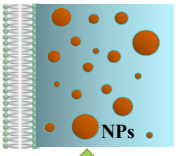
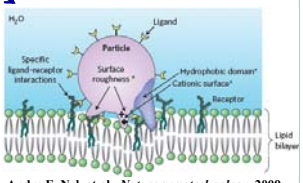
Bio-nano Interface



Biological consequences:
- Interfacial forces;
- Sorption processes;
- Surface accumulation, permeation, penetration, and cellular accumulation;
- Cellular damages (membrane disruption, machinery malfunction, genetic mutation)

Governing parameters:
Nanoparticles (NPs): Size, aggregation state, surface chemistry, hydrophobicity, surface geometry, surface roughness, surface charge, etc
Cell: Protein, lipo-polysaccharide, hydrophobicity, metabolic/signaling response, etc
Environment: pH, temperature, ionic strength, organic content, etc



Andre E. Nel, et al., *Nature nanotechnology*. 2009

Today's talk

Characterizing, Imaging, and Quantifying the Biological Interactions with Engineered Nanomaterials.

Questions to answer

How particle size impacts the biological interactions (e.g., interfacial forces, adsorption kinetics, cell surface disruption)?

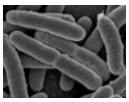
Nanomaterials and their Characterizations

α-Fe_2O_3
CeO_2
TiO_2
ZnO
Al_2O_3
CuO
SiO_2
QDs
Au
Ag

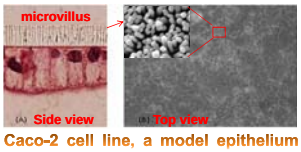
- Morphology, size, nanoelectric, and adhesive properties (TEM, SEM, and AFM);
- Surface energy, hydrophobicity, and crystallographic analysis (contact angle and HRTEM);
- Hydrodynamic sizes and zeta potential (DLS);
- Environmental behaviors: aggregation, metal ion release, and bioaccumulation (DLS and ICP-MS);

Bio-nano interactions

Biological systems

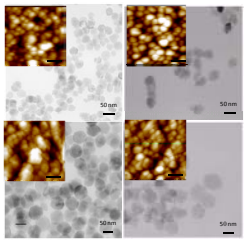


Escherichia coli (*E. coli*) cells



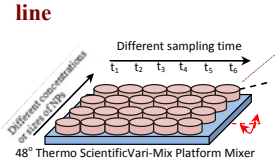
Caco-2 cell line, a model epithelium for human intestinal cells

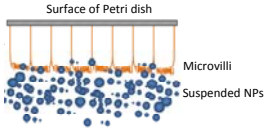
Nanoparticles



Hematite (α-Fe_2O_3) NPs, a reference nanomaterial

Bio-nano interactions: hematite NPs versus Caco-2 cell line



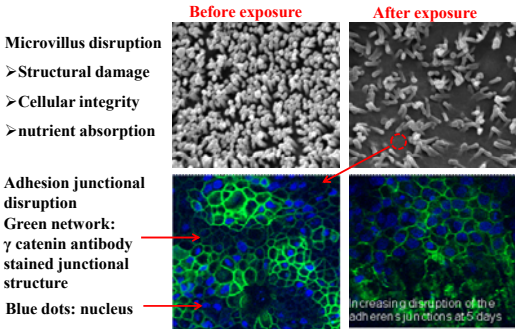


Caco-2 cell line

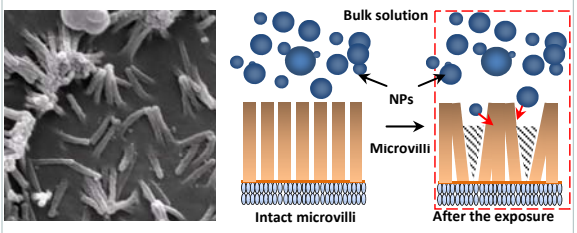
Evaluation

- Adsorption kinetics
- Membrane disruption by scanning electron microscopy
- Transepithelial Electrical Resistance (TEER) measurement and confocal microscopy to indicate cell penetration

Results: Bio-nano interactions using Caco-2 Cell line

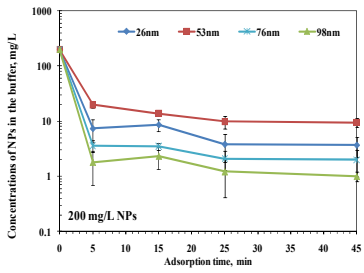


Structural disruption of microvilli on the cell surface



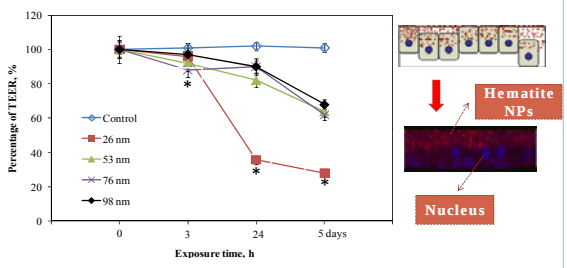
➢The left SEM image shows the morphological changes of microvilli on Caco-2 cell surfaces after the exposure to hematite NPs;
➢The right Carton shows the possible mechanism of depletion attraction by which the structures of microvilli were affected.

Results: Adsorption kinetics on Caco-2 cells



➢Adsorption kinetics show similar features (e.g., size dependency) as hematite NPs did with E. coli cells;
➢Large particles adsorbed faster by mass-based concentrations;
➢Small particles adsorbed faster in number-based concentrations.

Results: Size affects the disruption of adhesion junction and cell penetration



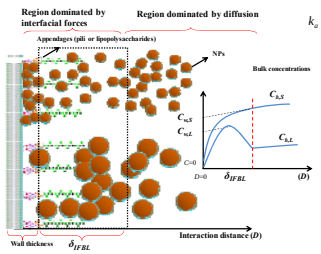
Small NPs penetrated cell lines faster and led to severer junctional disruption.

Conclusions

- Hematite NPs is ideal for use as a reference nanomaterial due to the high stability with uniform size distributions and low aggregation;
- Adsorption kinetics is size dependent, which can be interpreted by IFBL theory;
- The exposure to hematite NPs induced reorganization and distortion of surface structure damages (e.g., microvilli and flagella), and cell penetration.

Challenges and problems

What are the roles of interfacial forces and diffusion in the transport of nanoparticles toward biological system?
DLVO theory VS mass transport mechanisms



$$k_a = \frac{Diffusion}{\int_{D=0}^{D=\delta_{diffusion}} [(1+\frac{R_H}{D})\exp(U_{TOT}^{EDLVO}(D)/k_BT)-1]dD}$$

Combined effects are incorporated in IFBL theory

Next steps

➢Next year we will continue to extend our developed methodologies (e.g., models) to evaluate other types of NPs (e.g., CeO_2, Ag, and QDs) in their environmental and biological behaviors.

➢Furthermore, we will develop sophisticated imaging and quantifying techniques for the surface characterizations of NPs and their interactions with biological system at nanoscale .

13

Achievements

• 15 papers published in journals, such as JACS, Nano letters, and ACS Nano in 2009 to 2010;

• 6 manuscripts have been submitted.

• 20 invited talks or presentations in international wide conferences.

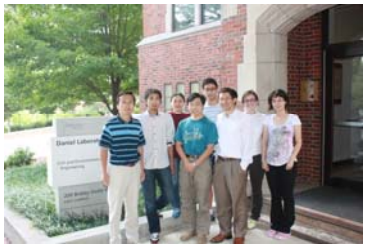
14

Dr. Yongsheng Chen's Group members

PhD students:
Wen Zhang, Kungang Li, Jia Yang, Wei Zhang, Nicole Sullivan.

Research engineers:
Ying Yao, Hao Jiang.

Visiting scholars:
Ying Huang, Rongjun Su, Yang Li.



15

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Semiconductor Research Corporation

16

AM Session 2: Effects at Sub-Cellular Level

Shaily Mahendra

Impacts of Quantum Dots on Gene Expression in *Pseudomonas aeruginosa*

Shaily Mahendra*[1,2], Yu Yang[2], Huiguang Zhu[3], Vicki Colvin[2,3], and Pedro J.J. Alvarez[2]
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Gene expression studies are valuable techniques for characterizing cellular responses to toxic substances as well as identifying mechanisms of toxicity. Global gene expression of *Pseudomonas aeruginosa* exposed to quantum dots (QDs) was investigated using whole transcriptome microarrays. Following exposure to 20 mg/L Qdot 655 ITK carboxyl-coated QDs, 54 genes were downregulated while 25 genes were upregulated. QDs artificially weathered by exposure to low pH, released Cd, Se, and Zn into the medium and caused repression of 100 genes and induction of 40 genes. Forty-four downregulated genes and 11 upregulated genes were found in both treatments. Quantitative PCR of impacted genes validated the microarray results ($R^2 = 0.92$).

Gene ontology analyses revealed that classes of inorganic ion transport and metabolism, energy production and conversion, nucleotide transport and metabolism, and DNA replication and repair were primarily upregulated. On the other hand, in the categories of carbohydrate, coenzyme, fatty acid and lipid transport and metabolism, cell motility, transcription, translation, and post-translational modification, the downregulated gene numbers were higher than those upregulated.

P. aeruginosa is a Gram-negative bacterium, which contains cation-antiporter Cd and Zn efflux pumps. Exposure to weathered QDs, which released heavy metals, caused relatively more negative impacts on gene expression. The categories of fatty acid, phospholipid, inorganic ion and coenzyme transport and metabolism, as well as transcription, cell motility, and energy production included more downregulated than upregulated genes. Weathered QDs also restrained aerobic respiration and energy production genes, amino acid synthesis, citric acid cycle, and acetyl-CoA assimilation. Although ammonium assimilation was inhibited, pathways of anaerobic respiration, fermentation, and denitrification were induced. This suggests that significant changes in cellular metabolism occurred in response to toxic stress.

This research will develop and disseminate critical data central to EPA's mission of environmental risk assessment and management. Characterizing ecotoxicological impacts of engineered nanomaterials such as QDs also will enhance future modeling efforts to support regulatory decisions, evaluate mitigation and cleanup strategies, and the development of durable QDs. Thus, this project will contribute to strengthening our scientific, engineering, research, education, and human resource base.

EPA Grant Number: R833858

Interagency Nanotechnology Implications Grantees Workshop
Portland, OR, Nov. 8, 2010

Impacts of Quantum Dots on Gene Expression in *Pseudomonas aeruginosa*

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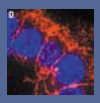
Acknowledgements
Yu Yang, Huiguang Zhu,
Vicki Colvin, & Pedro Alvarez

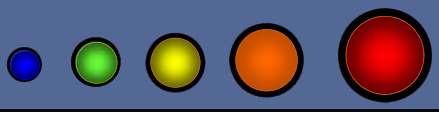
Quantum Dots

Biomedical Applications:
in-vivo imaging, immunoassays,
targeted gene and drug delivery



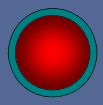
Wu et al., 2003

Electronics: solar cells, flat panel
LED displays, solid state lighting



Biocompatible Quantum Dots

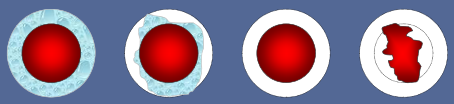
- Hydrophobic core/shell contains toxic metals (e.g., Cd and Se, Pb) surrounded by inorganic shell (e.g., ZnS)



- Can be stabilized in water by derivatizing the surface with amphiphilic organic coatings (may be elliptical)

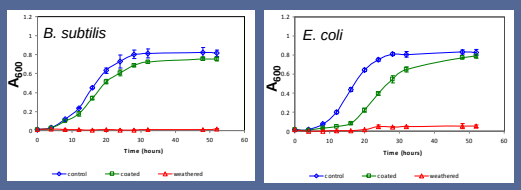
Quantum Dot Weathering

Hypothesis: *Toxicity of quantum dots is primarily due to free metal, and environmental weathering of the coating will increase their toxicity to cells*



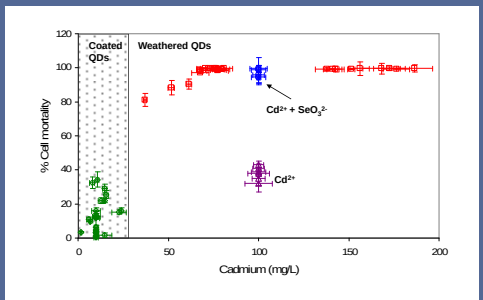
Capped QD | Degraded Coating | Exposed Core | Degraded Core

Coated QDs Retard Cell Growth; Weathered QDs Kill Bacteria



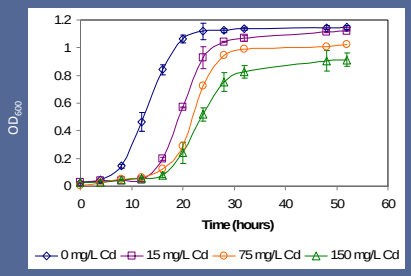
(Mahendra *et al.*, ES&T, 2008)

Cadmium and Selenite are Toxic



(Mahendra *et al.*, ES&T, 2008)

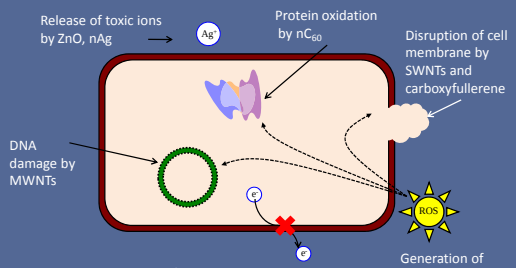
P. aeruginosa tolerated high Cd^{2+} from QDs



P. aeruginosa expresses Cd resistance via cation-antiporter efflux system.

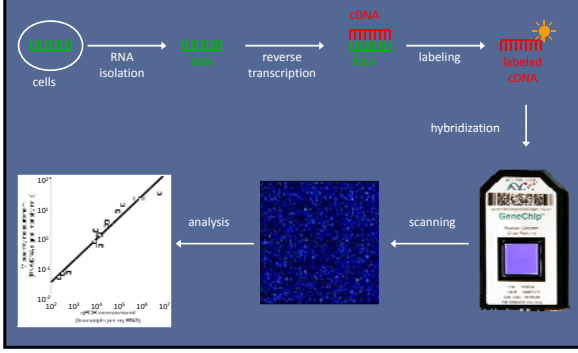
(Mahendra *et al.*, ES&T, 2008)

Bacterial Toxicity Mechanisms

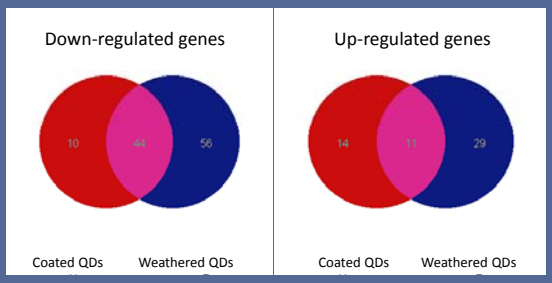


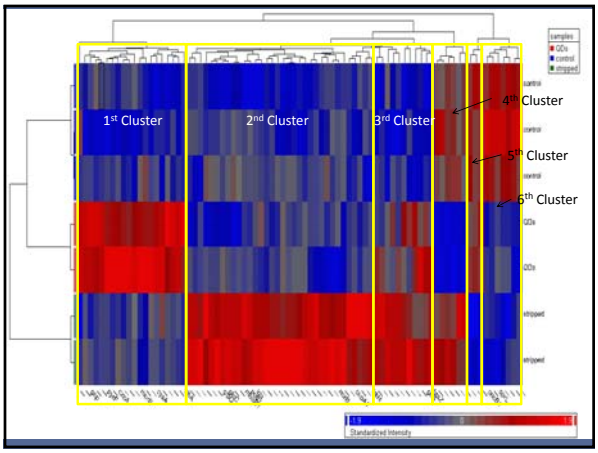
(Mahendra *et al.*, Chap 12 in Nanotech. Apps. For Clean Water, 2008)

Gene Expression - Microarrays



Global Gene Responses to QD Exposure





Summary

- Quantum dots exhibited antibacterial activity.
- Microarrays were used to understand QD toxicity mechanisms.
- Coated as well as weathered QDs affected gene expression in *P. aeruginosa.*
- Functional categories of amino acid metabolism, energy production, and carbohydrate metabolism were primarily regulated.

Terrance J. Kavanagh

Thiol Redox-Dependent Toxicity and Inflammation Caused by TOPO-PMAT Modified Quantum Dots

Terrance Kavanagh*[1], Dianne Botta[1], Collin White[1], Chad Weldy[1], Lisa McConnachie[1], Jasmine Wilkerson[1], Sean Gill[2], Xiaoge Hu[3], William Parks,[2] and Xiaohu Gao[3]
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Quantum dots (QDs) are semi-conductor fluorescent nanoparticles with potential uses in a variety of applications. Concerns have been expressed regarding their potential toxicity, specifically their capacity to induce oxidative stress. In this study, we assessed the effects of CdSe/ZnS core/shell QDs with a tri-n-octylphosphine oxide, poly(maleic anhydride-alt-1-tetradecene) (TOPO-PMAT) coating on the respiratory tract of mice. *In vitro* data in macrophages had shown that these QDs cause mild oxidative stress and secretion of pro-inflammatory cytokines, but this was dependent on the levels of the antioxidant tripeptide glutathione (GSH). We therefore investigated the pro-inflammatory effects of TOPO-PMAT QDs *in vivo* in mice genetically engineered to have deficiencies in GSH synthesis (GCLM null mice). Mice were exposed to QDs via nasal aspiration. Neutrophil counts in broncho-alveolar lavage fluid (BALF) increased in both wild-type (WT) as well as GCLM heterozygous (HT) mice, whereas GCLM null (KO) mice exhibited no increase in neutrophils. HT mice had a significantly higher level of neutrophils than WT mice. TOPO-PMAT gold nanoparticles had no effect on neutrophil influx in either WT or HT mice. Lung cadmium (Cd) levels peaked at 1 hr in HT mice, but were similar in WT mice at 0.5 hr, 1 hr and 3 hr. Cd levels in KO mice peaked at 0.5 hr. Levels of the pro-inflammatory cytokines KC and TNFa in BALF increased in the WT and HT mice, but not in KO mice. There was no change in matrix mettaloproteinase (MMP) activity in the lungs for any genotype. Neither WT nor HT mice had increased levels of myeloperoxidase (MPO – neutrophil marker). Interestingly, there was a decrease in MPO in the KO mice relative to untreated WT mice. We conclude that TOPO-PMAT QDs are pro-inflammatory in the respiratory tract of mice and are modulated by GSH-status. Because people are known to carry functional polymorphisms in GCLM which compromise GSH synthesis, GCLM HT and KO mice may be good models for investigating genetic susceptibility to QD-induced lung-inflammation in humans.

NIH/NIEHS Grant Numbers: P50ES015915, P30ES07033, T32ES07032, and R01ES016189

Thiol Redox-Dependent Toxicity and Inflammation Caused by TOPO-PMAT Modified Quantum Dots

Terrance J Kavanagh[1], Dianne Botta[1], Collin C White[1], Chad S Weldy[1], Lisa A McConnachie[1], Jasmine Wilkerson[1], Sean E Gill[2], Xiaoge Hu[3], William C Parks[2] and Xiaohu Gao[3]

Departments of [1]Environmental and Occupational Health Sciences, [2]Medicine, and [3]Bioengineering, University of Washington, Seattle, WA 98195

Overview

- Interactions of nanoparticles with biological systems, especially oxidative stress
- Role of glutathione (GSH) in preventing oxidative stress
- Quantum dot (QD) nanoparticles as a model system
- Effects of amphiphilic polymer coated QDs on multiple cultured mouse and human cell types
- Inflammatory response in normal and GSH depleted macrophages and in mice treated with QDs
- Ongoing studies

Quantum Dots (Qdots)

- Semiconductor nanocrystals
  - Highly resistant to photobleaching
  - Narrow fluorescence emission pattern
- Range in size from approximately 2 - 150 nm
- Metalloid crystalline core
  - Cadmium (Cd)
  - Selenium (Se)
  - Tellurium (Te)
  - Indium (In)
  - Mercury (Hg)
  - Lead (Pb
  - Arsenic (As)
- Cap or shell covering core
  - Zinc Sulfide (ZnS)
- Coatings
  - Biocompatible coatings
  - Amphiphilic polymer w/ functional groups
- Multiple uses
  - Biological imaging, photo-optronics, smart dyes
  - Gene and drug delivery

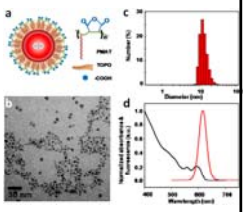
"Tunable" Fluorescence

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

Synthesis of Stable Aqueously Soluble Functionalized QDs

- Uncoated QDs often have poor solubility and are unstable in biological systems.
- We thus decided to mfg stable QDs for use as in vivo tracers.
- CdSe core
- ZnS shell
- TOPO: Tri-n-octylphosphine oxide capping ligand
- PMAT: Poly (maleic anhydride-alt-1-tetradecene); polymer with functional groups
- Exceptionally stable in aqueous solution (pH 7) and display red-orange fluorescence



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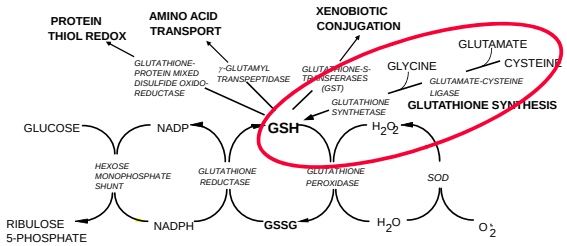
Quantum dot nanoparticles, oxidative stress and GSH

- QDs can release heavy metals (e.g. Cd, Zn) causing oxidative stress and toxicity in biological systems
- GSH is important in preventing oxidative damage to cellular macromolecules
- GSH has also been shown to be an important modulator of the immune response (lymphocyte proliferation; antigen presentation; T-helper cell polarization)
- GSH could thus be an important determinant of QD induced toxicity and inflammation

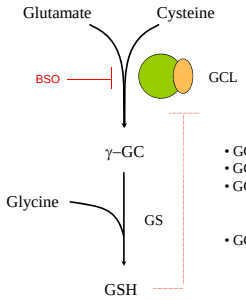
Glutathione (GSH)

- Tripeptide thiol (γ–glutamylcysteinylglycine)
- Antioxidant properties
- Important in scavenging free radicals
- Xenobiotic conjugation reactions (GSTs)
- Other important functions:
  - Amino acid transport
  - Protein thiol redox status
  - Cysteine storage
- Cellular GSH levels are controlled by:
  - Cysteine availability
  - Synthesis and utilization
  - Organ export/import

GLUTATHIONE METABOLISM



Glutathione Biosynthesis



GCLC 73 kDa

GCLM 31 kDa

- GCLC possesses all catalytic activity
- GCLC is feedback inhibited by GSH
- GCLM: lowers the K_m for glutamate increases the K_i for GSH possesses no catalytic activity
- GCL is inhibited by buthionine sulfoximine (BSO)

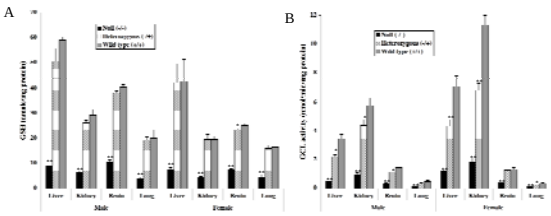
The Gclm null mouse: an *in vivo* model of GSH depletion

- To more thoroughly characterize the role of GCLM in GSH biosynthesis and oxidative stress, we made a Gclm knock-out mouse model.
- Humans are known to have polymorphisms in GCLM which predispose to heart disease, lung diseases, schizophrenia, and heavy metal body burden
- We tested the susceptibility of mice with varying amounts of GCLM production to nanoparticle induced lung injury by exposing them to Qdots

Gclm null mice have low GCL activity and low levels of GSH in most tissues

GSH levels

GCL activity



McConnachie et al., Tox Sci, 2007

Nasal Instillation of PMAT QDs

Lightly anesthetize mouse (ketamine/xylazine)

Instill 0.4 ul/gm of a 20 nM solution intranasally (~6 ug/kg Cd)

Sacrifice 8 or 24 hrs post instillation.

Collect BALF cells and fluid, serum, lungs, heart, spleen, kidney

Stain BALF cells for MΦ (F4/80+) and neutrophils (Gr1+)

Analyze cells by FACS for % Gr1+ cells

Measure total protein in BALF fluid

Possible reasons for lack of inflammation in Gclm null mice

- Failure of their MΦ to take up Qdots?
- Failure of their MΦ or airway epithelium to produce and/or secrete chemotactic peptides and cytokines?
- Failure of their MΦ to produce ROS (NADPH oxidase activity compromised?)
- Perhaps the lack of GSH has resulted in an adaptive response (up regulation of protective genes) which acts to squelch oxidative stress or the immune response?

Ongoing studies

- Mechanisms of PMAT Qdot uptake by MΦ
  - Scavenger Receptors; SRA, MARCO, LOX1
  - Calveoli, **clathrin**, endocytosis/macropinocytosis
- Examine markers of oxidative stress in the lung tissue, and BAL cells and fluid
- Chronic effects of exposure to PMAT-Qdots (e.g. pulmonary fibrosis?)
- DNA microarray analysis of gene expression for additional biomarkers of lung injury
- Systemic inflammation/markers of lung injury (plasma cytokines/chemokines; CC16; SPD; KL-6)
- Translocation of QDs and Cd to other organs
- Effects on olfactory epithelium and brain

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Emily Hu
Dave Eaton
François Baneyx
Mike Yost
Elaine Faustman
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Sengkeo Srinouanprachanh
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Patricia A. Holden

Bioavailability and Fates of CdSe and TiO_2 Nanoparticles in Eukaryotes and Bacteria

***Patricia Holden*[1], *Galen Stucky*[2], *and Jay Nadeau*[3]**
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This research addressed questions regarding interactions between specific engineered nanoparticles and cellular organisms, including: (1) what are the characteristics of nanoparticle uptake into cells?, (2) what is the stability of nanoparticles in association with cells?, (3) how do nanoparticles, including intact materials and products of instability, affect cells?, and (4) how do uptake, stability, and cellular effects vary with nanoparticles, cells, and environmental conditions? To address these questions, laboratory experiments were designed and performed using a variety of cells and nanoparticles. Research activities included nanoparticle synthesis and characterization, cell culturing, quantifying nanoparticle effects on growth and other cellular outcomes, analysis of nanoparticle and chemical constituent state during experiments, and using microscopy and spectroscopic methods as needed to assess cellular spatial interactions with nanoparticles at various levels of resolution. This research was aimed primarily at assessing the bioavailability of nanoparticles to cells and cellular outcomes of bioavailable nanoparticles, given that cellular effects including toxicity and cell-mediated nanoparticle alteration are predicated upon cells and nanoparticles interacting physicochemically. Major findings included that quantum dots (QDs) were toxic to bacteria, with oxidative stress and membrane damage explaining much of the observed nanoparticle-specific toxicity. Effects levels varied with gram positive versus gram negative bacteria, and between CdSe and CdTe-composed QDs.[1] Cadmium ions did not fully account for cellular effects of Cd-containing QDs.[2] Similarly to non-toxic gold nanoparticles that had been developed in this project as phylogenetic probes using oligonucleotide conjugation[3], QDs entered planktonic bacteria; however, differently, the QDs decomposed intracellularly plus generated cell-damaging reactive oxygen species (ROS).[2] Although cell exopolymers afforded some differential protective effects in planktonic culture[1], the exopolymer matrix of biofilm bacteria both in natural system mixed communities[4] and in laboratory pure cultures (manuscript in preparation) did not impede delivery of QDs to cells as assessed by staining for fluorescence and electron microscopy (EM) imaging[4] and by toxic effects to biofilm bacteria (manuscript in preparation). Photosensitization[5] of illuminated QDs and photoenhancement[6] of QDs using specific conjugates each led to ROS formation which damages cells in the light. Cellular toxicity also was shown to occur with direct electron transfer between cells and QDs; irradiated particles generated cell-damaging hydroxyl radicals.[7] Differences between gram positive and gram negative bacteria were observed[7], yet questions remain regarding the role of nano-bio interfacial charge transfer to cellular toxicity under dark conditions.

Intracellularization and bioaccumulation of QDs by bacteria[2] resulted in QD-containing bacterial prey that were subsequently studied for trophic transfer and QD biomagnification by protozoa. These studies showed that QDs were trophically transferred intact and were differently toxic to protozoan predators as compared to cadmium ions also packaged within bacterial prey. In contrast to QDs, nano-TiO_2 particles did not enter cells but associated externally on bacterial membranes, which led to the disagglomeration of large nanoparticle agglomerates outside of cells.[8] Association occurred in the dark, as did growth inhibition that appeared to scale inversely with nanoparticle size. Studies with these and other metal oxide nanoparticles are continuing to address outstanding questions regarding origins of apparent toxicity in dark conditions, including membrane damage and potentially cellular oxidation, plus indirect effects owing to the frequently observed affinity of metal oxide nanoparticles for cell envelopes. Overall, this research provides new and important insights into mechanisms of select nanoparticle toxicity to cells. This research suggests that nanoparticles are frequently growth-inhibitory, particularly under conditions that promote direct cell contact. Although not directly studied in this research, there may be broader implications to bacterially-driven processes in the environment as most

biogeochemical processes in nature are catalyzed through bacterial population growth that was studied in this research. The importance of this research to the U.S. Environmental Protection Agency is that it provides insights into the effects of nanoparticles on microbes that are responsible for biogeochemical processes, including pollutant transformations; this research also addresses fates of nanoparticles in the environment as affected by microbes that are omnipresent in soil, sediment, and water. The research is similarly important to the National Science Foundation with its focus on mechanisms of effects on microbes that could be important targets for rapid screening or biomarker development.

References:

1. Dumas EM, et al. Toxicity of CdTe quantum dots in bacterial strains. *IEEE Transactions on Nanobioscience* 2009;8(1):58-64.
2. Priester JH, et al. Effects of soluble cadmium salts versus CdSe quantum dots on the growth of planktonic Pseudomonas aeruginosa. *Environmental Science & Technology* 2009;43(7):2589-94.
3. Ehrhardt CJ, et al. An improved method for nanogold in situ hybridization visualized with environmental scanning electron microscopy. *Journal of Microscopy* 2009;236(1):5-10.
4. Clarke S, et al. Bacterial and mineral elements in an Arctic biofilm: a correlative study using fluorescence and electron microscopy. *Microscopy and Microanalysis* 2010;16(2):153-65.
5. Cooper DR, NM Dimitrijevic, JL Nadeau. Photosensitization of CdSe/ZnS QDs and reliability of assays for reactive oxygen species production. *Nanoscale* 2010;2(1):114-21.
6. Cooper DR, et al. Photoenhancement of lifetimes in CdSe/ZnS and CdTe quantum dot-dopamine conjugates. *Physical Chemistry Chemical Physics* 2009;11(21):4298-4310.
7. Dumas E, et al. Interfacial charge transfer between CdTe quantum dots and gram negative vs gram positive bacteria. *Environmental Science & Technology* 2010;44(4):1464-70.
8. Horst AM, et al. Dispersion of TiO_2 nanoparticle agglomerates by Pseudomonas aeruginosa. *Applied and Environmental Microbiology* 2010 (in press).

EPA Grant Number: R833323

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Bioavailability and Fates of CdSe and TiO_2 Nanoparticles in Eukaryotes and Bacteria

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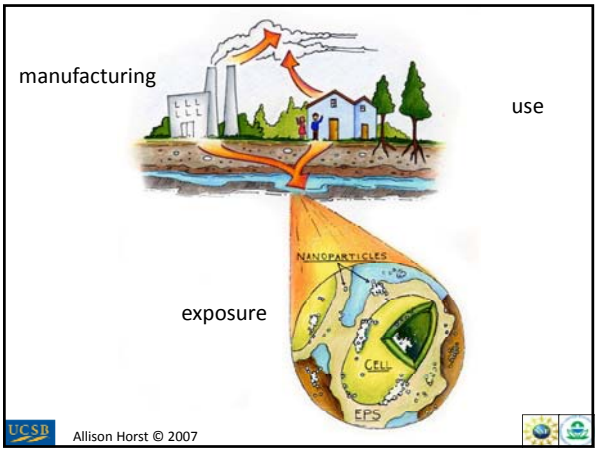
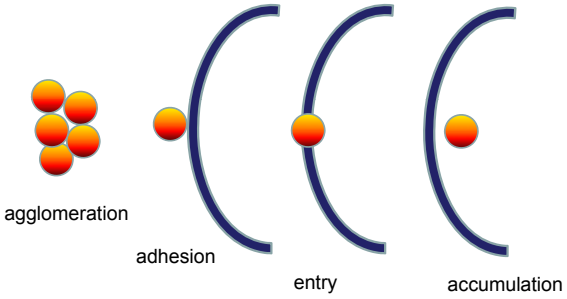


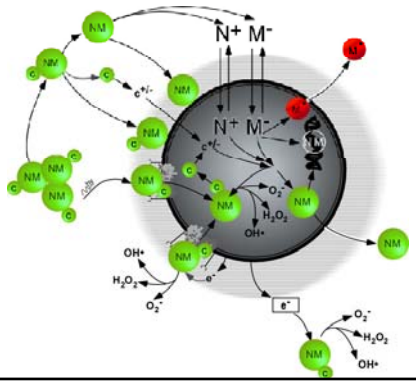
Bioavailability Continuum



Questions

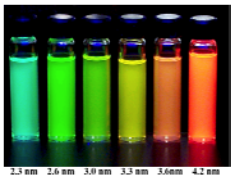
- When do nanoparticles enter cells?
- Do the particles stay intact?
- What are the cellular effects?
- What are the variables?

Hypothetical Interactions: Nanomaterials (NMs) and cells

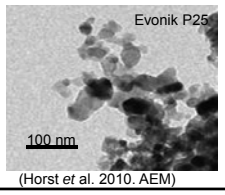


Nanparticles in this Research

- CdSe quantum dots (QDs)
  - laboratory-synthesized
  - bare and core shell (ZnS)
  - various conjugates
  - also CdTe



- TiO_2
  - Industrial (Evonik P25) and laboratory synthesized
  - 80% anatase / 20% rutile



(Horst *et* al. 2010. AEM)

Methods

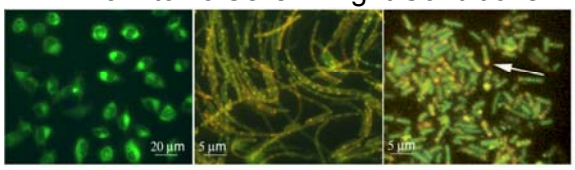
Nanomaterials

- Characterization
  - Microscopy (optical, TEM/EDS, STEM/EDS)
  - DLS, EPM (TiO_2)
  - ROS (DCFDA;SOSG for $^1O_2$; XTT for $O_2^{\cdot-}$; Na terephthalate for $^{\cdot}OH$; EPR spectroscopy)
  - TCSPC (lifetime fluorescence)
- Quantification
  - ICP-AES; AA
  - Dialysis / ultrafiltration
  - XANES (Se oxidation state)

Cells

- Exposure
  - Planktonic (growth, short term) & biofilms
  - Light / dark; oxygen/anoxic
- Effects
  - Growth (rate, extents)
  - Membrane integrity (as above); LIVE/DEAD
  - Membrane potential (BacLight: DiOC2)
  - Eukaryotic specific (e.g. MMP)
  - Metabolism (dehydrogenase; MTT).

Background: CdSe/ZnS QDs Enter Planktonic Cells in Light Conditions

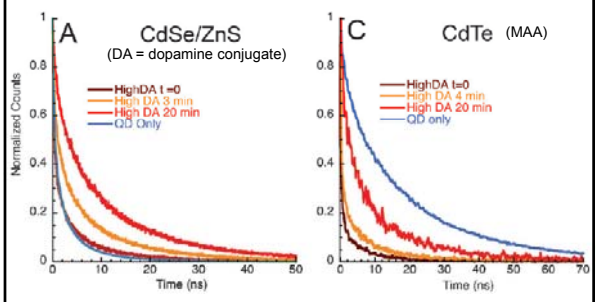


Mammalian A9 cells w/ green QD-dopamine. | *B. subtilis* w/ yellow QD-adenine. | Adenine auxotrophic *E. coli* w/ green QD-adenine.

→ Photoactivated uptake and fluorescence
→ Conjugate and receptor mediated
→ External binding prerequisite
→ Transient membrane damage
→Cellular processing
→Toxicity not from Cd(II)

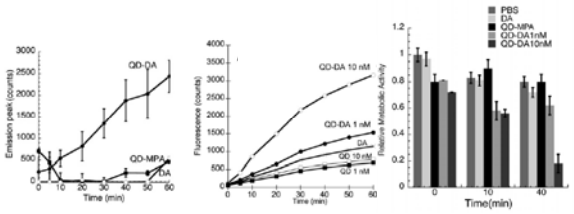
(Kloepfer et al., 2003; Kloepfer et al., 2005; Clarke et al., 2006)

QD fluorescence lifetimes vary with core, cap, conjugate



(Cooper et al. 2009. Phys. Chem. Chem. Phys).

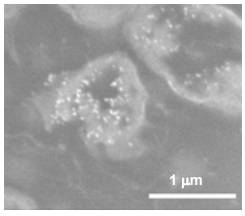
CdSe/ZnS QDs Photosensitized w/ Dopamine; PC12 Cells inhibited

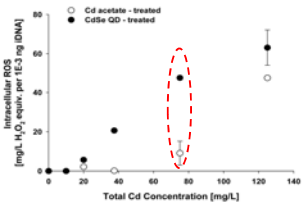


$O_2^{\cdot-}$ ↑ **intracellular ROS** ↑ **metabolism** ↓

(Cooper et al. Nanoscale. 2010)

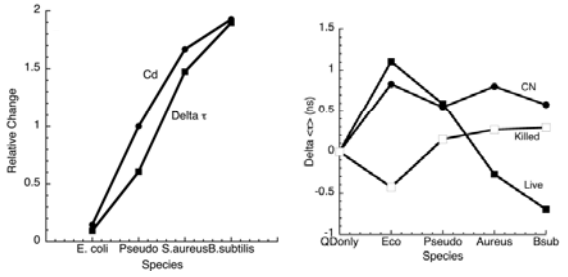
Bare CdSe QDs Enter & Toxic to *Pseudomonas* in Dark Conditions,





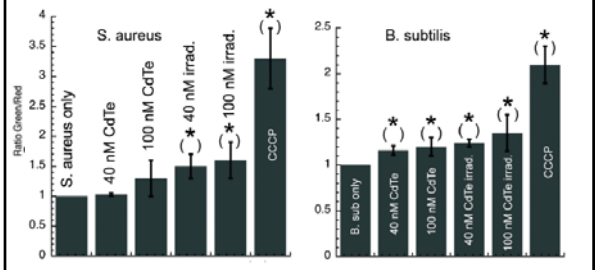
(Priester et al. ES&T 2009)

CdTe QDs differentially bind, transfer e^- to Bacteria Strains



(Dumas et al. ES&T. 2010)

G+ bacterial membranes depolarized, but growth not slowed

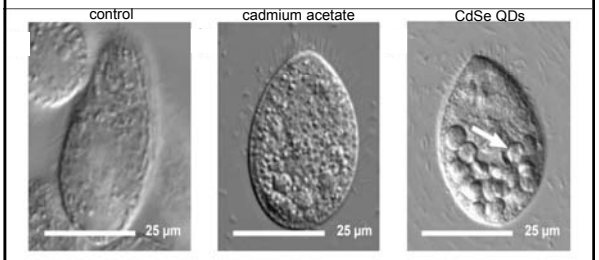


(Dumas et al. ES&T. 2010)

Quantum Dot summary

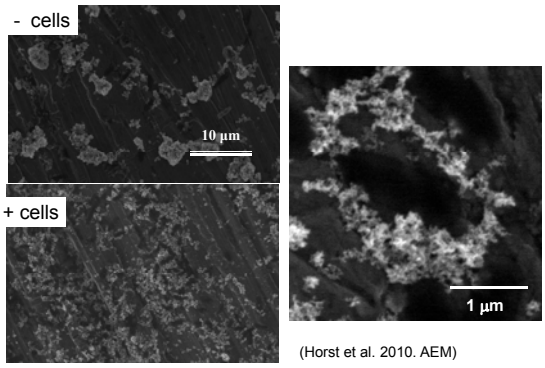
- QDs can enter cells
  - With ROS-mediated membrane damage
  - ROS form varies (light/dark; QD; conjugate)
  - Binding prerequisite
- QDs can enter intact
  - Cap slows dissolution
- Cells show consequences
  - Slow growth rate and lower yield
  - Membrane depolarization (not "toxic"?)

CdSe QDs trophically transferred from *Pseudomonas* to *Tetrahymena*



(Werlin et al. 2010 in revision)

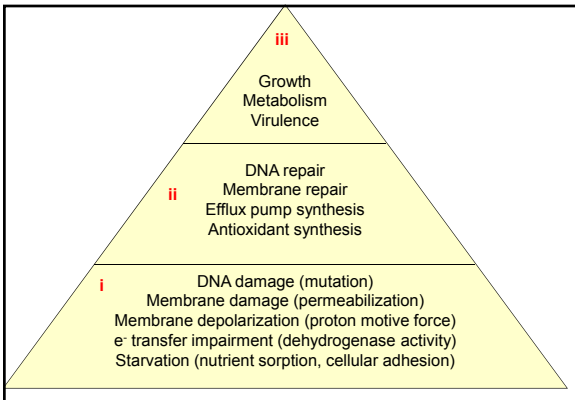
Pseudomonas binds & disagglomerates TiO_2



(Horst et al. 2010. AEM)

Summary & Next Steps

- SUMMARY:
  - QDs can damage & enter cells; e^- transfer
  - TiO_2 binds, but didn't enter
  - consequences to NP transport
  - variables: light/dark, strain, NP, cap/conjugate, oxygen
- NEXT STEPS:
  - High throughput studies: membrane effects
  - Quantify cell loading (dose)
  - Quantify bioprocessing



Poster WP190 Wednesday

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Warren Heideman

Using Zebrafish Embryos to Test Phototoxicity of TiO_2 Nanoparticles

***Warren Heideman*[1], *Ofek Bar-Ilan*[2], *Sarah Yang*[2], *Joel Pedersen*[2], *Robert Hamers*[2], *and Richard Peterson*[2]**
[1]*School of Pharmacy*, [2]*University of Wisconsin-Madison, Madison, WI*

The properties of nano-scale TiO_2 allow the production of an electron-hole pair in response to absorption of a photon of sufficient energy. In aqueous solutions, this can lead to the generation of Reactive Oxygen Species (ROS). Because ROS can react with a variety of essential cellular macromolecules, the production of ROS can be cytotoxic. With these facts in mind, we hypothesized that TiO_2 nanoparticles might produce toxicity *in vivo* if an exposed organism is illuminated. To test this hypothesis, we conducted dose-response experiments in which zebrafish embryos were exposed to a solution containing graded doses of commercially available TiO_2 nanoparticles. The fish were divided into two groups. In one group, the fish were illuminated with a bright light source using a 14h/10h light/dark cycle. This illumination was designed to simulate the slightly blue-shifted spectrum of sunlight, such as would be found at approximately 1 m below the surface of a clear body of water. The other group was kept in dim tungsten filament lighting using the same light/dark cycle. After 5 days of exposure, we observed toxicity that was clearly photo-dependent. In the illuminated group, we observed lethality with an LC_{50} in the upper ppm range. The non-illuminated group showed almost no lethality at any concentration tested. We found that embryos pre-exposed to TiO_2 nanoparticle solution and then washed into fresh water retained photosensitivity, consistent with a model in which the embryos absorb the TiO_2 nanoparticles internally.

The nominal individual particle size was 21 nm; however, the particles rapidly aggregated in solution to produce aggregates of approximately 1 micron in size. Nonetheless, these particles were internalized by the developing zebrafish. Studies of uptake using inductively coupled plasma optical emission spectrometry (ICP-OES) and transmission electron microscopy (TEM) showed uptake throughout the tissues of the developing zebrafish. Uptake and the potency of the nanoparticles were affected by hatching from the chorion, the protective egg shell. Artificial dechorionation produced increased TiO_2 uptake, and increased sensitivity to toxicity.

To test the hypothesis that light exposure would induce the production of ROS *in vivo*, we used a combination of chemical probes for ROS presence and measures of cellular damage induced by ROS. These showed light-dependent ROS production. We also developed a transgenic zebrafish line in which a GFP reporter is expressed from Antioxidant Response Elements (AREs). The *Tg(are:eGFP)* line showed elevated reporter expression when the fish were exposed to both the TiO_2 nanoparticles and illumination, but not in response to either stimulus alone. Together, these results demonstrate an *in vivo* test of our hypothesis that TiO_2 nanoparticles activated by light produce ROS and phototoxicity in a developing vertebrate.

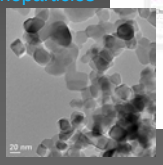
EPA Grant Number: RD-83386001

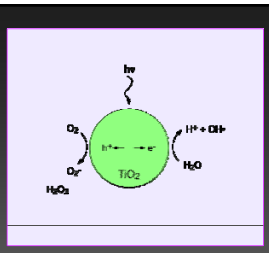
Using zebrafish embryos to test photo-dependent toxicity of titanium dioxide nanoparticles

Warren Heideman
University of Wisconsin-Madison

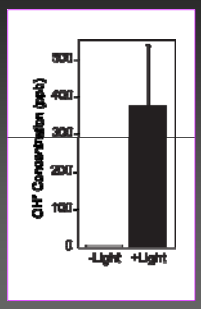
Titanium Dioxide

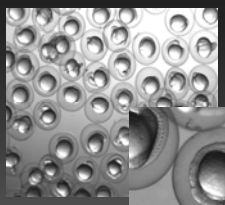
Nanoparticles



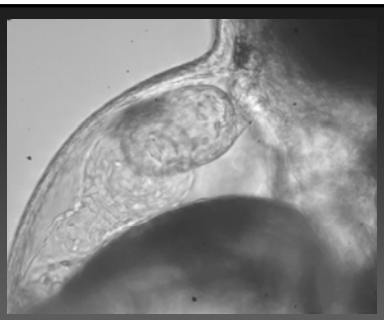


Light causes ROS production *in vitro*

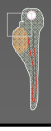




Does that matter *in vivo*?

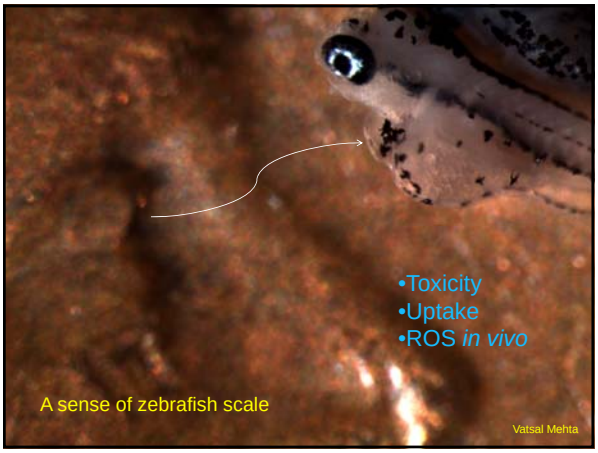


72 hpf

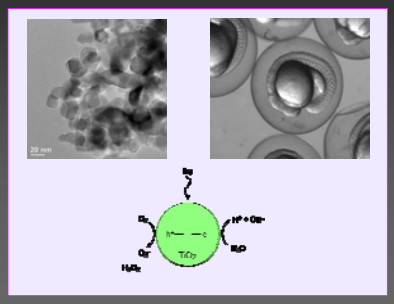


Zebrafish cardiotoxicity –if it were a mouse it would be dead.

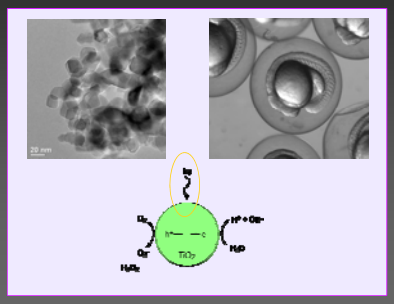
Vatsal Mehta

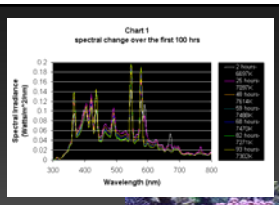


Testing TiO_2 nanoparticles *in vivo* requires:
- TiO_2 nanoparticles
- embryos



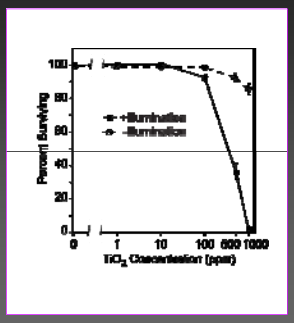
Testing TiO_2 nanoparticles *in vivo* requires:
- TiO_2 nanoparticles
- embryos
- light





Illumination comes from the aquarium hobby

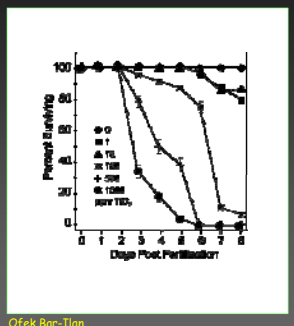
Toxicity



Zebrafish embryos exposed to TiO_2 nanoparticles and illumination do not survive

Ofek Bar-Ilan

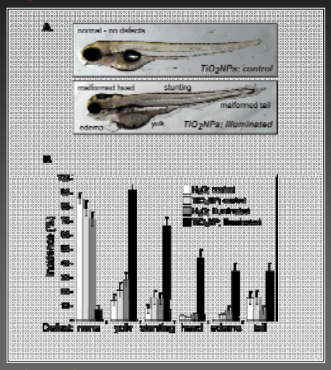
Toxicity



Zebrafish embryo survival *vs.* time and TiO_2 nanoparticle concentration

Ofek Bar-Ilan

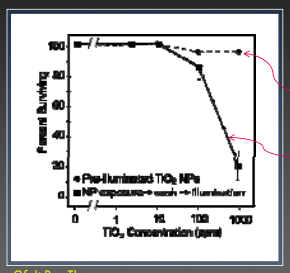
Toxicity



Defects associated with TiO_2 nanoparticle exposure and illumination

Ofek Bar-Ilan

Toxicity

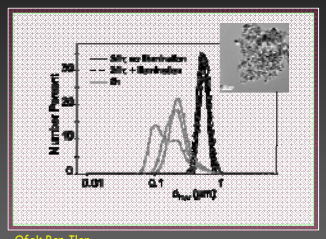


Ofek Bar-Ilan

Illuminate TiO_2 nanoparticles prior to exposing to embryos

Pre-expose embryos to TiO_2 nanoparticles, wash, then illuminate

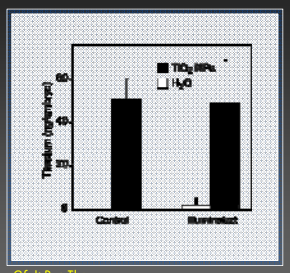
TiO_2 Uptake



Ofek Bar-Ilan

The TiO_2 nanoparticles have a pronounced tendency to aggregate

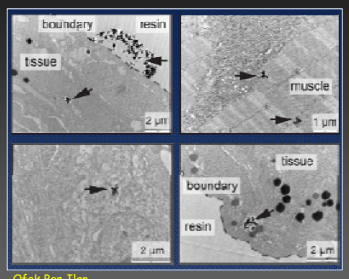
TiO_2 Uptake



Ofek Bar-Ilan

TiO_2 nanoparticle exposure adds measurable Ti to the fish.

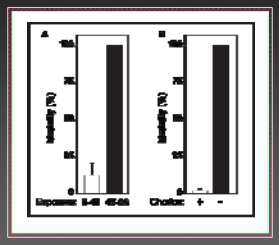
TiO_2 Uptake



Ofek Bar-Ilan

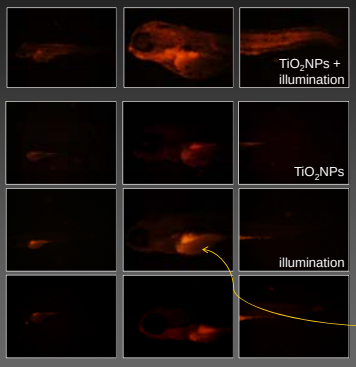
We find TiO_2 nanoparticles throughout the zebrafish embryo

TiO_2 Uptake



The egg chorion shields the embryos from toxicity

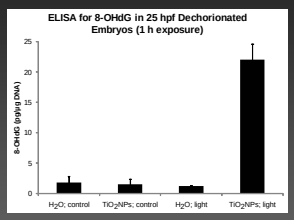
TiO_2 and ROS *in vivo*



Using DHE to detect superoxide production

yolk always shows autofluorescence

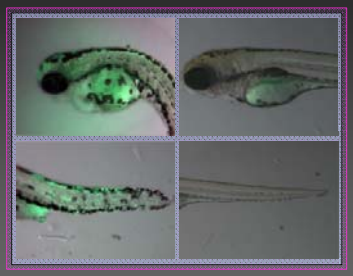
TiO_2 and ROS *in vivo*



DNA adducts are formed when TiO2 exposure is combined with illumination

TiO_2 and ROS *in vivo*

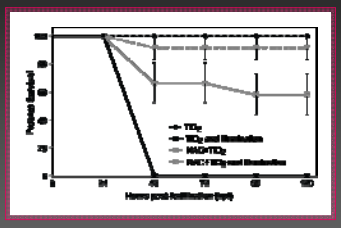
Exposed to TiO_2 nanoparticles



illuminated not illuminated

Using GFP as an ARE reporter shows activation by TiO_2 nanoparticles combined with illumination

TiO_2 and ROS *in vivo*



Preloading embryos with N-acetyl cysteine can prevent some of the effects of TiO_2 nanoparticle exposure

- The photochemistry of TiO2 nanoparticles predicted that the nanoparticles might cause phototoxicity due to ROS production. The uncertainty was whether this occurs *in vivo*.
- Using zebrafish embryos we show that TiO2 nanoparticles cause toxicity that is dependent on light and associated with uptake and ROS production.

- Zebrafish are not humans. However biological systems are strongly conserved. Mechanisms that work in zebrafish are often found in humans.

Richard E. Peterson

Kevin Lanham
Dorothy Nesbit
Olivia Bar-Ilan
Minsik Kim
Jessica Plavicki
Tracie Baker
Peter Hofsteen
Shaina Johnson
Felipe Burns

Joel Pederson
Robert Hamers
Kacie Louis
Sarah Yang

Christian Abnett
Eric Andreasen
Robert Tanguay
Cassandra Belair
Sean Severson
Amy Prasch
Sara Carney
Sue Bello
Adrian Hill
Tisha King-Heiden
Vatsal Mehta
Kong Xiong

US EPA RD-83386001

PM Session 1: Effects on Fish and Oysters

David S. Barber

Effects of Subchronic Exposure to Nanoparticulate Silver in Zebrafish

Robert Griffitt[1], Rachel Ryan[1], and David Barber[1]
[1]Center for Environmental and Human Toxicology, [2]Particle Engineering Research Center, University of Florida, Gainesville, FL

To examine the effects of subchronic exposure to nanoparticulate silver on zebrafish, we exposed adult female *Danio rerio* to nominal concentrations of 5, 15, 25, or 50 µg/L nanoparticulate silver for 28 days using a flowthrough system. A soluble silver treatment (5 µg/L nominal, ~2.5 µg/L measured) also was included. Samples were taken at days 7, 14, 21, and 28 for gene expression and tissue burden analysis, and at days 14 and 28 for histopathology analysis. Our results indicate that the use of flowthrough systems for chronic nanometal studies is a viable concept, as we were able to maintain measured concentrations of approximately 60 percent of nominal values over the course of the 28 day exposure. Dissolution of nanoparticulate silver were measured twice weekly throughout the exposure, with measured concentrations ranging from 0.5 to 1.0 µg/L, and there were no significant differences between treatments. Silver burdens of gills at conclusion of the study was concentration dependent with the 50 ppb nominal exposure producing burdens of 45 ± 9 ng Ag/mg wet wt, which was similar to that produced by silver nitrate (28 ± 6 ng Ag/mg wet wt). Microarray analysis of gills demonstrated that expression of 3,019 genes was significantly altered by silver exposure, mostly driven by 50 ppb nominal exposure. Clustering using LSMEANS places the 50 ppb and 25 ppb exposures together, 5 ppb and 15 ppb together, and all treatments separate from controls. These data demonstrate that subchronic exposure to nanosilver produces substantial effects on gill transcription, which does not appear to be driven solely by bulk release of dissolved silver as dissolved silver concentrations were comparable in all treatments.

NSF Grant Number: 0834075

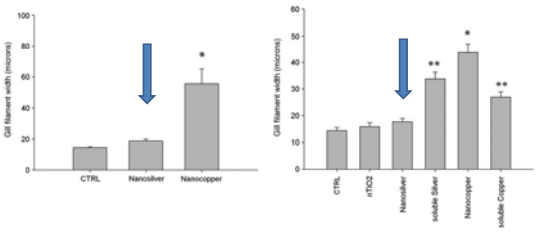
Effects of subchronic exposure to nanoparticulate silver in zebrafish

Joe Griffitt, Rachel Ryan
University of Southern Mississippi, Gulf Coast Research Laboratory

Andrew Kane, David Barber
University of Florida, Center for Environmental and Human Toxicology

Gill proliferation – acute exposure

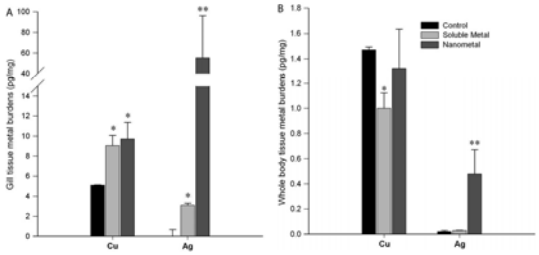
24 hours **48 hours**



Tox. Sci. 107(2) 404-415.

Silver accumulation – acute exposure

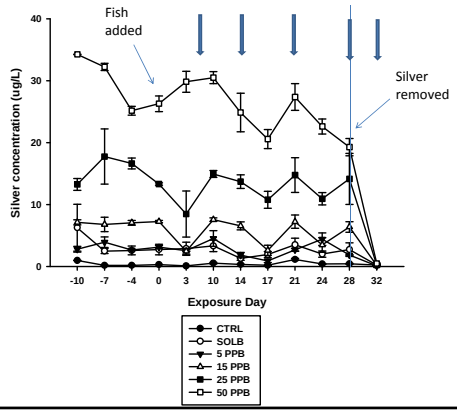
Gills +48 hours **Carcass +48 hours**



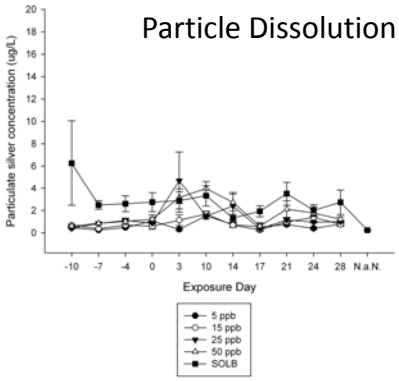
Tox. Sci. 107(2) 404-415.

Experimental Design

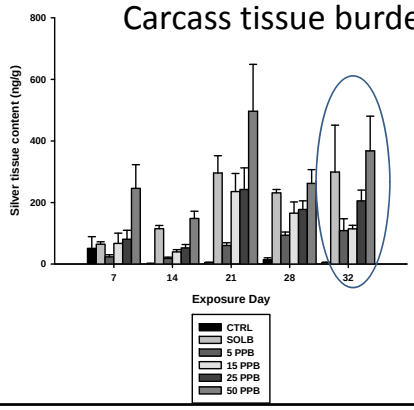
- QSI nanosilver (25nm primary particles)
- Stable suspension prepared by centrifugation
- 28d flow through exposures with 6 groups
  - 4 concentrations of nanosilver
    - 5 ug/L, 15 ug/L, 25 ug/L 50 ug/L
  - 1 Ag^{+} exposure (5 ug/L)
  - Control
- Sampled at Day 7, 14, 21, 28, 32



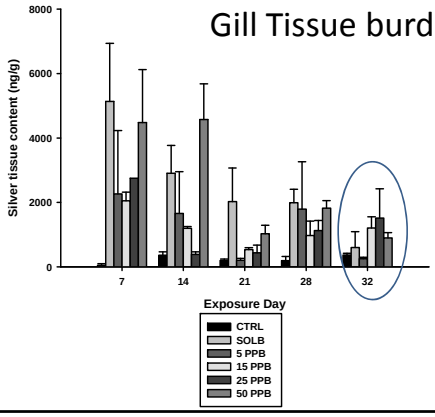
Particle Dissolution



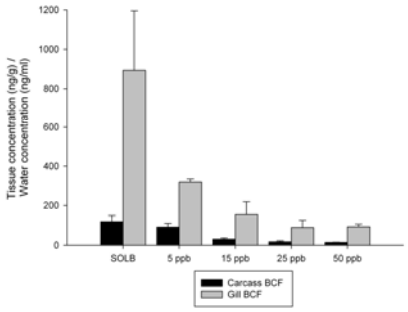
Carcass tissue burden



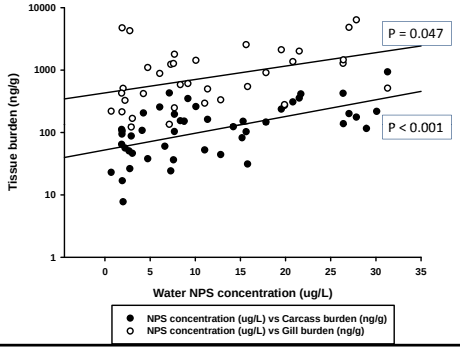
Gill Tissue burden



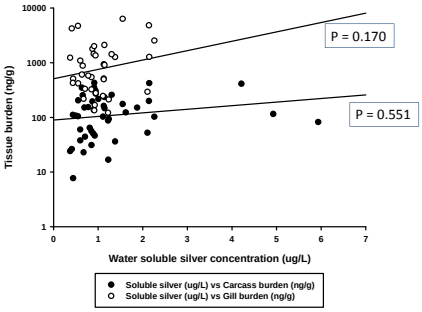
Silver tissue accumulation



Particulate silver and tissue burden



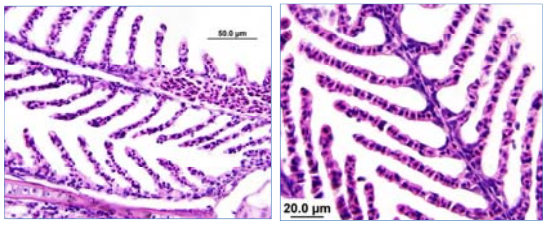
Soluble silver and tissue burden



Gill morphology

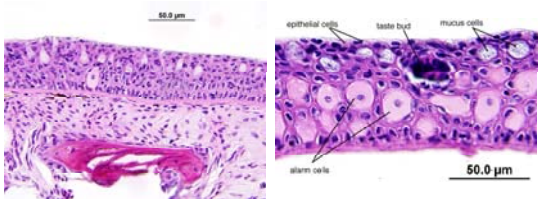
Control Gill – 28 days

50 ppb gill – 28 days



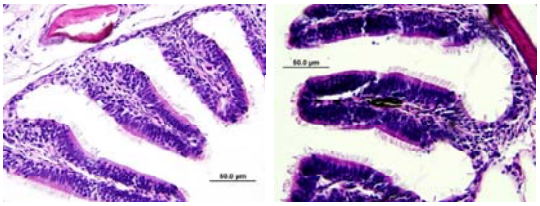
Skin morphology

- Control Gill – 28 days
- 50 ppb gill – 28 days

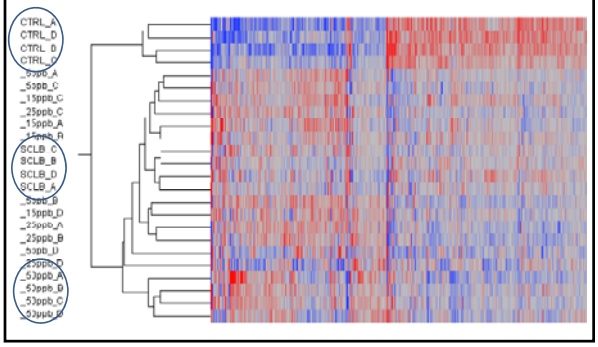


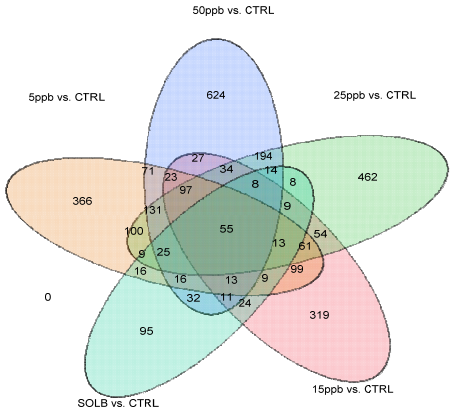
Nasal epithelium

- Control Gill – 28 days
- 50 ppb gill – 28 days



Transcriptional effects in gill at 28 days





GO analysis

GO TERM	Adjusted p-value
pseudouridine synthesis	0.0402
RNA modification	0.04442
skeletal system development	0.0402
ribosome biogenesis	0.00003509
rRNA processing	0.001303
RNA processing	0.006019
embryonic organ morphogenesis	0.04442
embryonic organ development	0.001303
DNA-dependent DNA replication	0.0402

Conclusions

- Zebrafish accumulate significant silver tissue burdens
  - Gill levels 10X higher than carcass levels
  - Remains for up to 4 days in the absence of AgNP
- Significant correlation between AgNP concentration and tissue burden
  - Not significant for soluble silver
- No observable effect on epithelial morphology
- Microarray data indicates significant alterations in gene expression patterns
  - Dose response pattern on number of genes affected.
  - GO analysis indicates two pathways
    - Organ development
    - Ribosome biogenesis

Acknowledgements

- Nancy Brown-Peterson, Idrissa Boube, Steve Manning (USM)
- April Feswick, Cody Smith (UF)
- National Science Foundation (BES 0540920)

Robert Tanguay

Refinements to the Use of Zebrafish for Nanomaterial-Biological Interaction Assessments

Lisa Truong[1,2], Tatiana Zaikova[2,3], Jim Hutchison[2,3], and Robert Tanguay[1,2]
[1]Department of Environmental and Molecular Toxicology, Environmental Health Sciences Center, Oregon State University, Corvallis, OR; [2]The Oregon Nanoscience and Microtechnologies Institute and the Safer Nanomaterials and Nanomanufacturing Initiative, Corvallis, OR; [3]Department of Chemistry, University of Oregon, Eugene, OR

With the increased usage and introduction of nanoparticles into industrial and consumer products, the concern about environment and health impacts remains unclear. It is largely unknown what environmental conditions and/or physico-chemical property influence how a nanoparticle will behave in complex environments. Often, under standard aqueous exposure conditions, nanoparticles precipitate and/or agglomerate complicating hazard identification. Ionic concentrations in aqueous media have a major influence on NP agglomeration rates and as a result, synthesis and storage of nanoparticles are often in ion-free water. This is problematic, however, for biological systems requiring buffering ions. Necessary suspension of nanoparticles in media appropriate for zebrafish embryo toxicity testing presents an assay development challenge.

Our group has developed rapid methods to assess nanomaterial responses in embryonic zebrafish. To date, we have assessed several classes of nanomaterials, including carbon nanotubes, fullerenes, silver and gold nanoparticles. Zebrafish offer inherent advantages, including rapid external development, optical clarity, genetic similarity to humans, and minimal material requirement (~1 mg). Additionally, zebrafish are native to brackish water. Our focus was to define the minimum ion concentration necessary to support normal embryonic development. We reasoned that if zebrafish could develop normally in low ionic concentration, more classes of NPs could be assessed with this rapid *in vivo* model because of reduced NP agglomeration during the exposure period. To determine the lowest tolerable salinity level ,embryos were developed in to 0, 0.16, 0.8, 4, 20, and 100 % zebrafish medium. Embryos in all groups developed normally when assessed at 120 hpf. The embryos were enzymatically removed from their chorions at 4 hours post fertilization (hpf). We anticipated that de-chorionated embryos would be more sensitive to developmental malformations. This was not the case. To determine if more subtle changes occurred, we also assessed larval behavioral. Exposure to both zero and 100% zebrafish medium did not induce statistically different behavior, reinforcing the morphological finding that plain RO water supports normal zebrafish development.

A standard protocol was used to characterize nanoparticle agglomeration under the different ionic concentrations. We selected a gold nanoparticle (AuNP) highly predisposed to agglomeration in 100% embryo medium and used UV-Vis spectroscopy to characterize the percentage of either a 10 or 50 µg/mL AuNP concentration remaining in 0, 0.16, 0.8, 4, 20, and 100 % medium over time. We found that at concentrations ≥ 4% zebrafish medium, both the 10 and 50 µg/mL AuNP precipitated almost immediately. At < 4% embryo medium, more than 80 percent of the nanoparticles remained in solution and monodispersed, confirming the ionic effect on agglomeration. Increasing ion concentration, and the resultant agglomeration, predictably affected AuNP toxicity. In the higher (4 – 100%) ionic strength medium, embryos exposed to 0.08, 0.4, 2, 10, or 50 µg/mL did not exhibit behavioral or morphological aberrations. In the lower ionic strength medium, embryos exhibited both developmental and behavioral responses to the range of AuNP concentrations. Our findings of normal zebrafish development in RO water and reduced toxicity of AuNPs in higher ionic strength medium have substantially refined the exposure conditions for more accurate nano-toxicology in the zebrafish.

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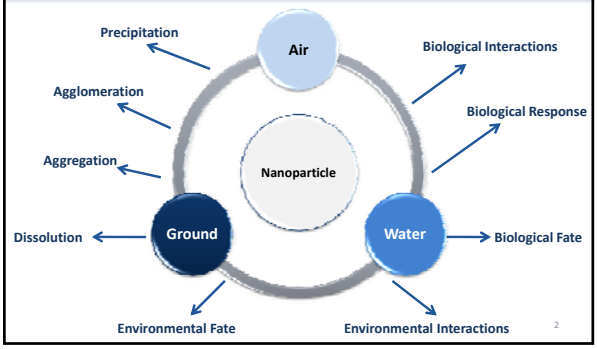
Refinements to the Use of Zebrafish for Nanomaterial-Biological Interaction Assessments

Lisa Truong[1,2], Tatiana Zaikova[2,3], Jim Hutchison[2,3], Robert Tanguay[1,2]

[1]Department of Environmental and Molecular Toxicology, Environmental Health Sciences Center, Oregon State University, Corvallis, OR, [2]The Oregon Nanoscience and Microtechnologies Institute and the Safer Nanomaterials and Nanomanufacturing Initiative, [3]Department of Chemistry, University of Oregon, Eugene, Oregon

EPA Nanotechnology Grantee Meeting – Nov 7-8, 2010

Physico-Chemical Properties Influence "Behavior" of Nanoparticles



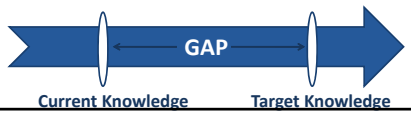
2

Knowledge Gap

Interaction of nanoparticles with environment and biological systems remains largely unknown

Missing toxicological data to understand biocompatibility

Identify risk associated with nanoparticle exposure



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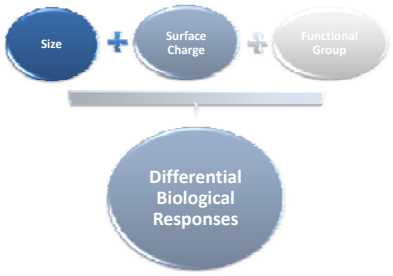
Research Goal

To determine what influence each NP parameter has on biological activity

4

Hypothesis



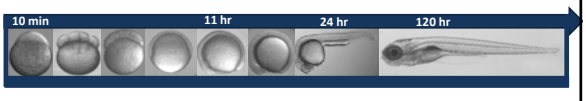
5

The Zebrafish Model

Vertebrates share many cellular, anatomical and physiological characteristics with humans

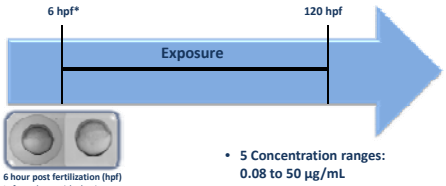
Early development is the period most well-conserved between species

Embryos are clear, which allows for non-invasive assessments over the course of development



6

High Throughput Screening
Experimental Design



6 hour post fertilization (hpf)
Left: embryo with chorion
Right: dechorionated embryo

- 5 Concentration ranges: 0.08 to 50 µg/mL
- 100 µL NP solution per well
- 1 embryo per well

* hours post fertilization

7

High Throughput Screening
Results

Assessed and evaluated over 200 nanoparticles

A large portion did not induce a biological response

Are there false negatives?

8

Assessment of NP Aggregation in Aqueous Media

NP properties change depending on aqueous environment/condition

Aggregation can occur in high ionic strength media

Biological response can be altered

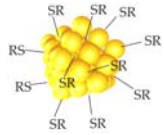
Necessary to characterize aggregation in test media and over-exposure period

9

Gold Nanoparticles (AuNPs)

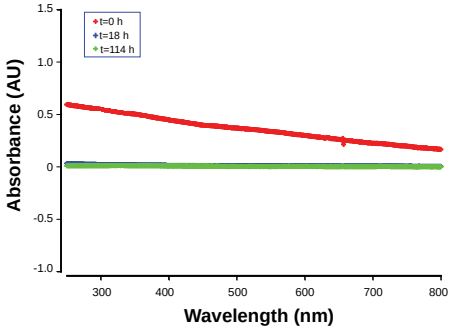
A diverse family of functionalized AuNPs has been prepared for 0.8-nm, 1.5-nm and 10-nm core sizes

$-(CH_2)_2SO_3^-Na^+$
$-(CH_2)_3SO_3^-Na^+$
$-(CH_2)_2N^+HMe_2Cl^-$
$-(CH_2)_2N^+Me_3Cl^-$
$-(CH_2)_{17}CH_3$
$-(CH_2)_{15}CH_3$
$-(CH_2)_{11}CH_3$
$-(CH_2)_9CH_3$
$-(CH_2)_8CH_3$
$-(CH_2)_7CH_3$
$-(CH_2)_5CH_3$
$-(CH_2)_2CH_3$
$-(CH_2)_2Si(OMe)_3$
$-CH_2COO^-Na^+$
$-(CH_2)_2COOH$
$-(CH_2)_{11}COOH$
$-(CH_2)_2OH$
$-(CH_2)_2PO(OH)_2$
$-[(CH_2)_2O]_3(CH_2)_2OH$
$-(CH_2)_2O(CH_2)_2OH$
$-[(CH_2)_2O]_2CH_2COOH$
-(CH2)2COGlyGlyOH
$-(CH_2)_2CONH(CH_2)_{14}CH_3$
$-(CH_2)_2O(CH_2)_2N^+Me_3^-OTs$
$-(CH_2)_2O(CH_2)_2O(CH_2)_2\ N^+Me_3\ OTs$
$-(CH_2)_2O(CH_2)_2O(CH_2)_2\ N^+Et_3\ OTs$



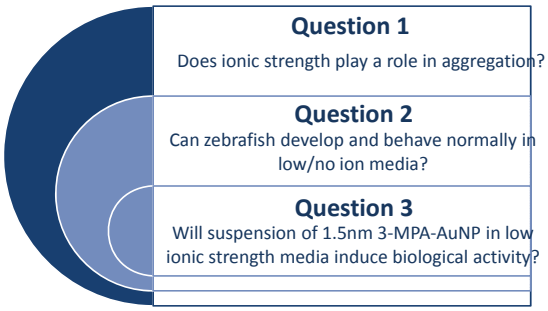
10

Characterization of 1.5nm 3-MPA in Test Media



11

Research Questions



Question 1
Does ionic strength play a role in aggregation?

Question 2
Can zebrafish develop and behave normally in low/no ion media?

Question 3
Will suspension of 1.5nm 3-MPA-AuNP in low ionic strength media induce biological activity?

12

Research Question 1

Specific Aim 1
Does ionic strength play a role in aggregation?

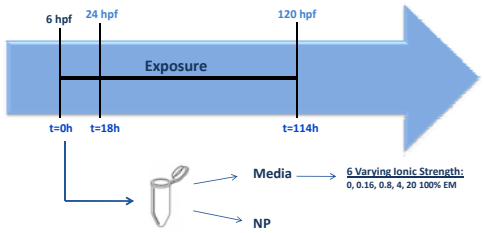
Specific Aim 2
Can zebrafish develop and behave normally in low/no ion media?

Specific Aim 3
Will suspension of 1.5nm 3-MPA-AuNP in low ionic strength media induce biological activity?

13

Q1: Does ionic strength play a role in aggregation?
Experimental Design

Size analysis using UV-Vis Spectroscopy



14

Q1: Does ionic strength play a role in aggregation?
Results

Percentage of Embryo Media (%)	10 (µg/mL)		50 (µg/mL)	
	18 hr	114 hr	18 hr	114 hr
100	5.4%	4.8%	3.4%	1.8%
20	85.4%	14.5%	24.9%	3.0%
4	78.5%	63.2%	90.4%	88.1%
0.8	98.6%	94.2%	95.8%	94.4%
0.16	94.9%	88.83%	93.5%	93.3%
0	98.5%	81.2%	96.7%	91.8%

15

Conclusions

High ionic strength media causes 1.5nm 3-MPA-AuNP aggregation

16

Research Question 2

Specific Aim 1
Does ionic strength play a role in aggregation?

Specific Aim 2
Can zebrafish develop and behave normally in low/no ion media?

Specific Aim 3
Will suspension of 1.5nm 3-MPA-AuNP in low ionic strength media induce biological activity?

17

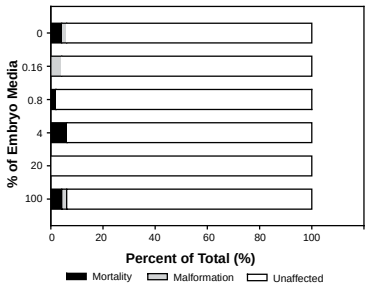
Q2: What Ion Concentration is Necessary?
Experimental Design

- 6 Ionic Concentration Media
  0, 0.16, 0.8, 4, 20, 100% EM
- 100 µL solution per well
- 1 embryo per well

- High Throughput Evaluation
- Behavioral Analysis

18

Q2: What Ion Concentration is Necessary?
Results



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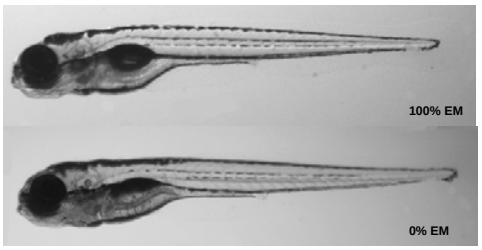
Q2: What Ion Concentration is Necessary?
Results



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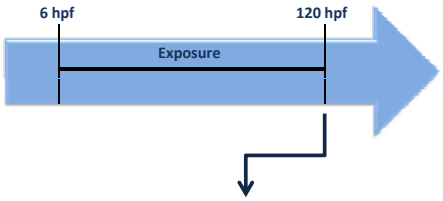
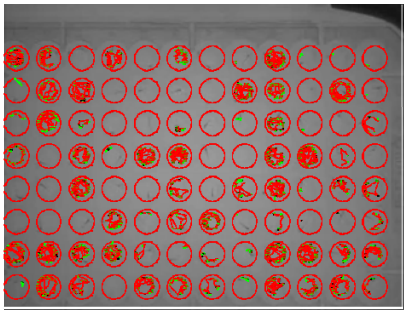
Q2: What Ion Concentration is Necessary?
Experimental Design



- High Throughput Evaluation
- Behavioral Analysis

21

Q2: What Ion Concentration is Necessary?
Experimental Design - Behavior



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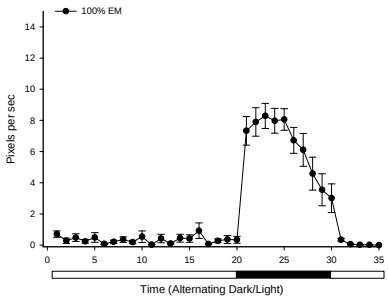
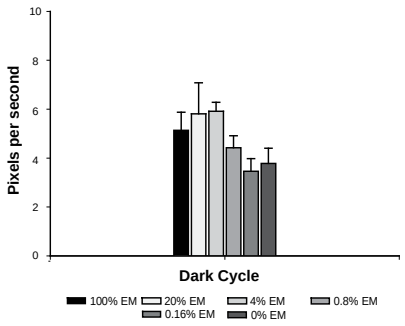
Q2: What Ion Concentration is Necessary?
Experimental Design - Behavior



23

Q2: What Ion Concentration is Necessary?
Results



24

Conclusions

High ionic strength media causes 1.5nm 3-MPA-AuNP aggregation

Zebrafish develop normally in low ionic media

25

Research Question 3

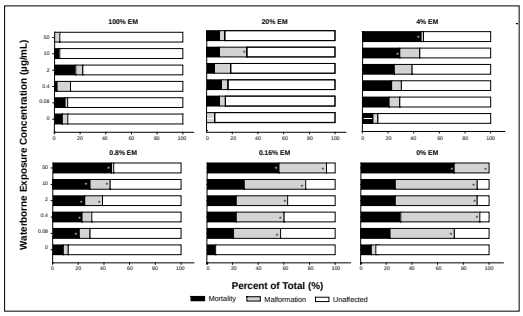
Specific Aim 1
Does ionic strength play a role in aggregation?

Specific Aim 2
Can zebrafish develop and behave normally in low/no ion media?

Specific Aim 3
Will suspension of 1.5nm 3-MPA-AuNP in low ionic strength media induce biological activity?

26

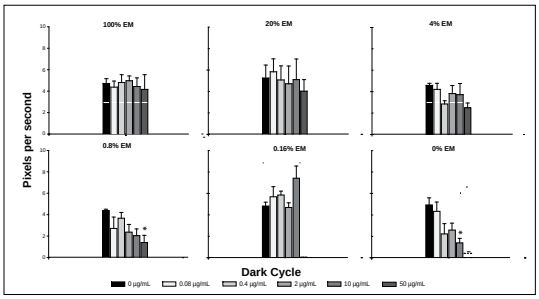
Q3: Biological Responses when NP is bioavailable?
Results



Data presented with * designate statistically significant values (One Way ANOVA – Dunnetts Post Hoc Test, p<0.05).

27

Q3: Biological Responses when NP is bioavailable?
Results



Data presented with * designate statistically significant values (One Way ANOVA – Dunnetts Post Hoc Test, p<0.05).

28

Conclusions

High ionic strength media causes 1.5nm 3-MPA-AuNP aggregation

Zebrafish develop normally in low ionic media

Low ionic strength media favors dispersion of 1.5nm 3-MPA-AuNP

1.5nm 3-MPA-AuNP are more toxic when dispersed

29

Implications

Every parameter must be taken into consideration when doing nanomaterial-biological interaction studies

Refinement of the current high throughput screening to include avoid false negatives and assess NPs deemed “problematic”

The zebrafish is a versatile model

30

Acknowledgements

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Dr. Tatiana Zaikova

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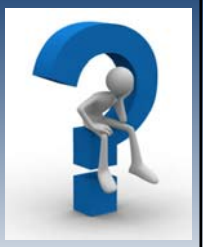
- Air Force Research Laboratory
  #FA8650-05-1-5041

ONAMI SNNI

31

QUESTIONS?



Devrah Arndt

Impacts of Functionalization of Fullerenes and Carbon Nanotubes on the Immune Response of Rainbow Trout

Rebecca Klaper[1], Devrah Arndt[1], Jian Chen[2], and Frederick Goetz[1]
[1]School of Freshwater Sciences, [2]Department of Chemistry,
University of Wisconsin-Milwaukee, Milwaukee, WI

The overall objective of this project is to assess the innate immune reaction of an aquatic model, the rainbow trout, to manufactured nanomaterials of varying chemistries at levels not inducing cellular toxicity. This research will create a mechanism with which to test other nanomaterials, provide data to support ecological risk assessments, and ultimately inform decisions as to which materials will be the safest to industrialize and use with respect to aquatic environments.

We investigated how structure and type of functionalization of manufactured nanomaterials could impact the immune response of the aquatic model species *Onchorycus mykiss* (rainbow trout). We examined cell viability as well as gene expression of genes associated with a pro-inflammatory or antiviral response in a well-studied trout macrophage primary cell culture system. There was a significant difference among different carbon nanotube-based nanomaterials in their level of pro-inflammatory gene expression behavior in macrophage cells and the dose at which they became stimulatory. All concentrations tested were sublethal to cells, yet almost all nanomaterials were stimulatory at some concentration. Functionalization to create water soluble particles caused a variable effect. Each nanotube type caused a dose-dependent response with the lowest exposures (0.05 to 1.0 μg/mL) having no stimulatory response and at the highest concentrations (5 μg/mL and 10 μg/mL) stimulating a response similar to the positive LPS positive control. Anionic functionalized multi-walled nanotubes and zwitterionic single-walled nanotubes were stimulatory at the lowest dose (0.5 μg/mL. Surfactants often used to suspend nanomaterials also were as stimulatory to the immune cells as the nanomaterials.

For fullerene-based particles, almost all nanomaterials we have tested caused an increase in candidate proinflammatory genes that is equivalent to stimulation of positive controls at the highest concentration of 10 μg/mL but as we decreased the concentration to 1.0 or 0.1 ug/mL, we began to see differences in inflammatory responses in a dose-dependent fashion. This would indicate that the dose that ultimately enters the organism will be extremely important to determine potential immune responses. Our data indicate that chemicals used for functionalization also may stimulate the immune response and that this response is equivalent to the nanoparticle alone.

We are now focusing on a broader suite of genes to monitor for each compound at these lower doses. These include individual genes known to be important for immune function as well as others that have been identified through microarray experiments.

This study is the first report of the effects of nanomaterials on the function of the immune system in a nonmammalian vertebrate. Because the innate immune system is the first to respond to the intrusion of foreign material, analysis of the effects of nanomaterials on cells of the innate immune system should provide valuable information on how these materials are perceived and affect an animal. Ultimately, such research will provide the means to determine which nanomaterials are most harmful to aquatic species and how particles may be altered or functionalized to decrease their toxicity.

References:

Klaper R, Arndt D, Setyowati K, Chen J, Goetz F. Structure and functionalization impacts the effects of carbon nanotubes on the immune system of an aquatic vertebrate model. *Aquatic Toxicology* 2010;100(2):211-7. Epub 2010 Jul 27.

Klaper R, Crago J, Arndt D, Goetz R, Chen J. Impact of nanomaterial structure and composition on the ecotoxicology of nanomaterials on aquatic species. Proceedings of the International Environmental Nanotechnology Conference-Applications and Implications, U.S. EPA, Chicago, IL, October 2008.

Klaper R. Ecological effects of nanomaterials: impacts from genomic to immune system in *Daphnia* and trout. NanoECO Meeting, March 3-8, 2008, Ascona, Switzerland.

Klaper R, Chen J, Goetz F. The cellular and gene expression effects of manufactured nanoparticles on primary cell cultures of rainbow trout macrophages. SETAC, November 11-15, 2007, Milwaukee, WI.

EPA Grant Number: R833319

The cellular and gene expression effects of manufactured nanoparticles on primary cell cultures of rainbow trout macrophages

Rebecca Klaper
Devrah Arndt, Frederick W. Goetz
Jian Chen

Great Lakes WATER Institute &
Department of Chemistry
University of Wisconsin-Milwaukee
Milwaukee, Wisconsin

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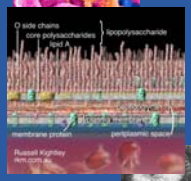
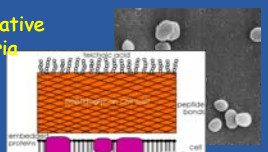
Grant # RD833319

1

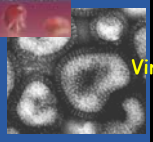
One Way Immune System Recognizes something as Foreign : Pathogen Associated Molecular Patterns (PAMPs)

Gram negative bacteria





Gram positive bacteria



Viruses

2

Hypotheses:

- May act as PAMPs

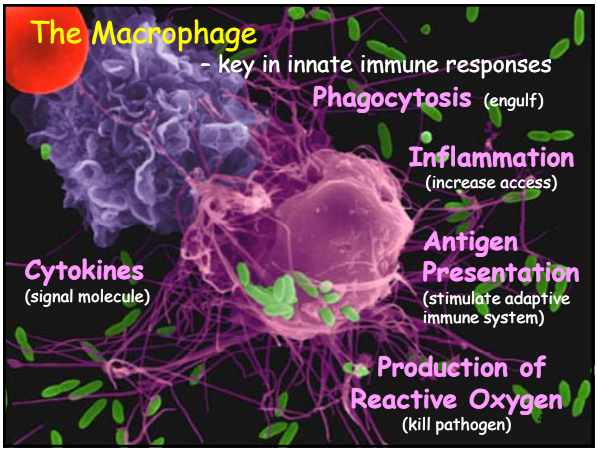
1)Nanoparticles should be considered foreign and will stimulate the immune system

2) Core structure will impact ability to stimulate immune system

3) Functionlization will impact ability to stimulate immune system

4) Nanomaterials will cause unique gene expression patterns that differ both from each other and from traditional stimulants (bacteria and viruses)

3



Overall Experimental Strategy

1. Produce nanoparticles of different types (fullerenes and tubes) and with different side groups (functionalizations, anionic, cationic)

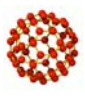
2. Test the nanoparticles directly on macrophages in culture

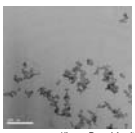
- Cell viability
- Gene expression
  - candidate genes
  - microarrays

5

Nanoparticle Types

C60-X
ZP=-42.2 mV
Z ave=103.7 nm





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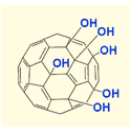
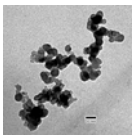
C60-OH(24)
ZP=-54.2 mV
Z ave=171.1 nm



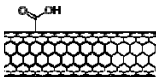


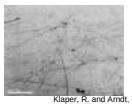
Klaper, R. and Arndt, D.

6

Nanoparticle Types

SWNT-COOH
ZP= -61.1 mV
Z ave=227.5 nm





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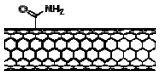
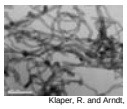
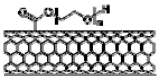
SWNT-CONH2
ZP= -52.4 mV
Z ave=177.1 nm

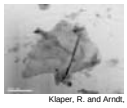




Klaper, R. and Arndt, D.

SWNT-PEG
ZP= -58.1 mV
Z ave=452.4 nm





Klaper, R. and Arndt, D.

7

Differentiation of monocytes to macrophages in vitro

Time in culture

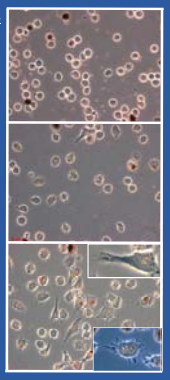
monocytes

24 h

72 h

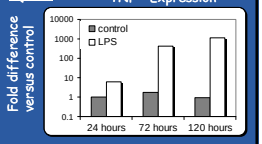
120 h

macrophages

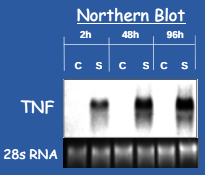


QPCR

TNF - Expression



Northern Blot



S = LPS (lipopolysaccharide)
C = control (no LPS)

8

Experimental Platform Trout Macrophage

1. Head kidneys isolated

2. Minced through 200 um mesh in DMEM (high glucose) + 10% FCS, glutamine, penicillin/streptomycin

3. Homogenate plated at 10 million cells/ml on poly D - lysine plates

4. Washed at 24 hrs, new medium added & incubated for 4 days

5. At 5 days total, remove old medium & stimulated

McKenzie, S., Planas, J. and F.W. Goetz. (2003). LPS-stimulated expression of a tumor necrosis factor-like mRNA in primary monocytes and *in vitro* differentiated macrophages. Developmental and Comparative Immunology 27:393-400.

9

Specific Experimental Scheme

1. Plate trout macrophages - incubate 5 days

Gene effects

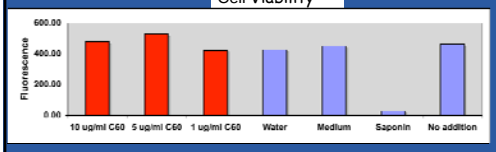
Cell viability

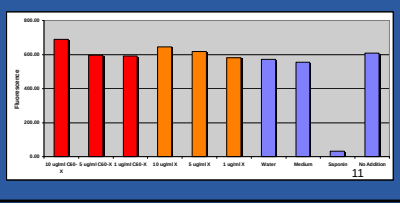
2. Remove medium and add nanoparticles
3. Incubate for 24 hours (proinflammatory) or 6 hours (proviral)
4. **Cell Viability**: Add QBlue Reagent and measure fluorescence
5. **Gene Effects**: Remove medium, add 1.0 ml Trizol, extract for RNA, prepare cDNA and QPCR for IL-1β, TNFα (proinflammatory) or IFNα, IP-10 (proviral)

10

Cell viability does not decline with nanomaterial exposures when not suspended with surfactants

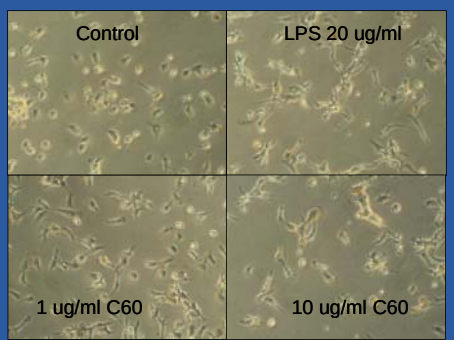
Cell Viability





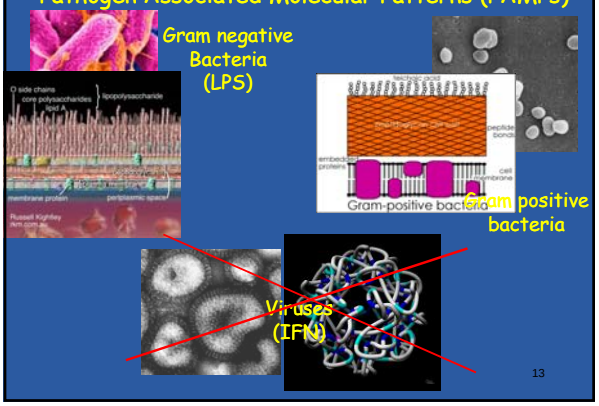
11

Cells following 24 hr nanomaterial stimulation: phagocytosis with nanomaterial exposures



12

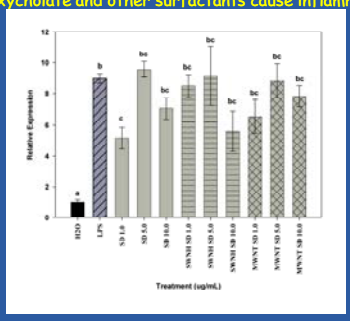
Pathogen Associated Molecular Patterns (PAMPs)



13

Expression of IL1B with nanoparticle exposure:

Sodium deoxycholate and other surfactants cause inflammatory response

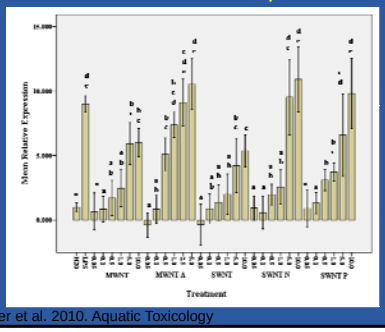


Klaper et al. 2010. Aquatic Toxicology

14

Expression of IL1B with nanoparticle exposure:

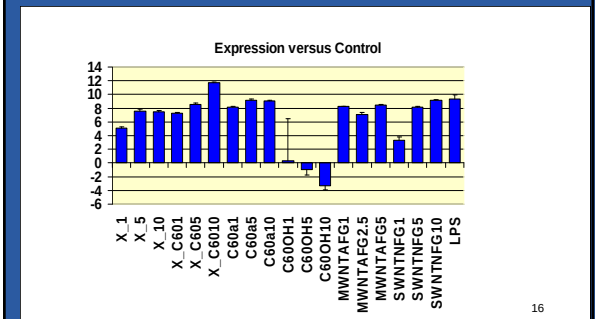
Single walled nanotubes inflammatory at lower concentrations
Functionalization increased reactivity



Klaper et al. 2010. Aquatic Toxicology

15

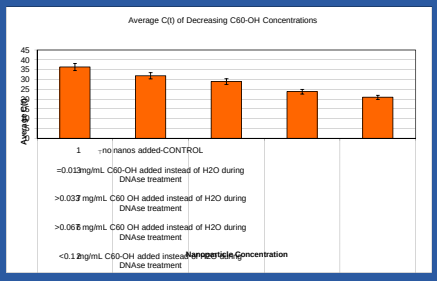
Expression of IL1B with nanoparticle exposure



16

Expression of Actin with nanoparticle exposure:

Adding C60-OH to RNA prior to cDNA synthesis = decrease in Actin expression measured

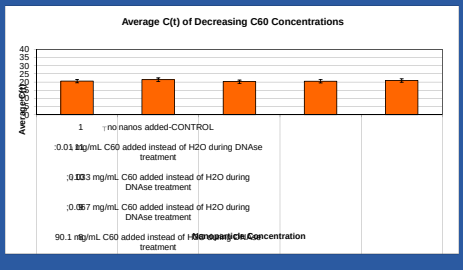


17

Klaper et al. in prep

Expression of Actin with nanoparticle exposure:

Adding C60 to RNA prior to cDNA synthesis = no impact on qPCR

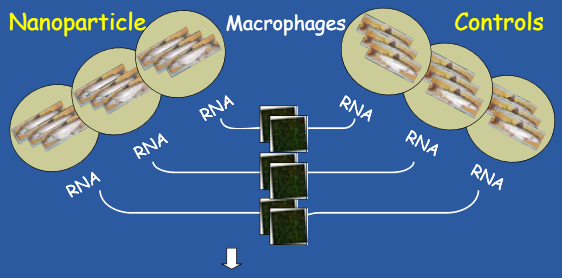


18

Klaper et al. in prep

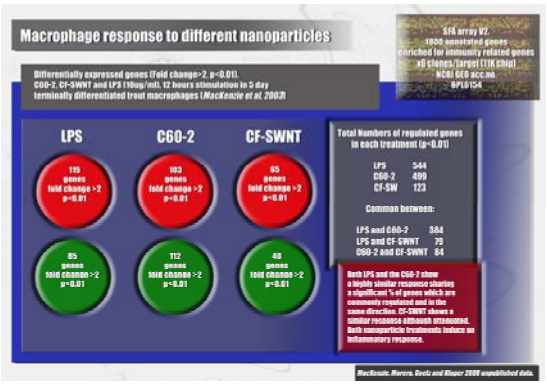
Comparative transcriptomics with microarrays

Trout immune/metabolic targeted array 1800 genes - spotted X 6 for each gene



Mean for 3 replicates ➡ Compare to database

Macrophage response to different nanoparticles



Macrophage response to different nanoparticles

Top 20 up-regulated genes common to all treatments (p<0.01). LPS treatment is the standard.
C60-2, CF-SWNT and LPS (10ug/ml), 12 hours stimulation in 5 day
terminally differentiated trout macrophages (MacKenzie et al. 2007)

Inflammatory Phenotype highlighted by induction of chemokines, ECM proteases, NFkBPCBPbeta profiles.

C60-2 (fold change)	CF-SWNT (fold change)	LPS (fold change)	Match Name
129.1	9.9	107.3	Dokuowa-227
92.0	20.3	36.0	TNF decoy receptor
35.8	10.5	94.7	CCAAT/enhancer binding protein beta
35.8	5.1	31.7	Leukocyte cell-derived chemotaxin 2
67.6	14.5	91.9	6014
35.1	92.6	20.1	Matrix metalloproteinase-9
13.3	5.8	11.0	NF-kappaB inhibitor alpha-1
19.3	13.5	10.9	Matrix metalloproteinase 9-1
7.3	8.7	10.4	Lysozyme C precursor
7.5	23.7	8.8	Galectin like 2
8.1	6.9	7.4	Transcription factor jun-B-1
6.7	6.8	7.1	Stomatin
6.8	7.9	7.0	Tyrosine-protein kinase HCK
5.9	14.3	7.0	Thioredoxin
6.3	10.6	6.7	Secretory granule proteoglycan core protein
9.4	5.5	6.7	Myristoylated alanine-rich protein kinase C substrate
9.1	8.6	6.3	Microsomal glutathione S-transferase 3
3.0	3.6	6.0	Glutathione peroxidase-gastrointestinal
4.0	4.5	5.1	Toll-interacting protein

MacKenzie, Morera, Goetz and Kloper 2008 unpublished data.

Conclusions

1. Trout macrophages are a sensitive tool to investigate the effects of nanoparticles (NP) on immune system

2. Nanomaterials are stimulatory of the immune system without complete cell toxicity

3. Level of stimulation depends on core structure AND surface chemistry of nanomaterials

4. Functionalization may increase toxicity

5. C60-OH may bind RNA and influence total gene expression in cells

6. Nanomaterials have unique gene expression signatures.

Acknowledgements

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Devrah Arndt - technical support
Kristen Setyowati

Amy Ringwood

Characterization of the Potential Toxicity of Metal Nanoparticles in Marine Ecosystems Using Oysters - Silver Nanoparticle Studies with Adults and Embryos

***Amy Ringwood*[1], *Melissa McCarthy*[1], *Nicole Levi-Polychenko*[2], *and David Carroll*[3]**
[1]University of North Carolina, Charlotte, NC; [2]Department of Plastic and Reconstructive Surgery, Wake Forest University Health Sciences, Winston-Salem, NC; [3]Wake Forest University, Center for Nanotechnology and Molecular Materials, Winston-Salem, NC

The use of silver and other metal nanoparticles continue to be incorporated into numerous consumer products. Metal nanoparticles may be introduced into aquatic environments during production processes and also as a result of release following their use in electronic and biological applications. The purpose of these ongoing studies is to characterize the toxicity of various metal nanoparticle preparations on oysters, *Crassostrea virginica*, a common estuarine species. Filter-feeding bivalve mollusks such as oysters spend their lives removing particles so they are very valuable as model species for characterizing nanoparticle bioavailability and interactions with basic cellular processes. Moreover, the adults release their gametes into the environment, so their embryos are also likely targets of nanoparticles. Therefore, the effects on lysosomal integrity, antioxidants, and oxidative damage, as well as the responses of different life history stages, are being investigated.

Adult oysters and newly fertilized oyster embryos were exposed to different preparations of silver (Ag) nanoparticles and dissolved Ag ($AgNO_3$) for 48 hours. For one set of studies, silver nanoparticle spheres and prisms were prepared with PVP; for another set of studies, silver nanoparticle spheres, prisms, and hexagonal plates were prepared with citrate. Gill and hepatopancreas tissues of adult oysters (both whole animal and isolated tissue exposures) were used to evaluate lysosomal destabilization, lipid peroxidation, and cellular antioxidant and detoxification responses (e.g., glutathione, catalase, superoxide dismutase, and metallothionein gene expression). Some studies with isolated hepatopancreas tissues also were conducted using an intracellular fluorescent probe to visually evaluate the production of reactive oxygen species (ROS) by microscopy. For the embryo studies, the percent normal development was determined. The intracellular fluorescent probe also was used to visually evaluate the production of ROS in the oyster larvae. The results of the lysosomal destabilization and lipid peroxidation assays with the adult oysters indicated differential toxicity with the different Ag nanoparticles. The prism preparations were consistently more toxic than either the spheres or plates. Based on the lipid peroxidation results, there was less toxicity with the PVP-coated particles. For the embryo studies, the prisms also were more toxic than the spheres or plates. Furthermore, the results of the fluorescent ROS studies with both oyster hepatopancreas cells and oyster larvae indicated higher levels of ROS in the prism exposed organisms.

This research program is designed to address a number of important issues regarding metal nanoparticle toxicity in marine organisms (e.g., nanoparticle characteristics associated with toxicity and adverse effects on fundamental cellular responses). These kinds of basic studies are essential for characterizing the potential risks and impacts of nanoengineered particles on estuarine and marine organisms.

EPA Grant Number: R833337

Characterization of the Potential Toxicity of Metal Nanoparticles in Marine Ecosystems using Oysters – Silver Nanoparticle Studies with Adults and Embryos

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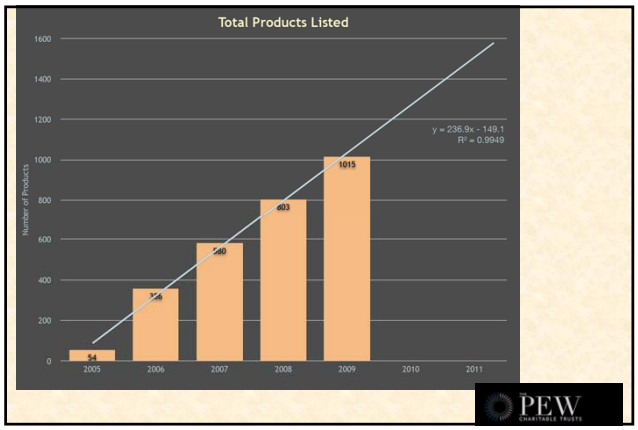
Lauren Marston, NC State University, Raleigh, NC

This research is funded by U.S. EPA-Science To Achieve Results (STAR) Program
Grant # RD833337

REU
NanoSure
UNCC

Nanoparticle Products



Oysters - *Crassostrea virginica*

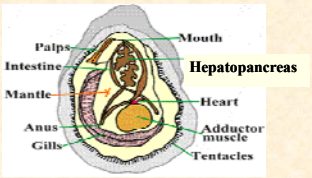
Filter Feeding Bivalves as Models

- Highly effective at removing particles
- High filtration rates
- Sample water column AND surface / resuspended sediments
- Extensive information regarding toxic responses to metals and organic contaminants

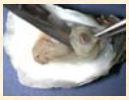
Oyster Nanoparticle Studies

- Adult Exposures
  - Lysosomal Destabilization
  - Lipid Peroxidation
  - Antioxidant Responses
  - Tissue / Cellular Accumulation
- Embryo Exposures
  - Normal Development
  - Antioxidant Responses

Oysters - *Crassostrea virginica*



Lysosomal Destabilization Assay



Dissect hepatopancreas

Incubate in neutral red for 1 hr and score > 50 cells

Incubate in $Ca^{++}Mg^{++}$-free saline, + trypsin on a shaker

Centrifuge gently thru 41 um nylon screen

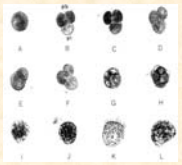
Oyster Hepatopancreas Cells

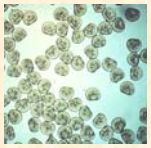
Stable Lysosomes

Destabilized Lysosomes

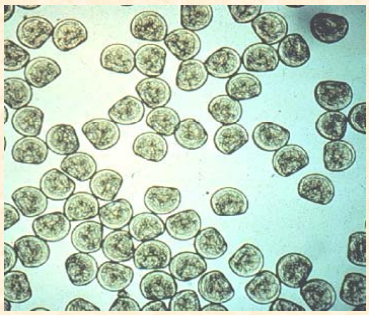
Oyster Nanoparticle Studies

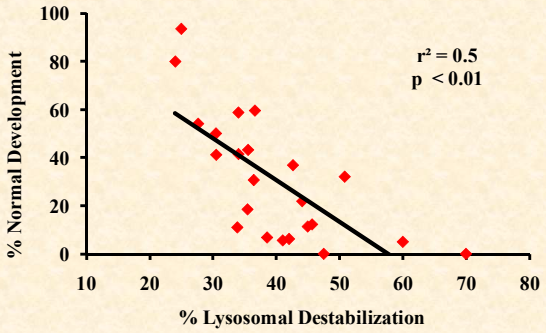
- Embryo Exposures
  - Normal Development
  - Metallothioneins





Oyster Veligers (48 hour)

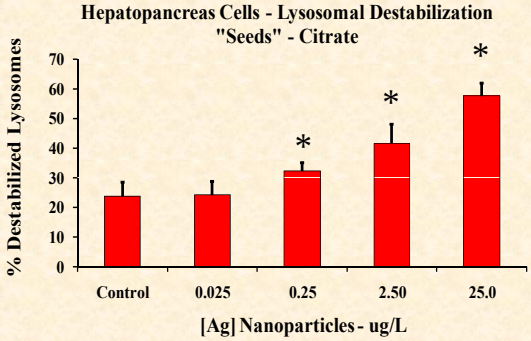


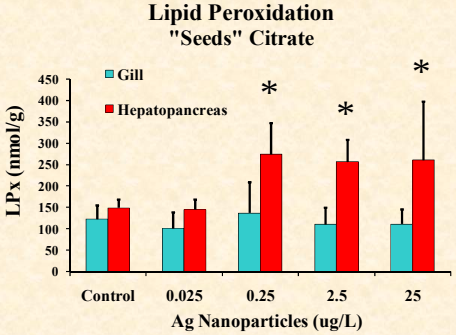


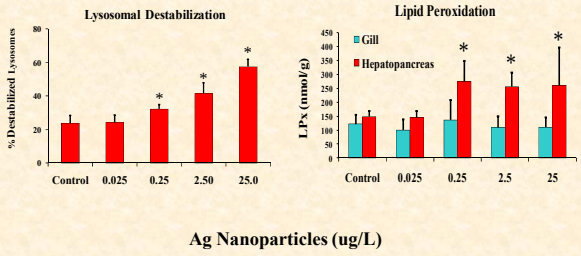
Ag Nanoparticle Studies

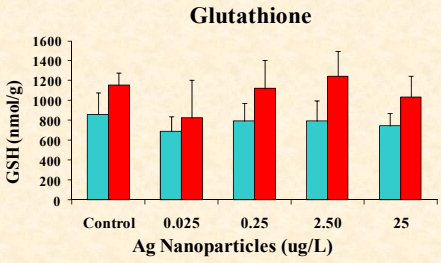
- "Seeds" – Citrate
- "Spheres" and "Prisms" – PVP (Polyvinylpyrrolidone)
- "Seeds", "Prisms", "Plates" - Citrate

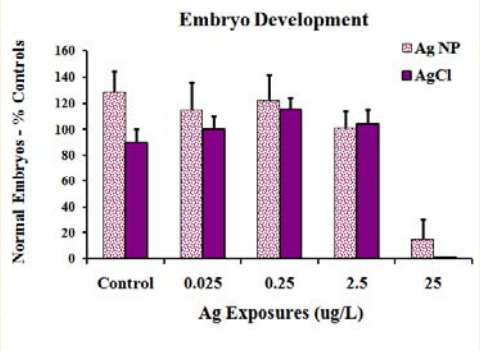
"Seeds" "Prisms" "Plates"









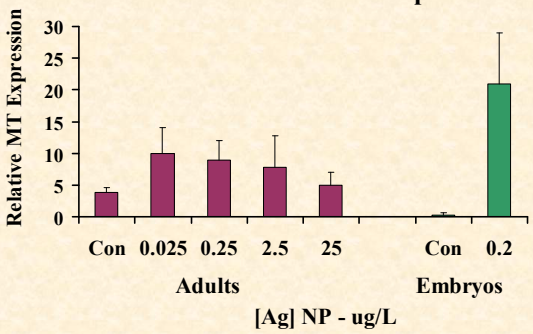


METALLOTHIONEINS (MT)

- Low molecular weight metal-binding proteins (6000 - 7000 D)
- High cysteine content (30%), (Cys - X - Cys)

MSDPCNCIETGTCACSDSCPATGCKCGPGCKCGDD-CKCAGCKVKCSCTSEGGCKCGEKCTGPATCKCGSGCSCKK

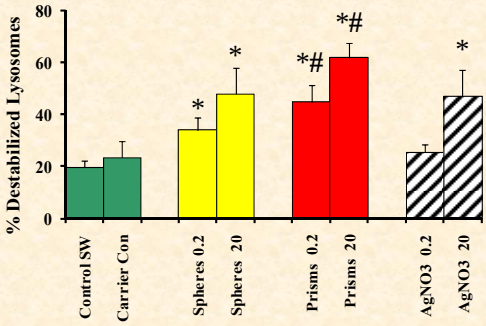
Metallothionein Gene Expression



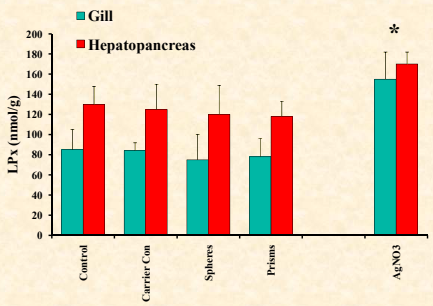
Ag Nanoparticle Studies

- "Seeds" – Citrate
- **"Spheres" and "Prisms" – PVP (Polyvinylpyrrolidone)**
- "Seeds", "Prisms", "Plates" - Citrate

Lysosomal Destabilization – Ag NPs (PVP)

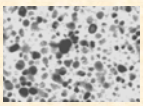


Ag NPs (PVP) (20 ug/L Ag)



Ag Nanoparticle Studies

- "Seeds" – Citrate
- "Spheres" and "Prisms" – PVP (Polyvinylpyrrolidone)
- **"Seeds", "Prisms", "Plates" - Citrate**

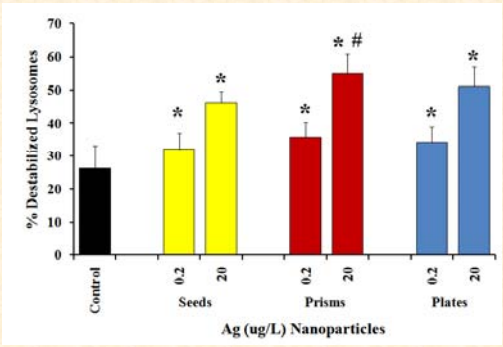


"Seeds"

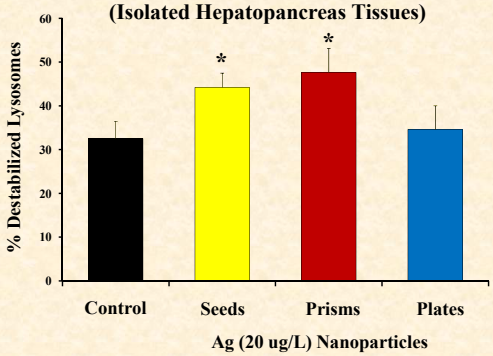
"Prisms"

"Plates"

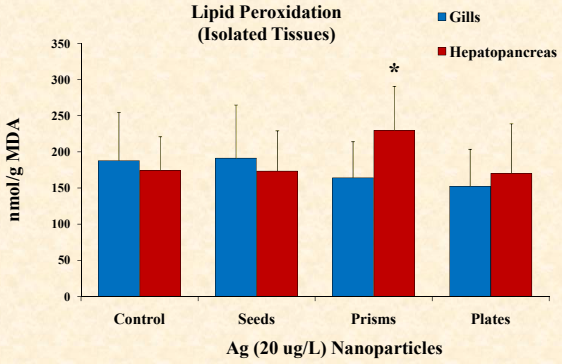
Lysosomal Destabilization – Ag NPs (Citrate)



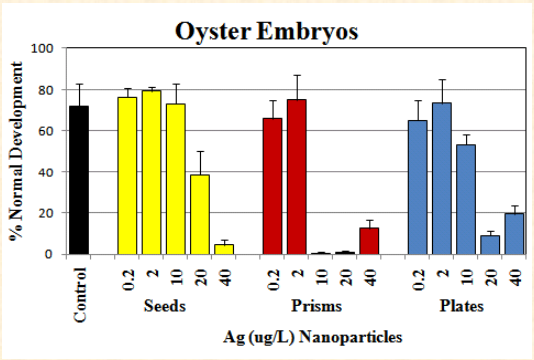
Lysosomal Destabilization (Isolated Hepatopancreas Tissues)



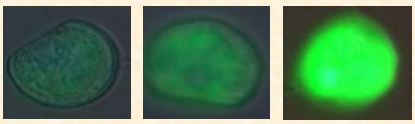
Lipid Peroxidation (Isolated Tissues)



Oyster Embryos



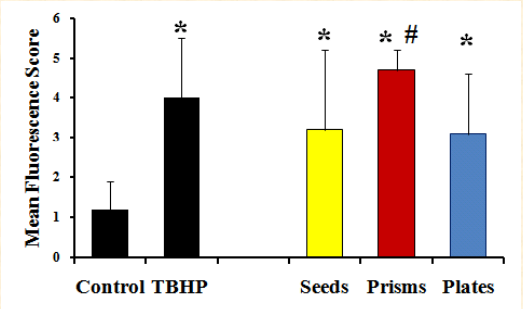
Embryo ROS Studies



1: Very low 3: Moderate 5: High

- Embryos (24 hr) were exposed to a 20 ppb concentration of AgNPs for 2 hr.
- Reactive oxygen species (ROS) production was assessed using a fluorescent probe (Carboxy H2DFDA, Molecular Probes)
- Embryo fluorescence was categorized as a 1, 3, or 5

Embryo ROS Studies



Summary

- Ag Nano "Prisms" were more toxic than "Spheres", and "Plates" in both adult and embryo oyster studies
- Mechanisms of toxicity associated with lysosomal dysfunction and oxidative stress.
- PVP coated particles may be slightly less toxic than citrate-based preparations.
- Oysters and other filter feeding bivalves are valuable model organisms for characterizing potential nanoparticle toxicity.

Edourd Manet

PM Session 2: Nanoparticles and Waste Treatment

Zhiqiang Hu

Bioavailability of Metallic Nanoparticles and Heavy Metals in Landfills

Yu Yang, Meng Xu, and Zhiqiang Hu
University of Missouri, Columbia, MO

Silver nanoparticles (AgNPs, nanosilver) released from the industry and consumer products will be likely disposed in landfills. The objectives of this research are to determine the bioavailability of nanoparticles and heavy metal species in bioreactor landfills as compared to traditional municipal solid waste landfills and to elucidate the mechanisms governing bioavailability as well as the mode of antimicrobial action by nanoparticles.

In this study, bioreactor landfill experiments were carried out to determine the impact of newly synthesized AgNPs (average particle sized = 21 nm) on the anaerobic/fermentation process in bench-scale bioreactor landfills. The solid waste taken from Columbia Sanitary Landfill (Columbia, MO) was exposed to AgNPs at the concentrations ranging from 1 to 10 ppm (mg/kg). The time course of cumulative biogas volume was recorded automatically, and the gas composition was determined by the gas tube method. At AgNPs concentrations of 1 ppm, there was no statistically significant difference of the cumulative gas volume or gas production rate between the nanosilver treated solid waste and the control. However, exposure of solid waste to nanosilver at a concentration of 10 ppm resulted in the reduced cumulative biogas volume ($p < 0.05$). Volatile fatty acid (VFA) accumulation and thereby consistently acidic condition (pH = 5.2) was observed in the leachate from the 10 ppm nanosilver treated bioreactor. The results suggest that AgNPs at low concentrations (1 ppm or below) have negligible impact on anaerobic waste decomposition and biogas production, but the concentration of nanosilver at 10 ppm result in reduced gas production and changes of methanogenic assemblages.

Quantitative PCR results demonstrated dominant methanogenic population shift from acetoclastic methanogens to hydrogenotrophic ones with nanosilver concentration. The bioreactor exposed to 10 ppm AgNPs had 40% acetoclastic methanogens in total, while the control bioreactor and the one treated with 1 ppm nanosilver had above 90% hydrogenotrophic methanogens, mainly *Methanobacteriales*. Total silver in the leachate decreased rapidly in 10 ppm nanosilver-treated bioreactor from 14.8 mg/L to below 2 mg/L. The concentrations of silver in leachates from the control and 1 ppm nanosilver treated bioreactor were approximately 2 mg/L.

Results of this project provide some of the first data on the bioavailability and risk assessment of metallic nanomaterials in solid waste disposal systems, especially under anaerobic conditions. Considering the potential release of nanomaterials in municipal landfills, the results of this project could help to better understand the transport, partitioning, and toxicity of nanoparticles to syntrophic anaerobic communities in municipal landfills.

EPA Grant Number: R833893

The Impact of Silver Nanoparticles on Anaerobic Processes in Bench-scale Bioreactor Landfills

Yu Yang, Meng Xu, Zhiqiang Hu
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University of Missouri
Columbia, MO 65211

Outline

- Introduction
- Materials and Methods
- Results and Discussion
- Summary

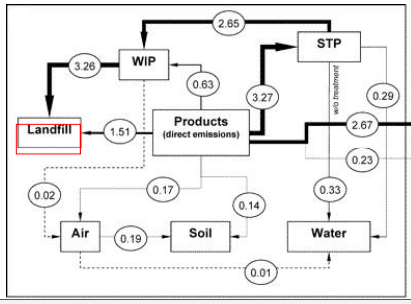
AgNPs as An Antimicrobial Agent

- Silver ions and silver nanoparticles AgNPs (nano silver): now both commonly used in consumer products.



- With a concentration factor of more than 100 in WWTP,the predicted silver concentrations in sludge is from 7 to 39 mg/kg (Blaser et al. 2008).
- In North America about 2200 Mg Ag/year were wasted through landfill, which was about 50% of the total wasted silver (Eckelman and Graedel 2007).

Fate of Nanosilver as Solid Wastes



Nanosilver flows from product to environments at high exposure scenario.
WIP; waste incineration plants, STP; sewage treatment plants. The number is a value in tons/year.
(Environ. Sci. Technol. 2008, *42*, (12), 4447–4453)

Silver Ion and Nanosilver Toxicity

- Ag^+
  - -Affected bacterial growth at 200 ppb (1.9 μM) under some pH.
  - -Reduced bacterial growth entirely at 2000 ppb (19 μM) *(Fabrega et al. 2009).*
  - -Interact with thiol groups of proteins, deactivate vital enzymes and inhibit DNA replication *(Klaine et al. 2008).*
- AgNPs
  - -Inhibited autotrophic bacterial growth by 86% at 1 mg/L *(Choi, 2008).*
  - -Highly toxic to zebrafish, daphnids and algal species with 48-h median lethal concentrations as 40 to 60 μg/L *(Griffitt et al. 2008).*
  - -Small size AgNPs (<10 nm) may enter the cells directly to release silver ions *(Morones et al. 2005).*

Sanitary Landfills

(i) Conventional Landfill:
- Storage/containment concept
- No recirculation
- Slowly and naturally degradation

(ii) Bioreactor Landfill:
- Leachate recirculation
- Increased degradation rate
- Improved the setting ability of solids, the recovery of landfill space
- Enhance the methane generation in the leachate

Major Biological Processes in Bioreactor Landfills

I. Hydrolysis

Degrade long chain polymers such as cellulose and hemicelluloses to simple organic molecules.

II. Acidogenesis/Acetogenesis

Amino Acids, long chain fatty acids and simple sugars are degraded during fermentation reactions, producing VFAs including acetic acids.

III. Methanogenesis

Convert primarily acetate and hydrogen plus carbon dioxide to methane.

- Hydrolysis and fermentation provide the substrate for methane generation.
- Methogenesis is very sensitive to reactor conditions
- Inhibition on methanogenesis may result from the interruption of hydrolysis and fermentation

Anaerobic Microorganisms: -Methanogens

- Methanogens: Important microorganisms for final biogas production; Good indicator of functional anaerobic bioreactor landfill.
- Acetoclastic Methanogens: *Methanosaeta* and *Methanosarcina* which convert acetate to methane and carbon dioxide
  $CH_3COOH \rightarrow CO_2 + CH_4$
- Hydrogenotrophic Methanogens: *Methanobacteriales*, *Methanococci* and *Methanomicrobiales*
  $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$

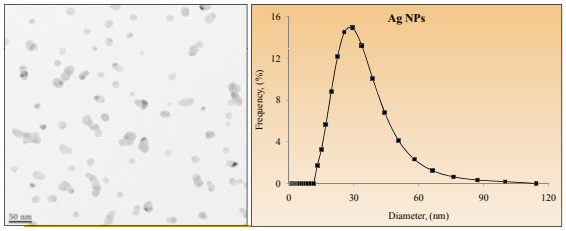
	Ks	μ (h^{-1})	Reference
Methanosaeta	30-40 mg/L	0.003	(Grady et al., 1999)
Methanosarcina	300 mg/L	0.014	
Methanococci	1.5 mM	0.009-0.03	(Demirel and Scherer 2008)
Methanomicrobiales	-	0.144	
Methanobacteriales	-	0.053-0.082	

Materials and Methods

AgNPs Fabrication and Characterization

➢**AgNPs:** AgNPs suspension (0.7 mM) were newly prepared using a chemical reduction method.

$2AgNO_3 + 2NaBH_4 \rightarrow 2Ag + H_2 + B_2H_6 + 2NaNO_3$



TEM

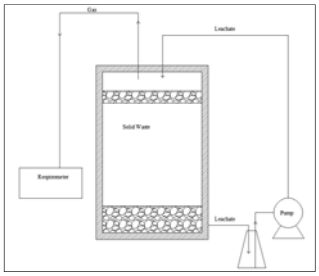
Dynamic Light Scattering: average, 29 nm

Bioreactor Landfill Experimental Design

➢ **Municipal Solid Waste (MSW):** Fresh MSW from the sanitary landfill site in Columbia, MO.

The solid composition (in weight): metal 0.9%, paper 14.1%, brick 17.7%, **wood and shredding** 4.7%, soil 36.7%, **organic waste** 15.6%, plastic bags 10.2%.

Bioreactor Setup



•Total Volume: 9 L

•2.9 kg MSW +1 L anaerobic sludge + Control/ 1ppm AgNPs/ 10 ppm AgNPs.

•Temperature, 37 °C. Recirculation rate, 5% of reactor volume.

Bench-scale Bioreactors

•The bioreactor landfills are operated with automatic biogas recording using Challenge resiprometer.

•The leachate collection bottles on the ground are not shown.

Sampling & Chemical/Microbial Analysis

➢ **Gas Production and Chemical Analysis:**

•Total volume of gas :AER-200 Respirometer
•Carbon dioxide : Gastec Tube 2HT (Gastec, Japan).
•20 mL leachate withdrawn every two weeks
(add 20 mL DI water back after sampling)
-pH, COD and NH_4^+, NO_2^- and NO_3^-
-Total silver : ICP-AES
-Volatile fatty acids (VFAs) (HPLC)

➢**DNA extraction and real time PCR**

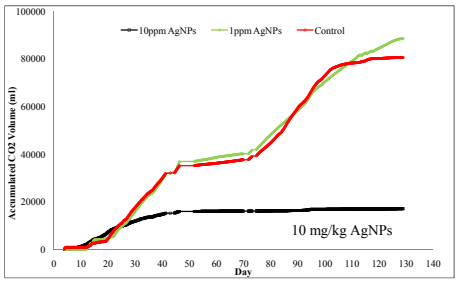
•The extracted DNA samples from leachates were stored at -20°C before use.
•Real time PCR assays were performed using ABI 7500 Real Time PCR System for methanogens.

Primers and Probes for qPCR

	Primer and Probe	Method	Reference
Methanosaeta	MS1b 585F (5'-CCGGCCGGATAAGTCTCTT GA-3')	SYBR Green	(Conklin, Stensel et al. 2006)
	Sae 835R (5'-GACAACGGTCGCACCGTGGCC-3')		
Methanosarcina	Mb1b 586F (5'-CGGTTTGGTCAGTC CTCCGG-3')		
	Sar 835R (5'-AGACACGGTCGCGCCATGCCT-3')		
Methanomicrobiales	MMB 282F(5'-ATCGRTACGG GTTGTGGG-3')	Taqman Probe	(Zhang, DiBaise et al. 2009)
	MMB 832R (5' -CACCTAACGCRCATHGTTTAC-3')		
	MMB 749-probe(5' -TYCGACAGTGAGGRACGAAAGCTG-3')		
Methanobacteriales	MBT 857f (5'-CGWAGGGAAGCTGTT AAGT-3')		
	MBT 1196R (5'-TACCGTCGTCCACTCCTT-3')		
	MBT 929-probe (5' -AGCAC CACAACGCGTGGA-3')		

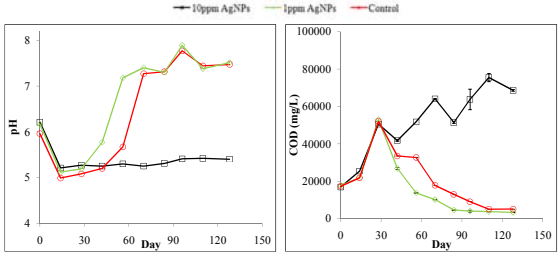
Results and Discussion

Cumulative Biogas Production



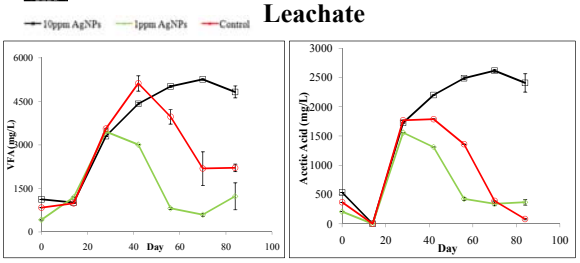
*A big difference of gas volume between the control, the reactors treated with 1 ppm AgNPs and the 10 ppm AgNPs.
* Solids treated with 1 ppm AgNPs show no inhibition on anaerobic process, while those treated at 10 ppm did affect biogas generation rate and volume.

Leachate pH and COD Changes



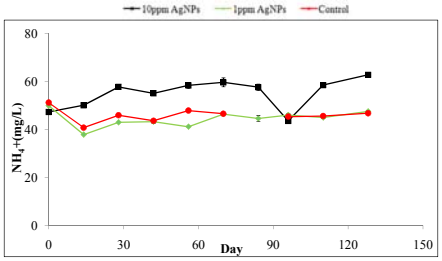
* The pH drop due to VFA accumulation in the bioreactor treated with 10 ppm AgNPs and the changes of leachate COD in 10 ppm AgNPs reactor confirmed the inhibitory effect of nanosilver on anaerobic biodegradation of solid waste.

Changes of VFAs and Acetic Acid in the Leachate



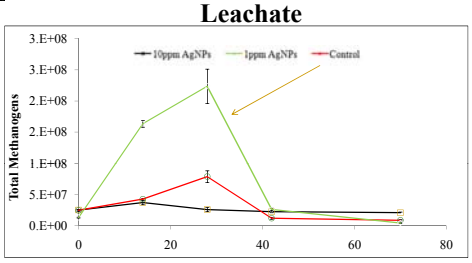
*The dynamic changes of VFAs and acetic acid from the reactor containing 10 ppm AgNPs confirmed the accumulation of VFAs and acetic acid, resulting in consistently low pH (5) in the leachate.
* Results are consistent with biogas production profile.

Leachate Ammonic Concentration

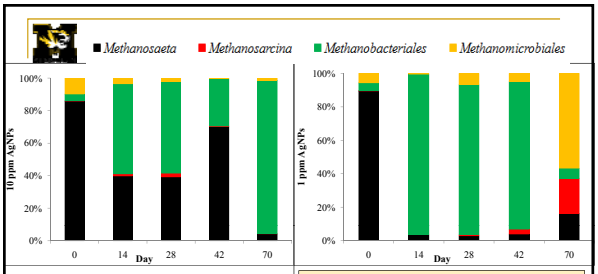


*Ammonia-N appeared to be constant at relatively low concentrations (~50 mg/L).

Changes of Methanogens Population in Leachate

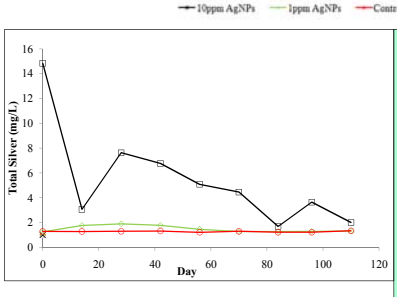


*Beginning: similar methanogen numbers , mainly from the anaerobic sludge added.
*At day 30, the control bioreactor had the highest methanogen numbers, about 8 and 2 times higher than 10 ppm AgNPs, 1 ppm AgNPs respectively.
*From day 40 to 70: almost the same total methanogenic population



• During the early stage of anaerobic decomposition (from day 14 to day 42), in control and bioreactor treated with 1 ppm AgNPs, *Methanobacteriales* dominated (at 90%).
•For comparison, in bioreactor treated with 10 ppm AgNPs, *Methanosaeta* was still above 40%.
•The methanogenic population continues to evolve in bench-scale bioreactor landfills.

Total Silver in Leachate



oTotal Ag in leachate from 10 ppm AgNPs treated bioreactor was about 14.8 mg/L, decreasing to 2 mg/L after about 100 days of operation

oTotal silver from the bioreactor treated with 1 ppm AgNPs and the control was around 1 mg/L, below TCLP maximum value 5 mg/L.

o Results indicate that silver could be precipitated or absorbed in landfill solid waste

Summary

- There was no significant difference of the cumulative gas production between the bioreactor treated with 1 ppm (mg Ag/kg solid) AgNPs and the control. But 10 ppm AgNPs resulted in the reduced biogas production, VFA accumulation and lower pH (around 5) in the leachate.
- qPCR results demonstrated dominant population shift from acetoclastic methanogens to hydrogenotrophic methanogens at the early stage of anaerobic solid degradation.
  - In early stage of solid degradation, leachate from bioreactor treated with10 ppm AgNPs had 40% acetoclastic methanogens in total, compared to other reactors including the control which had more than 90% hydrogenotrophic methanogens-mainly *Methanobacteriales*.
- After about 100 days, total silver concentrations in the leachate were all around 1-2 mg/L in bench-scale bioreactors.
- The results could be useful to the regulatory agencies and landfill operators for decision making and remedial actions.

Acknowledgements

Funded by EPA STAR Program
(#83389301)

Paul Westerhoff

Biological Fate and Electron Microscopy Detection of Nanoparticles During Wastewater Treatment

***Paul Westerhoff*, Bruce Rittmann, and Terry Alford**
Arizona State University, Tempe, AZ

The market for nanomaterials is increasing rapidly, and nanoparticles (NPs) present in consumer products, industrial wastes, biomedical applications, and so on will become significant in the near future for wastewater treatment, just as nutrients, pathogens, metals, and synthetic organic chemicals have been important for the last few decades. Wastewater (WW) treatment plant (WWTP) discharges (treated effluent, biosolids, and possibly aerosols) may become significant routes for NPs to enter the environment. Today, almost no information is available on the fate of manufactured NPs during biological wastewater treatment.

The goal of this project is to quantify interactions between manufactured NPs and WW biosolids. The objectives of this project are to: (1) quantify removal mechanisms and biotransformation of NPs by wastewater biomass/biosolids under different operational regimes (aerobic, anoxic, anaerobic); (2) verify that low NP dosages have minimal effect on WWTP operations; (3) develop microscopy techniques to rapidly scan for the presence of NPs in biological matrices and develop extraction techniques to separate NPs from biosolids; and (4) assess the relative significance of WWTP effluents and biosolids as significant environmental loadings of NPs.

Over the past year, a series of sequencing batch reactors (SBRs) containing either heterotrophic or nitrifying bacteria were operated with and without NP addition. The systems were operated for up to 120 days, and both hydraulic and sludge residence times were managed. NPs were applied twice per day. Silver (functionalized and non-functionalized), titanium dioxide (TiO_2), fullerene, and fullerols had no effect on heterotrophic bacteria performance (i.e., COD removal). At milligram per liter application dosages, silver NPs and silver ions impaired nitrifying bacteria. Functionalized nano-silver was removed from the SBR supernatant, compared with non-functionalized nano-sliver. Nano-TiO_2 was well removed (> 90%). Fullerols were removed less efficiently than aqueous fullerenes. In addition to SBRs, a number of batch isotherm-like experiments also have been completed with the same NPs and show removal results comparable with SBR findings, which suggest that simpler batch methods may be valuable for screening NP removal capability by wastewater biosolids. This research has been published and additional manuscripts are in preparation.

Papers on sources of NPs from textiles, a wide range of household products and cosmetics have been published or submitted. This is critical to provide NP content of commercial products that will enter sewage. Imaging of NPs in commercial products and full scale WWTPs and their biosolids have been published. We are currently analyzing titania solids in biosolids from the U.S. Environmental Protection Agency and U.S. Department of Agriculture archives.

EPA Grant Number: R833322

Biological Fate & Electron Microscopy Detection of NPs During Wastewater Treatment

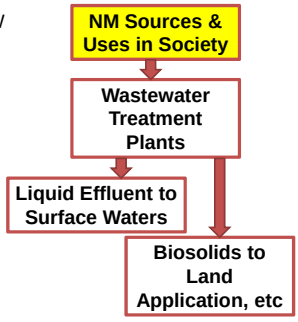
This research is funded by U.S. EPA - Science To Achieve Results (STAR) Program
Grant # RD833322

Paul Westerhoff
Bruce Rittmann & Terry Alford
Ayla Kiser, Yifei Wang, Troy Benn
Kiril Hristovski, David Ladner

November 2010

Project Goal

- Goal: to quantify interactions between manufactured NPs and WW biosolids:
  - We hypothesize that dense bacterial populations at WWTPs should effectively remove NPs from sewage, concentrate NPs into biosolids and/or possibly biotransform NPs.
  - The relatively low NP concentrations in sewage should have negligible impact on the WWTPs biological activity or performance.
  - Develop mechanistic models for NP removal in WWTPs



Release of Engineered Nanomaterials

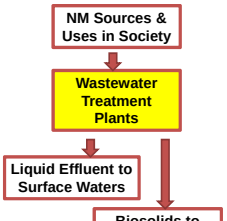
- Nanoparticle Silver Released into Water from Commercially Available Sock Fabrics (Benn & Westerhoff, *ES&T* 42:11:4133-4139 (2008))
  - Observed **release of silver materials from nano-silver impregnated socks**
  - Six types of socks contained up to a maximum of 1360 *µg-Ag/g-sock and leached* as much as 650 *µg of silver in 500 mL of distilled water.*
- The Release of (Nano)Silver from Consumer Products Used in the Home (Benn et al., *J. Environmental Quality,* 39:1-8 (2010))
  - Silver was quantified in a shirt, a medical mask and cloth, toothpaste, shampoo, detergent, a towel, a toy teddy bear, and two humidifiers.
  - **Silver concentrations ranged from 1.4 to 270,000 µg-Ag/g-product**.
  - Silver was released into water up to 45 µg-Ag/g-product, and **size fractions were both > & < 100 nm**
  - TCLP tests conducted to simulate release to landfills (0.13 to 54 µg-Ag/g-product)

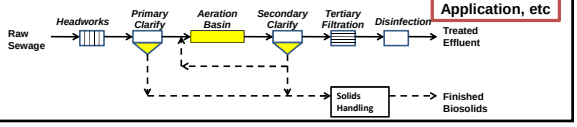
Release of Engineered NMs

- Detection of Fullerenes (C_{60} and C_{70}) in Commercial Cosmetics (Benn et al., submitted to JEM)
  - Five cosmetic products were evaluated for their fullerene content.
  - **A common cosmetic formulation that disperses fullerenes using polyvinylpyrrolidone (C_{60}-PVP) was characterized TEM**
  - LC/SM was used to separate and specifically detect fullerenes (C_{60} and C_{70}) from interfering substances typically present in cosmetics (e.g., castor oil).
  - Recovery of C_{60} from aqueous C_{60}-PVP using LLE and SPE approached 100% after accounting for LC-MS signal suppression caused by matrix interferences (acetic acid)
  - **C_{60} was detected in four commercial cosmetics ranging from 0.04 to 1.1 µg/g, and C_{70} was qualitatively detected in two samples**.
  - A single-use quantity of cosmetic (0.5 g) may contain up to 0.6 µg of C_{60} and demonstrates a pathway for human exposure to engineered fullerenes.
  - Fullerenes may enter the environment through wastewater systems after being released from cosmetics.

Nanomaterial Removal at WWTPs

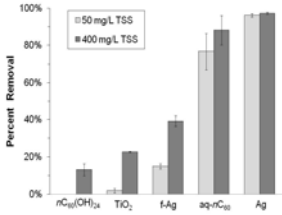
- Settling and Biosorption are dominant removal mechanisms
- Research evaluated:
  - Batch sorption to biomass
  - Continuous loading bioreactors
  - Occurrence at full-scale treatment plants





NM Sorption to Wastewater Biomass

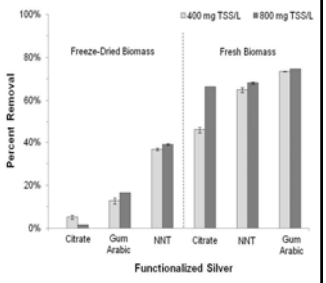
- Biosorption of nanoparticles on heterotrophic wastewater biomass (Kiser et al., *Water Research*, 44:14:4105-4114 (2010))
- Robust sorption method developed
- Surface properties were very important



C_{60}-PVP showed < 10% removal

Validate EPA Sorption Method

- OPPTS 835.1110 Activated Sludge Sorption Isotherm
- Validated method for organic pollutant (MB) using fresh and freeze-dried biomass
- **Method not valid for nanosilver, and likely other NMs**

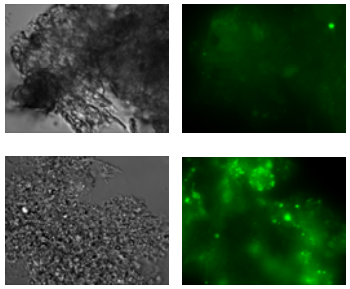


Materials courtesy of CEINt (Wiesner)

Freeze-Dried Biomass has different morphology

Freeze-Dried Biomass:

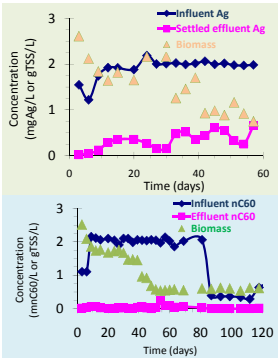
Fresh Biomass:



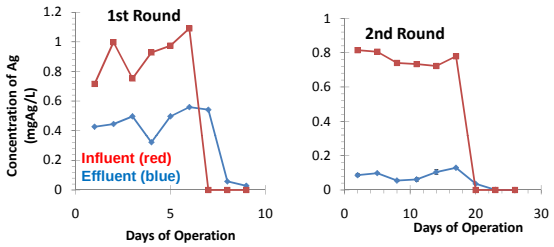
*** Biosorption of fluorescent latex spheres (20 nm sulfate coated) are far less on freeze-dried biomass**

Continuous NM Loading Study

- Sequencing batch reactors (SBRs) operated for weeks to months with daily renewal of simulated sewage + NMs
- Operated are realistic HRT and SRT
- Trends in removal for different types of NMs followed batch isotherms
- Functionalized nanosilver resulted in sludge bulking issues; removal decreased as TSS fell
- Varied nC60 loading and reduced biomass – still achieved very high nC60 removals
- No effect of heterotrophic removal of COD
- Minimal effect of nano-Ag on nitrification, compared to major effect from ionic silver

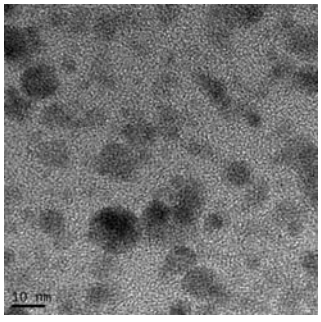


Another Example (f-Ag)

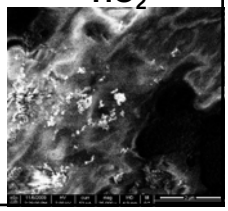


- Round 2 has 50% more biomass (TSS) than round 1

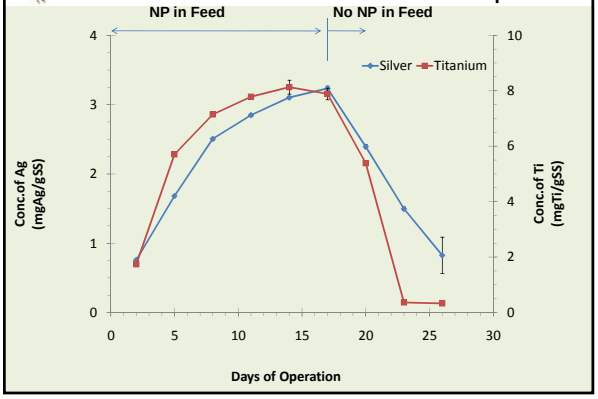
Nanomaterials Removed from Liquid Go to Biosolids (fn-Ag example)



TiO_2



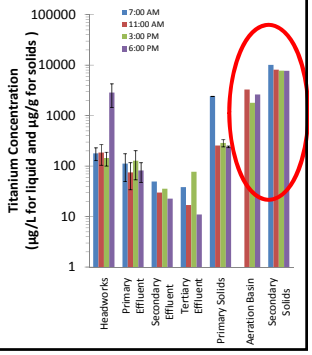
Nanomaterials Removed from Liquid



Occurrence at Full-Scale WWTPs

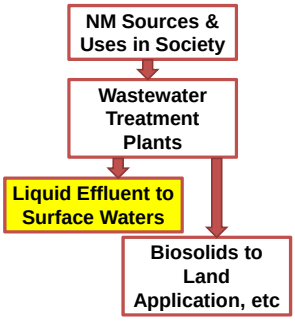
Titanium is found in biosolids at full-scale WWTPs already

- Titanium Nanomaterial Removal and Release from Wastewater Treatment Plants (Kiser et al., ES&T, 43:17:6757-6763 ,2009)
- >90% removal in coming titanium
- Three forms of titanium present:
  - Nanoscale
  - Microscale
  - Mixed element (clays)



Nanomaterials in WWTP Effluents

- Evaluate TiO2 presence at several WWTPs
- Evaluate membrane technologies to characterize or remove NMs

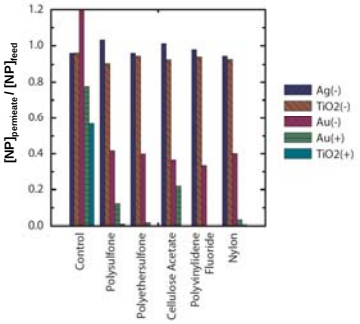


Titanium well removed at WWTPs in Arizona

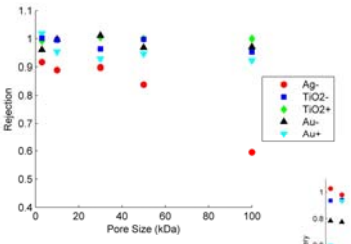
- Effluent Ti concentrations are similar to LCA model predictions
- Membrane bioreactors (MBR) have very low effluent Ti
- We isolated NPs in effluents also using roto-evap + dialysis (under analysis)

Different Facilities	Titanium Content of water (ugTi/L)	
	Headworks	Effluent
Activated sludge	615	5
Act. Sludge + filter	180	7
Activated sludge	363	3
Activated sludge	141	2
Activated sludge	581	18
Activated sludge		8
Activated sludge	233	2
Trickling filter	549	13
MBR	310	1
MBR	422	4
Average	377	6

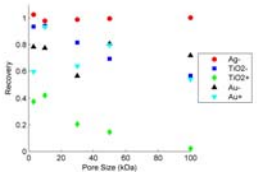
NM surface properties trumped membrane material properties in 0.22-µm syringe filtration experiments



Tighter Ultrafiltration Rejects NMs



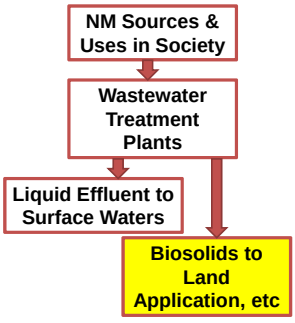
Amicon-Ultra centrifugal filtration device
(image from Sigma-Aldrich web site).



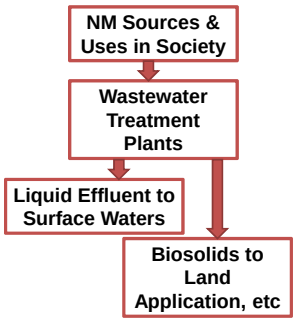
Rejection was high, but recovery indicates significant sorption

Nanomaterial in Biosolids

- Nanomaterials will accumulate in biosolids
- What do we do with WWTP biosolids:
  - 60% land applied
  - 22% incinerated
  - 17% landfilled
- Approximate content (not all "nano"):
  - 0.4 to 1 mgTi/g dry SS
  - 0.004 to 0.03 mgAg/ g dry SS
- Working with biosolids from EPA Inventory and local facilities



Summary & Needs



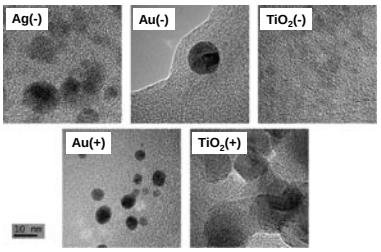
- Need better tools to differentiate engineered from "other" NPs in wastewaters
- Pollutant removal models for WWTPs are current not suitable for predicting NPs
- Better relationships between surface charge and core composition versus biosorption needed
- Fate of NMs in biosolids is poorly understood

Biological Fate & Electron Microscopy Detection of NPs During Wastewater Treatment

This research is funded by U.S. EPA - Science To Achieve Results (STAR) Program Grant # RD833322

Gordon Conference
2011 Gordon Research Conference
on
Environmental Nanotechnology
Waterville Valley Resort, Waterville Valley, NH,
May 29 - June 3, 2011

NPs used here were functionalized and small (even among *nano*particles.)

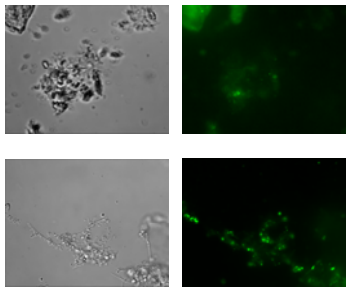


21

Fluorescence Images

Freeze-Dried Biomass:

Fresh Biomass:



*** Freeze-dried biomass shows less fluorescence than fresh biomass.**

P. Lee Ferguson

Analysis and Fate of Single-Walled Carbon Nanotubes and Their Manufacturing Byproducts in Estuarine Sediments and Benthic Organisms

***P. Lee Ferguson*[1] *and G. Thomas Chandler*[2]**
[1]Department of Civil and Environmental Engineering, Duke University, Durham, NC; [2]Department of Environmental Health Sciences, University of South Carolina, Columbia, SC

Single-walled carbon nanotubes (SWNT) have emerged as a promising material for commercial and industrial applications due to their outstanding electrical, optical, mechanical, and thermal properties. 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 have combined asymmetric flow field flow fractionation (A4F) with NIRF spectroscopy as a promising tool for determination of SWNT in the environment. Different purification, concentration, and separation methods are discussed to reduce matrix complexity and improve the detection limit of SWNT. In addition to concentration, structural information such as shape, length distribution, or agglomeration state of SWNT also must be identified and quantified to describe behavior and transport processes as well as biological interactions. NIRF spectral features of SWNT were retained after extraction from sediment, allowing diameter/chiral wrapping angle characterization for dilute solutions. Furthermore, A4F was applied as a separation method prior to NIRF spectroscopic analysis to determine SWNT length distribution and to reduce matrix complexity by separation of NOM and SWNT. We have utilized this comprehensive analytical approach to assess the fate and biological uptake of CoMoCAT SWNT in marine sediment microcosms and benthic deposit feeding organisms. SWNT were extracted from sediments and meiobenthic copepods and polychaete worms by ultrasonication in 2% surfactant solutions and individual surfactant-wrapped nanotubes were isolated from aggregates by ultracentrifugation. SWNT extracted from sediment and tissue in 2% sodium deoxycholate could be quantified down to 9 ng/mL, and detection was linear over > 3 orders of magnitude. Our results show that NIRF-spectroscopy is a valuable method for detection and characterization of surfactant-stabilized SWNT at trace concentrations in the aquatic environment.

EPA Grant Number: R833859

Analysis and fate of single-walled carbon nanotubes in estuarine sediments and benthic organisms

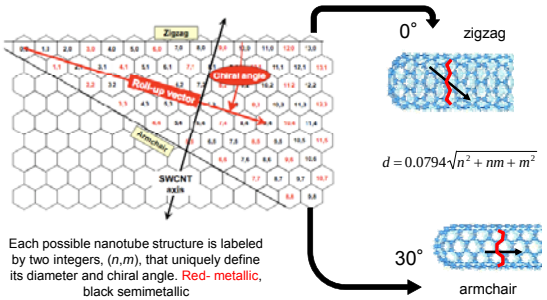
P. Lee Ferguson[1,2], Ashley N. Parks[1], P. Ariette Schierz[2], Kate Washburn, G. Thomas Chandler[3], Kay Ho[4], and Rob Burgess[4]

[1]Nicholas School of the Environment and [2]Department of Civil & Environmental Engineering, Duke University, Durham, NC
[3]Department of Environmental Health Sciences, University of South Carolina, Columbia, SC
[4]Atlantic Ecology Division, NHEERL, Narragansett, RI

SWNT as potential environmental contaminants

- SWNT composites have already made their way into the marketplace (composite sports equipment, nanoelectronic devices).
- Numerous companies now supply SWNTs on kilogram scale.
- Annual worldwide production of SWNT is estimated > 1,000 t by 2011.
- There are currently no reliable methods to detect SWNT in complex mixtures (e.g. sediment, tissue, ambient waters) at low concentrations.

SWNT have unique structural characteristics



$$d = 0.0794\sqrt{n^2 + nm + m^2}$$

Each possible nanotube structure is labeled by two integers, (*n*,*m*), that uniquely define its diameter and chiral angle. Red- metallic, black semimetallic

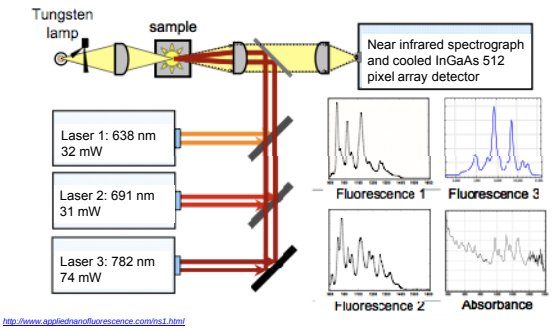
Adapted from *Fluorometric Characterization of SWCNT*, Weisman, RB, 2009.

RESEARCH OBJECTIVE:

Implement and apply near infrared fluorescence spectroscopy for qualitative and quantitative analysis of SWNT in complex environmental media

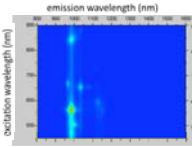
1. Develop sample preparation methods for isolating SWNT from sediment and tissue prior to near infrared fluorescence spectroscopy.
2. Explore asymmetric flow field flow fractionation coupled with NIRF spectroscopy for separating SWNT and reducing intereferences.
3. Apply AFFF-NIRF spectroscopy to analysis of SWNT uptake and accumulation in sediment-dwelling organisms.

Multi-laser NIR spectrofluorometer

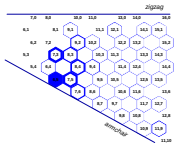


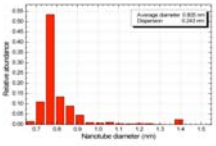
http://www.appliednanofluorescence.com/ns1.html

Qualitative characterization of CoMoCat SWNT type SG65 by NIRF spectroscopy



$$d = 0.0794\sqrt{n^2 + nm + m^2}$$

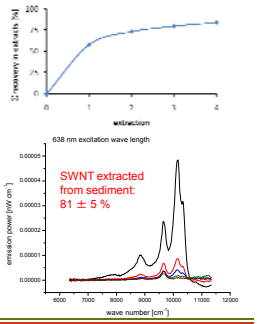




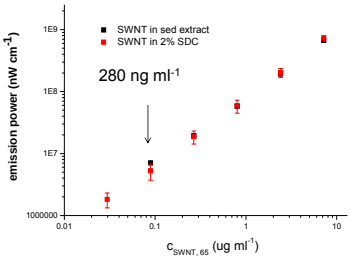
Detection of CoMoCat SWNT type SG65 in estuarine sediment by NIRF

- CoMoCat SWNT were spiked into estuarine sediment at 10 μg/g concentration.
- Sequential extractions were performed with 2% sodium deoxycholate (ultrasonication at 40 W for 10 minutes).

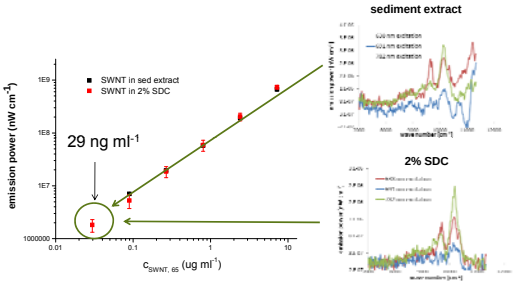
SWNT Diameter (nm)	Chiral species	Recovery % after 4 extractions
0.782	8,3	79
0.757	6,5	96
0.706	7,3	74
0.829	7,5	77
0.884	10,2	103
0.916	9,4	79
0.840	8,4	62
0.895	7,6	81
0.806	9,2	75



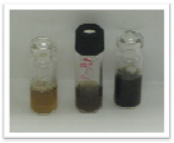
Quantitative performance of NIRF spectroscopy for SWNT in sediments



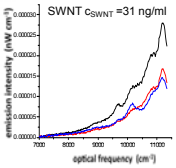
Quantitative performance of NIRF spectroscopy for SWNT in sediments



Challenges: Sample purification methods

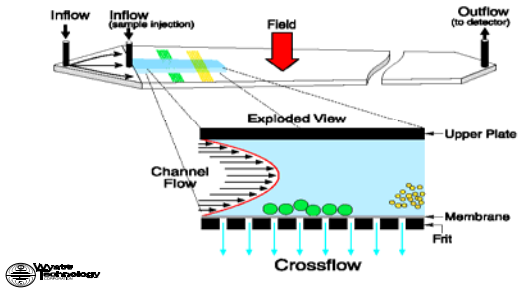


extracts of natural sediments



- **Oxidation** e.g. H_2O_2, $KMnO_4$, ..
- **Ultrafiltration** (Centriprep 100 kDa)
- **Ultracentrifugation**
- ***Asymmetric Flow Field Flow Fractionation***

Separation mechanism in asymmetric flow field flow fractionation AF4



Field flow fractionation coupled with NIRF spectroscopy

FFF channel

Field flow fractionation coupled with NIRF spectroscopy

FFF channel
⬇
DAD detector

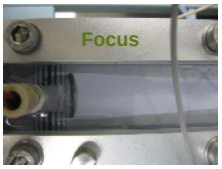
Field flow fractionation coupled with NIRF spectroscopy

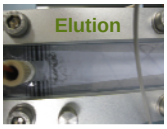
FFF channel
⬇
DAD detector
⬇
MALS detector

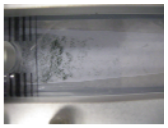
Field flow fractionation coupled with NIRF spectroscopy

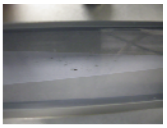
FFF channel
⬇
DAD detector
⬇
MALS detector
⬇
NIR Fluorescense detector

AF4 of CoMoCat SWNT

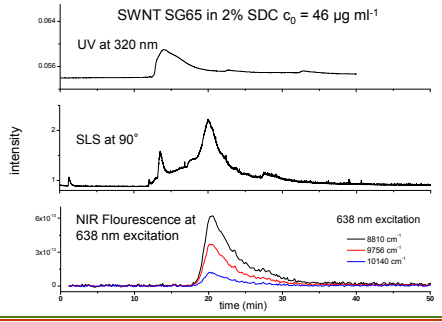




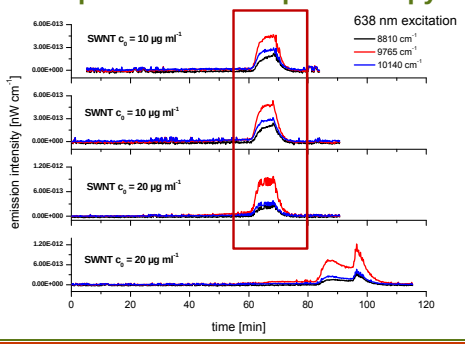




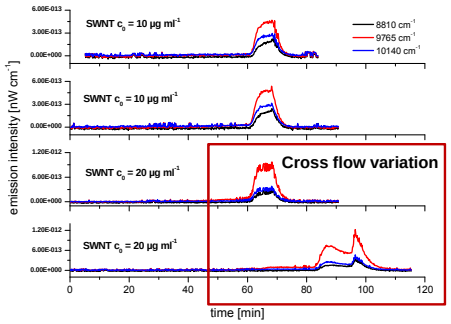
Qualitative detection of CoMoCat SWNT in 2% SDC by AF4 –NIRF spectroscopy



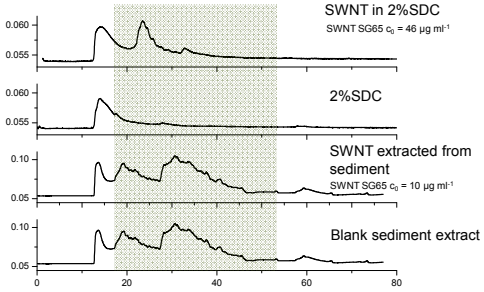
Separation of CoMoCat SWNT by AF4 coupled with NIRF spectroscopy



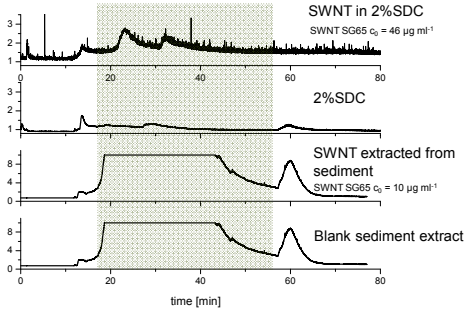
Separation of CoMoCat SWNT by AF4 coupled with NIRF spectroscopy



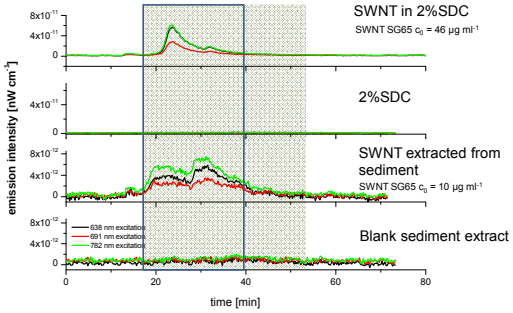
Detection of CoMoCat SWNT extracted from sediment by UV



Detection of CoMoCat SWNT in sediment by static light scattering



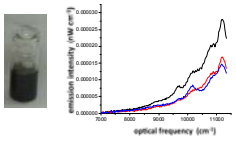
Detection of CoMoCat SWNT in sediment extracts by NIRF



CoMaCat SWNT are detectible in complex sediment extracts using AFFF-NIRF

sediment spiked 10 µg SWNT/g

NIRF static
Reference: sediment extract

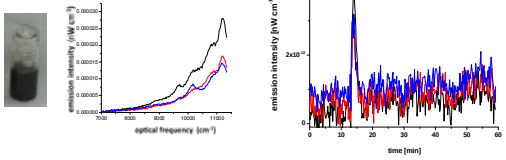


CoMaCat SWNT are detectible in complex sediment extracts using AFFF-NIRF

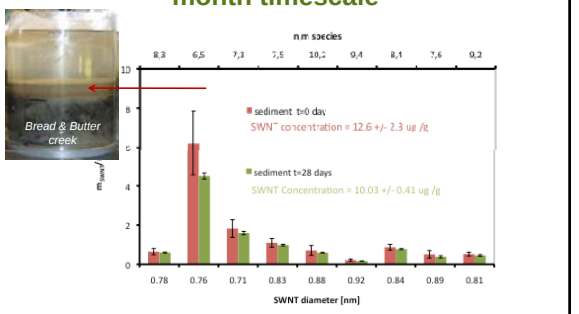
sediment spiked 10 µg SWNT/g

NIRF static
Reference: sediment extract

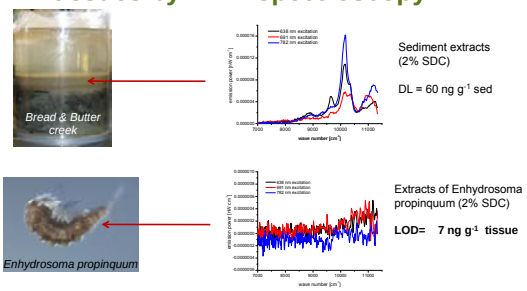
AFFF-NIRF
time resolved



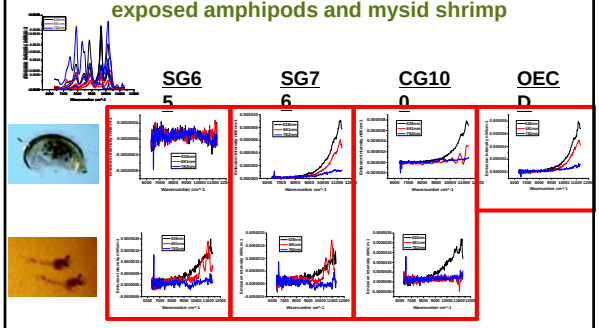
SWNT do not degrade in sediments over one month timescale



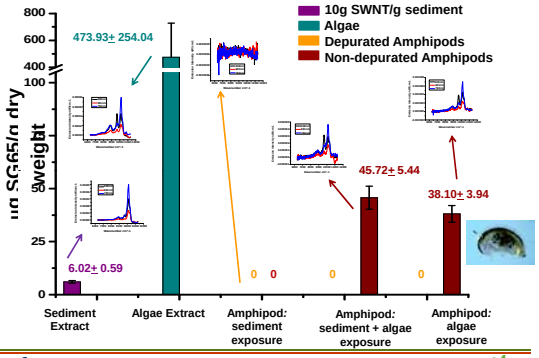
Detection of SWNT SG65 in sediments and tissues by NIRF spectroscopy



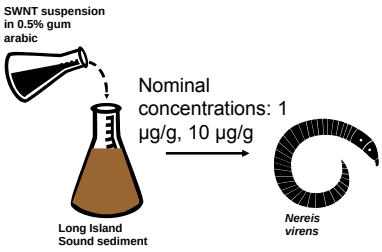
CoMoCat SWNT were undetectable in sediment-exposed amphipods and mysid shrimp



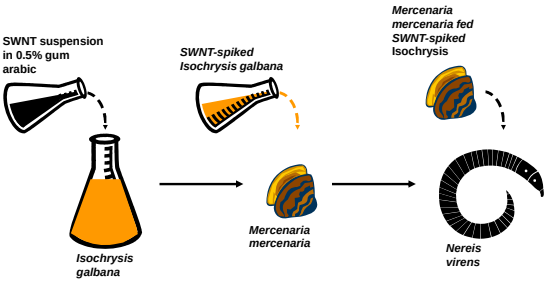
SWNT body burden measurements in sediment and/or food exposed organisms

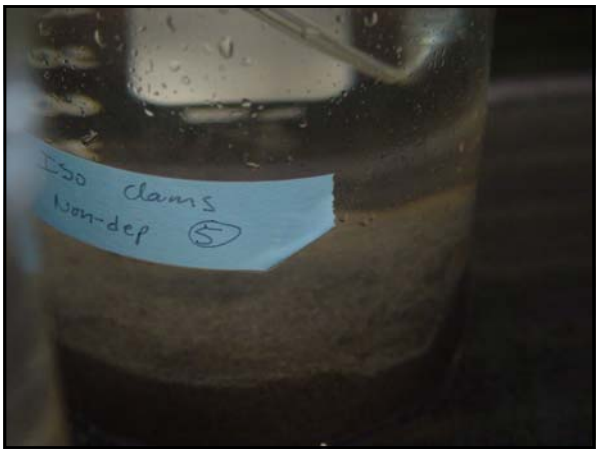


Accumulation of SWNT in benthic macroinvertebrates



SWNT bioaccumulation and trophic transfer





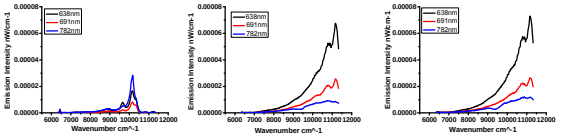
SWNT-spiked sediment exposure to *Nereis virens*

Nominal SWNT – spiked sediment concentration	Measured SWNT concentration: Batch A	Measured SWNT concentration: Batch B
10 µg/g	7.1±1.4 µg/g	10.4±1.6 µg/g

NIRF analysis of SWNT in spiked sediment

NIRF analysis of SWNT in depurated worms

NIRF analysis of SWNT in non-depurated worms



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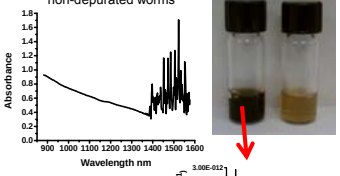
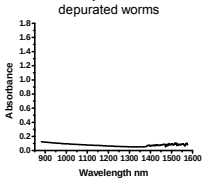
Direct NIRF analysis of worm extracts may be limited by internal filter effects

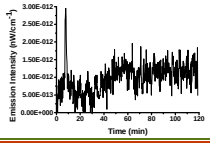
NIRF analysis of SWNT in non-depurated worms



NIRF analysis of SWNT in depurated worms



AFFF-NIRF analysis at 638nm excitation



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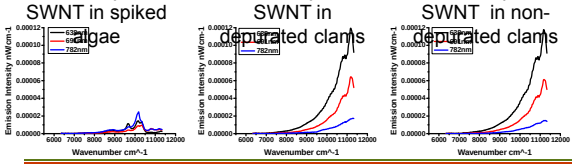
SWNT-spiked *Isochrysis galbana* exposure to *Mercenaria mercenaria*

Nominal SWNT – spiked algae concentration	Measured SWNT concentration: 7/30/10	Measured SWNT concentration: 8/2/10
1000 µg/g	94.5 µg/g	63.2 µg/g

NIRF analysis of SWNT in spiked algae

NIRF analysis of SWNT in depurated clams

NIRF analysis of SWNT in non-depurated clams



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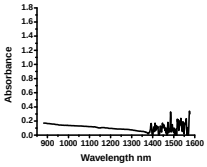
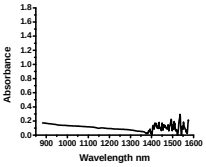
No internal filter artifacts present in NIRF analysis of clam extracts

NIRF analysis of SWNT in depurated clams



NIRF analysis of SWNT in non-depurated clams



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OUTLOOK

- **Additional microcosm-based experiments**
  - Track uptake of SWNT manufacturing byproducts in sediment-dwelling organisms as well as degradation in sediments
  - Investigate chirality/diameter-dependence of SWNT interaction with sediment and organisms
- **Survey environmental media (e.g. field sediment) for contamination with SWNT**

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Conclusions

- A novel and highly sensitive method for analysis of SWNT in sediments based on near infrared fluorescence spectroscopy has been developed.
- NIRF spectral features of SWNT were retained after extraction from sediment, allowing diameter/chirality characterization for dilute solutions.
- AFFF can be used as a clean up tool prior to NIRF analysis.
- SWNT do not appear to be highly bioaccumulative in estuarine invertebrates exposed via sediment or dietary routes.

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Acknowledgement

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CEINT Center for the Environmental Implications of NanoTechnology

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Robert A. Yokel

Safety/Toxicity Assessment of Ceria (A Model Engineered NP) to the Brain

Robert Yokel*[1], *Mo Dan*[1], *Rebecca Florence*[1], *Jason Unrine*[1], *Robert MacPhail*[2], *Michael Tseng*[3], *Uschi Graham*[1], *Rukhsana Sultana*[1], *Sarita Hardas*[1], *D. Allan Butterfield*[1] *Peng Wu*[1], *and Eric Grulke*[1]
[1]University of Kentucky, Lexington, KY; [2]U.S. Environmental Protection Agency, Research Triangle Park, NC; [3]University of Louisville, Louisville, KY

Nanoscale ceria has extensive commercial uses that can contribute to its environmental release, including its use as a diesel fuel additive. We are studying it as a model metal oxide engineered nanomaterial (ENM). Nanoscale ceria was nominated by the National Institute of Environmental Health Sciences for toxicological consideration and is on the priority list of the OECD for measurement, toxicology, and risk assessment studies.

The purpose of this study is to characterize the physico-chemical properties of a representative ENM that influences its distribution in blood, and into the brain compared to peripheral organs, biopersistence in those organs, and resultant effects.

Studies were conducted with in-house produced and characterized ~ 5, 15, 30, and 65 nm citrate-coated ceria ENM, compared to the cerium ion. Ceria ENM or the cerium ion was iv infused into rats to enable study of its distribution in blood and translocation from systemic circulation, as would occur following absorption into blood from any route of exposure. Blood was repeatedly sampled, an aliquot allowed to clot, and cerium determined by ICP-MS in serum and clot up to 4 h and in whole blood for much longer. To extend our prior work showing no appreciable reduction of cerium in mammalian reticuloendothelial tissues up to 30 days after a single administration of nanoscale ceria; determine the routes and rate of its excretion; and further characterize its distribution, persistence, and associated effects in the rat; a longer term study was conducted with 30 nm ceria. Rats were terminated 1, 7, 30, or 90 days after a single iv ceria ENM infusion, compared to cerium ion or vehicle controls. Rats were housed in metabolism cages for up to 2 weeks to quantify urinary and fecal cerium output, cage-side observations were recorded daily, and they were weighed weekly. Nine organs were weighed and samples of 14 tissues, blood, and CSF were collected for cerium determination by ICP-MS. Oxidative stress markers (protein carbonyls [PC], 3-nitrotyrosine [3-NT], and protein bound 4-hydroxy-2-trans-nonenal [HNE]), the glutathione antioxidant defense system (glutathione reductase and peroxidase), and antioxidant enzymes (Mn-SOD and catalase) were measured.

Ten minutes after infusion, < 1 percent of 15 to 65 nm ceria ENM, < 2 percent of a mixture of 30 nm cubic and rod ceria ENM, approximately 14 percent of the cerium ion, and approximately 33 percent of the 5 nm ceria ENM remained in blood. For all 4 ceria ENMs the elimination from blood was biphasic, with an initial half life of approximately 1 h and the second for the 5 to 30 nm ceria of approximately 100 to 200 h, and approximately 12 h for the 65 nm ceria. The 15 and 30 nm ceria predominantly associated with blood cells, whereas the 5 and 65 nm ceria and the cerium ion were approximately evenly distributed between serum and the clot fraction of blood. The 5 nm ceria ENM was not seen in BBB or brain cells. The amount of 15 to 65 nm ceria ENMs in brain samples was very small. Energy electron loss spectroscopy showed the ceria *in situ* to have similar valence (considerable Ce(III)) to the dosing material up to 30 days. The 30 nm ceria ENM was less acutely toxic than the cerium ion. Less than 1 percent of the ceria or cerium ion was excreted in the first week, of which 98 percent was in feces. Ceria was primarily retained in the spleen, liver, and bone marrow. Spleen weight was significantly increased in ceria-treated rats at several times after its infusion, and associated with visual evidence of abnormalities.

Thirty nm ceria was associated with blood cells to a greater extent than larger or smaller ENMs, consistent with reports showing this size is optimal for protein wrapping of ENMs. Ceria in blood is primarily cleared by

the reticuloendothelial tissues, in which it persists without significant decrease in mass amount for at least 3 months. Little enters the brain. Referring to nanoscale fiber-like structures, it has been stated: "The slower [they] are cleared (high bio-persistence), the higher is the probability of an adverse response" (European Parliament, Policy Department Economic and Scientific Policy "Nanomaterials in consumer products"). Our results support the concern about the long-term fate and adverse effects of inert nanoscale metal oxides that reach systemic circulation, from which they can distribute throughout the body, resulting in persistent retention and potential adverse effects in multiple organs.

These results of ENM translocation, biopersistence, and hazard identification in the mammal provide data for ENM risk characterization.

Reference:

Hardas SS, Butterfield DA, Sultana RL, Tseng MT, Dan M, Florence R, Unrine JM, Graham UM, Wu P, Grulke EA, Yokel RA. Brain distribution and toxicological evaluation of a systemically delivered engineered nanoscale ceria. *Toxicological Sciences* 2010;116(2):562-576, doi: 10.1093/toxsci/ kfq137.

EPA Grant Number: R833772

Safety/toxicity assessment of ceria (a model engineered NP) to the brain

The research team

- Robert A. Yokel and Mo Dan
  - Department of Pharmaceutical Sciences, College of Pharmacy & Graduate Center for Toxicology, University of Kentucky, Lexington, KY
- Jason Unrine
  - Department of Plant and Soil Sciences, U KY
- Michael T. Tseng
  - Departments of Anatomical Sciences & Neurobiology, University of Louisville, Louisville, KY

The research team

- Uschi M. Graham
  - Center for Applied Energy Research, U KY
- D. Allan Butterfield, Rukhsana Sultana, & Sarita Hardas
  - Department of Chemistry, U KY & (DAB) Center of Membrane Sciences, U KY
- Eric A. Grulke & Peng Wu
  - Chemical & Materials Engineering Department, U KY

Objective of this research

- Characterize the physico-chemical properties of a model engineered nanomaterial (ENM) that influence its biodistribution and effects, including:
  - distribution across the blood-brain barrier (BBB)
  - effects on oxidative stress endpoints in the brain
  - uptake into selected peripheral organs
  - persistence over time.

ENM studied

- Ceria (CeO_2, cerium dioxide, cerium oxide) was selected because:
  - it is an insoluble metal oxide that can be readily observed and quantified in tissue (electron microscopy, ICP-MS).
  - it has current commercial applications (a catalyst in diesel fuel and an abrasive in integrated circuit fabrication).
  - it has been reported to be cytotoxic as well as neuroprotective, representing the controversy about nanoscale materials.

ENM studied

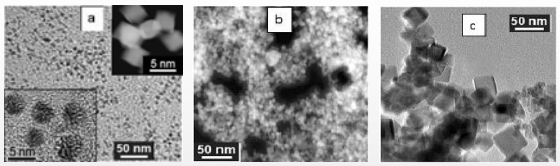
- We prepared citrate-coated ceria and characterized them using:
  - dynamic light scattering (size)
  - transmission electron microscopy (TEM) with an energy dispersive X-ray spectrometer (size and morphology)
  - X-ray diffraction (composition & crystallinity)
  - BET surface area analyzer (size)
  - zeta potential (surface charge & stabilizing agent)
  - FTIR (surface charge and stabilizing agent)
  - scanning TEM with electron energy loss spectroscopy (valence)
  - thermogravimetric analysis with mass spectrometry (surface citrate coating)

ENMs studied

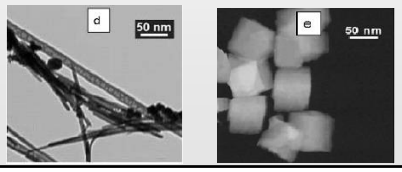
Ceria ENM size (nm)	shape	Zeta potential	Miller indices	Extent of surface citrate coating
5	polyhedral	-53 ± 7 mV at pH ~ 7.35	(111), (210), (200)	~ 40%
15	polyhedral	-57 ± 5 mV at pH ~ 7.3	(111), (210), (200)	~ 27%
30	cubic	-56 ± 8 mV at pH ~ 7.3	(111), (210), (200)	~ 18%
30	cubic + rods			
65	polyhedral	-22 ± 5 mV at pH ~7	(111), (210), (200)	~ 15%

HRTEM and STEM images of ceria ENMs

5 nm polyhedral | 15 nm polyhedral | 30 nm cube



30 nm cube & rod | 65 nm polyhedral



Objective: To assess the influence of size on ENM distribution, persistence, translocation and toxicity

- Citrate-coated ceria i.v. infused into un-anesthetized rats (0 or ~ 100 mg/kg); terminated 1 or 20 h or 30 days later.

Blood and tissue [cerium]

- Brain cortex cerium was always < 1% of the dose.
  - We did not see 5 nm ceria in brain, only in brain vasculature
- Spleen cerium concentration was greater than liver cerium concentration.
- Liver had the greatest mass amount of the ceria dose.
- There was little decrease in liver and spleen cerium up to 30 days.

Electron energy loss spectrometry characterization of ceria as synthesized and in situ

There was no observable change in the M5/M4 peak ratio (Ce(III)/Ce(IV) ratio) of 5 or 30 nm ceria in spleen agglomerates 30 days after ceria administration compared to the freshly prepared ceria ENM.

Ceria distribution in and elimination from blood

- Rats were iv infused with 5, 15, 30 or 65 nm citrate-coated ceria, an mixture of 30 nm cubic and rod citrate-coated ceria, or the cerium ion.
- After the infusion blood was drawn at 10, 30, 45, 60, 120 and 240 min.
- Cerium was determined in whole blood, plasma and clot.
- Whole blood cerium was determined in these and other rats up to 90 days after ceria dosing.

Ceria in blood

- Ten min after completion of the 1 h ceria infusion 30% of the 5 nm ceria was in blood; < 1% of the 15, 30 and 65 nm ceria.
- Compartmental pharmacokinetic analysis of whole blood cerium generally showed an initial $t_{½}$ of 1 h and a beta phase half-life of ~ 100 h.
- The 15 and 30 nm ceria predominantly associated with blood cells, whereas the 5 and 65 nm ceria were ~ evenly distributed between the two compartments.
- The greatest association of the 30 nm citrate-coated ceria with blood cells in the clot fraction is consistent with reports showing this size is optimal for protein wrapping of ENMs.

A 90 day survival study to assess longer term distribution, persistence and effects

- Single iv dosing of 87 mg 30 nm ceria/kg, 50 mg cerium ion/kg, or vehicle.
- Termination 1, 7, 30 or 90 days later.
- Fecal and urinary Ce excretion (metabolic cage).
- Weekly body weight.
- Weights and cerium concentration in multiple organs and fluids.
- Oxidative stress markers, histology (LM & EM).

- A single ceria ENM infusion resulted in modest decreased body weight gain.
- Less than 1% of the ceria ENM or cerium ion dose was eliminated in a week.
- Ceria was retained primarily in reticuloendothelial tissues. The liver contained ~ 20% and the spleen ~ 15% of the dose 90 days after ceria administration.
- No great decrease of the mass amount of ceria in liver and spleen occurred over 90 days.

Spleen pathology 30 and 90 days after ceria

– Splenomegaly: ~ 2-fold with 5 and 15 nm and 1.5-fold with 30 nm at 30 days.
– The red pulp 30 days after 5 nm ceria showed numerous densely stained lymphatic cells .
– The white pulp 90 days after 30 nm ceria showed ceria containing cell clusters.
– Granulomatous formations were seen 90 days after ceria.

Liver pathology 30 and 90 days after ceria

- 30 days after 5 nm ceria:
  - Non-uniform granuloma formations containing ceria-loaded Kupffer cells.
  - Mononucleated cell infiltration among the hepatic parenchyma and at perivascular sites.
  - Mononucleated cells appeared to encircle Kupffer cells.
  - No evidence of fibrosis or abscess formation.
- 90 days after 30 nm ceria:
  - Granulomatous formations seen.

Conclusions

- Citrate-coated 5 to 65 nm ceria does not enter the brain to any significant extent.
- It is primarily cleared by reticuloendothelial organs and sequestered in intracellular agglomerates.
- Some of these results are in Hardas et al Toxicological Sciences 2010 116(2):562-576.
- The Ce valence does not change *in situ* (in the first 30 days).
- There is little clearance of 5 to 65 nm ceria from reticuloendothelial organs, for 30 nm up to 90 days.
- The smaller the ceria ENM, the longer it remains in blood before being cleared.
- Maximal distribution into blood cells was seen with 30 nm ceria.

Conclusions

- Ceria ENM and the cerium ion are very slowly eliminated.
- Ceria ENM does not always behave like the cerium ion, in its distribution in blood or tissues.
- Referring to nanoscale fiber-like structures, it has been stated: “The slower [they] are cleared (high bio-persistence) the higher is the probability of an adverse response”. (European Parliament, Policy Department Economic and Scientific Policy “Nanomaterials in consumer products”).
- These results further support the concern about the long term fate and adverse effects of inert nanoscale metal oxides that reach systemic circulation, from which they can distribute throughout the body, resulting in persistent retention and potential adverse effects in multiple organs.

Future Plans

- Complete the histopathology, agglomeration extent and localization, cerium valence, and oxidative stress marker analyses as a function of time (1, 7, 30 and 90 days) after the 30 nm ceria infusion.

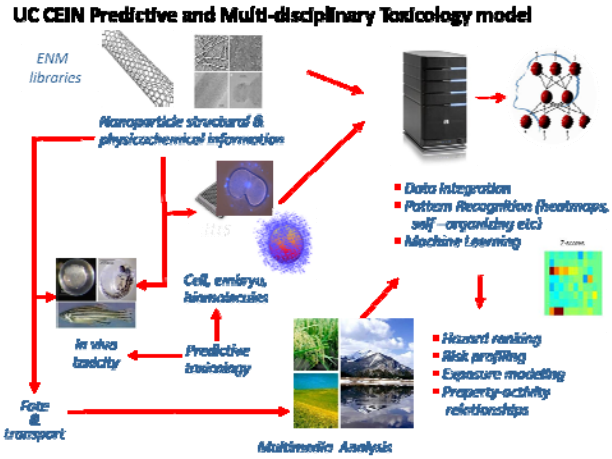
Handouts on Centers for Environmental Implications of Nanotechnology (CEIN)

UNIVERSITY OF CALIFORNIA

UC CEIN Center for Environmental Implications of Nanotechnology

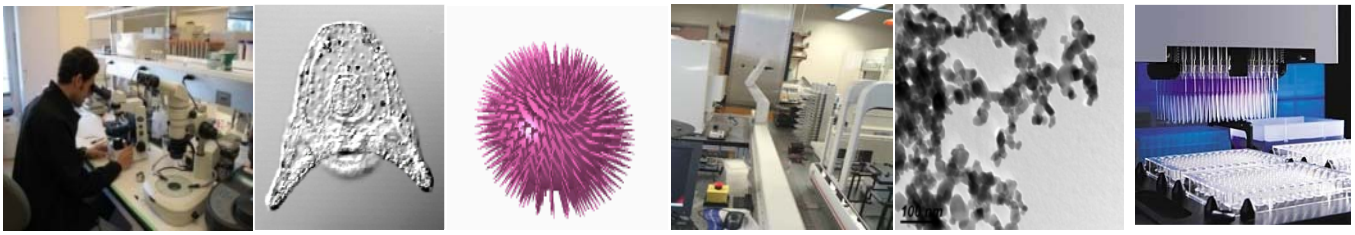
Mission Statement

The University of California Center for Environmental Implications of Nanotechnology (UC CEIN) was established to ensure that nanotechnology is introduced in a responsible and environmentally compatible manner, thereby allowing the US and International Communities to leverage the benefits of nanotechnology for global economic and social benefit. This mission is being accomplished by developing a series of decision tools based on models of predictive toxicology and risk ranking premised on selected nanomaterial property-activity relationships that determine fate, transport, exposure, and biological injury mechanisms at cellular, tissue, organism, and population levels. Since its founding in September 2008, the UC CEIN has successfully integrated the expertise of engineers, chemists, colloid and material scientists, ecologists, marine biologists, cell biologists, bacteriologists, toxicologists, computer scientists, and social scientists to create the predictive scientific platform that will inform us about the possible hazards and safe design of engineered nanomaterials (ENMs) that may come into contact with the environment.



The research of the UC CEIN is carried out by 46 distinct but highly interactive research projects across 7 interdisciplinary research groups (IRGs):

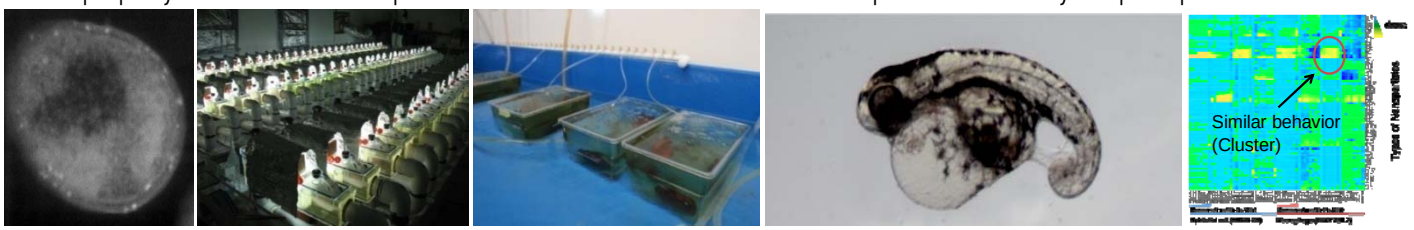
- ENM Standard Reference and Combinatorial Libraries and Physical-chemical Characterization (IRG 1)
- Studying ENM Interactions at the Molecular, Cellular, Organ, and System Levels (IRG 2)
- Organismal and Community Ecotoxicology (IRG 3)
- Nanoparticle Fate and Transport (IRG 4)
- High-Throughput Screening (HTS), Data Mining, and Quantitative-Structure Relationships for NM Properties and Nanotoxicity (IRG 5)
- Modeling of the Environmental Multimedia NM Distribution and Toxicity (IRG 6)
- Risk Perception of Potential Environmental Impacts of Nanotechnology (IRG 7)



Key Center Accomplishments

- UC CEIN plays a national leadership role in Nano EHS initiatives
- Compiled a nanomaterial library with 60+ different types of NP (characterized and introduced into active research) - Includes ZnO, CeO_2, TiO_2, CNT, Ag, Au, CdSe, Silica, Clays
- Developed a series of standardized dispersion protocols for relevant environmental conditions
- Successfully demonstrated the oxidative stress heirarchical paradigm for NPs with varying properties
- As an exercise of safe design, ZnO doped with Fe has been found to significantly reduce ZnO NP toxicity in mammalian cells
- Use of zebrafish embryos as an *in vivo* model for high-content screening has shown that *in vitro* toxicity testing results agree with HCS zebrafish *in vivo* studies, except when using silver nanoparticles
- Bacterial toxicity research shows that ZnO and CeO_2 inhibit growth more than TiO2, with gram positive bacteria found to be relatively more sensitive
- Exposure to Quantum Dots across trophic levels show significant bioaccumulation in bacteria and biomagnification in protozoa
- Testing of ZnO across ecosystems has shown that ZnO is consistently toxic, with toxicity resulting from exposure to Zn ions following ENM aggregation and Zn ion shedding
- Studies of mobility, persistence, bioavailability and reactivity of ENMs in environmental conditions are providing key characteristics of metal oxide NPs in seawater, freshwater, and terrestrial environments
- Efficient removal of ENM from aqueous systems can be archived by pH destabilization, coagulant dosing, sedimentation, and ultrafiltration

- Through validation of commercially available HTS technology, we have implemented gene reporter assays that provide readouts of known cellular signaling pathways. Preliminary results identify genotoxicity in a subset of ENMs
- A new efficient computer algorithm for feature selection ranking was developed for screening and ranking nanoparticle properties for the development of quantitative property-structure relationships
- An international survey of Industry NanoEHS is providing key insights into industry practices, perceived risks, and gaps in understanding, with "lack of information" being a key impediment to the implementation of Nano EHS programs in industry
- A Summer 2010 survey of nanotoxicology and regulatory experts will provide a vital comparative framework for future public and industry risk perception studies



Future Directions Include:

- Develop and characterize new libraries of Pt, Pd, SWCNT, Mesoporous Silica, and new derivations of metal oxides
- Continue ongoing cytotoxicity studies with ENM libraries and analyze data from in vitro and in vivo studies to rank NP toxicity, assess predictive power of in vitro studies, and begin building expert system required to generate structure activity relationships
- Expand marine organisms, cellular, and bacterial studies beyond the initial metal oxide NPs (ZnO, TiO_2, CeO_2)
- Adapt HTS methods of toxicity screening to marine and bacterial cells, demonstrating and documenting performance and challenges
- Results from ongoing HTS ecotoxicology experiments are being used to design mesocosm experiments in marine, terrestrial, and freshwater ecosystem studies
- Incorporation of CNT, Ag, QDs, Pt as well as NP of different sizes and morphologies into ongoing Fate and Transport studies
- Advance HTS gene knockout studies with yeast and bacterial strains
- Development of an automated high content screening method to enhance zebrafish *in vitro* toxicity studies
- As experimental data from across Center projects enters the Central Data Management system, models for multimedia transport and fate and nanoparticle structure activity relationship models will be refined and expanded. Development of a series of NP decision tools will commence with an initial focus on establishing questions needed to design model pathways
- Data from industry survey, public environmental risk perception survey, and survey of nanotoxicology and regulatory experts will help provide valuable knowledge about the societal implications and contexts for risk characterization

Education and Outreach

UC CEIN will serve to enhance our understanding of the environmental hazards of nanomaterials. Education and outreach programs to train scientists, develop safe handling guidance for nanomaterials, and develop methods to communicate the implications of our research to the public are key to the success of the Center. The knowledge generated by the Center will directly benefit scientists, public agencies, industrial stakeholders, and the general population.

UC CEIN is housed within the California NanoSystems Institute at the University of California, Los Angeles, with a second major hub at the University of California, Santa Barbara. Research partners include: UC Davis, UC Riverside, Columbia University, University of Texas, Nanyang Technological University, Northwestern University, the Molecular Foundry at Lawrence Berkeley National Laboratory, Lawrence Livermore National Laboratory, Sandia National Laboratory, University of Bremen, University of British Colombia, University College Dublin, Cardiff University, and the Universitat Rovira I Virgili.

For more information: Please visit the UC CEIN website: http://www.cein.ucla.edu

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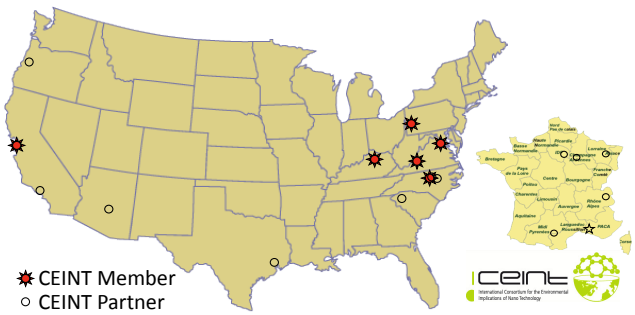
University of California Center for Environmental Implications of Nanotechnology (UC CEIN) is supported by the National Science Foundation and the U.S. Environmental Protection Agency (NSF: DBE-0830117)

CEINT | Center for the Environmental Implications of NanoTechnology

CEINT Goals

- Elucidate the fundamental *principles* that determine environmental behavior and effects of nanomaterials
- Provide guidance in assessing existing and future concerns surrounding the use of engineered nanomaterials
- Educate students and the general public regarding nanotechnology, nanoscale science, and the environment

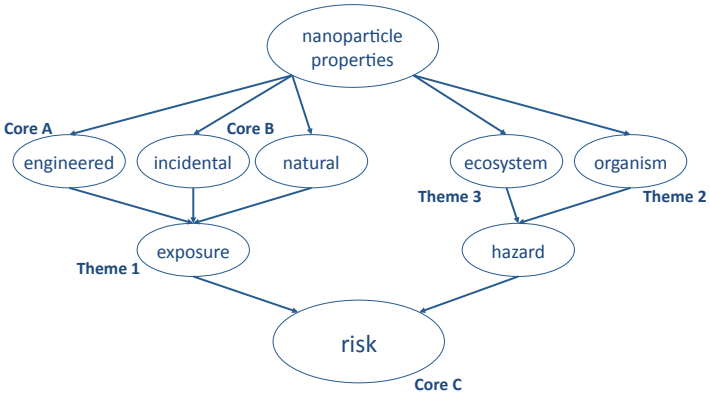


Research Focus Areas

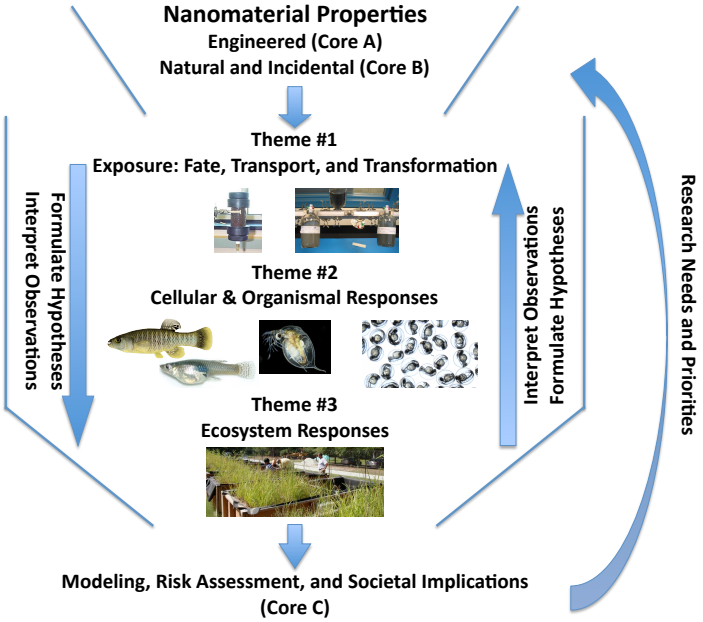
CEINT organizes a *comprehensive* effort looking at the environmental implications of nanotechnology with a focus on:

- Exposure: transport, fate, and transformation
- Effects in complex, real environments
- Risk assessment to inform decision-making

CEINT Research Themes and Cores



CEINT Research Approach



Key Findings to Date

1. Laboratory experiments in simplified systems were not sufficient to fully evaluate NM risks
   - Observed mesocosm effects from Ag NP exposure were not predicted based on findings from laboratory experiments
2. Particle coatings have a substantial role in all observed NP behaviors
   - Coatings (particularly organic macromolecular) directly impact fate, transport, toxicity, and effects observed for nanomaterials
3. NPs are ubiquitous in the environment
   - Natural NPs are found in impacted and unimpacted natural environments and in engineered environments
4. Different nanoparticle properties map to different toxicological endpoints

Significant Challenges and Approaches to Overcoming Them

1. Quantifying and speciating NMs in complex matrices at relevant environmental concentrations
    - Distinguishing between effects from dissolved and particulate Ag species
        - Working on methods to speciate Ag in complex media
    - Difficult to quantify and locate C- and Fe-based nanomaterials in natural samples because of background concentrations of these elements
        - Developing new detection methods and using advanced techniques, including synchrotron techniques (XANES and μ-XRF), and darkfield microscopy/hyperspectral imaging
    - Difficult to characterize nanoparticle macromolecular coatings
        - Developing methods to characterize the adsorbed macromolecules on NP surfaces in situ
2. Bioavailability and Toxicity
    - Separating dissolved and particulate toxicity is difficult – using multiple techniques to improve confidence in results
    - Difficult to determine uptake mechanisms (i.e. dissolved vs. particulate)-using advanced techniques, including synchrotron techniques (XANES and μ-XRF), and darkfield microscopy/hyperspectral imaging paired with various ICP-MS methods (e.g. laser ablation and flow field-flow fractionation)
3. Providing NPs of sufficient quality and consistency for Center researchers and for mesocosms
    - Avoiding commercially produced Ag NPs due to insufficient characterization and inconsistencies between batches
    - Dedicated personnel producing large quantities of materials with stringent QC protocols
    - Dramatic differences in the behavior and toxicity of the "same" materials (similar size, composition, coating) from commercial and internal sources
4. Insufficient and fragmentary information available for risk assessment
    - Reducing variance of estimates of nanoparticle production and environmental emissions
    - Using machine learning to identify nanoparticle properties responsible for toxicity and to identify the most meaningful measurements (units, distributions) of these properties
    - Prioritizing research needs based on value of information from research projects

Funding

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EPA United States Environmental Protection Agency

U.S. EPA NANOTECHNOLOGY GRANTEES MEETING

In Conjunction with the SETAC North America 31st Annual Meeting

Bridging Science with Communities

November 8 - 9, 2010 • Oregon Convention Center • Portland, OR

For more information on EPA's Nanotechnology Research Program, go to:

www.epa.gov/nanoscience
www.epa.gov/ncer/nano
www.epa.gov/ncer/nano/publications/nano_strategy_june2009.pdf

For more information on the National Science Foundation's Nanotechnology Programs, go to:

http://www.nsf.gov/crssprgm/nano/

For more information on the National Nanotechnology Initiative and other Federal Agencies, go to:

http://www.nano.gov
http://www.nano.gov/html/about/nniparticipants.html

EPA
United States
Environmental Protection
Agency

U.S. EPA Nanotechnology Grantees Meeting

Monday, November 8, and Tuesday, November 9, 2010

In Conjunction With
SETAC North America 31st Annual Meeting
Bridging Science With Communities

Where:

Oregon Convention Center
777 NE Martin Luther King, Jr. Blvd.
Rooms D135 and D136
Portland, OR 97232

Web Site:

http://www.scgcorp.com/nano2010

Meeting Contacts:

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The U.S. Environmental Protection Agency (EPA) Nanotechnology Grantees meeting will provide a forum for EPA-funded researchers to share their findings, problems, solutions and project plans, and to discuss strategies for addressing issues of common concern. The research focuses on what happens to nanoparticles and what impacts on aquatic organisms the particles have when they enter water environments.

This year, the EPA will hold its Nanotechnology Grantees Meeting in conjunction with the Society of Environmental Toxicology and Chemistry's (SETAC) North America 31st Annual Meeting, *Bridging Science With Communities*. Participants in the SETAC meeting and all others are welcome to attend.

Please register to attend.

To register, while space is available, go to:
http://www.scgcorp.com/nano2010/registration.asp