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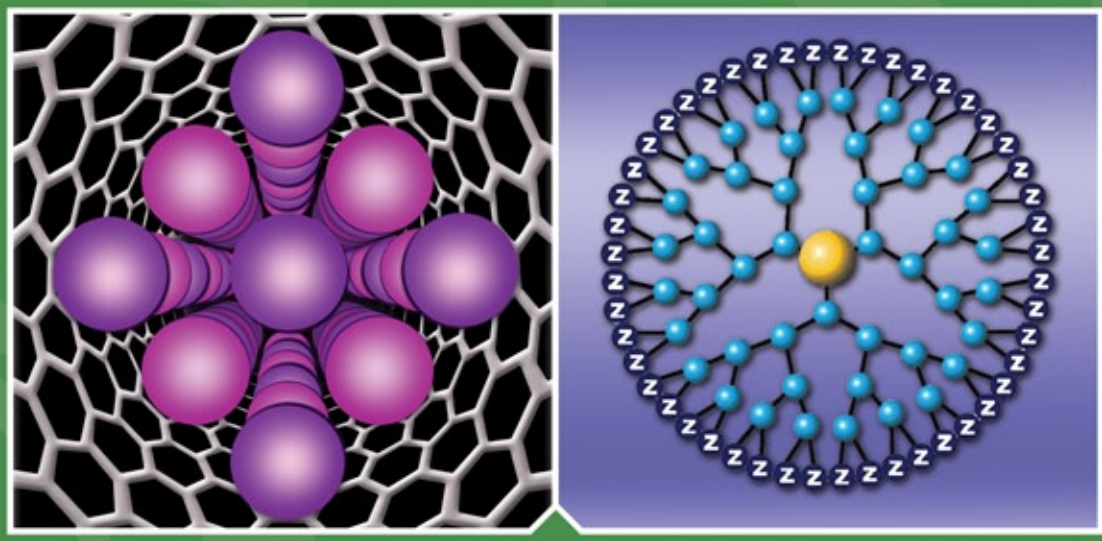

National Center for Environmental Research
Science To Achieve Results (STAR) Research Program

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Andrij Holian
Gi Soo Kang
David Pui
Tian Xia

Abstracts Taken From the EPA Web Site

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Yongsheng Chen
Mamadou Diallo
John Fortner
Terry Gordon
Patricia Heiden
Yan Jin
Rebecca Klaper
Gregory Mayer
Ashok Mulchandani
Elijah Petersen
Robert Tanguay
Chris Theodorakis
Paul Westerhoff
Xin-Rui Xia

Abstracts From the Meeting Last Year

David Barber
Peter Vikesland

11:00 a.m. Wednesday, November 19, 2008

Novel Supported Materials for Targeted Remediation of Chlorinated Compounds

Jingjing Zhan, Tonghua Zheng, Gerhard Piringer, Yunfeng Lu, Gary McPherson, and Vijay John
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Nanoscale zero-valent iron (ZVI) particles are a preferred option for the reductive dehalogenation of trichloroethylene (TCE). However, it is difficult to transport these particles to the source of contamination due to aggregation. This study describes a novel approach to the preparation of ZVI nanoparticles that are efficiently and effectively transported to contaminant sites. The technology developed involves the encapsulation of ZVI nanoparticles in porous sub-micron silica spheres that are easily functionalized with alkyl groups. These composite particles have the following characteristics: (1) they are in the optimal size range for transport through sediments; (2) dissolved TCE adsorbs to the organic groups thereby bringing tremendously increasing contaminant concentration near the ZVI sites; (3) they are reactive as access to the ZVI particles is possible; (4) when they reach bulk TCE sites, the alkyl groups extend out to stabilize the particles in the TCE bulk phase or at the water-TCE interface; and (5) the materials are environmentally benign. This research has demonstrated these concepts extensively through reactivity studies and column transport, capillary, and microcapillary transport studies. These iron/silica aerosol particles with controlled surface properties also have the potential to be applied efficiently for *in situ* remediation and permeable reactive barriers construction.

In extensions of the work, the researchers have shown that these particles function effectively as reactive adsorbents for TCE. This work will describe the synthesis of such composite nanoscale materials through an aerosol-assisted method and through solution methods to illustrate the versatility and ease of materials synthesis, scale up, and application. The research also will describe the development of carbon submicron particles that serve as supports for zerovalent iron with optimal transport and reactivity characteristics.

EPA Grant Number: GR832374

11:20 a.m. Wednesday, November 19, 2008

Synthesis and Application of a New Class of Stabilized Nanoscale Iron Particles for Rapid Destruction of Chlorinated Hydrocarbons in Soil and Groundwater

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Auburn University, Auburn, AL

The overall goal of this research project is to develop an *in situ* remediation technology using a new class of stabilized iron-based nanoparticles for the rapid destruction of chlorinated hydrocarbons in soil and groundwater. The specific objectives are to: (1) synthesize a new class of stabilized iron-based nanoparticles using low-cost and "green" stabilizers such as starch and cellulose; (2) test the stabilized nanoparticles for dechlorination of select contaminants (tetrachloro-ethylene, trichloroethylene (TCE), and polychlorinated biphenyls) in soil and groundwater; and (3) test the feasibility of an *in situ* remediation process that is based on the nanoparticles.

Building on the researchers' prior success in synthesizing cellulose-stabilized Fe-Pd nanoparticles of controlled size, the work in this stage focused on studying transport of the nanoparticles in porous media, testing the effectiveness of the nanoparticles for degradation of TCE sorbed in soils, and carrying out a pilot test at a Northern Alabama site to test the deliverability and effectiveness of stabilized Fe-Pd nanoparticles. Results revealed that the cellulose-stabilized nanoparticles (18.1 ± 2.5 nm) are highly mobile through four model porous media: coarse glass, fine glass, fine sand, and a loamy sand soil. The transport data can be interpreted using both classical filtration theory and a modified convection-dispersion equation with a first-order removal rate law. At full breakthrough, a constant concentration plateau (C/C_0) is reached, ranging from 0.99 for the glass beads to 0.69 for the soil. Although Brownian diffusion is the predominant mechanism for particle removal in all cases, gravitational sedimentation also plays an important role, accounting for 30 percent of the contact efficiency for the coarse glass beads and 6.7 percent for the soil. The attachment efficiency for CMC-Fe was found to be 1 to 2 orders of magnitude lower than reported for other surface-modified ZVI nanoparticles. The particle removal and travel distance are strongly dependent on interstitial flow velocity. Simulation results indicate that once delivered, nearly all nanoparticles are removed by soil matrix within 16 cm at a groundwater flow rate of 0.1 m/day. For the first time, this work demonstrated that the stabilized Fe-Pd nanoparticles can *in situ* effectively degrade TCE in soil pores. When treated with 120 mL (10 pore volumes) of a stabilized Fe/Pd suspension (Fe = 0.5 g/L, Pd/Fe = 0.1 wt%), greater than 38 percent of TCE contained in a fine sand column was completely dechlorinated. The investigators also observed that addition of surfactant may enhance or inhibit the dechlorination by the nanoparticles depending on the content of leachable soil organic matter. Long-term pilot tests confirmed the superb soil deliverability of the stabilized nanoparticles under field conditions. Following two consecutive injections of approximately 300 gallons (150 gallons each) of a Fe-Pd suspension (Fe = 0.5 g/L, Pd = 1% of Fe) into a heavily contaminated aquifer, the levels of PCE and TCE in two monitoring wells were consistently lowered by less than 85 percent for nearly 600 days, and to a lesser extent, PCBS, DCE, and VC concentrations also were lowered. The results also suggest that the injection of the stabilized nanoparticles induced and enhanced long-term biological dechlorination of various chlorinated solvents.

EPA Grant Number: GR832373

11:40 a.m. Wednesday, November 19, 2008

Nanoparticle Stability in Natural Waters and Its Implication for Metal Toxicity to Water Column and Benthic Organisms

James Ranville
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The overall goal of this project is to determine the potential ecotoxicological implications of nanoparticles (NPs), in particular metal-containing quantum dots (QDs). More specifically, this research is investigating the stability of QDs in surface waters as well as the relative aquatic toxicity of QDs compared to their constituent metals. The research focuses primarily on CdSe/ZnS QDs because of the high toxicity of Cd and Zn to aquatic species.

The researchers' approach is to perform acute and chronic toxicity testing of QDs using *Daphnia magna*. During these tests, the stability of the QDs was monitored using fluorescence and ICP-MS analysis of 0.02 μm and 0.003 μm filtrates. Several novel methods for QD detection and characterization also are being examined. QD uptake and distribution in *D. Magna* is being investigated by synchrotron XRF using the Brookhaven NLS.

Toxicity of QD was found to be influenced by QD size and surface coating. Comparison of QD toxicity to dissolved Cd and Zn found similar levels of toxicity, suggesting there is no significant enhanced toxicity of NPs over their constituent metals. Surface coating affected the short-term (48 hour) rate of dissolution of the QDs, with a non-ionic polymer (PEO) coated QD being more stable than an anionic (MUA) polymer-coated QD. In long-term (3 month) stability tests, both types of QDs were observed to degrade; however, the differences between surface coatings were still observed.

The significance of the initial results is that although toxicity due to QDs is seen, the level of the effect is not too dissimilar to what is seen for dissolved metals. This could suggest that risk assessments for dissolved metals could be applied to metal-containing NPs. Furthermore, under oxic conditions, the QDs appear to dissolve on the month time scale, suggesting they will not persist in the aquatic environment.

Future work on stability will include examination of aggregation of NPs with natural colloids under variable water chemistry conditions. Non-lethal toxicity tests (feeding and reproduction) will be performed with *D. magna*. Finally, acute and chronic tests on benthic organisms will be conducted.

EPA Grant Number: R833324

1:20 p.m. Wednesday, November 19, 2008

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

Gregory V. Lowry
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Nanomaterials such as zerovalent iron (nZVI) are used for groundwater remediation. Polyelectrolyte surface coatings are used to inhibit nZVI aggregation and enhance the transport of them in the subsurface. The polyelectrolyte coating also may affect the interaction of the particles with soil bacteria, and hence their potential toxicity. This study: (1) measured the rate and extent of desorption of polyelectrolyte coatings used to stabilize nZVI, including polyaspartate, carboxymethyl cellulose, and polystyrene sulfonate; and (2) determined the effect of polymer coatings and the oxidation of Fe^0 on the toxicity of nZVI to *Escherichia coli* under either aerobic or anaerobic conditions. Desorption of polyelectrolyte was very slow, with less than 30 wt percent of each polyelectrolyte desorbed after 4 months. The higher molecular weight polyelectrolyte had a greater adsorbed mass and a slower desorption rate for PAP and CMC. The nZVI mobility in sand columns after 8 months of desorption was similar to freshly modified nZVI, and significantly greater than unmodified nZVI aged for the same time under identical conditions. Based on these results, polyelectrolyte-modified nanoparticles will remain more mobile than their unmodified counterparts even after aging. This long-term mobility indicates a potential to reach sensitive receptors in the environment. However, coatings dramatically decreased the toxicity of nZVI to *E. coli*. Bare nZVI under anoxic conditions caused a log 3 inactivation of *E. coli* cells within 1 hour at 100 mg/L particle concentration. Polymer-coated particles with the same Fe^0 content were not toxic. Oxidized particles without Fe^0 also were not toxic to *E. coli*, indicating that redox activity correlated with toxicity. Because the coatings do not readily desorb, the potential for surface-modified nZVI toxicity will remain as that of coated nZVI, and the oxidation of nZVI in the subsurface by aging or by the interaction with DNAPL will further decrease the bactericidal effect.

EPA Grant Number: R833326

1:40 p.m. Wednesday, November 19, 2008

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

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[2]Savannah River National Laboratory, Aiken, SC

Risk from exposure to manufactured nanoparticles in terrestrial food webs depends on their propensity for uptake and retention by detritivorous soil organisms and subsequent trophic transfer to higher trophic levels as well as inherent particle toxicity. The overall objectives of this research are to: (1) investigate the relative roles of particle size and chemical composition in a series of nanosized metal particles (specifically Cu, Ag, Au) in determining soil bioavailability and oral uptake in a model soil detritivore; (2) elucidate mechanisms governing gastrointestinal uptake, tissue distribution, retention, and trophic transfer of nano-sized Cu, Ag, and Au along a simulated terrestrial food chain; and (3) investigate interactions among size and chemical composition of noble metal nanoparticles in determining bioavailability and toxic mode of action. Thus far, we have demonstrated the size-dependent uptake of Au, Ag and Cu nanoparticles in the earthworm *Eisenia fetida* from simulated soils. Bioaccumulation factors differed between exposure to metals as nanoparticles and equivalent concentrations of metal salts. The particles were absorbed from soil, taken up into internal tissues in the earthworms, and penetrated cell membranes as demonstrated by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), bulk ICP-MS analyses, synchrotron-based x-ray microanalysis, and transmission electron microscopy (TEM). Some evidence of increased mortality and decreased reproductive success associated with exposure to Au and Ag was observed. The research also examined changes in expression of genes related to oxidative stress and metal homeostasis. Although no significant differences from controls in expression of genes related to oxidative stress were observed, there were significant changes in expression of metallothionein as a result of exposure to Cu and Ag nanoparticles. The next phase of this research will investigate the kinetics of uptake and elimination of metal nanoparticles in earthworms as well as trophic transfer of nanomaterials along a simulated food chain consisting of soil, earthworms, and bullfrogs.

EPA Grant Number: R833335

2:00 p.m. Wednesday, November 19, 2008

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

Paul M. Bertsch[1], Brian Jackson[2], Andrew L. Neal[3], Phillip Williams[4], Travis Glenn[4], Nadine J. Kabengi[1], Benjamin Neely[5], Hongbo Ma[4], Jason M. Unrine[1], Pamela J. Morris[5], and Arthur Grider[6]
[1]Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY;
[2]Trace Element Analysis Core Laboratory, Dartmouth College, Hanover, NH;
[3]Biotechnology and Biological Sciences Research Council, Rothamsted Research, Harpenden, Hertfordshire, United Kingdom; [4]Department of Environmental Health Science, University of Georgia, Athens, GA; [5]Marine Biomedicine and Environmental Sciences Center, Medical University of South Carolina, Charleston, SC; [6]College of Family and Consumer Sciences, University of Georgia, Athens, GA

Decomposers and detritivores are central players relevant to potential ecological risks associated with the release of manufactured nanomaterials to the environment due to their intimate contact with soil and because they are at the base of the food chain. Key processes of interest include the role of ecological receptors on the uptake, transformation, and transfer from one trophic level to the next, as well as the lethal and sub-lethal toxicity endpoints of metal and metal oxide nanomaterials referenced to the dissolved ionic form of the metal.

The overall objectives of this research are to evaluate: (1) the bioavailability and toxicity of manufactured nanoparticles (ZnO-np) as a function of particle size to the model bacteria, *Burkholderia vietnamiensis* $PR1_{301}$ and the model detritivore *Caenorhabditis elegans* as referenced against aqueous Zn^{2+}; (2) the ability of manufactured ZnO-np to be transferred from one trophic level to the next as assessed in the simple food chain consisting of pre-exposed *PR1* and *C. elegans*; and (3) the synergistic or antagonistic effects of manufactured ZnO-np on the toxicity of Cu^{2+} to *PR1* and *C. elegans.* These three overall objectives are being approached in the context of the following four hypotheses:

Hypothesis 1: The bioavailability and toxicity of manufactured ZnO-np increases with decreasing particle size (i.e., 2 nm vs. 80 nm).
Hypothesis 2: The toxicity of ZnO-np to *PR1* and *C. elegans* is lower than an equivalent concentration of dissolved Zn^{2+}.
Hypothesis 3: The bioavailability and toxicity of ZnO-np introduced via trophic transfer differs from direct exposure.
Hypothesis 4: ZnO-np alter the bioavailability and toxicity of dissolved metals.

The first 2+ years of the project have been focused on the following activities:

(1) Characterization of the ZnO-np under physicochemical conditions representative of the exposure experiments (Kabengi et al., 2008. Electron beam interaction induces growth transformation in manufactured ZnO nanoparticles. *Microscopy and Microanalysis* [in revision]).

(2) Bioavailability and toxicity of ZnO-np to *B. vietnamiensis* $PR1_{301}$ and *C. elegans* as referenced to dissolved Zn^{2+}, including spatial analysis of Zn in tissues of *C. elegans* (Unrine et al., 2008. Bioavailability, trophic transfer, and toxicity of manufactured metal and metal oxide nanoparticles in terrestrial environments. In: Vicki H. Grassian (ed.). *Nanoscience and Nanotechnology*. John Wiley and Sons; Ma et al., 2008. Bioavailability and toxicity of manufactured ZnO nanoparticles in the nematode *Caenorhabditis elegans. Environmental Toxicology and Chemistry* [in press]; Neely et al., 2009. Cytotoxicity of engineered ZnO nanoparticles to *Burkholderia vietnamiensis* $PR1_{301}$: comparison to Zn^{2+} and the effects of counter-ion utilization. *Environmental Science and Technology* [in review]).

(3) Expanding research based on initial results to include the model earthworm *Eisenia fetida* and an acetate utilizer metal sensitive bacteria *Cupriavidus Necator* (two manuscripts in preparation).

(4) Initiating experiments on the trophic transfer of ZnO-np from pre-exposed bacteria to nematodes.

(5) Examination of Cu^{2+} toxicity to *C. elegans* in the presence and absence of ZnO-np.

Characterization studies have revealed that acetate used in the synthesis and stabilization of the 2 nm ZnO-np inhibits surface reactivity through the passivation of surface sites. The removal of acetate leads to aggregation of the ZnO-np primary particles but promotes greater surface reactivity. The 80 nm particles, which are not synthesized in a high acetate background, are far more difficult to stabilize but have greater surface reactivity. The results of TEM characterization of the 2 nm ZnO-np has revealed that particle growth is induced in the e-beam due to acetate degradation, leading to anomalous size estimates of primary particles (4-8 nm) compared to dynamic laser light scattering (1-2 nm). Acetate utilization (as a C source) also was demonstrated in microbial exposure experiments and the loss of acetate resulted in the destabilization/ aggregation/agglomeration of primary particles.

In ZnO-exposure experiments, it has been demonstrated that the EC_{50} for lethality, behavior, and reproduction to the nematode model *C. elegans* was not different from dissolved Zn^{2+} for the 2 nm ZnO (s-ZnO-np) particles, whereas there was no observed toxicity for the 80 nm (l-ZnO-np) or 1.2 µm ZnO (bulk-ZnO) particles. Although no differences in the three toxicity endpoints were observed between dissolved Zn^{2+} and the s-ZnO-np, there were differences in the spatial distribution of Zn and gene expression (metallothionien-2) in exposed organisms as elucidated by micro-X-ray fluorescence spectroscopy and epifluorescence microscopy. Likewise, the growth rate of the bacterial models *B. vietnamiensis* $PR1_{301}$ and *C. necator* displayed no difference between the s-ZnO-np and Zn^{2+}. However, higher acetate utilization rates were observed for *C. necator* in the presence of Zn^{2+} compared to s-ZnO-np, and there was evidence for greater membrane damage for the s-ZnO-np exposed bacteria. This suggests greater Zn bioavailability from Zn^{2+} compared to s-ZnO-np and different toxicity mechanisms. Ongoing work on protein expression in *C. necator* has provided evidence for differences in the up- and downregulation of specific proteins between the s-ZnO-np and the Zn^{2+} exposed organisms. Identification of key proteins exhibiting differential expression is underway.

Experiments designed to examine the synergistic/antagonistic effects of s-ZnO-np on metal toxicity have provided evidence that s-ZnO-np reduce Cu^{2+} toxicity at a Zn concentration above 100 mg L^{-1} as compared to Zn^{2+}. Feeding s-ZnO-np exposed bacteria to nematodes has not provided evidence for significant trophic transfer of the s-ZnO-np; however, this may be more related to experimental challenges using GFP expression as the primary assessment endpoint.

The results of these studies suggest that the size of ZnO-np is a critical parameter controlling bioavailability and observed effects using several ecologically relevant endpoints to decomposers and detritivores, with smaller particles being more bioavailable along with concomitant observed effects. The results also indicate that, although the observed effects of ecologically relevant endpoints (growth, behavior, reproduction) between s-ZnO-np and Zn^{2+} expressed as a common total Zn concentration are not significant, there are differences in Zn distributions within organisms (nematodes and earthworms) as well as in gene and protein expression (nematodes and bacteria). This suggests that there may be differences in the mechanisms of toxicity between s-ZnO-np and Zn^{2+}.

EPA Grant Number: R832530

2:20 p.m. Wednesday, November 19, 2008

Bioavailability and Fates of CdSe and TiO_2 Nanoparticles in Eukaryotes and Bacteria

Patricia A. Holden[1], Galen Stucky[2], and Jay L. Nadeau[3]
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Semiconductor nanocrystals differ in important ways from bulk semiconductor materials. Their increased band gap means that they function as strong oxidizing and/or reducing agents, and their small size allows them to pass into living cells. Conjugation of biomolecules to the crystal surface can alter any or all of these properties. In preliminary experiments, we observed that only bioconjugated CdSe quantum dots are taken up by bacteria and eukaryotic cells. Intracellular fluorescence varies, apparently by electron transfer-mediated quenching and nanoparticle breakdown. Bare quantum dots are as toxic to growing bacteria in part due to Cd^{2+}, implying possible extracellular breakdown, but subsequent fates and toxicity relationships are unknown. Particle size dependencies are implied, but insufficiently understood for use in risk analysis. A systematic inquiry into size- and chemistry-dependent uptake and fate processes is needed. This research is focused on quantifying cellular-scale processes that affect nanoparticle entry, stability, and toxicity for a variety of bacterial and eukaryotic cells. This project is concentrating on two nanoparticles: CdSe whose metals are toxic, and TiO_2 whose toxicity arises solely from its size and electron transfer activity. Both short-term labeling and longer term growth experiments are being performed to quantify particle entry into cells and toxicity; also under study is the energy transfer between nanoparticles and energized membranes as a mechanism. The relative importance of near-cell breakdown, whole-particle electron scavanging, and intracellular particle reformation as fates are being quantified. This project also is addressing how nanoparticles and cells may cooperate in transmembrane transport as well as toxicity. This research is focused on predicting cellular-scale exposure and toxicity for bacteria and eukaryotes in soil and water.

Nanotechnology Research Grants Investigating Environmental and Human Health Effects of Manufactured Nanomaterials: a Joint Research Solicitation—EPA, NSF, NIOSH, NIEHS, EPA-G2006-STAR-F2.

EPA Grant Number: R833323

3:20 p.m. Wednesday, November 19, 2008

Microbial Impacts of Engineered Nanoparticles

Shaily Mahendra, Delina Y. Lyon, Dong Li, Mark Wiesner, and Pedro J.J. Alvarez
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The rapid growth in production and use of nanomaterials in commercial products has raised concerns about their beneficial and harmful effects on the environment. An evaluation of potential environmental impacts needs to consider how they will interact with microorganisms, which are at the foundation of all known ecosystems and participate in primary production, nutrient cycling, and waste decomposition. They also serve as good indicators of the potential effects on higher organisms. In this research, representatives of two classes of nanomaterials, fullerenes, and metal-containing TiO_2, ZnO, and Fe(0), were evaluated for their effects on bacteria and viruses.

Buckminsterfullerene water suspensions (nC_{60}) exerted potent antimicrobial activity similar to that of nano-silver. The antimicrobial activity of nano-sized ZnO, TiO_2, Fe(0), and SiO_2 was significantly lower. Multiple samples of nC_{60} prepared using various methods caused time-dependent and dose-dependent antibacterial activity towards bacterial pure cultures. However, the effect of nC_{60} on soil microbial communities was negligible. Although neither sunlight nor oxygen eliminated the long-term antibacterial activity of nC_{60}, its toxicity was increased by smaller particle size in a manner disproportionate to the increase in surface area to volume ratio. However, toxicity was significantly mitigated by salts, which promoted coagulation and precipitation. Natural organic matter present in soil effectively sorbed nC_{60} and reduced its bioavailability and, consequently, its antibacterial activity. This indicates the need to consider nC_{60} interactions with common constituents in environmental matrices to obtain representative results of potential impacts. Although eukaryotic cell damage by fullerenes has been attributed to reactive oxygen species (ROS), no evidence of ROS-mediated damage in bacteria killed by nC_{60} was observed in this study. Instead, flow cytometry studies with dyes that assess membrane potential and reductase activity suggested that nC_{60} acts as a direct oxidant that interferes with energy transduction. Furthermore, the colorimetric methods used to evaluate ROS production and damage were confounded by interactions between nC_{60} and the reagents that yield false positives, revealing a need to re-evaluate previous studies that concluded that toxicity is due to ROS damage. In contrast, polyhydroxylated fullerene (fullerol) produced ROS through UV photosensitization. Inactivation of MS2 bacteriophage increased in the presence of fullerol-derived ROS as compared with UV-A illumination alone. These results suggest a potential for fullerenes to impact microbial populations in both natural and engineered systems.

In toto, this research identifies the mechanisms of antibacterial activity of nC_{60} and antiviral mechanisms of fullerol, and provides a methodology by which the potential environmental impacts of other nanomaterials can be evaluated.

EPA Grant Number: R832534

3:40 p.m. Wednesday, November 19, 2008

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

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

This work is supported by National Science Foundation grant BES-0540920.

4:00 p.m. Wednesday, November 19, 2008

Characterization of the Potential Toxicity of Metal Nanoparticles in Marine Ecosystems Using Oysters

Amy H. Ringwood[1], Melissa McCarthy[1], David Carroll[2], and Joel Berry[2]
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The fate and effects of nanoparticles on aquatic organisms are important environmental concerns that must be addressed as the production and uses of nanoparticles continue to increase. The purpose of these ongoing studies is to characterize the toxicity of various metal nanoparticle preparations on oysters, *Crassostrea virginica*, a common estuarine species. As filter-feeders, oysters are a very valuable model species for characterizing nanoparticle bioavailability and interactions with basic cellular processes. This research project is designed to address a number of important issues regarding metal nanoparticle toxicity in marine organisms (e.g., morphological changes of metal nanoparticles in seawater, adverse effects on fundamental cellular responses related to lysosomal integrity, effects on antioxidants and oxidative damage, the relative sensitivity of different life history stages, and cellular and tissue accumulation patterns). The results of these studies will be used to evaluate the following overall hypotheses:

H_1: Metal nanoparticle morphology and size are important determinants of toxicity.
H_2: Embryonic and larval stages are more sensitive than adult forms.
H_3: Oxidative damage is a common mechanism of cellular toxicity.

The results of recent studies with silver nanoparticles, approximately 15-20 nm seeds in which laboratory exposure studies were conducted with adult and embryonic oysters, are presented here. The potential for hepatotoxicity was evaluated using a lysosomal destabilization assay, and lipid peroxidation assays were used to assess oxidative damage in both gill and hepatopancreas tissues. For the embryo assays, newly fertilized oyster embryos were exposed to the nanoparticles and the percent normal development after 48 hours was assessed. These studies were used to address issues such as the relative sensitivity of embryos compared to adults, tissue distribution, and cellular accumulation and effects. Generally, embryos tended to be slightly less sensitive than adults, and hepatopancreas tissues were more sensitive than gills. Atomic absorption spectrometry was used to verify the accumulation of the nanoparticles. Significant relationships were observed between tissue Ag levels and toxicity as well as with exposure concentrations. These kinds of basic studies are essential for addressing the potential impacts of nanoengineered particles on fundamental cellular processes as well as aquatic organisms.

EPA Grant Number: RD833337

4:20 p.m. Wednesday, November 19, 2008

Pulmonary and Systemic Inhalation Toxicity of Multi-Walled and Single-Walled Carbon Nanotubes

Jacob McDonald, Leah Mitchell, Scott Burchiel, Randy Vander Wal, and Andrew Giggliotti
Lovelace Respiratory Research Institute, Albuquerque, NM

Inhalation of multi-walled carbon nanotubes (MWCNTs) and single-walled carbon nanotubes (SWCNT) at particle concentrations up to 1 mg/m^3 did not result in significant lung inflammation or tissue damage, but caused systemic immune function alterations. C57BL/6 adult (10-12 week) male mice were exposed by whole-body inhalation to control air or 0.3 or 1 mg/m^3 respirable aggregates of MWCNTs or SWCNTs for 14 days, with either immediate sacrifice or sacrifice of a recovery group 30 days after the end of exposure. Histopathology of lungs from exposed animals showed alveolar macrophages containing significant amounts of black particles; however, there was minimal to no inflammation or tissue damage observed. Bronchial alveolar lavage fluid also demonstrated particle-laden macrophages; however, white blood cell counts were not increased compared to controls. Both types of carbon nanotubes caused systemic immunosuppression after 14 days and after recovery. Immunosuppression was characterized by reduced T-cell-dependent antibody response to sheep erythrocytes as well as T-cell proliferative ability in the presence of the mitogen Concanavalin A (Con A).

EPA Grant Number: R832527

4:40 p.m. Wednesday, November 19, 2008

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

Christopher Theodorakis[1], Elizabeth Carraway[2], and George Cobb[3]
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The objectives of this research project are to determine the environmental hazard associated with selected metal oxide nanoparticles (Fe_2O_3, ZnO, CuO, and TiO_2) in terms of acute and chronic toxicity to fathead minnows (*Pimephase promelas*) and the African clawed frog (*Xenopus laevis*). The hypotheses are that nanoparticle exposure will affect the survival, growth, development, egg hatchability, and metamorphosis of these organisms in a dose-dependent fashion, and differences in relative toxicity (LC50, EC50, NOEC, LOEC) of these nanoparticles coincide with the relative toxicity of their soluble salts or oxides.

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

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

EPA Grant Number: R832842

8:40 a.m. Thursday, November 20, 2008

Conducting-Polymer Nanowire Immunosensor Arrays for Microbial Pathogens

Ashok Mulchandani, Wilfred Chen, Nosang V. Myung, and Marylynn V. Yates
University of California at Riverside, Riverside, CA

A promising approach for the direct (label-free) electrical detection of biological macromolecules uses one-dimensional (1-D) nanostructures such as nanowires and nanotubes, configured as field-effect transistors that change conductance upon binding of charged macromolecules to receptors linked to the device surfaces. Combined with simple, rapid and label-free detection, these nanosensors also are attractive due to the small size, low power requirement, and most of all, the possibility of developing high-density arrays for simultaneous analyses of multiple species. Although current nanosensors based on carbon nanotubes and silicon nanowires has elucidated the power of 1-D nanostructures as biosensors, they have low throughput and limited controllability and are unattractive for fabrication of high-density sensor arrays. More importantly, surface modifications, typically required to incorporate specific antibodies, have to be performed post-synthesis and post-assembly, limiting our ability to address individually each nanostructured sensing element with the desired specificity.

The overall objective of this research project is to develop a novel technique for the facile fabrication of bioreceptor (antibody) -functionalized nanowires that are individually addressable and scalable to high-density biosensor arrays, and to demonstrate its application for label-free, real-time, rapid, sensitive, and cost-effective detection of multiple pathogens in water. Electropolymerization of conducting polymers between two contact electrodes is a versatile method for fabricating nanowire biosensor arrays with the required controllability. The benign conditions of electropolymerization enable the sequential deposition of conducting-polymer nanowires with embedded antibodies onto a patterned electrode platform, providing a revolutionary route to create a "truly" high-density and individually addressable nanowire biosensor arrays. The nanowire immunosensor arrays utility will be used to simultaneously quantify three important model pathogens, poliovirus, hepatitis A virus (HAV), and rotarvirus.

The researchers will use their recently reported (Ramanathan et al., 2004) simple yet powerful facile technique of electrochemical polymerization of biomolecule-friendly conducting polymers, such as polypyrrole, in prefabricated channels of tailor-made aspect ratio between two contact electrodes at site-specific positions to synthesize nanowires of tailor-made properties for fabricating individually addressable high-density nanowire biosensor arrays. Detection of pathogens will be achieved by the extremely sensitive modulation of the electrical conductance of the nanowires brought about by the change in the electrostatic charges from binding of the pathogens to the antibodies. Effects of monomer concentration, dopant type and concentration, aspect ratio, and electrochemical polymerization mode on the sensitivity, selectivity, and durability of poliovirus, HAV, and rotavirus antibodies-functionalized polypyrrole nanowires as label-free bioaffinity sensors of these important model viral pathogens in water will be investigated to establish optimum synthesis conditions of biomolecules-functionalized nanowires to successfully realize our innovation to practice.

The lack of methods for routine rapid and sensitive detection and quantification of specific pathogens has limited the amount of information available on their occurrence in drinking water and other environmental samples. The nanowire biosensor arrays developed in this study would improve the ability to provide rapid and ultrasensitive quantification of pathogens. The end results of this research will be a nanoelectronic sensor for rapid, sensitive, selective, and reliable detection of multiple important viruses simultaneously that will be useful not only for water and environmental monitoring but also homeland security, health care, and food safety. Additionally, the technique of hierarchical assembly of high-density nanowire arrays developed in this research also will find application in the rapidly advancing fields of proteomics and genomics.

EPA Grant Number: GR832375

9:00 a.m. Thursday, November 20, 2008

Carbon Nanotubes: Environmental Dispersion States, Transport, Fate, and Bioavailability

Walter J. Weber and Qingguo Huang
University of Michigan, Ann Arbor, MI

The overarching goal of this research project is to evaluate factors that control the environmental dispersion states, transport, fate, and bioavailability of carbon nanotubes, thereby providing a foundation for human and ecological risk assessment. Specifically, single-walled and multi-walled 14C-labeled carbon nanotubes will be synthesized, purified, and characterized using techniques previously established in our laboratory. These radio-labeled materials will then be used to systematically investigate: (1) the dispersion states of these nanomaterials under typical environmental conditions, (2) their transport behaviors within and through a series of different types of soil and sediment media, and (3) their bioavailability to selected critical aquatic and terrestrial food-chain organisms.

The researchers have developed and refined a means for producing single-walled and multi-walled 14C-labeled carbon nanotubes by using radioactively labeled methane as a feedstock for the synthesis of carbon nanotubes via chemical vapor deposition methods. Carbon nanotubes will be mixed with natural organic matter and subjected to a wide range of aquatic conditions (i.e., pH, ionic strength, etc.) to elucidate their dispersion state in natural environments. Carbon nanotube transport through a series of soil and sediment sorbent materials having different geochemical properties will be tested in dynamic column studies, and relationships among the breakthrough behaviors and the properties of both the nanotubes and the geosorbent materials will be analyzed. Carbon nanotube bioavailability to a fish, an aquatic worm, and an earthworm will be tested in lab-scale systems to examine the potentials of these nanomaterials to enter food chains in different environments, and factors controlling ecological bioavailability will be determined.

The proposed study will: (1) provide fundamental information regarding carbon nanotube dispersion states, transport, fate, and bioavailability in different environmental systems; (2) identify factors controlling these environmental behaviors; and (3) establish deterministic models capable of predicting behaviors under different environmental conditions. This information is critically needed by the U.S. EPA and the research community for rigorous assessments of the environmental fate, transport, and ecological risks of carbon nanotubes in various soil/water/sediment systems.

EPA Grant Number: R833321

9:40 a.m. Thursday, November 20, 2008

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

Peter J. Vikesland, Linsey C. Marr, Joerg Jinschek, Laura K. Duncan, Behnoush Yeganeh, and Xiaojun Chang
Department of Civil and Environmental Engineering, Virginia Polytechnic Institute, Blacksburg, VA

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

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

NSF Award Number: 0537117

10:00 a.m. Thursday, November 20, 2008

Transport and Retention of Nanoscale Fullerene Aggregates in Quartz Sands and Natural Soils

Kurt D. Pennell[1,2], Joseph B. Hughes[1], Linda M. Abriola[3], Yonggang Wang[1], and Yusong Li[4]
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[2]Department of Neurology, Center for Neurodegenerative Disease, Emory University, Atlanta, GA;
[3]Department of Civil and Environmental Engineering, Tufts University, Medford, MA;
[4]Department of Civil Engineering, University of Nebraska-Lincoln, Lincoln, NE

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

Stable aqueous suspensions of nC_{60} aggregates were prepared by dissolving fullerene in tetrahydrofuran (THF), which was mixed with an equal volume of water, evaporated at 75°C, and sparged with N_2 gas. Batch and column experiments were performed to assess the aggregation and transport behavior of fullerene nanoparticles in water-saturated quartz sands and natural soils as a function of electrolyte concentration and species. As the electrolyte concentration was increased from 1 to 100 mM, the change in nC_{60} particle diameter was minimal in the presence of NaCl but increased by more than seven-fold in the presence of $CaCl_2$. The latter effect was attributed to the agglomeration of individual nC_{60} aggregates, consistent with a net attractive force between the nanoparticles and suppression of the electrical double layer. At low ionic strength (3.05 mM), nC_{60} aggregates were readily transported through 40 to 50 mesh Ottawa sand, appearing in the column effluent after introducing less than 1.5 pore volumes of an nC_{60} suspension, with approximately 30 percent and less than 10 percent of injected mass retained in the presence of $CaCl_2$ or NaCl, respectively. At higher ionic strength (30.05 mM) and in finer Ottawa sand (100-140 mesh), greater than 95 percent of the introduced nC_{60} particles were retained in column regardless of the electrolyte species. Approximately 50 percent of the deposited nC_{60} particles were recovered from 100 to 140 Ottawa sand after sequential introduction of de-ionized water adjusted to pH 10 and 12. These results indicate that nC_{60} transport and retention in water-saturated quartz sands is strongly dependent on electrolyte conditions, and that release of deposited nC_{60} aggregates requires substantial changes in surface charge, consistent with retention in a primary energy minimum.

Introduction of up to 65 pore volumes of nC_{60} suspensions containing 1 mM $CaCl_2$ into columns packed with either Appling soil or Webster soil resulted in 100 percent retention of the injected nC_{60} mass. Retention of nC_{60} aggregates occurred primarily within 6 cm of the column inlet, with solid phase concentrations approaching 130 μg/g. The addition of Suwannee River humic acid (20 mg/L) to the nC_{60} suspension resulted in slightly enhanced nC_{60} mobility, although effluent breakthrough was not observed. However, when nC_{60} suspensions were prepared with 1,000 mg/L polyethoxylate (20) sorbitan monooleate (Tween 80), nC_{60} aggregates were readily transported through Appling soil, with less than 40 percent of injected mass retained. These results clearly demonstrate that Appling soil and Webster soil possess a large retention capacity for nC_{60} aggregates, but that nC_{60} transport can be greatly enhanced in the presence of stabilizing agents.

A mathematical model that incorporates nonequilibrium attachment kinetics and a maximum retention capacity was utilized to simulate experimental nC_{60} effluent breakthrough curves and deposition profiles as a function of quartz sand size fraction and flow rate. Fitted maximum retention capacities (S_{max}) ranged from 0.44 to 13.99 μg/g, and were found to be correlated with normalized mass flux. The resulting correlation

provides a means to estimate S_{max} as a function of flow velocity, nanoparticle size, and grain size of the porous medium. Collision efficiency factors, estimated from fitted attachment rate coefficients, were relatively constant (ca. 0.14) over the range of conditions considered. The fitted attachment rate coefficients, however, are more than one order of magnitude larger than the theoretical collision efficiency factor computed from the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory (0.009). Subsequent analyses suggest that neither physical straining nor attraction to the secondary minimum was responsible for this discrepancy. Patch-wise surface charge heterogeneity is shown to be the likely contributor to the observed deviations from classical DLVO theory. These findings indicate that modifications to clean-bed filtration theory and consideration of surface heterogeneity are necessary to accurately predict nC_{60} transport behavior in saturated porous media.

EPA Grant Number: R832535

10:40 a.m. Thursday, November 20, 2008

Photochemical Fate of Manufactured Carbon Nanomaterials in the Aquatic Environment

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

The photochemical transformation of aqueous C_{60} clusters (nC_{60}) in sunlight (West Lafayette, IN, 86° 55' W, 40° 26' N) and lamp light (λ = 300-400 nm) has been investigated. Upon exposure to light, the brown to yellow color of nC_{60} was lost gradually and the cluster size decreased as the irradiation time increased. TOC analysis indicated that nC_{60} products/intermediates were soluble in the aqueous phase and C_{60} may have mineralized or partially mineralized. The rate of C_{60} loss in sunlight was faster for smaller clusters compared to larger clusters (i.e., $k_{obs} = 3.66 \times 10^{-2}$ h^{-1} and 1.42×10^{-2} h^{-1} for C_{60} loss from 150-nm and 500-nm nC_{60} clusters, corresponding to half-lives of 18.9 h and 40.8 h, respectively, at the same initial C_{60} concentration). Dark control samples showed no loss, confirming phototransformation as the underlying degradation process. The presence of 10 mg/L fulvic acid, changes in pH, and the preparation method of nC_{60} clusters had negligible effects on the reaction rate. Deoxygenation resulted in a decreased loss rate, indicating O_2 played a role in the phototransformation mechanism. These findings suggest that release of nC_{60} into surface waters will result in photochemical production of currently unknown intermediate compounds.

EPA Grant Number: R833340

11:00 a.m. Thursday, November 20, 2008

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

Bo Zhang[1], Min Cho[1], John D. Fortner[2], Jaesang Lee[3], Ching-Hua Huang[1], Joseph B. Hughes[1], and Jae-Hong Kim[1]
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The oxidative reactivity of THF derivatives formed during THF/nC_{60} synthesis was evaluated with indigo dye as a model compound. The results showed that the formation of previously undetected oxidizing agents during THF/nC_{60} synthesis accounted for the degradation of indigo dye by THF/nC_{60} (THF/nC_{60}/unwashed), while THF/nC_{60} after vigorous washing (THF/nC_{60}/washed) and nC_{60} prepared without the use of THF were not reactive.

γ-Butyrolactone (GBL) was detected by GC-MS in the THF/nC_{60}/unwashed as one of THF derivatives, but showed no reactivity with indigo dye. An organic peroxide was detected in the THF/nC_{60}/unwashed by HPLC, and was reactive with indigo dye. This compound also was found to account for the elevated antibacterial and bactericidal activities of THF/nC_{60}/unwashed on *Escherichia coli*. Analysis by LC/(+ESI)MS and 1H NMR showed that the detected THF peroxide was tetrahydro-2-(tetrahydrofuran-2-ylperoxy)furan. The formation of THF peroxide during the preparation of aqueous stable C_{60} aggregates provides another potential explanation for the reactivity and oxidative stress mechanisms of the THF/nC_{60} system reported in the literature, although it does not exclude the potential reactivity and toxicity of nC_{60} itself.

EPA Grant Number: R832526

11:20 a.m. Thursday, November 20, 2008

Role of Particle Agglomeration in Nanoparticle Toxicity

Terry Gordon, Lung Chi Chen, and Beverly S. Cohen
New York University, Nelson Institute of Environmental Medicine–Tuxedo, Tuxedo, NY

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

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

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

EPA Grant Number: R832528

11:40 a.m. Thursday, November 20, 2008

Potential Environmental Implications of Manufactured Nanomaterials: Toxicity, Mobility, and Nanowastes in Aquatic and Soil Systems

Jean-Claude Bonzongo, Dmitry Kopelevich, and Gabriel Bitton
University of Florida, Gainesville, FL

The potential effects of manufactured nanomaterials (MNs) were evaluated by testing the hypothesis that: "*chemical elements used in the production of MNs could lead to environmental dysfunctions due to: (1) the potential toxicity of these elements and their derivatives; (2) the small size-driven mobility of MNs through heterogeneous porous media and ultimate contamination of aquifers; (3) their toxicity to microorganisms and the resulting negative impacts on key environmental microbial-catalyzed reactions; and (4) the large surface area which would allow MNs to act as carriers/delivers of pollutants adsorbed onto them.*" To address this broad hypothesis, three well-established small-scale toxicity tests (i.e., the *Ceriodaphnia dubia* acute toxicity test, the *Pseudokirchneriella subcapitata* chronic toxicity test, and MetPLATE™) were used. In addition, studies at the system level were conducted using a combination of column and batch experiments to investigate the transport behavior of MNs in heterogeneous porous media and the interactions of MNs with microbial-catalyzed oxidation of organic matter in sediments. Finally, in addition to the above experimental work, molecular dynamics simulations were performed to investigate the potential interactions between NMs and cellular membrane components. The major findings of this research are briefly summarized as follows.

Carbon- (i.e., C_{60}, single-walled carbon nanotubes) and metal- (i.e., nanometals including *n*Ag, *n*Cu, *n*Co, *n*Ni, *n*Al and CdSe quantum dots) based nanomaterials were used in different laboratory experiments. All tested MNs showed some degree of toxicity response to either one or more of the above three microbiotests, with *n*Cu and *n*Ag being the most toxic. The use of experimental conditions that mimic likely scenarios of MNs' introduction to aquatic systems showed that toxicity response of test model organisms to MNs under such conditions would be affected by key water quality parameters such as organic matter content and solution chemistry. Column studies of SWNTs transport in heterogeneous porous soils showed that soil characteristics and the chemical composition of MN suspensions affect transport behaviors, and that the latter can be quantitatively predicted by use of mathematical models such as the convection-dispersion equation. Finally, the use of sediment slurries spiked with either each type of MNs or pollutant (i.e., mercury) bound to MNs allowed the assessment of: (1) the impact of MNs on microbially catalyzed oxidation of organic matter; and (2) the potential for Hg-bound to SiO_2-TiO_2 nanocomposites obtained from flue gas remediation studies to become available in sedimentary environments as a function of pH. Overall, these findings help shed light on the potential environmental implications of MNs. However, several questions remain unanswered, as these short-term laboratory investigations may not be able to predict the environmental fate/transport and implications of MNs on a long-term basis. On the other hand, the use of prediction modeling tools can help address the above concern.

These modeling studies were performed using a coarse-grained molecular dynamics (CGMD) model, which approximates small groups of atoms as a single united atom. So far, our modeling efforts have been limited to the interactions between carbon-based nanomaterials and cell membranes. The latter are modeled as lipid bilayers, thereby neglecting other constituents of the membrane such as membrane proteins. This model for cell membranes is consistent with the experimental indication that interaction of NMs with membrane lipids plays a dominant role in mechanisms of cytotoxicity. For model carbon-based NM (i.e., C_{60} and carbon nanotubes), we observed an extremely small barrier for the permeation of these NM into the hydrophobic interior of a lipid bilayer. On the other hand, the calculated residence time of these NM within the bilayer interior is very large, which could possibly lead to destabilizing interactions between NM and the membrane. To assess possible mechanisms of the membrane disruption by NM, we performed computational studies of physical properties of a membrane with embedded NMs. This analysis indicates that carbon-based nanoparticles do not lead to changes in the membrane bending and lipid tilt moduli (i.e., these nanoparticles do

not affect the membrane deformations). Another possible effect of NM on a cellular membrane is a change of the lateral pressure profile within the membrane, which may affect function of mechano-sensitive membrane proteins. It was observed that relatively small carbon-based nanoparticles do not alter the lateral pressure profile. This analysis is being extended to larger nanoparticles (nanotubes of larger diameter and longer length) as well as nanoparticles containing charged and/or hydrophilic groups, which may disrupt the membrane through interactions with lipid head groups.

EPA Grant Number: R832635

12:00 p.m. Thursday, November 20, 2008

Structure-Function Relationships in Engineered Nanomaterial Toxicity

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

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

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

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

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

EPA Grant Number: R832536

2:00 p.m. Thursday, November 20, 2008

Aquatic Toxicity of Carbon-Based Nanomaterials at Sediment-Water Interfaces

Joseph N. Mwangi[1], Ning Wang[3], Christopher G. Ingersoll[3], Doug K. Hardesty[3], Eric L. Brunson[3], Hao Li [2], and Baolin Deng[1]
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[2]USGS, Columbia Environmental Research Center, Columbia, MO

Carbon nanotubes (CNTs) are relatively insoluble in water and are likely to accumulate in sediments if released into the aquatic environment. The potential impacts of CNTs released into the environment are largely unknown. The objective of this study was to evaluate the potential toxicity of commercially available or modified CNTs to sediment-dwelling invertebrates. Short-term 14-d water-only tests were conducted by exposing the amphipod (*Hyalella azteca*), the midge (*Chironomus dilutus*), the oligochaete (*Lumbriculus variegatus*), and rainbow mussels (*Villosa iris*) to a thin layer of five types of CNT materials with periodic replacement of water. A 14-d whole sediment toxicity test was conducted by exposing amphipods to CNTs spiked into silica sand and Florissant soil (99:1 sediment to CNTs ratio on dry weight basis). In the water only tests, the survival of the invertebrates was significantly reduced in three as-produced CNT and not in two modified CNT samples relative to the control. The growth of some test organisms also was found significantly reduced with exposure to CNTs. The survival and growth of the amphipods in whole sediment toxicity tests for the two types of sediment were significantly reduced relative to the control. Light microscopy photographs and transmission electron microscopy (TEM) images of surviving organisms at the end of the exposures demonstrated the presence of CNTs in the gut of the amphipods, midge, and oligochaete. The CNTs appeared to smother the organisms and may interfere with their ability to feed. Other mechanisms may exist for the demonstrated toxicity such as by dissolution of toxic metals from the CNTs. Additional whole sediment tests will be conducted to determine the dose-response relationships of selected nanomaterials spiked into sediment.

EPA Grant Number: RD833316

2:20 p.m. Thursday, November 20, 2008

Aquatic Toxicity of Waste Stream Nanoparticles

Terry Gordon, Lung Chi Chen, and Isaac Wirgin
New York University, New York, NY

The objective of this study is to determine the biological consequences of nanoparticle contamination of the aquatic environment. The investigators hypothesize that there will be a particle-type dependent difference in the developmental toxicity of manufactured nanoparticles in aquatic species, and in testing this hypothesis, we will: (1) measure the differential toxicity of several types of nanoparticles in an estuarine species of fish, Atlantic tomcod; and (2) identify whether the embryo and larval stages of development of tomcod are particularly susceptible to carbon nanoparticle versus nanotube toxicity. A number of investigators have clearly demonstrated that nanoparticle toxicity in the mammalian lung is governed, in part, by particle size. The investigators' previous studies have demonstrated that freshly formed nanoparticles produce lung injury and inflammation in mice and the extent of adverse effects is influenced by particle type as well as genetic host factors. Little research has been published, however, on whether these physico-chemical properties of nanoparticles influence their toxicity in aquatic species. Thus, while a considerable data base has been established to understand the influence of physico-chemical properties of nanoparticle toxicity in a gaseous medium, it will be critical to understand the ability of various nanoparticles to produce toxicity once they have entered the waste stream and the aquatic environment. In the proposed studies, a group of particle toxicologists will collaborate with a fish toxicologist to explore the toxicity of a variety of manufactured nanoparticles in an established fish model of aquatic toxicity.

To test the hypothesis that there is a particle-type dependent difference in the aquatic toxicity of manufactured nanomaterials, the researchers will expand their preliminary results to examine the aquatic toxicity of a wide range of nanoparticles. The primary approach is to study the toxicity of particles present in nanoparticle manufacturers' waste products because they have the greatest opportunity of entering the aquatic environment. The investigators propose to study nanoparticle toxicity in tomcod fish at sensitive developmental stages: embryo and larval stages. The proposed endpoints will include: (1) basic toxicity endpoints (e.g., survival and time to hatching); (2) developmental morphology; (3) behavior (larval activity); and 4) gene expression changes.

As determined in preliminary studies, we expect that nanoparticle toxicity will be influenced by a variety of exposure conditions, including particle type (e.g., carbon toner particle vs. fullerene vs. nanotube), particle concentration, stage of manufacturing process (e.g., raw soot precursor material vs. purified final material vs. sludge waste product), and the natural composition of the aqueous medium. By careful analysis of the several endpoints included in the proposed developmental toxicity experiments, this work will provide key information regarding the toxicity of emerging nanoparticle technologies, and the data obtained in the proposed aquatic studies can be used readily for extrapolation to ambient environments. In summary, the results from this project address a number of research needs, including toxicity and exposure assessment.

EPA Grant Number: R833317

2:40 p.m. Thursday, November 20, 2008

Ecotoxicology of Fullerenes (C_{60}) in Fish

Theodore B. Henry[1,2,7], June-Woo Park[1], Shaun Ard[3], Fu-Min Menn[1], Robert N. Compton[3,4], and Gary S. Sayler[1,5,6]
[1]Center for Environmental Biotechnology; [2]Department of Forestry, Wildlife and Fisheries; [3]Department of Physics; [4]Department of Chemistry; [5]Department of Microbiology; [6]Department of Ecology and Evolutionary Biology, The University of Tennessee, Knoxville, TN; [7]Ecotoxicology and Stress Biology Research Center, University of Plymouth, Plymouth, Devon, United Kingdom

Establishing the toxicity of nanoparticles (NPs) is essential to protect human and environmental health and to guide appropriately the development of nanotechnology. The researchers' investigations involve assessment of the ecotoxicology of un-derivatized C_{60} in model fish species and attempt to link particle characteristics to toxicological effects. Larval zebrafish were exposed to the following treatments: (1) C_{60} aggregates generated by stirring and sonication (72 h) of C_{60} in water (12.5 mg C_{60}/500 mL water); (2) C_{60} aggregates generated by established methods with tetrahydrofuran (THF) vehicle; (3) THF vehicle (i.e., method 2 without C_{60} added); and (4) "fish water" control. The Affymetrix zebrafish array was used to assess changes in gene expression (14,900 gene transcripts), and results indicated that changes in expression were related to decomposition products of THF rather than to toxicity from C_{60}. Subsequently, the researchers investigated the interaction of other contaminants with C_{60} aggregates and have determined that aggregate characteristics (e.g., size and charge) can change in the presence of a co-contaminant and that C_{60} can alter contaminant bioavailability in zebrafish. A separate objective was to assess dietary toxicity of C_{60} (500 mg/kg food) in rainbow trout exposed for 6 weeks. Effects of dietary exposure were evaluated by organ histopathology, measurements of oxidative stress, and effects on osmoregulation. Results of this exposure indicate minimal toxicity from C_{60}; however, assessment of the actual uptake of C_{60} and distribution among tissues is ongoing.

EPA Grant Number: R833333

3:40 p.m. Thursday, November 20, 2008

Effects of Nanomaterials on Human Blood Coagulation

Peter L. Perrotta[1] and Perena Gouma[2]
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Common human diseases including myocardial infarction and stroke are caused by abnormalities of blood coagulation that predispose to thrombosis (clots). These diseases are influenced by environmental factors, but not all risk factors for clotting disorders are known. Because nanomaterials that enter the workplace or home could have short- and/or long-term effects on the blood coagulation system, the researchers are studying the effects of nanosized materials on the blood coagulation system using a variety of techniques. An important part of these studies involved documenting adequate dispersion of nanoparticles within biological media. Interestingly, nanoparticle (NP) size can be verified in plasma-containing solutions by dynamic light scattering (DLS) when the nanoparticles are of uniform size and shape. Using these well-dispersed NP-plasma suspensions for clotting studies, it appears that NPs have the effect of shortening clotting times *in vitro*. They also are capable of altering the ability to generate thrombin, the most physiologically relevant clotting enzyme. Based on the importance of thrombin in human coagulation, the investigators have explored several sensor strategies for detecting clotting proteins like thrombin. The investigators recently have begun to study plasma obtained from rats exposed to ultrafine and nanometer-sized particles through inhalation. Differences in endogenous thrombin potential (ETP) and fibrinogen levels can be identified between exposed and control animals. In addition, global proteomic profiling techniques (differential gel electrophoresis, DIGE) and more targeted multiplexed (Luminex) panels have demonstrated significant alterations in rat proteins involved in the coagulation and inflammatory systems.

EPA Grant Number: R832843

4:00 p.m. Thursday, November 20, 2008

Engineered Nanomaterial Ecological Effects Research Within ORD's National Health and Environmental Effects Research Laboratory

Stephen A. Diamond[1], Christian Andersen[2], Amanda Brennan[1], Robert Burgess[3], Kay Ho[3], Sarah Hoheisel[1], Mark G. Johnson[2], David R. Mount[1], and Paul Rygiewicz[2]
[1]Mid-Continent Ecology Division, U.S. Environmental Protection Agency, Duluth, MN; [2]Western Ecology Division, EPA, Corvallis, OR; [3]Atlantic Ecology Division, EPA, Narragansett, RI

Ecological effects of manufactured nanomaterials are being investigated at three of the EPA's ecological research laboratories: the Atlantic, Mid-Continent, and Western Ecology Divisions. These efforts are focused on and guided by EPA's regulatory needs. Accomplishments to date include the review of ecological effects test guidelines to ascertain their applicability or adequacy for testing nanomaterials. Scientists from the ecology divisions, along with scientists from seven other countries, reviewed 25 harmonized test guidelines, five additional test guidelines, and a guidance document on testing difficult substances published by the Organization for Economic and Cooperative Development (OECD). These efforts and additional test guideline reviews will be summarized. Initial nanomaterials research has included development of consistent and repeatable approaches for conducting nano-scale TiO_2 toxicity assays in freshwater systems; methods that will likely be applied to nanoscaled silver and other nanomaterials. Through collaboration with the Army Corp of Engineers and academic researchers' studies on suspension and toxicity of C_{60} fullerenes and effects of carbon nanotubes on plant vigor are either complete, or nearing completion. The C_{60} research is notable for its focus on the relationship of natural organic matter, C_{60} particle size and stability, as well as the effect of solar radiation on both processes, and toxicity. Ecology division researchers also are in the planning stages of studies that will link closely fate processes with toxicity of nanoscaled silver. These results and planned research will be presented within a framework of EPA's regulatory needs and international collaborations within the OECD.

EPA Grant Number: R832843

4:20 p.m. Thursday, November 20, 2008

Innate Immune Response of an Aquatic Vertebrate Model to Manufactured Nanoparticles Assessed Using Genomic Markers

Rebecca Klaper, Jian Chen, and Frederick Goetz
University of Wisconsin–Milwaukee, Milwaukee, WI

The innate immune system is one of the first physiological systems to interact with foreign materials and therefore will be key to understanding how organisms will be affected by exposure to nanomaterials. Recent studies have indicated that the innate immune system of fish responds to certain pathogen patterns differently than that of mammals. Therefore, the response of the mammalian immune system may not necessarily be representative of the immune reaction of aquatic vertebrates such as fish. Past cellular studies have concentrated on general cytotoxicity.

The overall objective of this research 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 study 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. Our hypothesis is: nanomaterials of dissimilar chemical composition will stimulate different patterns of trout macrophage gene expression, and nanomaterials of similar chemical characteristics (e.g., charge, shape, and functional group) may be grouped with respect to their bioactivity, expressed as a particular gene response pattern. Specifically, the chemical properties of nanomaterials will impact the genomic response of the immune system: nanomaterials of dissimilar chemical composition will stimulate different patterns of macrophage gene expression and the response will be dose-dependent.

A range of water-soluble C_{60} and carbon nanotubes with different chemical compositions and surface chemistries will be synthesized and tested for their effects on trout macrophages. A trout primary macrophage cell culture system will be used to determine the: (1) dose versus cell viability for each synthesized nanomaterial type; (2) level of expression (by quantitative PCR) of marker genes associated with inflammatory, antiviral, and anti-inflammatory responses with respect to nanomaterial dose at levels that have no deleterious effect on cell viability; and (3) global patterns of gene expression for those materials that cause significant changes in marker genes using custom trout immune microarrays.

Methods developed here will improve risk assessment by creating a mechanism to test other nanoparticles prior to commercial release. The goal of this project will be to help identify nanomaterials with the least negative environmental impact for environmentally conscious manufacturing. Risk managers will use this data to identify particles for restricted release to limit harm to aquatic species.

EPA Grant Number: R833319

8:40 a.m. Friday, November 21, 2008

Nanostructured Membranes for Filtration, Disinfection, and Remediation of Aqueous and Gaseous Systems

Kevin Kit
Department of Materials Science and Engineering, The University of Tennessee, Knoxville, TN

Nanofiber filtration media comprised primarily of biopolymer chitosan were produced by electrospinning. Electrospinning of pure chitosan has proved to be difficult due to limited solubility and high degree of intermolecular hydrogen bonding. The investigators have been able to form nanometer-sized fibers without bead defects by electrospinning chitosan blends with synthetic polymers poly(ethylene oxide) and poly(acrylamide) with up to 95 percent chitosan in blend fibers. The processing window was expanded by modifying the spinning apparatus to operate at elevated temperatures. Fiber morphology was affected by polymer molecular weight, blend ratios, polymer concentration, and spinning solution temperature.

The physical (aerosols, polymer beads), chemical (chromium IV), and microbial (*Escherichia coli K-25*) filtration efficiencies of the fabricated nanofibrous filter media were characterized. Surface chemistry of these blend fibers was characterized using X-ray Photoelectron Spectroscopy. Surface properties of blend fibers showed a strong correlation with the structure and morphology of the fibers. Much higher chromium binding capacities compared to similar blend ratio chitosan films were observed. Nanofibrous filter media has been fabricated by electrospinning a layer of chitosan nanofibers onto a non-woven spun bonded polypropylene fabric. These coated filter media have been tested for their metal binding and antimicrobial properties, and results showed applicability towards effectively filtering heavy metals and bacteria from waste media. The filtration performance of these nanofibrous filter media has been tested against latex polystyrene beads, and aerosol particles and filtration efficiencies of these media were a function of pore size, fiber diameter, and size of filtrate.

EPA Grant Number: GR832372

9:00 a.m. Friday, November 21, 2008

Comparative Life Cycle Analysis of Nano and Bulk Materials in Photovoltaic Energy Generation

Vasilis Fthenakis, S. Gualtero, and H.C. Kim
Center for Life Cycle Analysis, Earth and Environmental Engineering Department, Columbia University, New York, NY

Life-cycle analysis (LCA) is used to assess potential environmental impacts from the rapidly growing implementation of photovoltaic (PV) systems. Nano materials are investigated for use in photovoltaic and other energy generation applications. The information derived from the LCA of bulk material-based PV are extrapolated to the processes used for their nanomaterial equivalents. For each of the life stages of PV (i.e., material production, cell/module manufacture, installation, operation/maintenance, recycling, and disposal), resource utilization, process efficiencies, extra controls/steps, conversion efficiencies, recyclability, and the environmental fate of the micro and the nonmaterial alternatives will be investigated. This way, data and relationships will be built that will enable the quantification of the environmental effects of nanomaterials from existing micromaterial life-cycle inventory data.

EPA Grant Number: R833334

9:20 a.m. Friday, November 21, 2008

The Life Cycle of Nanomanufacturing Technologies

Thomas L. Theis, Hatice Sengul, and Allan Fluharty
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A significant component of the driving force behind the evolution and acceleration of nanotechnology lies in the prevalence of diverse manufacturing routes for nanoscale products. All nanoscale products must proceed through various manufacturing stages to produce a material or device with nanoscale dimensions. This research project explores manufacturing routes of nanoscale products with special attention focused on those attributes that are likely to have significant environmental implications.

Nanomanufacturing methods are usually classified into one of two groups: "top-down," which is achieved by carving or grinding methods (such as lithography, etching, and milling); or "bottom-up" in which matter is assembled at atomic scale through nucleation and/or growth from liquid, solid or gas precursors by chemical reactions or physical processes (using techniques such as sol-gel or epitaxy). "Top-down" manufacturing is the more common approach used today to produce nanoproducts; it is generally believed that such techniques are more waste-producing than "bottom-up" techniques. In contrast, it is often suggested that "bottom-up" nanomanufacturing technologies should be the ultimate tools for sustainable manufacturing because they allow for the customized design of reactions and processes at the molecular level that minimize unwanted wastes.

Regardless of the specific product or type of manufacturing process, certain general statements can be made about the sources of relatively high waste-to-product ratios and potential environmental impacts of manufacturing processes. Nanomanufacturing involves:

- Strict purity requirements and less tolerance for contamination during processing than more conventional manufacturing processes;
- Low process yields or material efficiencies;
- Repeated processing, postprocessing, or reprocessing steps of a single product or batch during manufacturing;
- Use of toxic/basic/acidic chemicals and organic solvents;
- Need for moderate to high vacuum and other specialized environments such as high heat or cryogenic processing;
- Use or generation of greenhouse gases;
- High water consumption; and
- Chemical exposure potential in the workplace and through technological/natural disasters.

NSF Award Number: 0646336.

9:40 a.m. Friday, November 21, 2008

Evaluating the Impacts of Nanomanufacturing Via Thermodynamic and Life Cycle Analysis

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

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

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

EPA Grant Number: R832532

10:20 a.m. Friday, November 21, 2008

Impact of Physiochemical Properties on Skin Absorption of Manufactured Nanomaterials

Xin-Rui Xia, Nancy A. Monteiro-Riviere, and Jim E. Riviere
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The wide applications of manufactured nanomaterials will create enormous potential for human exposure and environmental release. Skin, as the largest organ protecting the body from exogenous toxins and particulates, will be a major portal of entry for nanomaterials. The investigators' preliminary study has shown that fullerene nanoparticles can penetrate deep into the stratum corneum (the primary barrier of the skin) and be modulated by solvents and ion-pairing agents. Currently, there is no method available for quantitative assessment of the skin absorption of the manufactured nanomaterials.

The objective of this research project is to establish a structure-permeability relationship for skin absorption of manufactured nanomaterials for safety evaluation and risk assessment. Four dominant physiochemical properties (particle size, surface charge, hydrophobicity, and solvent effects) in skin absorption will be studied. Fullerene and its derivatives will be used as model nanomaterials. The absorption and disposition kinetics and dose-response relationships will be measured experimentally for quantitative model development.

The novelty of this project is to study one parameter of interest (e.g., size) while keeping other parameters (e.g., surface charges and hydrophobicity) constant, in contrast to most of the current research focusing on the toxicological effects of the nanomaterials. Three well-developed experimental methods will be used in consideration of throughput, cost, and biological complexity. Diffusion experiments will provide *in vitro* absorption kinetic information by measuring the nanomaterial flux across the skin. Tape-stripping is designed to provide *in vitro* disposition kinetic information of the nanomaterials in the stratum corneum. An isolated perfused porcine skin flap (IPPSF) technique will provide *ex vivo* absorption kinetic information that has proved to be effective for human *in vivo* prediction.

The ion-pairing effects, solvent effects, and the impact of particle size and hydrophobicity on skin absorption of nanomaterials will be quantitatively measured to provide three sets of absorption kinetic data: *in vitro* absorption, *ex vivo* absorption, and *in vitro* disposition kinetics. The quantitative data obtained in this project will be used to develop quantitative structure-permeability relationships based on the physiochemical properties of nanomaterials, which will define a general applicable approach for quantitative risk assessment and safety evaluation of manufactured nanomaterials.

EPA Grant Number: R833328

10:40 a.m. Friday, November 21, 2008

Safety/Toxicity Assessment of Ceria (A Model Engineered NP) to the Brain

Robert A. Yokel[1,2], Rebecca L. Florence[1], Jason Unrine[3], Michael T. Tseng[8], Uschi M. Graham[4], Rukhsana Sultana[5], D. Allan Butterfield[5,6], Peng Wu[7], and Eric A. Grulke[7]
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Objective: The objective of this research project is to characterize the biodistribution and toxicity of nanoscale ceria that had entered blood.

Rationale: Ceria was chosen as a model insoluble and stable metal oxide tracer with extensive engineered nanomaterial (ENM) applications.

Material: A commercial 5 percent crystalline aqueous ceria dispersion, mean size approximately 30 nm (by particle size determination); primary size approximately 3 to 5 nm (by high resolution transmission electron microscopy [HR-TEM]); surface area approximately 13 m^2/g.

Procedures: The effect of saline and 10 percent sucrose on ceria agglomeration was assessed. Ceria was i.v. infused into un-anesthetized rats (0, 50, 250 or 750 mg/kg), which were terminated 1 hour or 20 hours later. Its biodistribution was assessed by microscopy and ICP-AES/ICP-MS cerium analysis. The potential to produce toxicity was assessed by microscopy. Neurotoxic or neuroprotective potential was assessed by 4-hydroxy-2-nonenal (HNE), 3-nitrotyrosine (3-NT), and protein carbonyls in frontal cortex (FC), hippocampus (HC), and cerebellum (CB). Five minutes prior to termination anesthetized rats were given i.v. Evans blue (EB)-albumin and Na fluorescein (Na_2F) as blood-brain barrier (BBB) integrity markers.

Results: Saline and 10 percent sucrose caused ceria agglomeration *in vitro*. Fresh blood incubated with ceria for 1 hour showed primary and agglomerated ceria by EM and energy-dispersive X-ray spectroscopy. Systemic ceria $t_{½}$ in the rat was less than 1 hour. Brain EB and Na_2F increased somewhat in rats terminated at 20 hours, but was less consistent in 1-hour rats. Tissue [Ce] in rats terminated at 1 hour and 20 hours was dose-dependent (spleen > liver > brain > blood serum). At 20 hours, 4-HNE increased in the HC; 3-NT changed little in FC, HC or CB; and protein carbonyls decreased in the CB. No significant effects were seen at 1 hour.

Conclusions: Ceria was cleared by peripheral reticuloendothelial tissues. Much less ceria entered the BBB cells or the brain. The results provide a foundation to study the impact of the physico-chemical properties of ENMs on peripheral organ distribution, brain entry, and neurotoxic or neuroprotective potential.

EPA Grant Number: R833772

11:00 a.m. Friday, November 21, 2008

Nanotechnology: A Novel Approach To Prevent Biocide Leaching

Patricia Heiden[1], Benjamin Dawson-Andoh[2], and Laurent Matuana[3]
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The primary objective of this research project is to develop a practical and effective approach to prepare biocide-loaded nanoparticles (organic and copper-based biocides) that can be efficiently introduced into wood to reduce or eliminate biocide leach into sensitive environments. Preventing biocide loss to leach also is expected to increase the useful lifetime of wood products while using less biocide. To accomplish this objective, the nanoparticle must be constructed to serve as a protective reservoir for the biocide that prevents its loss by leach or by degradation, but that also releases biocide into the wood in a controlled manner at a rate that maintains the minimal amount of biocide required within the wood for wood preservation.

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

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

EPA Grant Number: GR832371

11:20 a.m. Friday, November 21, 2008

Internalization and Fate of Individual Manufactured Nanomaterial Within Living Cells

Galya Orr, David J. Panther, Kaylyn J. Cassens, Jaclyn L. Phillips, Barbara J. Tarasevich, and Joel G. Pounds
Pacific Northwest National Laboratory, Richland, WA

The cellular interactions and intracellular fate of manufactured submicrometer and nanoscale materials dictate the cellular response and ultimately determine the level of toxicity or biocompatibility. However, the cellular interactions and pathways of particles with specific sets of properties are largely unknown. In addition, little is known about the cellular interactions and pathways of individual or small nanoparticle aggregates, as they are likely to be presented to cells *in vivo*, mostly because of their tendency to agglomerate under experimental conditions. In this study, the researchers investigated the initial interactions and internalization pathways of individual precipitated amorphous silica particles with specific surface properties and size by following one particle at a time. Using time lapse fluorescence microscopy, it was found that both 100 nm and 500 nm particles can take advantage of the actin turnover machinery within microvilli to advance their way into alveolar type II epithelial cells, an expected target cell for inhaled submicrometer and nanoscale materials. This pathway is strictly dependent on the positive surface charge of the particles and on the integrity of the actin filaments unraveling charge-dependent coupling of the particles with the intracellular environment across the cell membrane. To identify the molecules that capture the particles at the cell surface, the researchers therefore searched for a negatively charged, transmembrane molecule that could mediate the coupling of the particles with the actin filaments. Using flow cytometry, time lapse fluorescence, and laser confocal microscopy, it was found that syndecan I, a transmembrane heparan sulfate proteoglycan, mediates the initial interactions of the particles at the cell surface, their coupling with the intracellular environment, and their internalization pathway. Together, the findings reveal a new mechanism by which positive surface charge supports particle recruitment by polarized epithelial cells bearing microvilli, and identify a critical role for syndecan I in the cellular interactions and subsequent potential toxicity of these particles.

EPA Grant Number: R833338

11:40 a.m. Friday, November 21, 2008

Methodology Development for Manufactured Nanomaterial Bioaccumulation Test

Yongsheng Chen[1], Yung Chang[1], John C. Crittenden[1], Qiang Hu[1], C.P. Huang[2], and Milton Sommerfeld[1]
[1]Arizona State University, Tempe, AZ; [2]University of Delaware, Newark, DE

Because of their small size and high specific surface area, manufactured nanomaterials have enhanced mobility and, potentially, greater toxicity as they have almost unrestricted access into aquatic organisms and the human body. However, there are no data available on whether these manufactured nanomaterials are toxic within months or years. So, these nanomaterials could constitute a new class of non-biodegradable pollutants and may bioaccumulate in the food chain. Consequently, it is imperative to develop a suitable methodology to evaluate the potential risks of bioaccumulation of manufactured nanomaterials in aquatic organisms so that we can understand their potential impacts and avoid serious environmental consequences, such as with DDT (dichlor-diphenyl-trichloroethane) and PCBs (polychlorinated biphenyls). The objectives of this research project are to: (1) develop suitable manufactured nanomaterial bioaccumulation testing procedures to ensure data accuracy and precision, test replication, and the comparative value of test results; (2) evaluate how the forms of these manufactured nanomaterials affect the potential bioavailability and bioconcentration factor (BCF) in phytoplankton; 3) determine the potential biomagnification of manufactured nanomaterials in zooplankton; and 4) determine the potential biomagnification of manufactured nanomaterials in fish.

This research project brings together a multidisciplinary team, which includes nanomaterial engineers and chemists, physiologists, and molecular biologists. A hypothesis of whether manufactured nanomaterials can be accumulated in aquatic organisms will be tested. The bioconcentration, bioaccumulation, and biomagnification of manufactured nanomaterials will be evaluated in a simulated food chain and aquatic organisms including algae, daphnia, and zebrafish. Advanced analysis techniques and methods, including image shape analyzing particle counter, transmission electron microscopy (TEM), secondary ion mass spectrometer (SIMS), and electron microscopy, will be employed for analysis of nanomaterial size, exploration of bioavailability and dispersion pathways of nanomaterials entering into cells of an aquatic organism, and determination of the ratio of nanomaterials dispersed in the organs of an organism.

Any risk assessment requires basic information on toxicity to biota and the likelihood of uptake into the food chain. This study will provide essential nanomaterial bioaccumulation testing procedures and fundamental data on the movement and transformation capabilities of nanomaterials in aquatic organisms and the first evidence that such nanomaterials can or cannot be biologically accumulated in aquatic organisms. This research would ultimately allow us to better understand the consequences of manufactured nanomaterials in the environment.

EPA Grant Number: R833327

2:00 p.m. Friday, November 21, 2008

Agglomeration, Retention, and Transport Behavior of Manufactured Nanoparticles in Variably Saturated Porous Media

Yan Jin and John Xiao
University of Delaware, Newark, DE

The production of significant and increasing quantities of synthetic nanomaterials and the very limited knowledge on their potential environmental and health effects have caused increasing public concerns. The overall objective of this research project is to develop an understanding of the fate of nanoparticles released into the subsurface environments. The hypothesis of this study is that nanoparticles are likely to be mobile and have the potential to contaminate water resources either as contaminants themselves or by facilitating the transport of other toxic substances. The investigators propose to conduct a comprehensive study to systematically investigate the major processes that control the movement of nanoparticles in the subsurface under environmentally relevant conditions. Our specific objectives are to: (1) determine agglomeration behavior of nanoparticles under different solution chemistry (pH, ionic strength, and presence of dissolved humic material); (2) measure mobility of nanoparticles in model porous media under both saturated and unsaturated flow conditions; and (3) experimentally elucidate the attachment and retention mechanisms of nanoparticles at various interfaces at the pore scale.

TiO_2 and Fe nanoparticles will be used as models representing two major categories of nanoparticles that have been used or have the potential to be used in large quantities commercially. Agglomeration of nanoparticles will be evaluated in batch experiments by dynamic light scattering. Transport and potential transformation will be studied with a series of laboratory column experiments using model sand of various surface properties. Sorption and reaction models will be combined with transport models to describe the transport experiments quantitatively. An innovative approach of using confocal microscopy to visualize and analyze particle-particle and particle-interface interactions in micromodels will provide resolution high enough to reveal detailed particle arrangement in bulk solution and at interfaces to elucidate the mechanisms involved in particle attachment and retention at the pore scale.

This study integrates experiments across disciplines (environmental soil physics/hydrology and physics/material science) and scales (column, batch, and pore scale). The results of this study will lead to better understanding of particle-particle and particle-interface interactions at the microscopic level, as well as particle agglomeration, retention, and movement in porous media under various chemical (pH, ionic strength, presence of dissolved humic material) and physical (variable water content) conditions at the macroscopic scale. The investigators expect to provide conclusive evidence about the conditions under which transport of nanoparticles is expected and the quantitative magnitude of the process. Such information will contribute to the overall understanding of how nanomaterials interact with the natural environment and provide a scientific basis for determining exposure pathways and developing exposure guidelines, which is the first element in risk assessment to quantify potential human health effects.

EPA Grant Number: R833318

2:20 p.m. Friday, November 21, 2008

Biological Fate and Electron Microscopy Detection of Nanoparticles During Wastewater Treatment

Paul Westerhoff, Terry Alford, and Bruce Rittman
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. Waste water 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 research project is to quantify interactions between manufactured NPs and WW biosolids. We will model their fate with a mechanistic model that reflects and helps us gain mechanistic understanding. 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.

This project involves environmental engineers and spectroscopy experts who will quantify the removal of four classes of manufactured NPs (metal-oxide, quantum dots, C_{60} fullerenes, carbon nanotubes) during WW treatment. The unique size and surface characteristics of these NPs are expected to behave differently from greater than 1 μm sized particles currently in wastewaters. The relative importance of four NP removal mechanisms will be quantified: (1) adsorption to the outer cell walls; (2) enmeshment into the extracellular polymeric substances (EPS); (3) partitioning into the cytoplasm; and (4) cellular uptake and synthesis. Batch adsorption experiments will use NPs with whole biosolids, cellular biomass only, and EPS from three types of biological reactors (aerobic heterotrophic, aerobic heterotrophic and autotrophic nitrifying, and anaerobic methanogenic) and from full-scale WWTP reactors. NP application to the same three types of laboratory bioreactors operated in a semi-continuous mode will validate adsorption onto biosolids and quantify the NP biotransformation and toxicity to the biological community/activity. Imaging techniques (environmental SEM, TEM) will be developed to understand "where" NPs reside with biosolids. Techniques to extract NPs from complex biological matrices also will be explored. Finally, NP removal reactions will be incorporated into existing mechanistic WWTP models.

This research project addresses three broad questions:
(1) What mechanisms remove NPs?
(2) Can NPs be imaged within bacteria and WWTP biosolids?
(3) Do NPs affect biological WW treatment?

Data and mechanistic interpretation/modeling directly supports all four of the stated U.S. Environmental Protection Agency interests from the Request for Proposals. Experiments will assess the toxicity and biological effects of NPs on the three common mixed WW bacterial communities. The project quantifies the fate (biosorption, biotransformation) of manufactured NPs in contact with complex biological matrices (i.e., WW biosolids). This study will be among the first to apply imaging and extraction procedures for NPs in complex biological matrices. By understanding NP removal in WWTPs, this project helps identify potential NP exposure pathways (effluent discharge to rivers, lakes; land application of biosolids; biosolids incineration) to the environment and provides insight for considerations during life-cycle assessments (e.g., additional treatment requirements at WWTPs).

EPA Grant Number: R833322

2:40 p.m. Friday, November 21, 2008

Genomics-Based Determination of Nanoparticle Toxicity: Structure-Function Analysis

Alan T. Bakalinsky[1], Alex Hadduck[1], Vihangi Hindagolla[1], Mark Smith[1], Bin Xie[2], and Qilin Li[2]
[1]Department of Food Science and Technology, Oregon State University, Corvallis, OR; [2]Department of Civil and Environmental Engineering, Rice University, Houston TX

The researchers' long-term goal is to determine mechanisms by which manufactured nanomaterials may cause cytotoxicity in realistic environments of exposure. To assess potential toxicity and to determine mechanisms through which two such materials may elicit toxic responses, cell yield and survival of the yeast *Saccharomyces cerevisiae* and *Escherichia coli* were determined in the presence of underivatized fullerene and functionalized gold nanoparticles. Three independent batches of aqueous fullerene nanoparticles solubilized initially in toluene (tol/nC_{60}) or THF (THF/nC_{60}) or directly in water (aq/nC_{60}) at about 30 μg/mL exhibited no observable effect on cell yield of either wild-type yeast or *E. coli* in minimal medium relative to control cells. In contrast, cell yield of 3 among 48 yeast cell wall mutants tested (*ecm30*, *ecm17*, and *get2)* was better in the presence of tol/nC_{60} than in its absence. In 27 separate exposures of wild-type yeast at different cell concentrations to this same dose of nC_{60} prepared from all three lots of the three types of fullerene, yeast survival relative to control cells was unaffected 50 percent of the time, was better 20 percent of the time, and worse 30 percent of the time. Survival of *E. coli* exposed to this same dose of tol/nC_{60} in 0.9 percent saline was worse or the same as that of a control about 70 or 30 percent of the time, respectively. No striking differences were observed in either zeta potential or particle size of the one tol/nC_{60} lot that exhibited greater toxicity than the other tol/nC_{60} lots.

Yeast cell yield was unaffected by exposure to 100 μg/mL of functionalized Au nanoparticles (Au-TMAT) carrying a positive charge and containing an 11 atom core 0.8 nm in diameter. In contrast, yeast survival was reduced by exposure to Au-TMAT concentrations of less than 1 μg/mL. A specific amount of these particles appeared to kill a fixed number of cells rather than a fixed fraction of cells. For example, 1 μg killed about 100,000 cells regardless of the number of cells exposed. To identify genes and mechanisms implicated in Au-TMAT-mediated killing, a yeast gene deletion library was screened for mutants resistant to Au-TMAT relative to the wild-type parent strain. Six resistant clones were isolated from the initial screen of 2,500 mutants, which constitute about one-half of the library. Loss of *GYL1*, YMR155W, *DDR48*, and YGR207C was found to result in Au-TMAT resistance, suggesting that these genes play roles in mediating Au-TMAT toxicity.

EPA Grant Number: R833325

3:00 p.m. Friday, November 21, 2008

Biological Activity of Mineral Fibers and Carbon Particulates: Implications for Nanoparticle Toxicity and the Role of Surface Chemistry

Prabir K. Dutta[1], Amber Nagy[2], Brian Peebles[1], and W. James Waldman[2]
Departments of [1]Chemistry and [2]Pathology, The Ohio State University, Columbus, OH

In this presentation, the researchers' work on the correlations between biological activity and physicochemical characteristics of minerals and particulates, including the biological response (oxidative burst), mutagenicity, and the chemical reactivity (Fenton reaction) of zeolite minerals and oxidative stress and inflammatory responses of carbon particulates, will be summarized. Zeolites, with well-defined crystal structures, serve as model systems for asbestos and other toxic minerals. For assessment of biological response, phagocytosis as well as the oxidative burst has been studied. For determining chemical reactivity, the researchers have focused on the ability of the iron-exchanged forms of the zeolites to produce hydroxyl radicals from H_2O_2 (Fenton reaction). Mutagenic potential of erionite and mordenite and how this mutagenic potential is modulated by iron has been examined. The impact of carbon-based particulate physicochemical characteristics on their ability to induce oxidative stress and inflammatory responses will be reported. Internalization of particulates by freshly isolated and differentiated human monocyte-derived macrophages (MDM) is being examined. To determine the impact of particulate physicochemical characteristics on their inflammatory potential, inflammatory endothelial adhesion molecule expression by immunofluorescence flow cytometry is being examined. Fenton activity of particulates is being assayed by measurement of their ability to catalyze the decomposition of hydrogen peroxide to hydroxyl radicals by spin trapping with 5,5-dimethylpyroline-N-oxide (DMPO).

NSF Award Number: 0532250

3:20 p.m. Friday, November 21, 2008

A Rapid *In Vivo* System for Determining Toxicity of Manufactured Nanomaterials

Robert L. Tanguay and Stacey Harper
Oregon State University, Corvallis, OR

Rapid growth of the nanotechnology industry is resulting in increased exposure of humans and the environment to nanomaterials prior to the scientific investigation of potential risks. It is clear that there is a need to develop rapid, relevant, and efficient testing strategies to assess these emerging materials of concern. Here, the researchers propose an *in vivo* system for rapidly assessing the toxicity of nanomaterials at multiple levels of biological organization (i.e., molecular, cellular, systems, and organismal). Early developmental life stages often are uniquely sensitive to environmental insult, due in part to the enormous changes in cellular differentiation, proliferation, and migration required to form the required cell types, tissues, and organs. Molecular signaling underlies all of these processes. Most toxic responses result from disruption of proper molecular signaling, thus, early developmental life stages are perhaps the ideal life stage to determine if chemicals or nanomaterials are toxic. The hypothesis of this study is that the inherent properties of some engineered nanomaterials make them potentially toxic. To test this hypothesis, we specifically propose to (1) further develop our *in vivo* zebrafish toxicity assay to define the *in vivo* responses to nanomaterials, and (2) begin to define structural properties of nanomaterials that lead to adverse biological consequences.

The investigators propose a three-tiered approach exploiting the advantages of the embryonic zebrafish model to assess the toxicity of nanomaterials. **Tier 1:** Rapid screening experiments will be conducted to assess the toxicity of a wide range of structurally well-characterized nanomaterials commercially available or produced by researchers of the Oregon Nanoscience and Microtechnologies Institute (ONAMI). Nanomaterials found to elicit significant adverse effects will proceed to Tier 2 testing. **Tier 2:** Potential cellular targets and modes of action will be defined *in vivo* using a suite of transgenic fluorescent zebrafish and indicators of cellular oxidative state. Nanomaterials will be grouped according to structural indices and effects. Representative nanomaterials from each group will be selected for Tier 3 testing. **Tier 3:** Global gene expression profiles will be used to define the genomic responses to nanomaterials. Data from these studies will be used to define structure-activity relationships using a Nanomaterials Effects Database that the investigators have created to collate, organize, and analyze data on nanomaterial effects across species and exposure scenarios.

The successful completion of these studies will fill important gaps in our understanding of the human health risk posed by exposure to nanomaterials. The proposed research will deliver (1) a validated *in vivo* system for rapidly assessing existing and future novel nanomaterials, and (2) data on nanomaterial structure effects relationships.

EPA Grant Number: R833320

3:40 p.m. Friday, November 21, 2008

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

Mamadou S. Diallo, William A. Goddard, and Jose Luis Riechmann
California Institute of Technology, Pasadena, CA

Dendrimers are relatively monodisperse and highly branched nanoparticles that can be designed to: (1) chelate metal ions; (2) encapsulate metal clusters; (3) bind organic solutes or bioactive compounds; and (4) become soluble in appropriate media or bind onto appropriate surfaces. Because of these unique properties, dendrimers are providing unprecedented opportunities to develop functional nanomaterials for a variety of applications, including chemical separations and catalysis, chemical sensing, medical imaging, DNA/drug delivery, and water purification. As the U.S. Environmental Protection Agency begins its assessment of the impact of nanotechnology on human health and the environment, there is a critical need for data and quantitative tools for assessing the environmental fate and toxicity of nanomaterials such as dendrimers. The overall objective of this research project is to advance our fundamental understanding of the relationships between the affinity of ethylene diamine (EDA) core poly(amidoamine) (PAMAM) dendrimers to cell membranes and their vascular and ingestion toxicity using: (1) n-octanol and solid-supported phosphatidylcholine lipid bilayers as model cell membranes; and (2) endothelial and kidney cells as model human cells.

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

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

EPA Grant Number: R832525

4:20 p.m. Friday, November 21, 2008

Nanoparticle Toxicity in Zebrafish

Gregory D. Mayer[1], Jay L. Nadeau[2], Anja Nohe[1], and V. Smorodin[1]
[1]University of Maine, Bangor, ME; [2]McGill University, Montreal, Quebec, Canada

The overlying objective of this research project is to investigate the toxicity of semiconductor nanostructures using an *in vivo* developmental system (zebrafish, *Danio rerio*, embryos). The approach will monitor, in real time, the effects of particle composition, size, and charge on uptake and accumulation of nanostructures in multiple tissues. Additionally, the investigators will monitor the release of ions from the particles using a transgenic zebrafish model that expresses green fluorescent protein (GFP) in the presence of metal ions. These data will be correlated to altered embryo development after particle exposure, and the effects will be extrapolated to human health. Finally, the researchers will develop a model to predict particle toxicity that will help to evaluate potential health risks of the release of semiconductor nanoparticles into the environment.

To effectively determine how particle composition, size, and charge affect toxicity, researchers will begin by refining techniques of synthesis and characterization to alter one variable at a time. These well-characterized particles then will be applied to cultured zebrafish, zebrafish embryos, or embryonic cells. Uptake, accumulation, and ion release in cells and whole embryos will be quantitatively measured in real time by multicolor confocal microscopy that will simultaneously detect the nanoparticles, GFP, and co-transfected fluorescent organelle markers. Additionally, the force of adhesion of the range of particles to cell membranes and the embryo will be investigated using laser tweezers. All obtained data will be used to develop a model for the prediction of cellular uptake and resulting cellular toxicity based on the physical properties of the particles and the cell membranes that they encounter.

The investigators expect the toxicity of semiconductor nanoparticles to depend on their size, charge, and composition. However, because of the unique properties that arise from their small size and quantum confinement, the exact dependence of toxicity on each of these factors is likely to be surprising and to be poorly predictable from the behavior of the bulk materials. Also, it is expected that the nanoparticles will increase mortality and developmental abnormalities in zebrafish. Calculation of LC50s, hatch success, uptake routes, and acute and developmental toxicity endpoints will help validate the proposed model. The resulting data are expected to be of value for prediction of risks of nanoparticle release, especially into aqueous environments where the particles would have direct access to developing and adult organisms.

EPA Grant Number: R833339

4:40 p.m. Friday, November 21, 2008

Zinc Oxide Nanoparticles: It's the Contact That Kills

John M. Veranth, N. Shane Cutler, and Philip J. Moos
Department of Pharmacology and Toxicology, College of Pharmacy, University of Utah, Salt Lake City, UT

Previously, the investigators evaluated the toxicity and transcriptional responses to six lower-cost, high production-volume manufactured nanoparticles (carbon black, SiO_2, Al_2O_3, TiO_2, ZnO, and Fe_20_3) in colon cell lines. These manufactured nanoparticles are used in cosmetics, dental products, sunscreen, food additives, and dyes, making general population and occupational exposure likely. This research project has focused on a model of bowel inflammation and uses RKO and CaCo human colon-derived cell lines with and without activation by TNFα. The central hypothesis being tested is that ingested manufactured nanoparticles are taken up by inflamed colon cells, translocate to the nucleus, and alter gene transcription, thereby further increasing inflammation and leading ultimately to the development of pathological conditions including cancer.

In initial experiments, the metal oxide nanoparticles (Al_2O_3, TiO_2, SiO_2, and Fe_2O_3) were not toxic, carbon black showed modest toxicity, primarily at the highest concentrations, and ZnO displayed the most toxicity. The TNFα pretreatment did not dramatically alter the sensitivity of the RKO and CaCo-2 cells to any of the PM. In separate experiments, samples were prepared from all nanoPM and representative microarray experiments were run. The investigators are following up with selective QPCR as a validation method. TiO_2 and ZnO displayed transcriptional effects, with ZnO having the most pronounced effect. The data suggest that multiple pathways are activated by the ZnO, including: stress response pathways, Zn metabolism and transport genes, and genes that suggest alterations in redox pathways.

NanoZnO displayed the most toxicity and demonstrated the most pronounced transcriptional response. This transcriptional response suggested that part of the exposure to nanoZnO was exposure to elemental Zn, and therefore, perhaps the toxicity was merely Zn toxicity. Therefore, the investigators sought to determine if the nanoZnO toxicity was due to the dissolution of ZnO to elemental Zn and the mechanism of the cell death upon exposure to the nanoZnO. In addition, two size ranges of ZnO particulate matter were utilized to evaluate the effects of size/surface area. The researchers set out to determine if: (1) cell and particulate matter contact was required for ZnO toxicity; and (2) if ZnO dissolution to free Zn was dependent on the cells. A set of three experimental conditions were used: (1) a dialysis device with a 10 kD cutoff was used to separate the ZnO from cellular contact to ensure no ZnO particulate matter could interact directly with cells; (2) transwells with 0.4 micron pores that would allow greater interactions with cellular products but still separate the cells and the particulate matter were used; and (3) ZnO particulate matter was placed in direct contact with the cells. The Zn concentrations were measured in the media by ICP spectrometry and cell viability by PI exclusion. The ZnO toxicity was only observed when the particles were in contact with the cells, but the Zn levels in the media were equally high in the transwell and direct contact experiments, suggesting that contact and potentially uptake is required for cellular toxicity. The investigators have found that ZnO induces apoptosis by inducing superoxide production in the mitochondria and disruption of the mitochondrial potential. In addition, all of the toxic effects are dependent on particle size, as the larger ZnO particulate matter always demonstrated reduced toxicity compared to the smaller ZnO nanoparticles.

EPA Grant Number: R833336

5:00 p.m. Friday, November 21, 2008

Mass-Mobility Relationships for Silica Nanoparticle Agglomerates: Implications for Transport and Morphological Properties

Jacob H. Scheckman[1], Jaimie Hamilton[2], Sotiris E. Pratsinis[3], and Peter H. McMurry[1]
[1]Particle Technology Laboratory, Department of Mechanical Engineering, University of Minnesota, Minneapolis, MN; [2]Loyola Marymount University, Los Angeles, CA; [3]Particle Technology Laboratory, Department of Mechanical and Process Engineering, ETH Zurich, Zurich, Switzerland

Transport and physical/chemical properties of nanoparticle agglomerates depend on primary particle size, fractal dimension, and the number of primary particles in the agglomerate. Agglomerate properties were determined by tandem measurements of mobility (Differential mobility analyzer, DMA), mass (Aerosol particle mass analyzer, APM), and morphology (Electron microscopy, SEM/TEM). Of particular interest are the effects of agglomerate structure on lung deposition. To investigate this, deposition of silica agglomerates through a physical model simulating lung generation 22 was compared to that of spheres.

Nanoparticle agglomerates of silica were generated by oxidizing hexamethyldisiloxane in a methane/ oxygen diffusion flame. Particles leaving the flame were classified by electrical mobility size with a DMA, and their mass was measured with the APM. The measured relationship between mass and mobility was used to determine the fractal dimension. The effects of oxygen flow rate and mass production rate on single particle mass, fractal dimension, and dynamic shape factor were characterized. Electron microscopy was used to determine primary particle size and give qualitative information on particle morphology.

The generated particles were chain agglomerates with clearly defined primary particles. Average primary size ranged from 12 to 93 nm. Fractal dimensions ranged from 1.76 to 2.39. Increasing the oxygen flow rate was shown to decrease the primary particle size and the fractal dimension and increase the dynamic shape factor. Increasing the production rate was shown to increase the primary particle size and mass of the product particles without affecting the fractal dimension, and to decrease the dynamic shape factor. The effects of oxygen flow rate and production rate on primary particle size were in agreement with the literature.

Deposition patterns were determined for particles passing through a capillary tube bundle with tube diameters simulating lung generation 22 by measuring the particle concentration upstream and downstream of the model with two identical condensation particle counters. Silica agglomerates with a fractal dimension of 2.0 and primary particle size of approximately 53 nm were compared to spheres produced by atomizing oleic acid. When expressed in terms of electrical mobility equivalent diameter, deposition efficiency was the same for the agglomerates and the spheres. Similar experiments measuring deposition in other regions of the lung with additional fractal dimensions and with additional primary particle sizes are planned.

NSF Grant Number: BES-0646507

Appendices

Interagency Environmental Nanotechnology Grantees Workshop

Sheraton Tampa Riverwalk Hotel
Tampa, FL

November 19 - 21, 2008

REVISED AGENDA

DAY 1, Wednesday, November 19, 2008

7:30 – 8:15 a.m.	**Registration**
8:15 – 8:20 a.m.	**Welcome** *Nora Savage, National Center for Environmental Research (NCER), U.S. Environmental Protection Agency (EPA)*
8:20 – 8:50 a.m.	**EPA and Nanotechnology** *Christopher Zarba, Deputy Director, NCER, EPA*
8:50 – 9:10 a.m.	**National Science Foundation (NSF)** *Mihail (Mike) Roco, Senior Advisor for Nanotechnology, NSF*
9:10 – 9:30 a.m.	**National Institute for Occupational Safety and Health (NIOSH)** *William (Allen) Robison, NIOSH*
9:30 – 9:50 a.m.	**National Institute of Environment Health Sciences (NIEHS)** *Srikanth Nadadur, Program Administrator, NIEHS*
9:50 – 10:20 a.m.	***BREAK***
10:20 – 10:40 a.m.	**U.S. Department of Energy (DOE) Nanotechnology Characterization Facilities** *Neal D. Shinn, Sandia National Laboratories*
	Metals, Metal Oxides Remediation and Exposure
10:40 – 11:00 a.m.	**Engineered Nanomaterials Fate and Transport Research Within the Office of Research and Development's (ORD) National Exposure Research Laboratory (NERL)** *Michele Conlon, EPA, NERL*

DAY 1, Wednesday, November 19, 2008 (continued)

Metals, Metal Oxides
Remediation and Exposure (continued)

11:00 – 11:20 a.m. **Novel Nanostructured Catalysts for Environmental Remediation of Chlorinated Compounds**
Vijay John, Tulane University
Yunfeng Lu, University of California, Los Angeles

11:20 – 11:40 a.m. **Synthesis and Application of a New Class of Stabilized Nanoscale Iron Particles for Rapid Destruction of Chlorinated Hydrocarbons in Soil and Groundwater**
Dongye Zhao, Auburn University

11:40 – 12:00 p.m. **Nanoparticle Stability in Natural Waters and Its Implication for Metal Toxicity to Water Column and Benthic Organisms**
James Ranville, Colorado School of Mines

12:00 – 1:20 p.m. ***LUNCH (on your own)***

Metals, Metal Oxides
Fate/Transport

1:20 – 1:40 p.m. **The Effect of Surface Coatings on the Environmental and Microbial Fate of Nano-Iron and Fe-Oxide Nanoparticles**
Greg Lowry, Carnegie Mellon University

1:40 – 2:00 p.m. **The Fate and Effects of Nanosized Metal Particles Along a Simulated Terrestrial Food Chain Investigated Using Genomic and Microscopic Techniques**
Jason Unrine, University of Kentucky

2:00 – 2:20 p.m. **The Bioavailability, Toxicity, and Trophic Transfer of Manufactured ZnO_2 Nanoparticles: A View From the Bottom**
Paul Bertsch, University of Georgia

2:20 – 2:40 p.m. **Bioavailability and Fates of CdSe and TiO_2 Nanoparticles in Eukaryotes and Bacteria**
Patricia Holden, University of California, Santa Barbara

2:40 – 3:00 p.m. ***BREAK***

DAY 1, Wednesday, November 19, 2008 (continued)

Metals, Metal Oxides Toxicity

3:00 – 3:20 p.m. **Engineered Nanomaterial Health Effects Research Within ORD's National Health and Environmental Effects Research Laboratory (NHEERL)**
Kevin Dreher, EPA, NHEERL

3:20 – 3:40 p.m. **Engineered Nanomaterial Ecological Effects Research Within ORD's National Health and Environmental Effects Research Laboratory**
Steve Diamond, EPA, NHEERL

3:40 – 4:00 p.m. **Microbial Impacts of Engineered Nanoparticles**
Shaily Mahendra, Rice University

4:00 – 4:20 p.m. **Characterization of the Potential Toxicity of Metal Nanoparticles in Marine Ecosystems Using Oysters**
Amy Ringwood, University of North Carolina at Charlotte

4:20 – 4:40 p.m. **Acute and Developmental Toxicity of Metal Oxide Nanoparticles to Fish and Frogs**
Chris Theodorakis, Southern Illinois University

Other Nanomaterials Sensors and Treatment

4:40 – 5:00 p.m. **A Novel Approach To Prevent Biocide Leaching**
Patricia Heiden, Michigan Technological University

5:00 p.m. ***ADJOURN – DAY 1***

DAY 2, Thursday, November 20, 2008

7:30 – 8:30 a.m. **Registration**

8:30 – 8:40 a.m. **Welcome and Announcements**

Carbon-Based Sensors and Exposure

8:40 – 9:00 a.m. **Conducting-Polymer Nanowire Immunosensor Arrays for Microbial Pathogens**
Ashok Mulchandani, University of California, Riverside

DAY 2, Thursday, November 20, 2008 (continued)

Carbon-Based Fate/Transport

9:00 – 9:20 a.m. **Carbon Nanotubes: Environmental Dispersion States, Transport, Fate, and Bioavailability**
Elijah Petersen, University of Michigan

9:20 – 9:40 a.m. **Aggregation and Deposition Behavior of Carbon Nanotubes in Aquatic Environments**
Menachem Elimelech, Yale University

9:40 – 10:00 a.m. **Cross-Media Environmental Transport, Transformation, and Fate of Manufactured Carbonaceous Nanomaterials**
Peter Vikesland, Virginia Polytechnic Institute and State University

10:00 – 10:20 a.m. **Fate and Transport of C_{60} Nanomaterials in Unsaturated and Saturated Soils**
Kurt Pennell, Georgia Institute of Technology

10:20 – 10:40 a.m. ***BREAK***

10:40 – 11:00 a.m. **Photochemical Fate of Manufactured Carbon Nanomaterials in the Aquatic Environment**
Chad Jafvert, Purdue University

11:00 – 11:20 a.m. **Fate and Transformation of C_{60} Nanoparticles in Water Treatment Processes**
Jaehong Kim, Georgia Institute of Technology

Carbon-Based Toxicity

11:20 – 11:40 a.m. **Role of Particle Agglomeration in Nanoparticle Toxicity**
Terry Gordon, New York University School of Medicine

11:40 – 12:00 p.m. **Assessment of the Environmental Impacts of Nanotechnology on Organisms and Ecosystems**
Jean-Claude Bonzongo, University of Florida

12:00 – 12:20 p.m. **Structure-Function Relationships in Engineered Nanomaterial Toxicity**
John Fortner, Rice University

DAY 2, Thursday, November 20, 2008 (continued)

Carbon-Based Toxicity (continued)

12:20 – 1:40 p.m. ***LUNCH (on your own)***

1:40 – 2:00 p.m. **Long-Term Cardiovascular Effects of Inhaled Nanoparticles**
Gi Soo Kang, New York University

2:00 – 2:20 p.m. **Aquatic Toxicity of Carbon-Based Nanomaterials at Sediment-Water Interfaces**
Baolin Deng, University of Missouri–Columbia

2:20 – 2:40 p.m. **Aquatic Toxicity of Waste Stream Nanoparticles**
Judy Blatt-Nichols, New York University School of Medicine

2:40 – 3:00 p.m. **Ecotoxicology of Underivatized Fullerenes (C_{60}) in Fish**
Theodore Henry, University of Tennessee

3:00 – 3:20 p.m. ***BREAK***

3:20 – 3:40 p.m. **Development of Methods and Models for Nanoparticle Toxicity Screening: Application to Fullerenes and Comparative Nanoscale Particles**
Tian Xia, University of California, Los Angeles

3:40 – 4:00 p.m. **Effects of Nanomaterials on Human Blood Coagulation**
Peter Perrotta, West Virginia University

4:00 – 4:20 p.m. **Uptake and Toxicity of Metallic Nanoparticles in FreshwaterFish**
David Barber, University of Florida

4:20 – 4:40 p.m. **Innate Immune Responses of an Aquatic Vertebrate Model to Manufactured Nanoparticles Assessed Using Genomic Markers**
Rebecca Klaper, University of Wisconsin–Milwaukee

Metals, Metal Oxides Toxicity (continued)

4:40 – 5:00 p.m. **Chemical Fate, Biopersistence, and Toxicology of Inhaled Metal Oxide Nanoscale Materials**
Jacob McDonald, Lovelace Respiratory Research Institute

5:00 p.m. ***ADJOURN – DAY 2***

DAY 3, Friday, November 21, 2008

7:30 – 8:30 a.m. **Registration**

8:30 – 8:40 a.m. **Welcome and Announcements**

Other Nanomaterials
Life Cycle Analysis and Remediation

8:40 – 9:00 a.m. **Nanostructured Membranes for Filtration, Disinfection, and Remediation of Aqueous and Gaseous Systems**
Kevin Kit, University of Tennessee

9:00 – 9:20 a.m. **Comparative Life Cycle Analysis of Nano and Bulk Materials in Photovoltaic Energy Generation**
Vasilis Fthenakis, Columbia University

9:20 – 9:40 a.m. **The Life Cycle of Nanomanufacturing Technologies**
Thomas Theis, University of Illinois

9:40 – 10:00 a.m. **Evaluating the Impacts of Nanomanufacturing Via Thermodynamic and Life Cycle Analysis**
Bhavik Bakshi, The Ohio State University

10:00 – 10:20 a.m. ***BREAK***

Other Nanomaterials
Exposure

10:20 – 10:40 a.m. **Impact of Physiochemical Properties on Skin Absorption of Manufactured Nanomaterials**
Xin-Rui Xia, North Carolina State University

10:40 – 11:00 a.m. **Safety/Toxicity Assessment of Ceria (A Model Engineered NP) to the Brain**
Robert Yokel, University of Kentucky

Other Nanomaterials
Fate/Transport

11:00 – 11:20 a.m. **Agglomeration, Retention, and Transport Behavior of Manufactured Nanoparticles in Variably Saturated Porous Media**
Yan Jin, University of Delaware

DAY 3, Friday, November 21, 2008 (continued)

Other Nanomaterials Fate/Transport (continued)

11:20 – 11:40 a.m. **Internalization and Fate of Individual Manufactured Nanomaterial Within Living Cells**
Galya Orr, Pacific Northwest National Laboratory

11:40 – 12:00 p.m. **Methodology Development for Manufactured Nanomaterial Bioaccumulation Test**
Yongsheng Chen, Arizona State University

12:00 – 12:20 p.m. **Experimental and Numerical Simulation of the Fate of Airborne Nanoparticles From a Leak in a Manufacturing Process To Assess Worker Exposure**
David Pui, University of Minnesota

12:20 – 12:40 p.m. **Nanoparticle Disruption of Cell Function**
Andrij Holian, University of Montana

12:40 – 2:00 p.m. ***LUNCH (on your own)***

2:00 – 2:20 p.m. **Biological Fate and Electron Microscopy Detection of NPs During Wastewater Treatment**
Paul Westerhoff, Arizona State University

Other Nanomaterials Toxicity

2:20 – 2:40 p.m. **Genomics-Based Determination of Nanoparticle Toxicity: Structure-Function Analysis**
Alan Bakalinsky, Oregon State University

2:40 – 3:00 p.m. **Role of Surface Chemistry in the Toxicological Properties of Manufactured Nanoparticles**
Prabir Dutta, The Ohio State University

3:00 – 3:20 p.m. **A Rapid *In Vivo* System for Determining Toxicity of Manufactured Nanomaterials**
Robert Tanguay, Oregon State University

3:20 – 3:40 p.m. **Cellular Uptake and Toxicity of Dendritic Nanomaterials: An Integrated Physicochemical and Toxicogenomics Study**
Mamadou Diallo, California Institute of Technology

DAY 3, Friday, November 21, 2008 (continued)

3:40 – 4:00 p.m.	**BREAK**
4:00 – 4:20 p.m.	**Effects of Ingested Nanoparticles on Gene Regulation in the Colon** *John Veranth, University of Utah*
4:20 – 4:40 p.m.	**Nanoparticle Toxicity in Zebrafish** *Gregory Mayer, Texas Tech University*
4:40 – 5:00 p.m.	**Lung Deposition of Highly Agglomerated Nanoparticles** *Jacob Scheckman, University of Minnesota*
5:00 p.m.	***ADJOURN***

Interagency Environmental Nanotechnology Grantees Workshop
November 19 - 21, 2008

Sheraton Tampa Riverwalk Hotel
200 North Ashley Drive
Tampa, FL

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Nanotechnology

W. Allen Robison, Ph.D.

National Institute for Occupational Safety and Health
Office of Extramural Research

19 November 2008 Tampa, FL

Purpose

- Increase knowledge of nanotechnology and manufactured nanomaterials
- Occupational Safety and Health
- Applications/Implications
- Complements intramural program

Background

2001-2008

- R01, R03, R21, R43/44 funding mechanisms
- Program Announcements
- Joint Request for Applications
  (EPA, NIEHS, NSF)

NIOSH Extramural Funding

- 2001/2002 $850K (R43/44 NIOSH)
  - 2004 $100K (R43 NIOSH)
  - 2005 $1.46M (R01 NIOSH)
  - 2005 $789K (RFA EPA lead)
  - 2006 $100K (R43 NIOSH)
  - 2006 $359K (RFA EPA lead)
  - 2007 $500K (RFA NIEHS lead)
  - 2008 $800K (joint RFA + NIOSH)
    (About $5M total)

NIOSH Extramural Funding

- 2007
  Four active grants R01 extramural grants
  Two end in 2008
- 2008
  New R01, R03 and R44 grants

Types of Research Funded

- 13 different projects
- Sensors for Portable Monitors
- Novel Protection Garments
- Lung Oxidative Stress/Inflammation
  - (lung cells, macrophages)

Types of Research Funded

- Workplace Assessment Methods (air)
- Monitoring Airborne Carbon Nanotube Particles
  - (characterizing)
- Role of Surface Chemistry in Toxicology
  - (oxidative stress, inflammation)
- Toxicity of Inhaled Nanoparticles

For More Information

- **Progress Toward Safe Nanotechnology in the Workplace**
- DHHS (NIOSH) Publication No. 2007-123
- NIOSH Nanotechnology Research Center
- Summary of extramural projects in appendix
- **www.cdc.gov/niosh/topics/nanotech**

For More Information

- W. Allen Robison (WRobison@cdc.gov)
- 404.498.2530
- www.cdc.gov/niosh/oep

Other Web Sites

- **www2a.cdc.gov/niosh-nil**
- **www.cdc.gov/niosh/r2p/**
- **www.cdc.gov/niosh/programs/**
- **www.cdc.gov/niosh/topics/nanotech/ultrares.html**
- **www.cdc.gov/niosh/topics/nanotech/critical.html**

NIEHS
National Institute of Environmental Health Sciences

NIEHS Activities on Nanotechnology: Applications and Implications

Sri Nadadur, Ph.D.,
Division of Extramural Research & Training
National Institute of Environmental Health Sciences
National Institutes of Health, RTP, NC

Interagency Nanotechnology Grantee Meeting, Tampa, FL Nov2008

NIEHS
National Institute of Environmental Health Sciences

NIEHS Nanotechnology Health Implications

Exposure Research

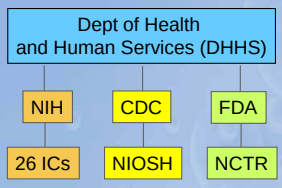
- Routes of exposure and systemic distribution
- Correlate physical and chemical characteristics of ENM with biological response
- Identify biomarkers of exposure and biological response
- Develop models to evaluate and predict biological response

Basic Research

- Interaction of ENM with biomolecules
- Transmembrane transport, cellular uptake, subcellular localization and retention
- Identify cell and organ-specific toxicity response pathway
- Effect of structural and surface modifications

NIEHS
National Institute of Environmental Health Sciences

NIH Research Interests in Nanotechnology



- **Nano Delivery Systems**
- **Bioimaging & Informatics**
- **Organ-tissue nano-engineering**
- **Medical Devices**
- **Biocompatibility and Toxicity**
- **Environmental health & safety**

NIEHS
National Institute of Environmental Health Sciences

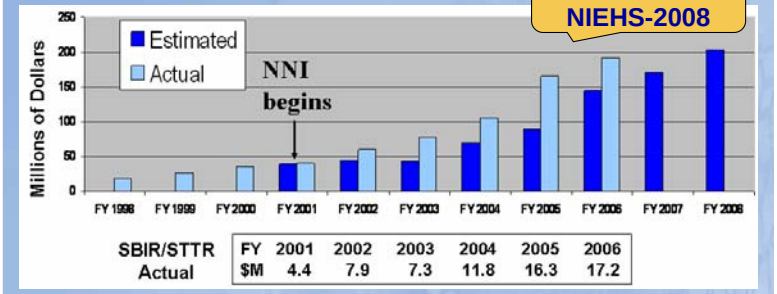
Extramural Research Program-Health Implications

- **Types of ENM**
  - Carbon (C60CS, fullerenes, SWNT, MWNT, CB), QDs, metal, silica, polystyrene,
- **Routes of Exposure**
  - Respiratory, dermal, gastric, ocular
- **Physico-chemical characterization**
  - Size, shape, structure, surface area, charge, aggregation, surface ligands, functional groups
- **Metabolism, Transport**
  - Cell/organ-specific transport, bio-persistence, biotransformation, elimination
- **Molecular mechanisms of toxicity**
  - Interaction with macromolecules, signaling pathways, stress pathways, immune function, xenobiotic metabolism, DNA repair, epigenetics, etc.,
- **Biomarkers of Exposure/response**
  - High throughput approaches (genomics, proteomics, metabonomics)

NIEHS
National Institute of Environmental Health Sciences

NIH- Nanotechnology Research Funding

30 Millions NIEHS-2008



SBIR/STTR Actual	FY	2001	2002	2003	2004	2005	2006
	$M	4.4	7.9	7.3	11.8	16.3	17.2

NIEHS
National Institute of Environmental Health Sciences

Extramural Research Program-Health Implications

➢Comparative in vitro toxicity screening for oxidative stress response of commercially available nanoparticles- -Andre Nel, UCLA

➢Cardiovascular toxicity of nickel nanoparticles and particles coated with sulfuric acid studied following inhalational exposure – Lung-chi Chen, NYU

➢Comparative toxicology of CNP (C60CS, SWNT, MWNT)- alterations in macrophage membrane function for lung inflammation and injury- AndriJ Hoilan, University of Montana

➢Understand relative influence of nanomaterial characteristics on nanomaterial biological interactions at multiple levels of biological organization- Rob Taugary, Oregon State Univ.

Nan

Nanotech arrays to recognize gasoline and diesel combustion products—*Ashok Mulchandani, U. of California at Riverside*

Polymer wire "tuning fork" to detect Volatile organics—*NJ Tao, Arizona State U.*

- En
  - Sensor arrays - Primarily affinity based
  - Functional profiling to detect unknown toxins
- Biological Sensors
  - Develop technologies to link exposure to disease etiology
  - Mechanistic research (cellular dynamics, signal pathways)
  - Biomarker detection (in vivo imaging, sensor arrays)

Nanoparticle based immunoassay systems for detection of N-Fibrinogen - Yuhe Lin, PNNL

Contact: David Balshaw: balshaw@niehs.nih.gov

NIEHS Nanotechnology Programs

Implications **Applications**

Interaction of Engineered Nanomaterials with Biological Systems

Structure **Activity**

Predictive Models

Risk Assessment

Nanotechnology - Remediation efforts

➢**2008 RFA-** ***Development and Application of Nanotechnology-based Tools to Understand Mechanisms of Bioremediation***

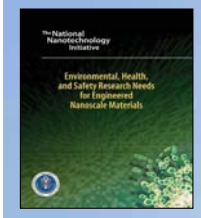
➢**Sensors (for As, PCBs)**

➢

- Mechanisms of Hg adsorption from mixed pollutant streams – **carbon nanotubes** – *R. Hurt, Brown U*
- Dechlorination of TCE by nanosized metallic systems and by chelate-modified hydroxyl radical reaction – **bi-metallic (Fe/Ni) nanoparticles** - *D Bhattachatyya, U Kentucky*
- Ground water As decontamination using nanoparticle and granular zero-valent iron – nZVI-based permeable reactive barriers – *D Sedlak, UC Berkeley*
- Activated carbon as a multi-functional amendment treatment to treat PCBs and mercury – **nZVI impregnated AC** – *R Luthy, Stanford U**

Contact: Heather henry, henryh@niehs.nih.gov

NIEHS Next Step: Building the NanoHealth and Safety Enterprise



- ➢ Build on the NIH investment and expertise
- ➢ Invite stakeholder participation
- ➢ Target questions within a shared research strategy
- ➢ Harmonize with US goals for commercialization and innovation

NTP Nanotechnology Safety Research

Nanoscale titanium dioxide (sunscreens)

- **Dermal penetration studies, *in vivo* and *in vitro***
- **Phototoxicology and photocarcinogenicity**

Quantum dots

- **Pharmacokinetics studies**
- **Dermal studies**

Carbon fullerenes

- **Oral and pulmonary toxicity studies**
- **Material formulation and characterization in progress**

Dendrimers

- **Pharmacokinetics and biocompatibility**
- **Collaborative efforts with NCL**

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

NanoHealth and Safety Enterprise

- Characterizaton of Materials
- Biochemical -Interactions
- Pathophysiological Mechanisms
- Training Program

NIEHS
National Institute of Environmental Health Sciences

Targeted Research Projects

Implications

Applications

Interaction of Engineered Nanoscale Materials with Biological Systems

Dose Metrics

Uptake by Route of Exposure

Interaction with Biological Fluids

Informatics Resource (NIBIB)

NIEHS
National Institute of Environmental Health Sciences

Potential Research Products

Biologically and clinically relevant design principles

Curated data sharing framework

Network of research partners

Strategic product design and development

Shorter time from concept to manufacture

Data for hazard identification

Standards setting

Computational Models for Safe Design

High Throughput Screening

Reactive Composites for Targeted Remediation of TCE

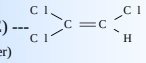
Jingjing Zhan, Tonghua Zheng, Bhanu Sunkara,Gerhard Piringer, Gary McPherson, Yunfeng Lu*, Vijay John*

Department of Chemical & Biomolecular Engineering
Tulane University. New Orleans, LA

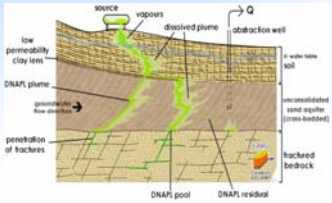
Supported by EPA Grant GR832374

Background

- Dense nonaqueous phase liquid (DNAPL)
  - Density greater than water (plumes transport to lower sediment layers)
  - Low (but finite) solubility in water.
- Example: trichloroethylene (TCE) ---



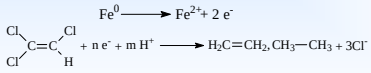
  - Density: 1.46 g/mL (heavier than water)
  - Solubility: 1100 ppm in water
  - Toxicity: Organ damage, carcinogen



Excerpted from: http://www.dnapl.group.shef.ac.uk/main.htm

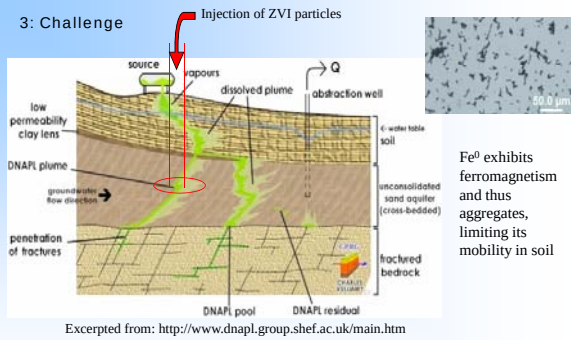
2: Chemical Remediation of TCE

- Zero valent nanoscale iron (ZVI) is an effective reductant for remediation of TCE with the following advantages:
  - environmental friendly
  - high efficiency
  - low cost
- Mechanism



Y.Liu, et al *Environ.Sci.Technol.* **2005**, 39, 1338

3: Challenge



Fe^0 exhibits ferromagnetism and thus aggregates, limiting its mobility in soil

Excerpted from: http://www.dnapl.group.shef.ac.uk/main.htm

- ZVI particles have poor mobility, hence a low efficiency in in-situ remediation.

Objective

Effective in-situ remediation of TCE requires the successful delivery of reactive iron particles through soil. Our goal is to engineer reactive particles that have good mobility through soils and directly target TCE.

Outline

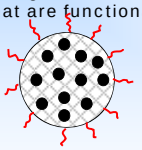
- Synthesis of ZVI containing composite particles.
- Reaction Characteristics
- Transport characteristics of the particles
- Partitioning characteristics between the bulk aqueous and the DNAPL phase

I: Particle synthesis

1: What are particle design requirements?

- Particles that are reactive to TCE
- Particles that will partition to TCE or to the TCE/water interface
- Particles that are of the correct size range for optimal mobility through sediments

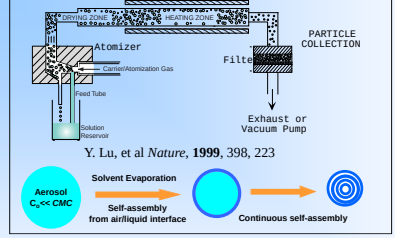
2: Idea: Incorporating nano-scale iron into porous submicron Silica particles that are functionalized with alkyl groups



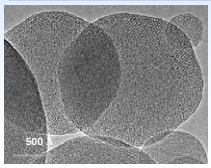
Characteristics:

- Nanoscale zero valent iron ensures high reactivity;
- Silica can be functionalized easily by varying the precursor;
- Silica is environmentally friendly.

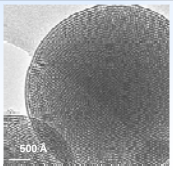
Aerosol Process to Mesostructured Silicas



Y. Lu, et al *Nature*, **1999**, 398, 223

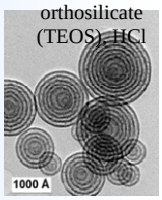


Hexagonal



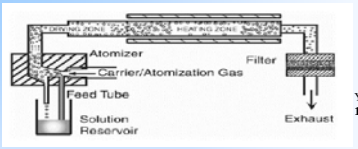
Cubic

Precursor solution: Surfactant, ethanol, tetraethyl orthosilicate (TEOS), HCl



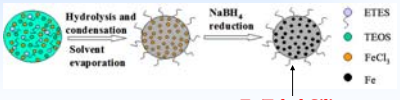
Lamellar

3: Particle preparation



Y. Lu, et al *Nature* **1999,** *398*, 223

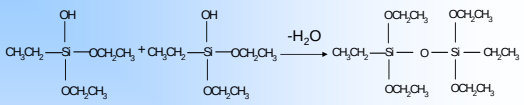
Aerosol technology: a simple, rapid method to obtain particles. Can be easily scaled up.

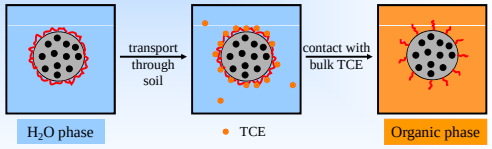


Fe/Ethyl-Silica

Precursor solution: 4.0g $FeCl_3$•$6H_2O$, 15ml H_2O, 3.0g TEOS and 1.2g ETES.
(TEOS: tetraethyl orthosilicate; ETES: ethyl triethoxysilane)

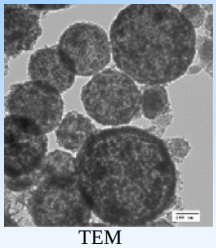
4: Reason to use functionalized silicas





• **Hypothesis**: Organic functional groups adsorb dissolved TCE facilitating contact with ZVI and also extend in the organic phase (TCE) to help particle stability.

Characterization



TEM

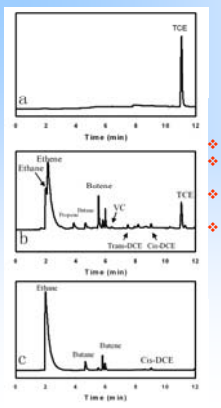
BET surface area (m^2/g)	**229.6**
Pore volume (cm^3/g)	**0.05**
Particle density (g/cm^3)	**4.0**

❖ **Fe/Ethyl-Silica particles are spherical with nanoiron throughout the silica matrix;**

❖ **Fe/Ethyl-Silica particles are porous**.

Zheng et al., *ES&T*, 2008

GC Spectra of Reaction Products

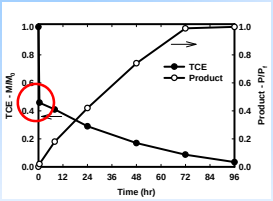


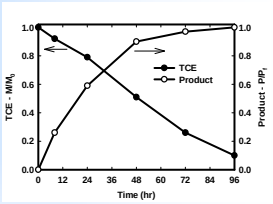
(a) at hr 0
(b) after 8 hr reaction with Fe/Ethyl-Silica
(c) after 1 hr reaction with Pd/Fe/Ethyl-Silica

❖ **Reaction is relatively slow for Fe/Ethyl-Silica**
❖ **Almost all the TCE disappeared after 1 hr of reaction with Pd/Fe/Ethyl-Silica**
❖ **Palladized particles produce more saturated ethane instead of ethene**
❖ **Trace amounts of chlorinated hydrocarbons are evident - disappears at long reaction times.**

Effect of Surface Modification

(a) Fe/ Ethyl- Silica **(b) Fe/Silica**

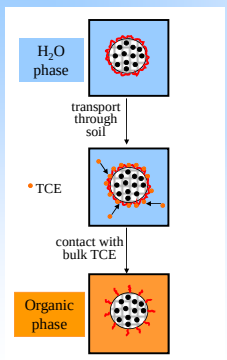


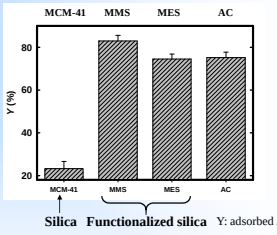


a) **TCE disappears faster for Fe/Ethyl-Silica particles in the first few hours of reaction.**
b) **Product evolution rates are comparable for Fe/Silica and Fe/Ethyl-Silica particles;**

❖ **Modification with ethyl functional groups leads to the adsorption of TCE on Fe/Ethyl-Silica particles.**

Reasons to use functionalized silicas



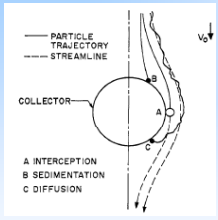


Silica **Functionalized silica** Y: adsorbed / initial

• Organic functional groups adsorb dissolved TCE, facilitating contact with ZVI and also extend in the organic phase (TCE) to help particle stability.

II: Transport properties

Filtration theory



Basic transport mechanisms in filtration

Kuan-Mu Yao; Habibian, M. T.; O'Melia, C. R.. *Environ. Sci. Technol.* **1971,** *5*, 1105-1112.

•**Diffusion** - influenced by interparticle van der Waals interactions .

•**Sedimentation** - gravitational effects

•**Interception** - Particles following flow streamlines come into contact with a sediment grain (collector).

Small Particles – Diffusion dominated

Large Particles – Dominated by sedimentation and interception

Tufenkji-Elimelech model

Tufenkji, N.; Elimelech, M.. *Environ. Sci. Technol.* **2004,** *38*, (2), 529-536.

$$\eta_0 = 2.4A_S^{1/3}N_R^{-0.081}N_{Pe}^{-0.715}N_{vdW}^{0.052} + 0.55A_S N_R^{1.675}N_A^{0.125} + 0.22N_R^{-0.24}N_G^{1.11}N_{vdW}^{0.053}$$

Diffusion Interception Sedimentation

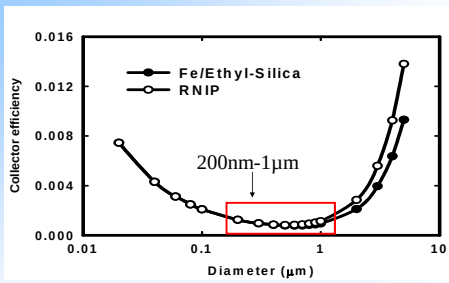
Commercial Reactive Nanoscale Iron Particles (RNIPs)

$$\eta_0 = 5.543\times10^{-9}d_P^{-0.796} + 1.391\times10^{5}d_P^{1.425} + 3.08\times10^{8}d_P^{1.98}$$

Fe/Ethyl-Silica particles

$$\eta_0 = 5.543\times10^{-9}d_P^{-0.796} + 1.391\times10^{5}d_P^{1.425} + 1.67\times10^{8}d_P^{1.98}$$

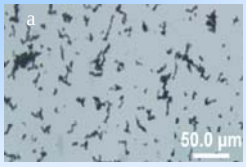
• based on differences in particle densities



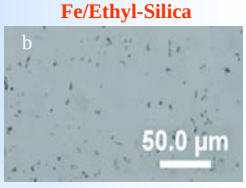
• 200nm-1µm is the correct size range for optimal mobility through sediments

Aggregation Characteristics

Bare-RNIP (Toda Kogyo Corp.)



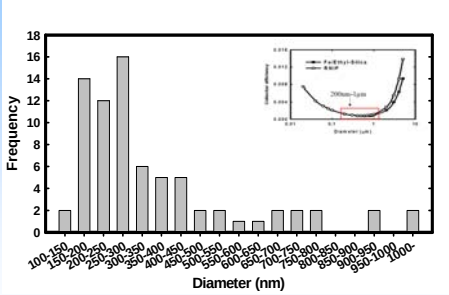
Fe/Ethyl-Silica



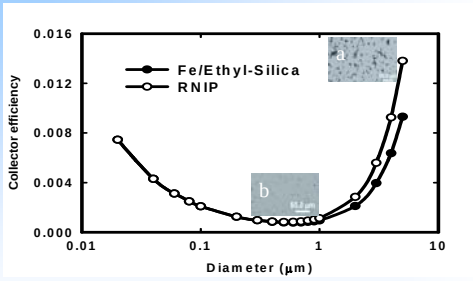
(In accordance to the result previously reported : Phenrat, T. et al *Environ. Sci. Tech.* **2007**, *41*, 284)

• RNIP aggregates significantly due to magnetic interactions. Incorporation of ZVI into the silica matrix substantially decreases aggregation.

Size distribution



• Almost all Fe/Ethyl-Silica particles are in the size range for optimal mobility.

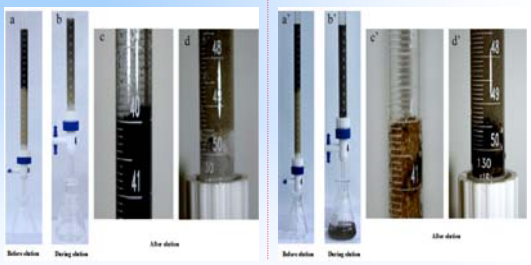


The iron-silica partilces have optimal collector efficiency

Column test

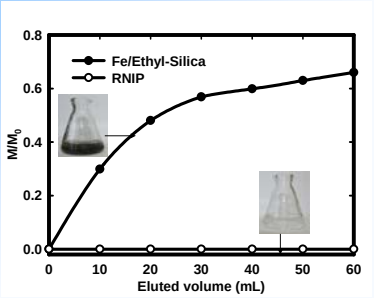
1.2cm(i.d.) burette packed with Ottawa Sand

Bare-RNIP | Fe/Ethyl-Silica



- Fe/Ethyl-Silica suspension transports through the soil readily, while most of RNIP particles are retained at the top of the column.

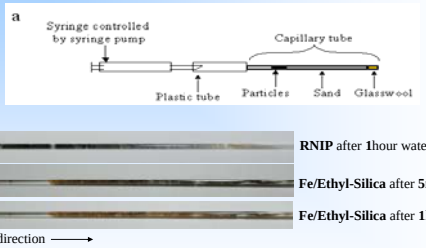
Effluent analysis



- ~66% of Fe/Ethyl-Silica particles are eluted through the sediment, while RNIP does not elute.

Capillary experiment

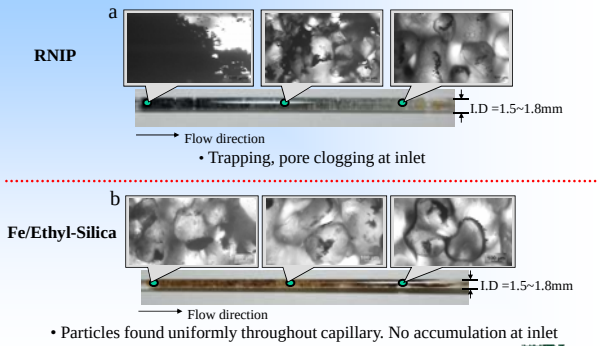
1.5 mm(i.d.) Capillary packed with Ottawa Sand



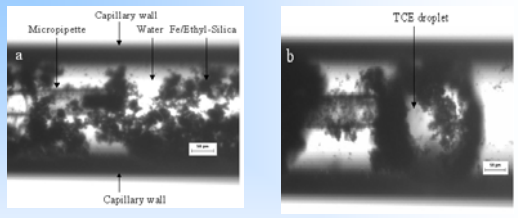
- Bare RNIP accumulates at capillary inlet.
- Fe/Ethyl-Silica Particles move through the capillary.

Zhan et al., *ES&T*, 2008

Optical Microscopy of Capillary



- Trapping, pore clogging at inlet
- Particles found uniformly throughout capillary. No accumulation at inlet



Before injection | After injection

Injection of a TCE droplet into a microcapillary containing Fe-EthylSilica particles.

Summary

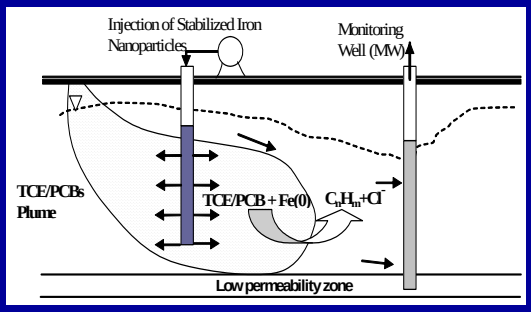
- **Synthesis of adsorptive-reactive Fe/Ethyl-Silica composite particles.**
- **The Fe/Ethyl-Silica particles are in the correct size range for optimal mobility through model soils.**
- **Fe/Ethyl-Silica particles may preferentially accumulate and localize at the TCE/water interface, making dechlorination more efficient.**
- **Adsorption of TCE on the particles leads to a dramatic reduction in solution TCE concentration.**
- **The composite particles can be used in in-situ remediation and in the development of reactive barriers.**

Synthesis and Application of Polysaccharide-Stabilized Fe-Pd Nanoparticles for *in situ* Dechlorination in Soil and Groundwater

Progress Report III: Nov 19, 2008

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

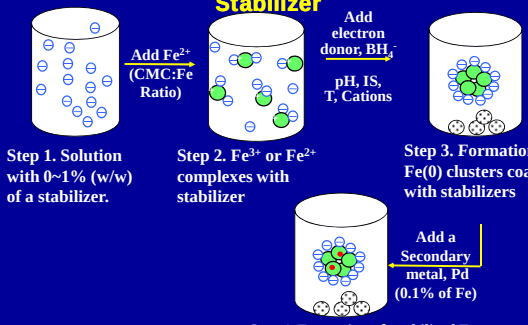
In situ Degradation of Chlorinated Solvents by ZVI Nanoparticles



Primary Accomplishments in Year 3

- Conducted batch and column tests for degradation of TCE sorbed/trapped in soils using CMC-stabilized ZVI nanoparticles
- Tested and modeled transport behaviors of CMC-stabilized ZVI nanoparticles in porous media
- Pilot-tested in situ dechlorination in soils using CMC-stabilized ZVI nanoparticles

Size-Controlled Synthesis of ZVI Nanoparticles Using Carboxymethyl Cellulose (CMC) as a Stabilizer



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

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

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

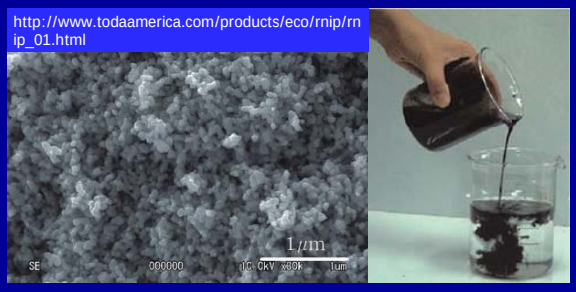
Step 4. Formation of stabilized Fe-Pd bimetallic nanoparticles.

He et al., Industrial & Engineering Chem. Res. 2007, 46(1), 29-34.

Stabilized vs Non-Stabilized ZVI Nanoparticles

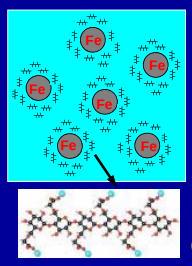
Commercially Available Iron "Nanoparticles", RNIP (Toda America Inc.)

http://www.todaamerica.com/products/eco/rnip/rnip_01.html

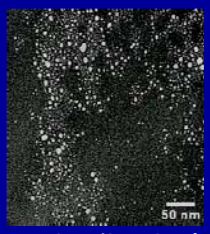


Toda claims: "RNIP are zero valent iron solids with an average particle size of 70 nm."
"RNIP are available as a water-based slurry."

ZVI Nanoparticles Stabilized with a CMC (90k M.W.)

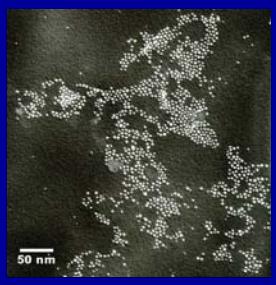


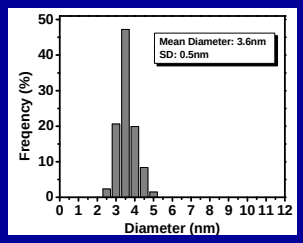
CMC



D = 4.3 nm (SD=1.8 nm)

CMC can Facilitate Synthesis of Nearly Mono-disperse Pd Nanoparticles that can Catalyze TCE Degradation

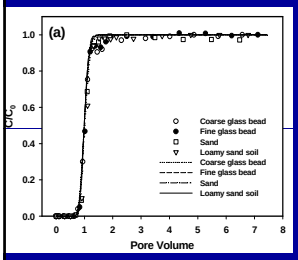


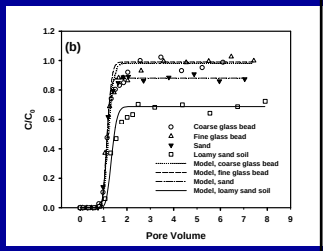


Pd = 0.2 mM; CMC = 0.15 wt%, Temp. = 95 °C

Column Set-up for Studying Transport of CMC-ZVI Nanoparticles in Four Porous Media

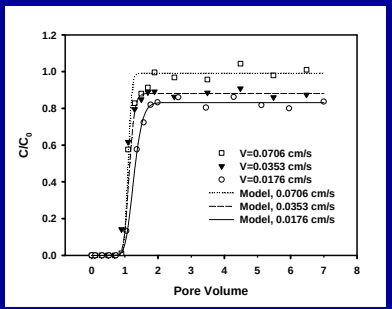
Breakthrough Curves of Br^- and CMC-Stabilized ZVI Nanoparticles through Four Porous Media





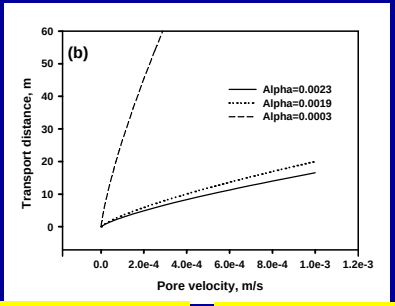
Br^- = 50 mg/L; Fe = 0.2 g/L; Empty Bed Contact Time (EBCT) was 28 min. Lines are model simulation.

Breakthrough Curves of CMC-Stabilized ZVI Nanoparticles through Sand at Various Velocities



Fe = 0.2 g/L; Lines are model simulations.

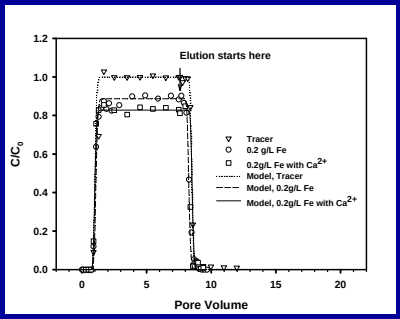
Predicted Maximum Travel Distance of CMC-Fe Nanoparticles in Sand as a Function of Pore Liquid Velocity



$$L_{max} = -\frac{2}{3(1-f)\alpha\eta_0}\frac{d_c}{}\ln(0.01)$$

$$\alpha = \frac{\eta}{\eta_0} = -\frac{2}{3(1-f)L\eta_0}\frac{d_c}{}\ln(C_e/C_0)$$

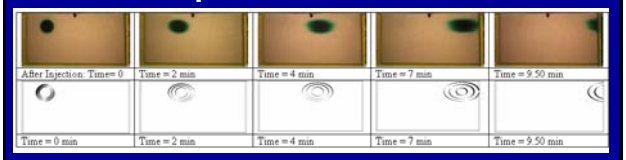
Breakthrough and Elution Curves of CMC-Fe Nanoparticles through a Sand Bed



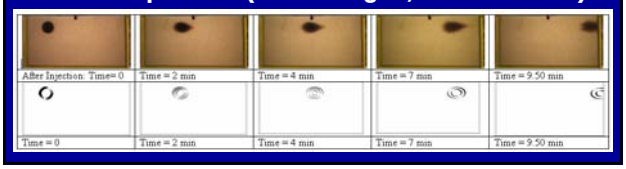
Influent Fe = 0.2 g/L; EBCT = 28 min; Pore liquid velocity = 0.0353 cm/s; Ca = 40 mM.

Transport in a 2-D Sand Box (Kanel et al. *ES&T*, 2008)

- Tracer transport



- ZVI Nanoparticle (Fe = 0.2 g/L, CMC=0.16%)



Column Set-up for in-situ Degradation of TCE in a Sand Column

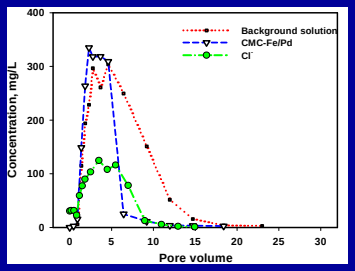
- TCE was spiked in the sand bed and 0.5 g/L ZVI nanoparticle suspension was pumped through the column

(a)

(b)

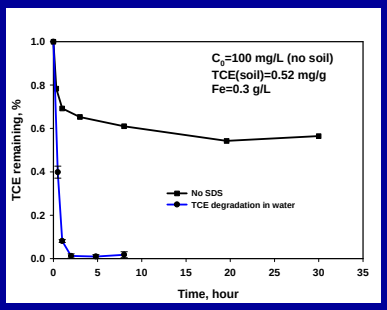
(a) Column setup for in-situ TCE degradation; (b) A close-up of the column as Fe/Pd nanoparticle suspension was introduced.

In situ Degradation of TCE Spiked in a Sand Column

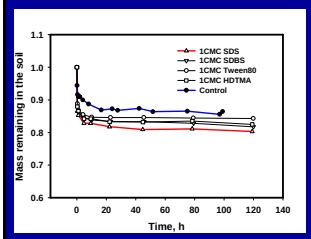


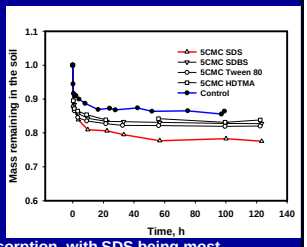
Degradation of TCE and generation of Cl^- in a sand column during the nanoparticle treatment
(TCE = 15 mg, Fe = 0.5 g/L, Pd/Fe = 0.1 wt%, Cellulose = 0.4 wt.%, suspension pore velocity = 0.0118 cm/s, EBCT = 84 min)

Degradation of TCE Sorbed in an Organics-Rich Soil Compared to TCE in Water (Batch Tests)



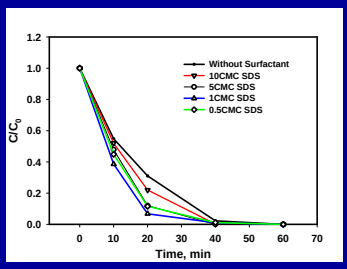
Surfactant-Enhanced TCE Desorption from an Organic Soil





- All surfactants enhanced TCE desorption, with SDS being most effective, resulting in a TCE mass desorption of ~18% in 120 h at 1xCMC surfactant dosage
- TCE desorbed was increased to 22% at 5xCMC SDS
- TCE_0 in Soil = 0.52 mg/g

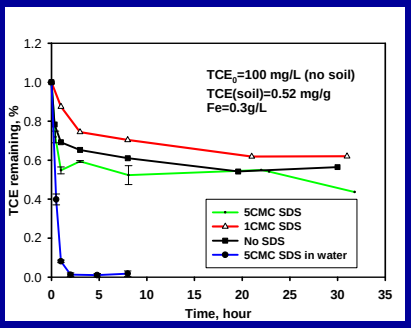
Effects of Surfactants on TCE Degradation in Aqueous Phase



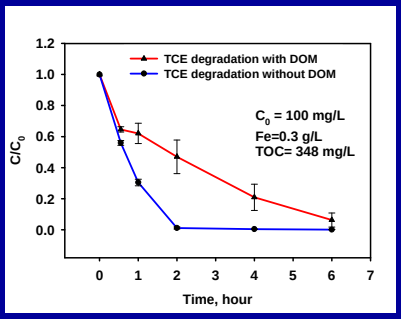
- SDS enhances TCE degradation: 1xCMC >0.5CMC≈ 5xCMC >10xCMC. At 1xCMC SDS, the rate constant was increased by a factor of ~1.7 than without surfactant

Test conditions: TCE=10mg/L, Fe=0.1g/L, Pd/Fe=0.1wt%, Cellulose=0.2 wt%

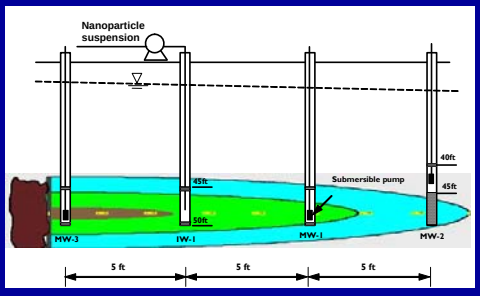
Effect of SDS on Degradation of TCE Sorbed in an Organics-Rich Soil



Effect of DOM on TCE Degradation in Water



Field Assessment of CMC-Stabilized Fe-Pd Nanoparticles at an Alabama Site



A sectional view of the aquifer and location of injection well and monitoring wells. ($K=7x10^{-5}$ m/s or 20 ft/d)

The Nanoparticle Tank and Injection Setup

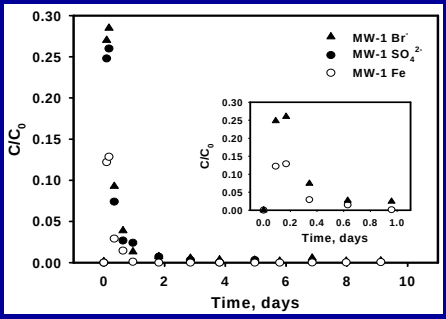
Suspension of CMC-Stabilized Fe-Pd Nanoparticles before Injection

Slurry of ZVI Nanoparticles (0.33 g/L)

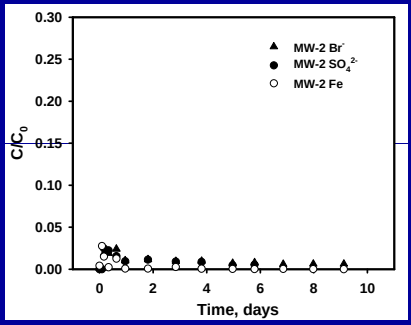
The Monitoring Well

Monitoring well MW-1

Concentration Histories of Iron Nanoparticles in MW-1 Compared to Tracers

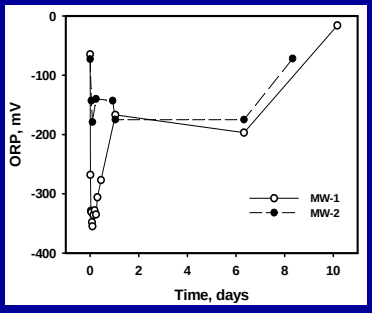


Concentration Histories of Iron Nanoparticles in MW-2 Compared to Tracers

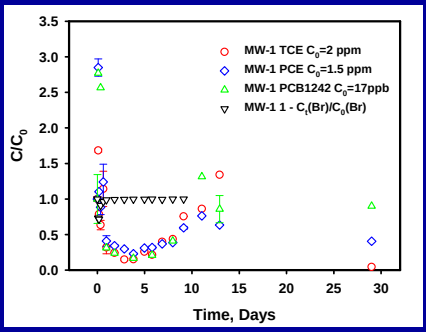


Note: Little ZVI was detected in the up-gradient well

Changes of Oxidation and Reduction Potential (ORP) in Monitoring Wells



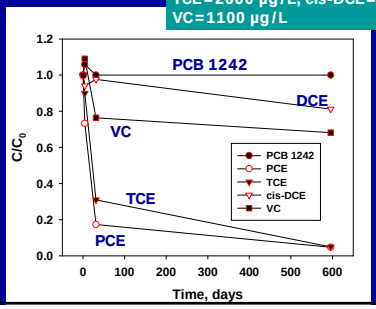
Concentration Evolution of PCE, TCE and PCB 1242 in Groundwater from MW-1 Following Injection of 150 G (0.2 g/L) Fe-Pd Nanoparticle Suspension



Note: Little degradation was detected in the up-gradient well

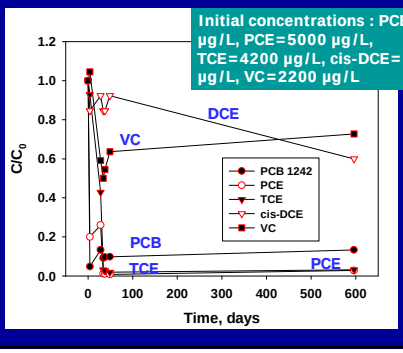
Concentration Evolution of PCE, TCE and PCB 1242 in MW-1 following two Injections of (150 G 0.2 g/L +150 G0.5 g/L) Fe-Pd Nanoparticle Suspension

Initial concentrations: PCB= 17.0 µg/L, PCE=1500 µg/L, TCE=2000 µg/L, cis-DCE=8500 µg/L, VC=1100 µg/L



Concentration Evolution of PCE, TCE and PCB 1242 in MW-2 Following two Injections of (150 G 0.2 g/L +150 G0.5 g/L) Fe-Pd Nanoparticle Suspension

Initial concentrations : PCB= 60.0 µg/L, PCE=5000 µg/L, TCE=4200 µg/L, cis-DCE=13000 µg/L, VC=2200 µg/L



Summary

- CMC can facilitate size-controlled synthesis of ZVI nanoparticles
- Transport of CMC-stabilized Fe nanoparticles are controllable and can be modeled by CDE & filtration theory
- CMC-stabilized ZVI can degrade TCE in soil, but must overcome mass transfer and sorption limitation and DOM inhibition

Acknowledgements

- USEPA STAR Grant (GR832373)
- Dr. Nora Savage – EPA Project Manager
- Golder Consultants, Atlanta
- Dr. Gupta in Chemical Engineering Department for DLS analysis

Characteristics, Stability, and Aquatic Toxicity of CdSe/ZnS Quantum Dots

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Department of Chemistry & Geochemistry
Colorado School of Mines
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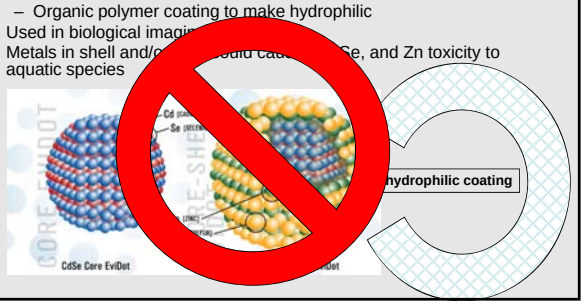
Presented at EPA Nanotechnology PI meeting, Nov. 19th, 2008

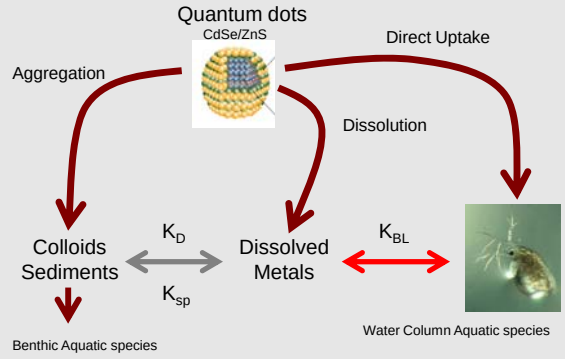
This research is funded by U.S.EPA - Science To Achieve Results (STAR) Program
Grant # RD-83332401-0

CdSe/ZnS Quantum Dots

- Bright, photostable fluorophores
- Basic Structure
  - Metalloid core, CdSe
    - Wurtzite crystal structure, 1:1 Cd/Se mole ratio
  - Protective shell, ZnS
  - Organic polymer coating to make hydrophilic
- Used in biological imagi
- Metals in shell and/o Se, and Zn toxicity to aquatic species



Environmental Fate of Metals in Quantum Dots



Research Approach

- Characterization
  - Core
    - UV-Vis absorption, Fluorescence
  - Core/Shell
    - TEM
  - Core/Shell/Polymer (Hydrodynamic)
    - Light scattering, Metal ratios (ICP-AES/MS), Metal size distributions (FFF-ICP-MS)
- Stability
  - Aggregation, dissolution
    - Short-term (48 hours)
    - Long-term
- Toxicity/Uptake
  - Acute (48-hr) *D. Magna*
  - Brookhaven National Light Source: μ-XRF

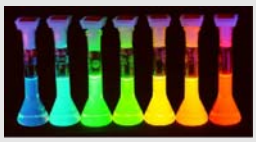
Materials

- Four types of QDs used in experiments
  - Two core sizes, two coatings (PEO, MUA)
- Optical properties depend on core size
  - UV-Vis Absorbance used to size core

$$\text{CdSe: } D = (1.6122 \times 10^{-9})\lambda^4 - (2.6575 \times 10^{-6})\lambda^3 + (1.6242 \times 10^{-3})\lambda^2 - (0.4277)\lambda + (41.57)$$

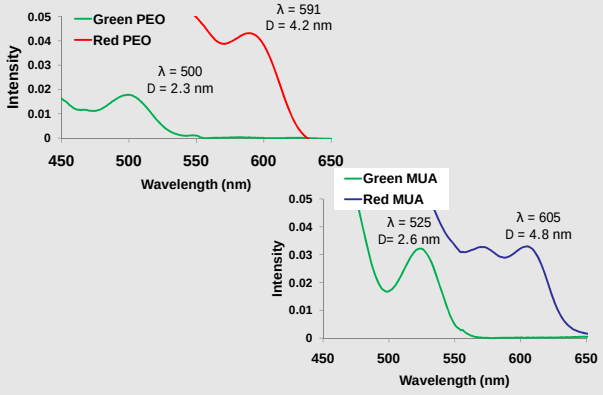
Yu et al., 2003

  - Fluorescence

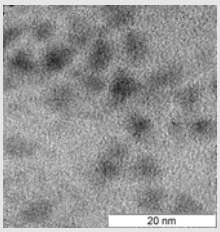


Evident Technologies Inc

Characterization: UV Absorbance of 4 QDs

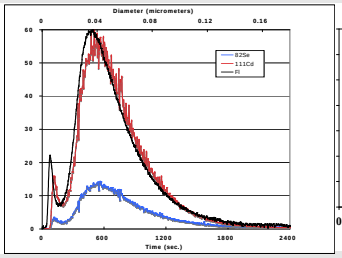


TEM

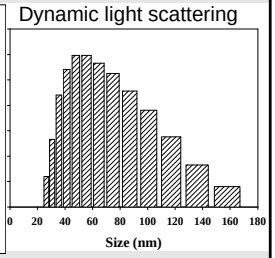


- Red EviTag PEO coating
- Polymer coating not observable
- Core/shell size about 5-7nm
- UV wavelength max of 609nm gives about 5 nm core
- ZnS coating computed from chemical analysis to be about 1.2 nm

Hydrodynamic Size (Core/Shell/Polymer)
Fl FFF and DLS Characterization
Fl-FFF-ICP-MS



Dynamic light scattering



DLS requires higher concentration than FFF

Fluorescence comes from CdSe Core

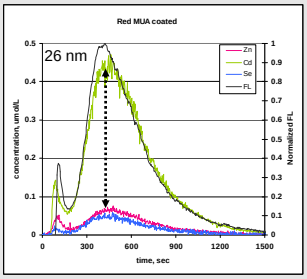
ICP-AES/MS: Metal Ratios

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
	c	*1.6*	*1.5*	*2.1*	*5.8*
MUA	a	23	0.14	ND	0.23
	b	22	0.14	11	0.23
	c	*9.1*	*0.16*	*7.1*	*0.26*

a. ICP-AES: QD in hard water
b. ICP-AES: QD in DI water
c. Integrated signal from FFF-ICP-MS

- Cd:Se ratio not 1:1
- Excess Cd, especially for MUA QDs
- Zn/Cd higher for smaller (green) QD

Elemental Size Distribution by Fl FFF-ICP-MS

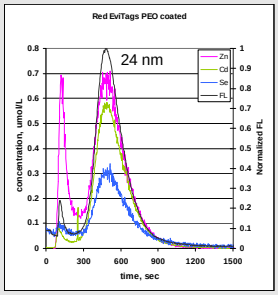


Possible explanation:
Cd associated with polymer due to poor washing during synthesis

Element Ratios

	Void	QD Peak
Zn/Cd	0.34	0.16
Cd/Se	8.4	9.1

Elemental Size Distribution by Fl FFF-ICP-MS



Possible explanation:

No dissolved Zn detected in a 3K Dalton filtrate

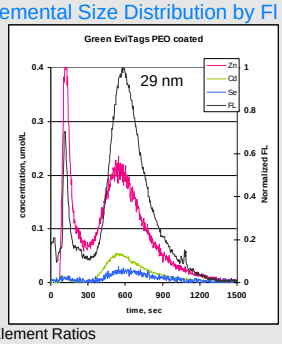
Zn associated with free polymer

Very little Fl in void peak

Element Ratios

	Void	QD Peak
Zn/Cd	8.1	1.2
Cd/Se		1.6

Elemental Size Distribution by Fl FFF-ICP-MS



Possible explanation:

No dissolved Zn detected in a 3K Dalton filtrate

Zn associated with free polymer or ZnS

Some Fl in void peak

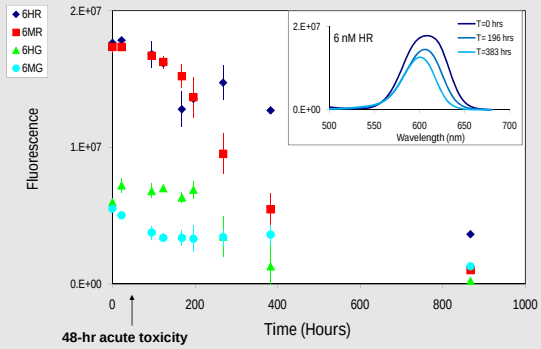
Element Ratios

	Void	QD Peak
Zn/Cd	40.3	4.3
Cd/Se		2.1

FFF-ICP-MS Discussion

- Large excess of Cd associated with QD
  - Possibly associated with polymer coating
- Zn in Void peak
  - Unlikely to be dissolved
  - Low fluorescence: Zn possibly associated with unattached polymer
  - High fluorescence: Zn possibly present as ZnS
- What are the implications for stability and toxicity?

Long Term Stability: Loss of fluorescence (PEO QDs)



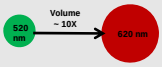
Acute Toxicity: Methods

48 hr acute toxicity tests
- USEPA Standard Test Protocol
- Mortality (ie immobile) endpoint
- EPA hard water

Daphnia magna obtained from cultures maintained in our lab at 20ºC and a 16:8 hr day:night cycle

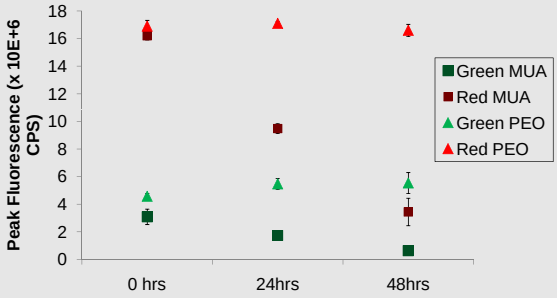
PEO and MUA coated CdSe/ZnS quantum dots
Exposure Concentration Range
- 0.1 – 30 x 10^{-9} mol QDs/L

Two different size dots were tested
- Green Dots – 2.5 nm core diameter
- Red Dots – 5 nm core diameter

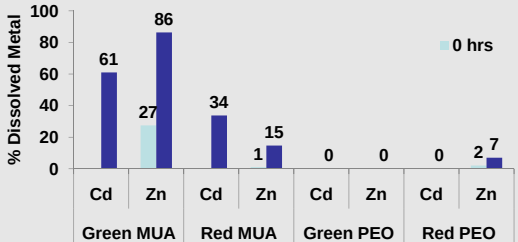


Stability of QDs monitored throughout 48hr test
- Fluorescence: 0, 24 and 48 hrs
- ICP-AES metals analysis of solutions (total metals in solution): 0 and 48 hrs
- 3kDa filtrations w/ ICP-AES metals analysis (dissolved metals): 0 and 48hrs

Acute Toxicity: Fluorescence intensity (CPS) throughout acute toxicity test

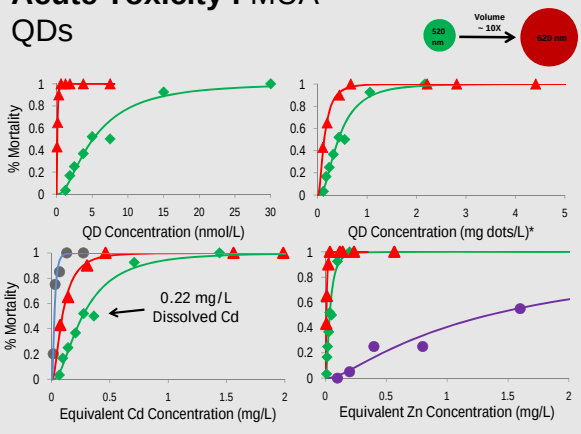


Acute Toxicity: Percent dissolved metals at beginning (0 hrs) and end (48hrs) of acute toxicity tests for a 7.5 nmol/L QD solution



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

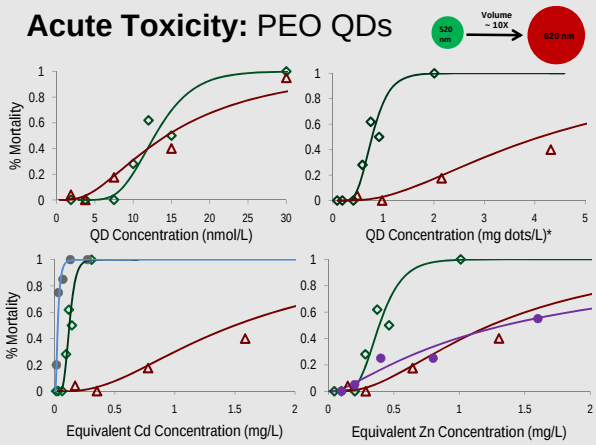
Acute Toxicity : MUA QDs



MUA Toxicity Discussion

- Toxicity seems to be a mass based phenomenon
- Dissolved metals present at 48 hrs (i.e. MUA QDs release metals)
- There is enough Cd to cause observed death (not enough total Zn)
- Rate of metal release is important

Acute Toxicity: PEO QDs

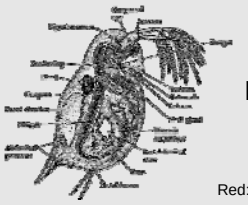


PEO Toxicity Discussion

- Toxicity seems to be a particle number phenomenon
- Distinct differences in toxicity are observed when toxicity curves are plotted on a mass basis (smaller QDs are more toxic)
- No detectable dissolved metals found in solution at 48 hrs, yet toxicity is observed
  - If metal toxicity, metals must be released in the daphnid gut
- Cd is not completely bioavailable, as dissolved Cd is more toxic than both PEO QDs on an equivalent Cd basis
- Dissolved Zn is potentially the toxic agent for the Red PEO QDs, as the two dose-response curves overlap

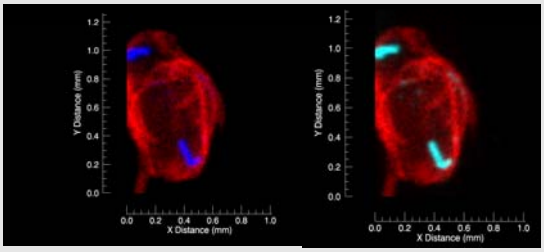
Acute Toxicity: Conclusions

- Stability has a strong influence on QD toxicity
  - The stability & toxicity may be related to impurities more than the actual QD core/shell
- Dissolved Cd can explain observed toxicity for MUA QDs
- However, no dissolved metals in PEO QDs at 48hrs suggests an alternate pathway
  - Metals are released after QDs are ingested
  - Toxicity due to the particle
  - Impurities in the QD stock solution



Uptake & Metal Availability: μ-XRF Brookhaven National Light Source

Red: Ca Blue: Zn Cyan: Se



Future Work

- Stability Experiments
  - Aggregation experiments with model environmental colloids
  - Further dissolution rate experiments with more well-defined QDs
- Toxicity Experiments
  - Sub-lethal tests with *D. Magna* at low QD concentrations
    - Feeding and fecundity endpoints
  - Tests with benthic species (*H. azteca*)
- Characterization
  - Further application of FFF-ICP-MS to QD synthesis through collaborations with QD researchers

Acknowledgements

Funding

This research is funded by U.S.EPA-Science To Achieve Results (STAR) Program Grant # RD-83332401-0

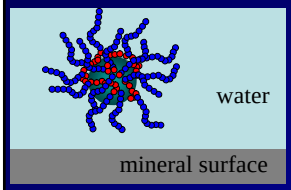
DOE ERSP Grant: ER64419
Edna Bailey Sussman Fellowship

Student Investigators

FFF work
- Sungyun Lee
- Emily Lesher

Tox & Stability
- Jesse Forth
- Heather Pace

Effect of surface coatings on the fate of NZVI and Fe-oxide NPs



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EPA STAR Grantees Meeting
November 19-21, 2008

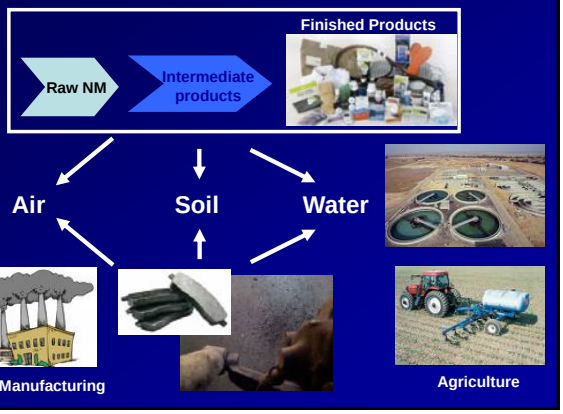
SERDP A Partnership to Improve the Environment

Collaborative Effort

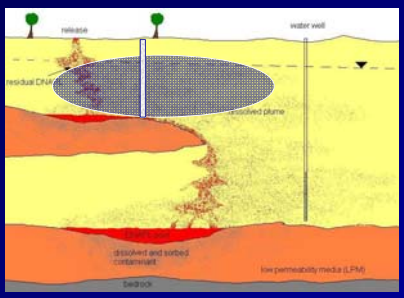
- Robert Tilton-CHE/BME
- Ned Minkley-Microbiologist
- Pedro Alvarez-Env. Eng. and microbiologist
- Chris Kim-geochemist
- Ph.D. students

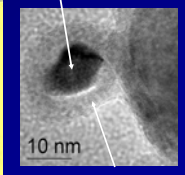


Nanomaterial Sources

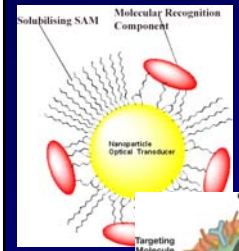


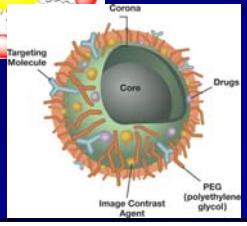
Nanoparticle-based Groundwater Treatment



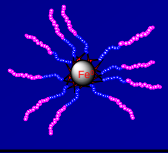


Most Nanomaterials are Coated



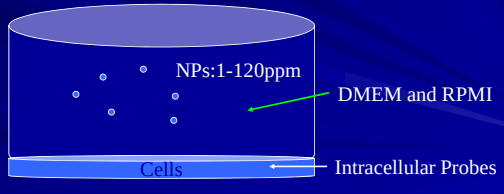


- Coatings provide…
  - Dispersion stability
  - Functionality
  - Targeting capabilities
  - Biocompatibility



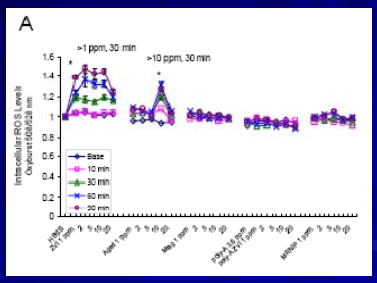
Polyaspartate coating Decreases ROS and Cytotoxicity in Glial Cells (BV2) and Neurons (N27)

Fluorescent and chemiluminescent probes to measure OS-specific endpoints
OxyBURST® H_2HFF Green BSA—H_2O_2
Lucigenin and MitoSOX™ Red—O^{2-}
MitoTracker® Red-membrane permeability/mitochondrial respiration
ENLITEN-intracellular ATP



Long et al. (2006). *ES&T* 40 (14) 4346; Phenrat et al., *ES&T* (in press)

Coatings Decrease OS response by Microglia (BV2) and Cytotoxicity to Neurons (N27)

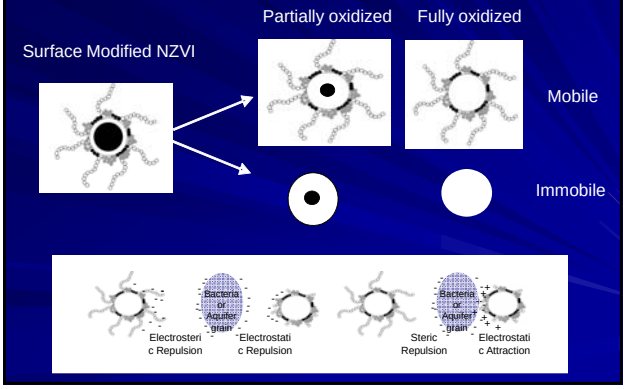


N27 Cytotoxicity : fresh nZVI > SM-RNIP > "aged" nZVI ≈ magnetite

ROS : fresh nZVI > "aged" nZVI ≈ magnetite > SM-RNIP

Phenrat et al., *ES&T* (in press)

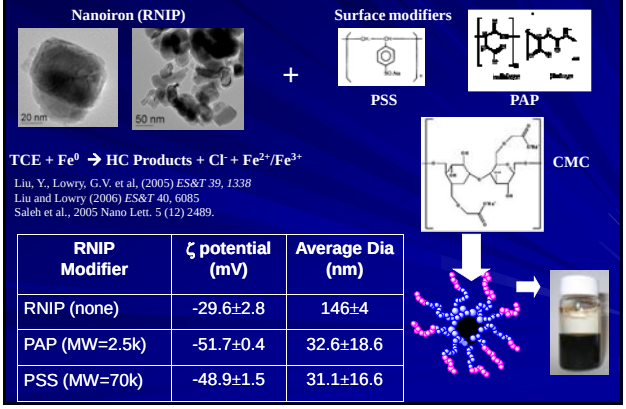
Fate of NZVI and effect of coatings on mobility and interaction with bacteria



Key Questions

- What is the oxidation rate of NZVI in the environment?
  - Geochemical effects
  - Microbial effects
- What is the fate of the coatings?
  - Resistance to desorption
  - Effect on mobility
- Do aging and coatings affect bactericidal properties?
- Is there synergy between NZVI, coatings, and bacteria that enhance remediation?
  - Coatings as a carbon source
  - H_2 as electron donor

Functionalized Reactive Nanoiron (NZVI)



TCE + Fe^0 → HC Products + Cl^- + Fe^{2+}/Fe^{3+}

Liu, Y., Lowry, G.V. et al, (2005) *ES&T 39, 1338*
Liu and Lowry (2006) *ES&T* 40, 6085
Saleh et al., 2005 Nano Lett. 5 (12) 2489.

RNIP Modifier	ζ potential (mV)	Average Dia (nm)
RNIP (none)	-29.6±2.8	146±4
PAP (MW=2.5k)	-51.7±0.4	32.6±18.6
PSS (MW=70k)	-48.9±1.5	31.1±16.6

Slow desorption of polymeric surface modifiers

Objective: investigate the **rate** and **extent** of **desorption** of adsorbed polyelectrolyte from NZVI over a 4-month period

- Effect of molecular weight
- Effect of the type of surface interaction (specific vs. non-specific)

Kim et al., 2009 *ES&T 43* (10) 3824.

Methods

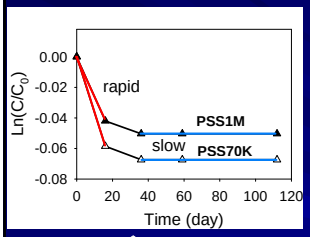
- PAP (2.5K, 10K), PSS (70K, 1M) and CMC (90K, 700K) were adsorbed to RNIP for 5 days in an end over end rotator at 30rpm.
- **Adsorption and desorption :**
  Analyzed by UV-Vis absorbance (PSS) or total organic carbon (CMC, PAP) to determine the adsorbed mass (mg adsorbed polymer/m^2 of NZVI) and desorbed mass over 4 months.
- **Transport of aged materials:**
  - 12.5-cm saturated silica sand column using 1 g/L NZVI (θ=0.33, V_{ave} =1.08 x 10^{-3} m/s)
  - Compared mobility of freshly modified particles and after 8 months of aging

Desorption of Polyelectrolytes from NZVI

Coating	Adsorbed mass (mg/m^2) [a]	2 weeks [a] (% remaining)	4-6 weeks (% remaining)	8 weeks [a] (% remaining)	16 weeks [a] (% remaining)
PAP2.5K	0.85±0.2	90.9 ± 3.0	86 ± 4.7	81.7 ± 5.5	**73.9 ± 8.1**
PAP10K	1.47±0.1	94.5 ± 2.3	91.5 ± 2.5	90.0 ± 2.0	**87.2 ± 2.8**
PSS70K	2.89±0.6	94.3 ± 0.5	93.5 ± 0.6	93.5 ± 0.6	**93.5 ± 0.6**
PSS1M	2.55±0.5	95.9 ± 4.1	95.1 ± 4.7	95.1 ± 4.7	**95.1 ± 4.7**
CMC90K	2.09±0.0	87.6 ± 2.1	83.1 ± 2.9	81.1 ± 3.1	**79.7 ± 3.3**
CMC700K	3.71±0.4	93.8 ± 0.4	91.5 ± 0.8	90.4 ± 0.9	**89.6 ± 1.2**

Kim et al., 2009 *ES&T 43* (10) 3824.

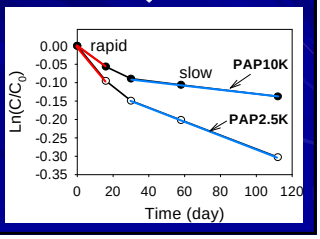
Rate of Desorption



Kim et al., 2009 *ES&T 43* (10) 3824.

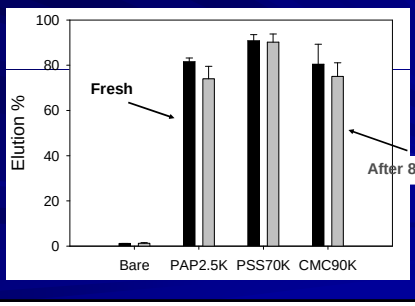
PAP

PSS



Particles remain mobile after 8 months allowed for desorption

Kim et al., 2009 *ES&T 43* (10) 3824.



Coatings Affect Bactericidal Properties of NZVI

Objectives: Determine how the following conditions affect the toxicity of NZVI

- Polymer and NOM coatings
- Oxidation state of NZVI
- Environmental conditions
  - (aerobic or anaerobic)

Summary of Findings

- High MW coatings do not readily desorb from NZVI
  - <30% desorbed after 4 months
  - Rate is a function of MW and interaction with surface
  - NZVI remains potentially mobile after 8 months compared to bare NZVI
- Coatings and O_2 decrease bactericidal effects
  - Coatings inhibit NZVI contact with cells
  - Presence of DO has greater effect on toxicity than oxidation of particles (Fe^0 content)
    - possibly due to surface passivation of the particles

Thank You

- Questions?

Center for Environmental Implications of NanoTechnology

- 4 Core Institutions: Duke, CMU, Va Tech, Howard
- U Kentucky, Stanford, Rice University, NC State, Colorado School of Mines, Clemson
- 5 years-$14.4 M from NSF + EPA
- 25 faculty currently funded
- 17 International partners on 3 continents
- Collaborators with 5 US government entities

CEINT

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

PIs: Jason Unrine[1], Olga Tsyusko[1], Paul Bertsch[1] , Andrew Neal[2]
Postdocs: W. Aaron Shoults-Wilson[1], Simona Hunyadi[1,3]
Graduate Student: Jonathan Judy[1]
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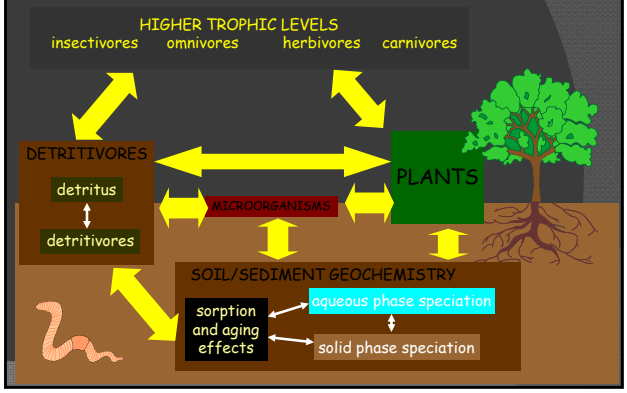
2.Rothamsted Research Center, Harpenden, UK.
3.Savannah River National Laboratory, Aiken, SC
4.Toxicology Excellence for Risk Assessment, Cincinnati, OH; Antioch College, Yellow Springs, OH.

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

Grant No. 9732138. Research Experiences for Undergraduates

Fate, transport, and implications of trophic transfer of manufactured nanoparticles in the environment



Overall project objectives

- Determine interactions between particle size and particle composition in determining ADME and toxicity in earthworms and amphibians.
- Investigate the plausibility of nanomaterial trophic transfer along a simulated laboratory food chain.
- Determine if simulated environmental and biological modifications influence bioavailability and toxicity.

Hypotheses

- Nanomaterials have relatively low bioavailability in soils.
- Uptake from soils, toxicity and distribution of nanomaterials within organisms is size and material dependent.
- Biological responses are related to the release of metal ions.

Approach

- Focus on both mechanistic *and* ecologically meaningful endpoints.
- Focus on exposure scenarios that are more environmentally relevant.
- Systematically address structure activity relationships.

NANOPARTICLE → COMMUNITY, POPULATION, INDIVIDUAL, ORGAN SYSTEM, TISSUE, CELL, MOLECULE

Structure Activity Relationships

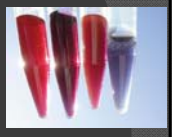
Half Reaction	E^o (V)	Group 11 "Noble Metals"
$Cu^{2+} + 2e^- \rightarrow Cu^0$	+0.34	Cu
$Ag^+ + e^- \rightarrow Ag^0$	+0.74	Ag
$Au^{3+} + 3e^- \rightarrow Au^0$	+1.52	Au

Stability ↓ Polydispersity

Particle Size

Test Materials

- Au (4, 18, 20 and 55 nm colloidal spheres; $HAuCl_4$; citrate capped).
- Ag (20 and 55 nm colloidal spheres; $AgNO_3$; citrate capped).
- Cu (20-40 nm and <100 nm powder; $CuSO_4$; Sigma).



4 nm 18 nm

Test material characterization

- AF4-UV/VIS-DLS-ICP-MS
  - Primary particle and agglomerate size.
- TEM
  - Primary particle shape and size
- ICP-MS/XRF
  - Particle purity and concentration
- Ion Chromatography
  - Surfactants
- XANES
  - Oxidation state
- PALS
  - Electrophoretic mobility

Phase 1 – Small Au particles

Artificial soil mesocosms
Eisenia fetida

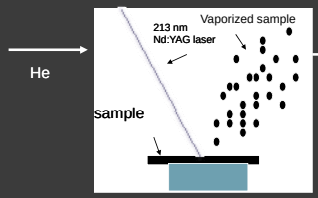
68 % silica sand
10 % peat moss
20% kaolin
2 % limestone
35% moisture content

4 nm Au | 18 nm Au | Citrate | $HAuCl_4$

300 g of soil
10 animals per mesocosm

Exposure concentration = ~ 3.5 mg kg^{-1}

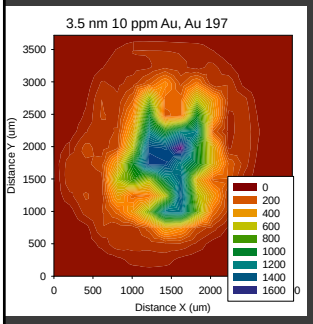
Laser Ablation-ICPMS

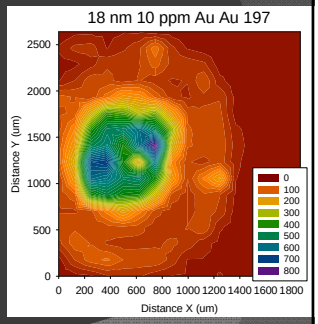


Laser Ablation Cell

Spot Size ~ 5-400 µm

Uptake of nanoscale Au in *Eisenia fetida*



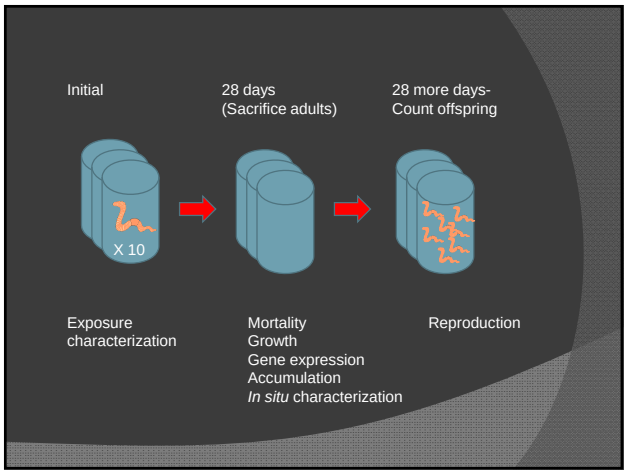


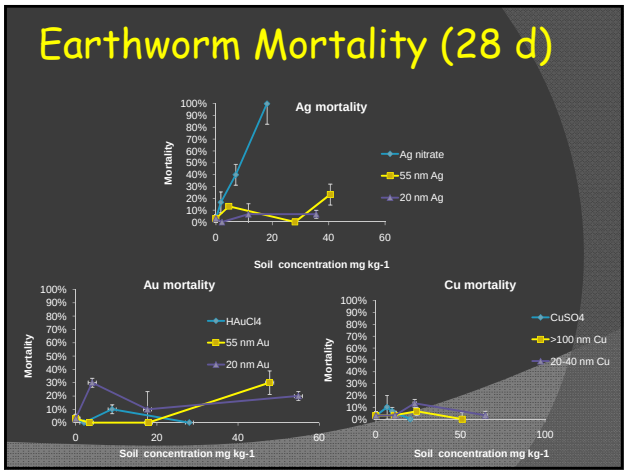
Phase 2 – earthworm subchronic toxicity and reproduction

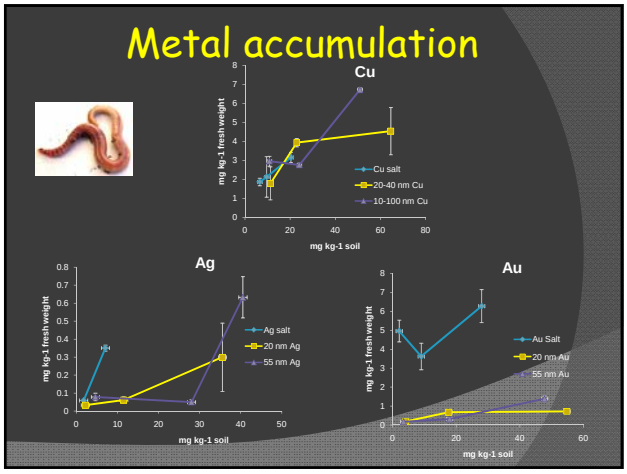
3-way completely randomized design

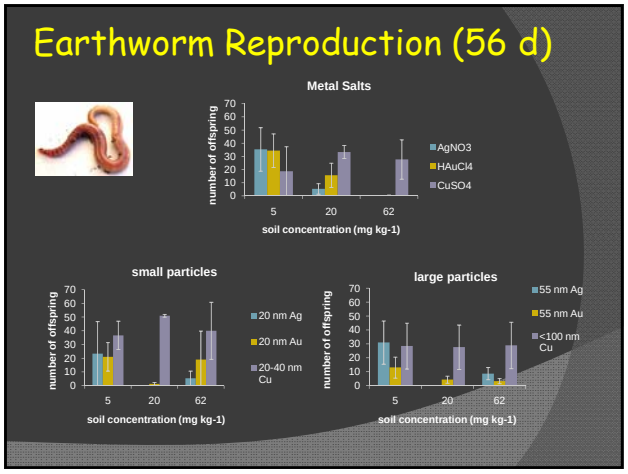
Particle size – 2 size classes (20 and 55 nm or 20-40 and <100)
Particle concentration – 3 nominal concentrations (5, 20 or 62 mg kg^{-1})
Particle composition – 3 compositions (Cu, Ag or Au)
Controls – citrate, H_2O, $HAuCl_4$, $CuSO_4$, $AgNO_3$
Replicates – 3 mesocosms, 10 animals per mesocosm

Total mesocosms = 87
Total treatments = 29
Total animals - 870



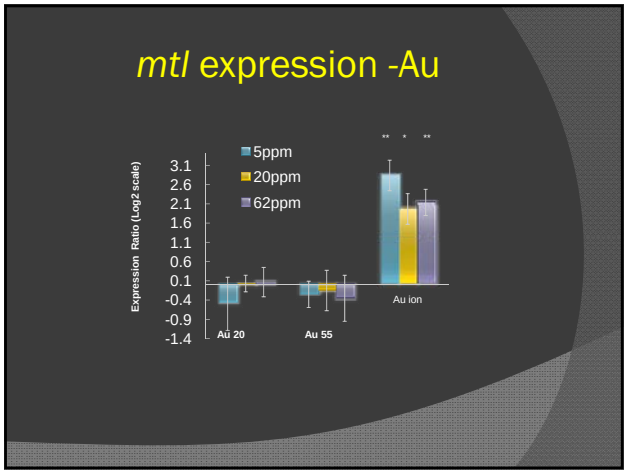




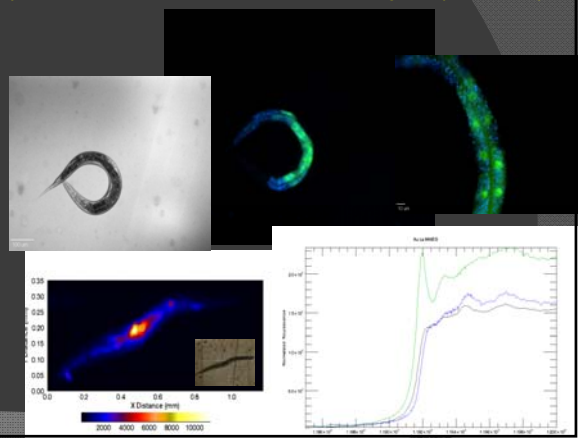


Gene expression – Q-RT-PCR

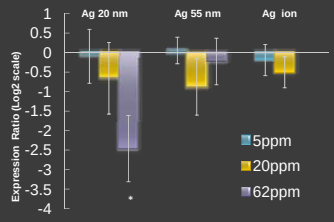
- Metal homeostasis
  - Metallothionein
- Oxidative stress
  - Catalase
  - Superoxide dismutase
  - Glutathione peroxidase
- Molecular chaperones
  - Heat shock proteins 60 and 70
  - Ubiquitin
- Housekeeping gene
  - Beta-actin



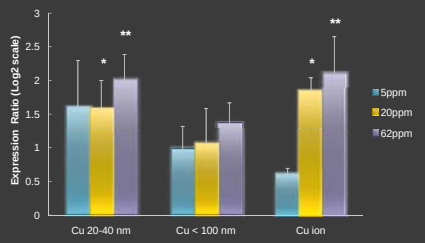
Uptake of 3.5 nm Au in *Caenorhabditis elegans* (mtl2::GFP)



mtl expression -Ag

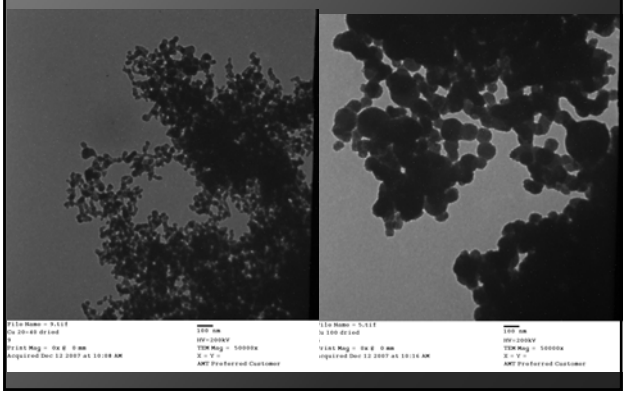


mtl expression - Cu

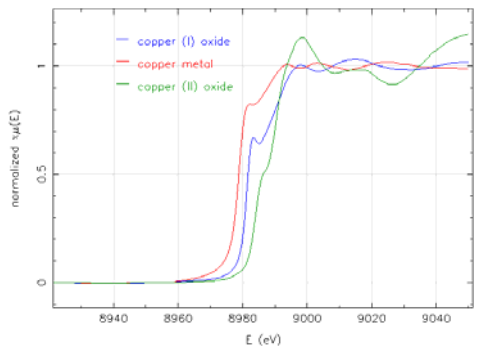


* 0.01< P < 0.05 ** P ≤ 0.01

20-40 nm Cu **<100 nm Cu**

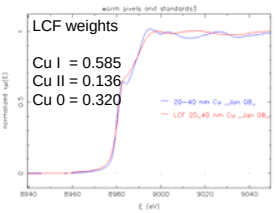


Copper K-edge XANES

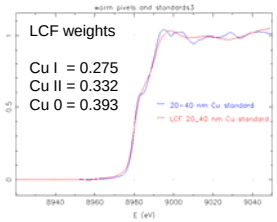


20-40 nm Cu powder oxidation

T = 1 month

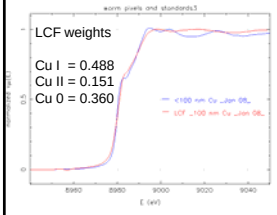


T = 10 months

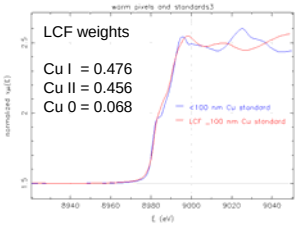


<100 nm Cu powder oxidation

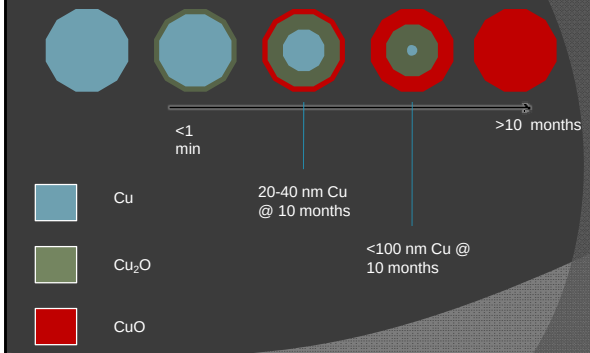
T = 1 month



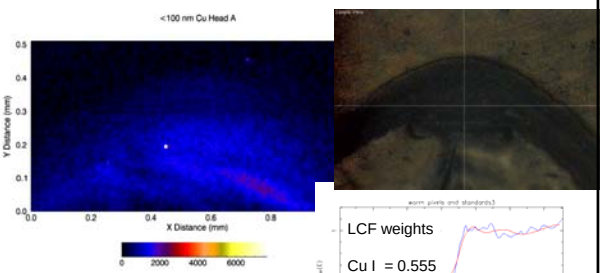
T = 10 months



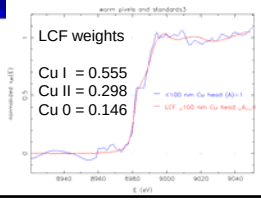
Cu oxidation process



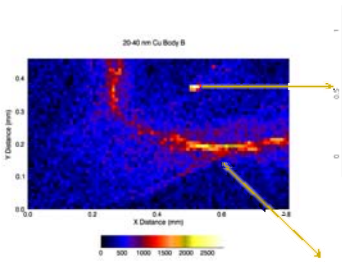
<100 nm Cu in blood vessel



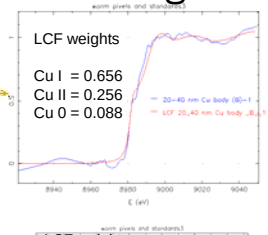
Cu Kα Fluorescence

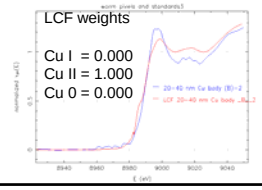


20-40 nm Cu exposed worm gut



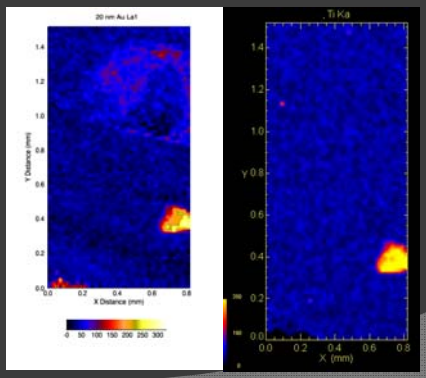
Cu Ka Fluorescence





Distribution of 20 nm Au in the earthworm cross-section

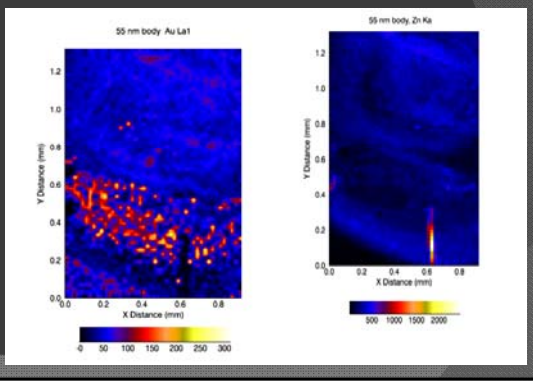
Au Lα



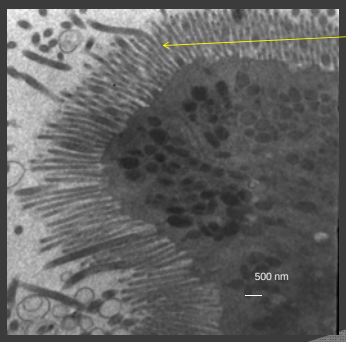
Ti Kα

Distribution of 55 nm Au in the earthworm cross-section

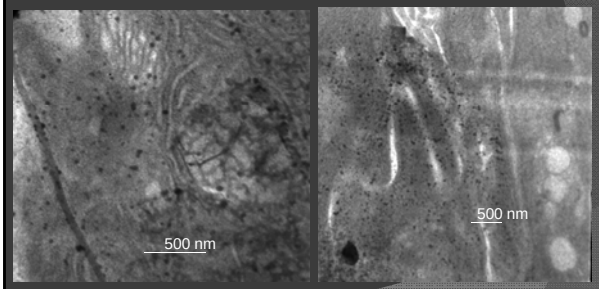
Au Lα Zn Kα

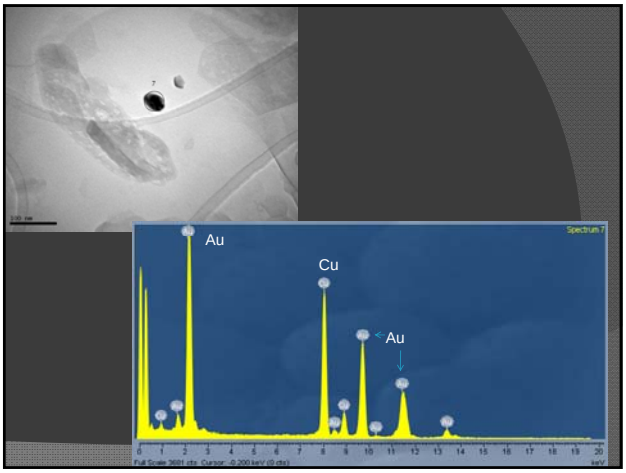


Control gut absorptive cell

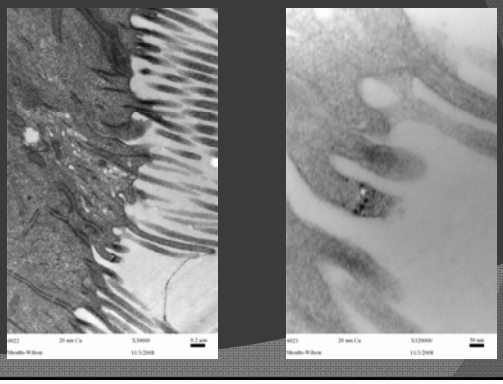


Gut cells of worms exposed to 55 nm Au showing intracellular nanoparticles

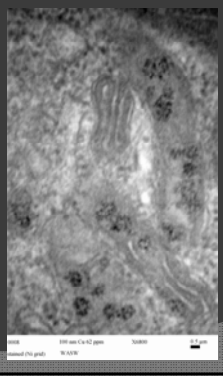




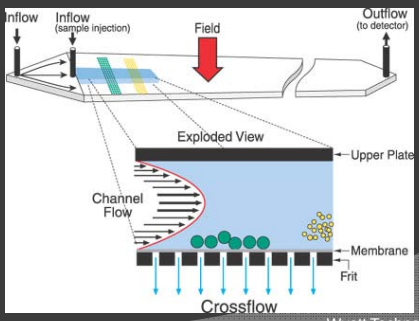
20-40 nm Cu



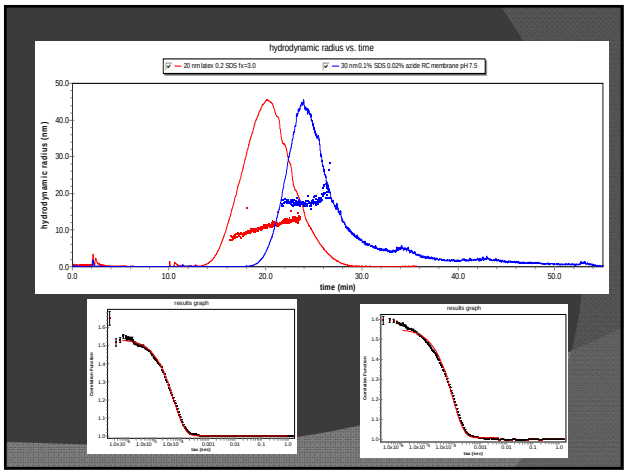
<100 nm Cu

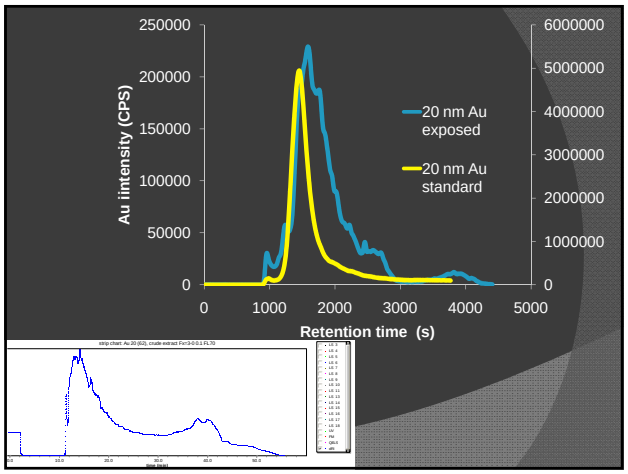


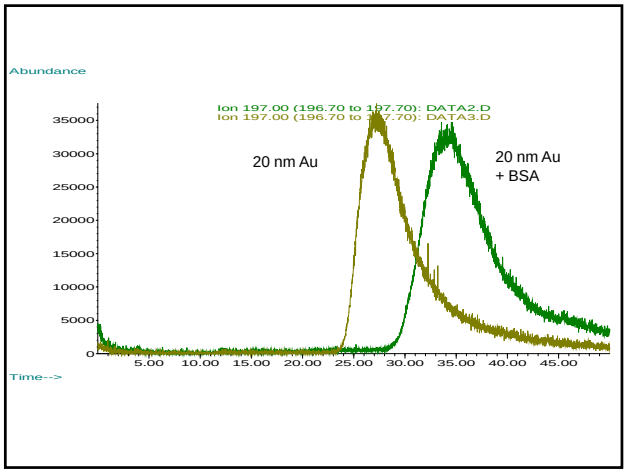
FI-FFF-UV/VIS-DLS-ICPMS



Wyatt Technology Corp.







Future directions

- Determine uptake and elimination rates in earthworms.
- Toxicity of smaller particles at higher concentrations.
- Further develop methods for in situ characterization of particles/metals in soils and tissues.
- Add another trophic level (amphibians).

PUBLICATION

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*. Vicki H. Grassian, Ed. John Wiley & Sons, Hoboken, NJ. pp 345-360.

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

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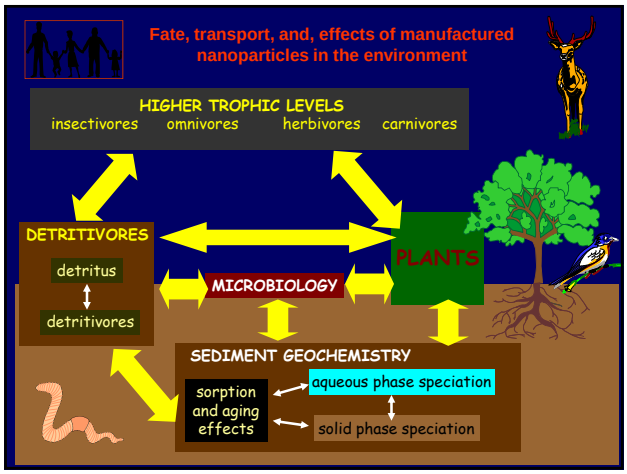
The University of Georgia
Savannah River Ecology Laboratory



OBJECTIVES to evaluate:

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

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

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

HYPOTHESES

1: The bioavailability and toxicity of manufactured NPs increases with decreasing particle size (i.e. 2 nm vs. 80 nm)

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

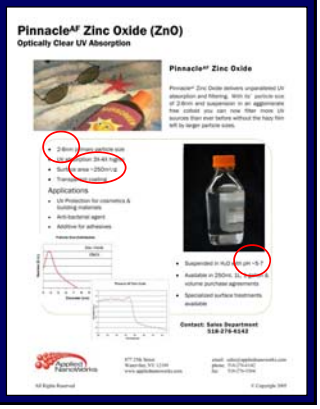
3: The bioavailability and toxicity of NPs introduced via trophic transfer differs from direct exposure

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

Characterization – the critical first phase

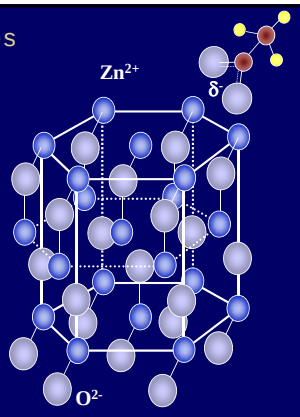
	Batch 1	Batch 2
pH	6.25	4.5
[Zn] g L^{-1}	56.0	72.0
[Zn] moles L^{-1}	0.86	1.11
[Acetate] moles L^{-1}	2.33 M	3.08 M
PZNC	ND	pH 6-7

BET SA=5.7 m^2/g



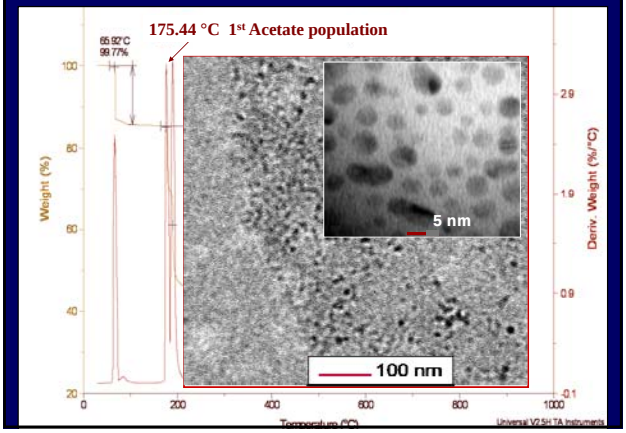
ZnO nanoparticles

- Versatile nanomaterial
- inexpensive to produce
- Found in pigments, rubber additives, sunscreens, personal care products, biological and chemical sensors, varistors, transducers, photoelectrodes, and catalysts

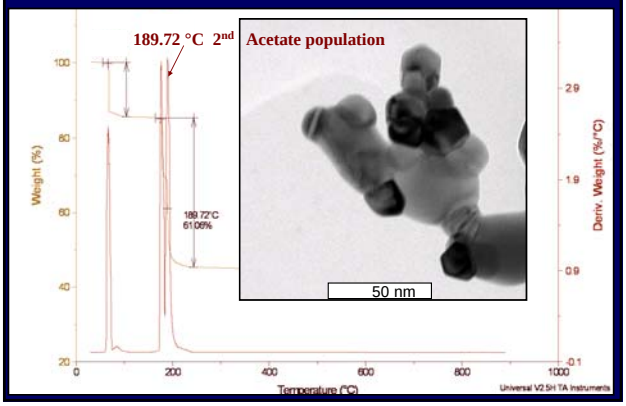


Crystalline model of ZnO with acetate coordinating a Zn atom (adapted from Wang 2004)

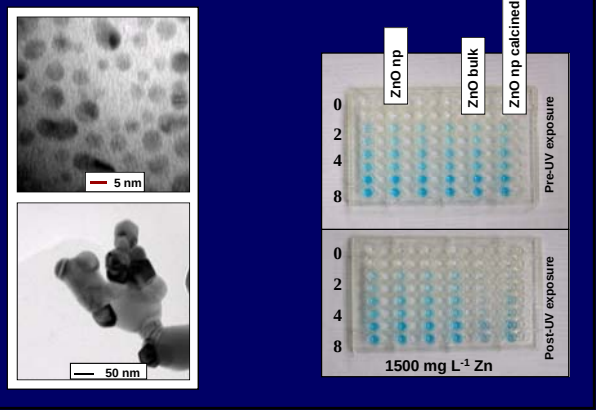
HR-TGA-Air dried ZnO-np



TGA-Air dried ZnO-np



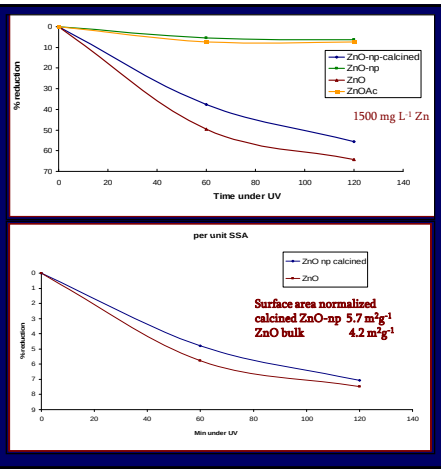
Characterization: Acetate Importance



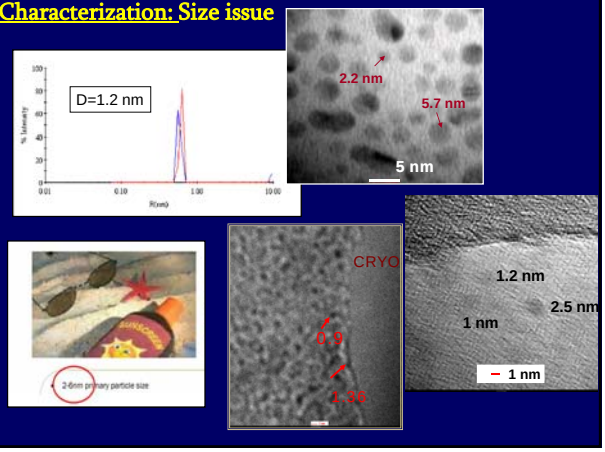
Methylene Blue assay for ZnO surface reactivity

Recent results:

Toxicity of ZnO-np to *C. elegans* under UV irradiation can be predicted from MB assay

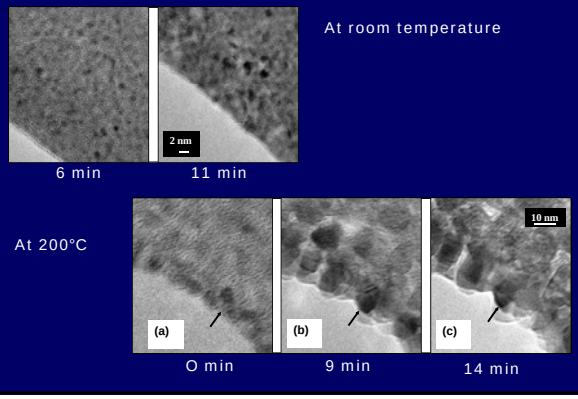


Characterization: Size issue

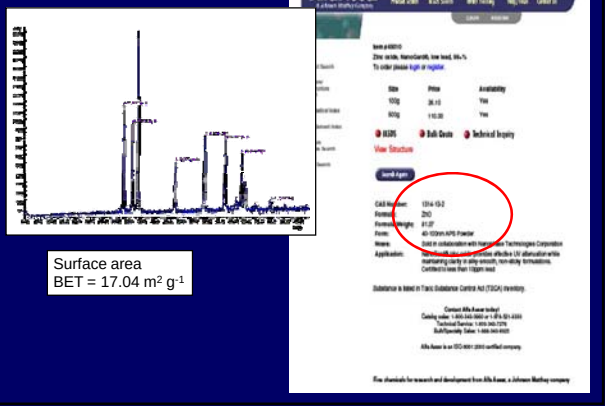


Characterization: Changing nature – artifact of TEM observation

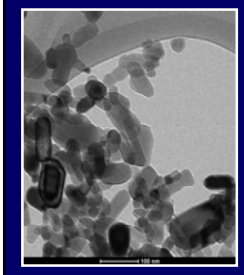
Kabengi , Bertsch, Shields and Wu



Large ZnO-np: 80 nm

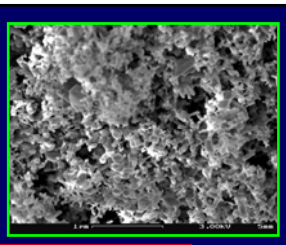


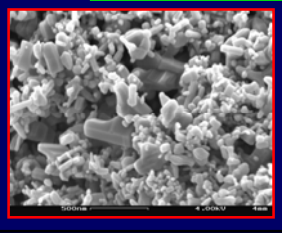
Characterization: Size ~80 nm



TEM in DDI

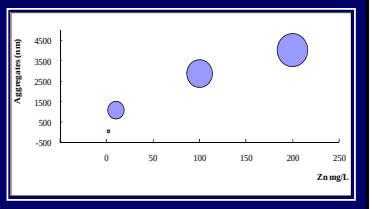
SEM-dry

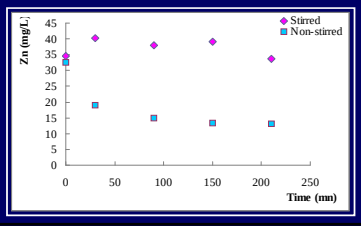




SEM- aq

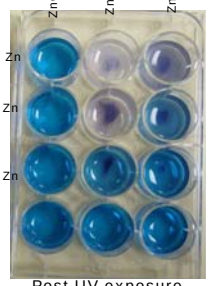
Aggregation of 40-100 nm ZnO particles with increasing concentration



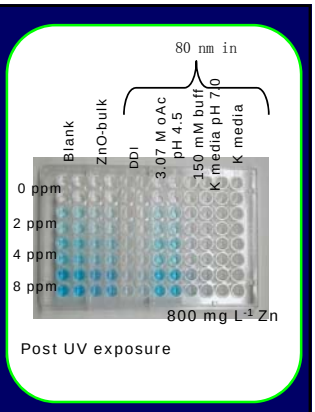


Characterization: Reactivity

10 ppm MB

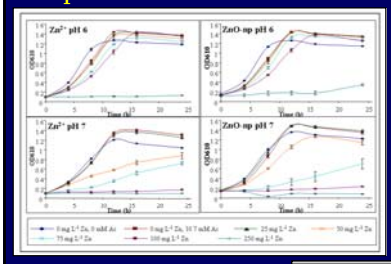


Post UV exposure



Post UV exposure

Nanoparticle-Bacterium Interactions- *B. vietnamiensis* PR1

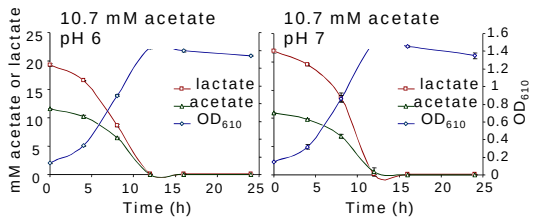


- Acetate concentrations were normalized to 10.7 mM across conditions.
- PR1 was unable to grown with 10.7 mM acetate at pH 5
- Growth unaffected by:
  100 mg L^{-1} Zn at pH 6
  25 mg L^{-1} Zn at pH 7

- Calculated EC_{50} based on OD_{610} at 24 hours, using 0 mg L^{-1} metal as a reference
- EC_{50} of Zn^{2+} and ZnO-np values are not sig. different at pH 6 or 7

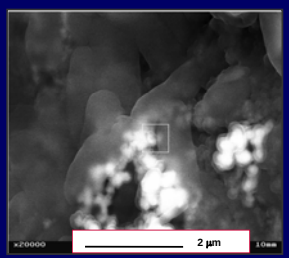
pH 6	
Aqueous Zn^{+2}	163 ± 9.6 mg L^{-1}
ZnO-np	188.6 ± 8.5 mg L^{-1}
pH 7	
Aqueous Zn^{+2}	96.6 ± 14.1 mg L^{-1}
ZnO-np	104.4 ± 13.5 mg L^{-1}

Acetate and Lactate Utilization



- Acetate and lactate are 95% depleted by 12 h and had similar utilization patterns
- Degradation of acetate (NP counter-ion) may affect NP stability

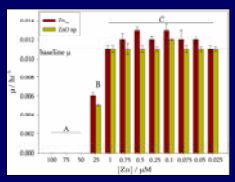
Floc formation in bacterial culture

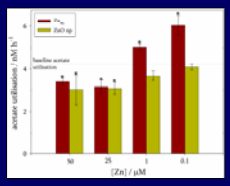


- At 250 mg L^{-1} ZnO-np (pH6), significant flocculation of primary particles at 24 hours associated with cell growth
- Flocs do not form at 250 mg L^{-1} ZnO-np in the absence of PR1 suggestive of biological induction
- Flocs could result from acetate degradation and/or exopolymer secretion

Nanoparticle-Bacteria interaction

Cupriavidus necator-metal sensitive



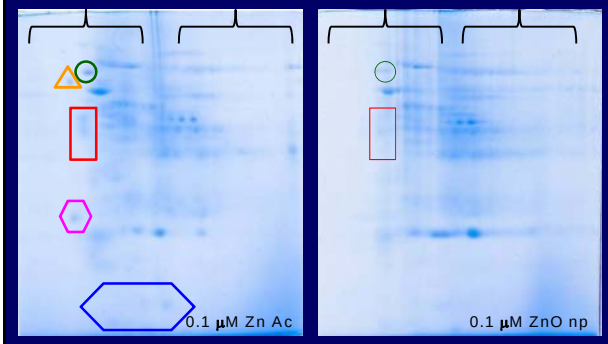


- No significant difference between $Zn^{2+}_{aq.}$ and ZnO-np growth rates
- Higher OAc utilization rates with $Zn^{2+}_{aq.}$ compared to ZnO-np
- Evidence for bioavailability of Zn ion, but not ZnO-np
- Epifluorescence microscopy indicates an increased number of cells with compromised membranes associated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

PROTEOMICS 2-D gels- Arthur Grider - UGA

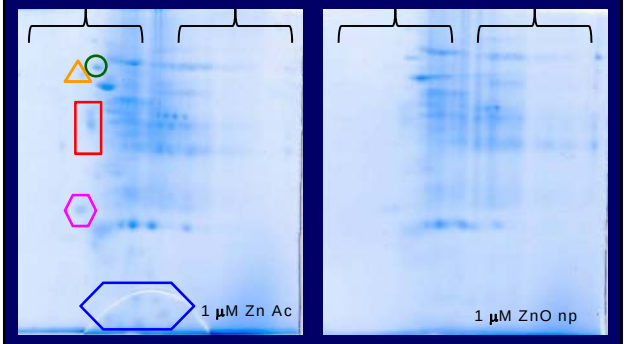
0.1 µM Zn Ac versus 0.1 µM ZnO np

Acidic Side Basic Side Acidic Side Basic Side

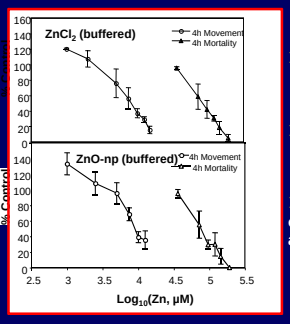


1 µM Zn Ac versus 1 µM ZnO np

Acidic Side Basic Side Acidic Side Basic Side

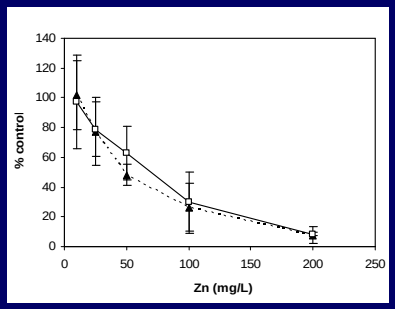


Behavior & Lethality *C. elegans* exposed to $ZnCl_2$ and ZnO-np

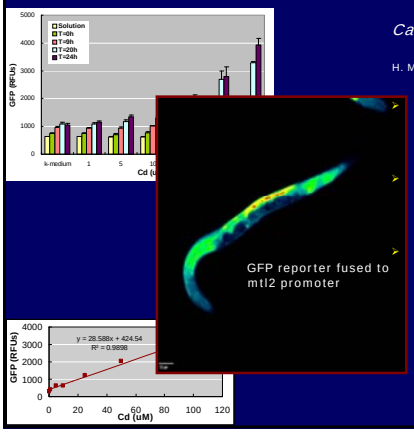


- $ZnCl_2$: EC50= 8.54 mM
  LC50=83.1mM
- ZnO-np: EC50= 9.42 mM
  LC50= 79.1 mM
- No significant difference in EC50 or LC50 was found between $ZnCl_2$ and ZnO-np

Concentration-response relationships for reproduction of *C. elegans* on exposure to ZnO-np (▲) or $ZnCl_2$ (□) (error bar denotes standard error, n=3): EC50(ZnO-np)= 53 mg/L Zn (0.8 mM); EC50($ZnCl_2$)=60 mg/L Zn (0.91 mM).



Genome-enabled environmental analysis-bioavailable metals



Caenorhabditis elegans
transgenic mtl2::GFP
H. Ma, T. Glenn, and P. Williams

- Simple organism (approx. 959 cells), genome sequence complete
- Feeds on bacteria and other particles < 5 µm in diameter
- GFP induction shows time and metal concentration-dependence.

ZnO-np vs. Zn^{2+} exposed nematodes

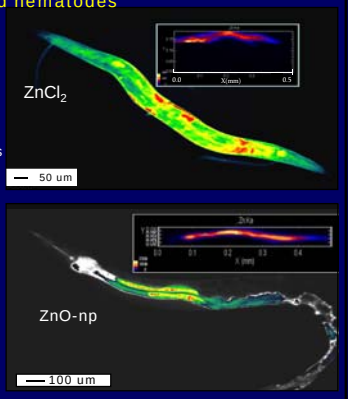
- XRF-

  Maximum Zn intensities are independent of exposure concentration

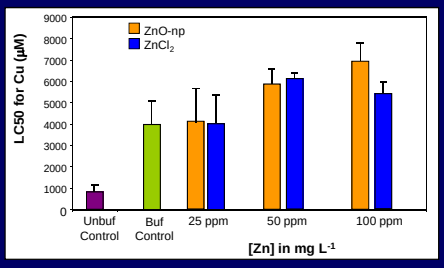
  Areas of maximum intensity are more evenly distributed as exposure concentration increases and at lower concentrations for ZnO nanoparticle exposed worms

- Mtl2::GFP

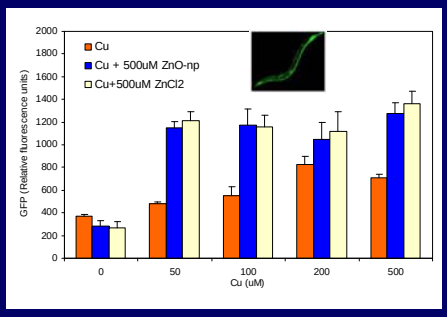
  The maximum intensities in $ZnCl_2$ exposed nematodes are approximately twice as high as in ZnO-np exposed nematodes and there is more even GFP expression for $ZnCl_2$, suggesting differential bioavailability or tissue dependent reactivity, e.g., dissolution.



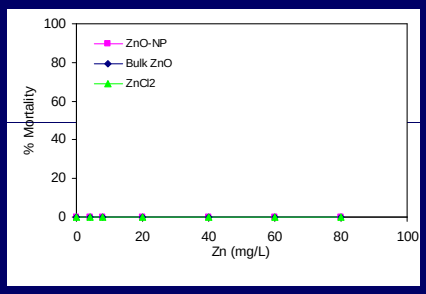
Effects of ZnO-np and $ZnCl_2$ on Cu toxicity



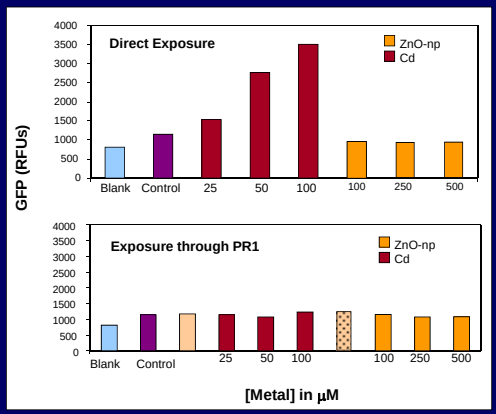
Effects of ZnO-np or $ZnCl_2$ on Cu toxicity: mtl-2 expression in transgenic organisms



Toxicity of larger ZnO-NP (40-100nm): 4h lethality

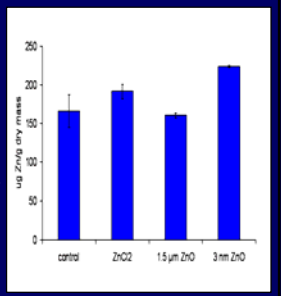


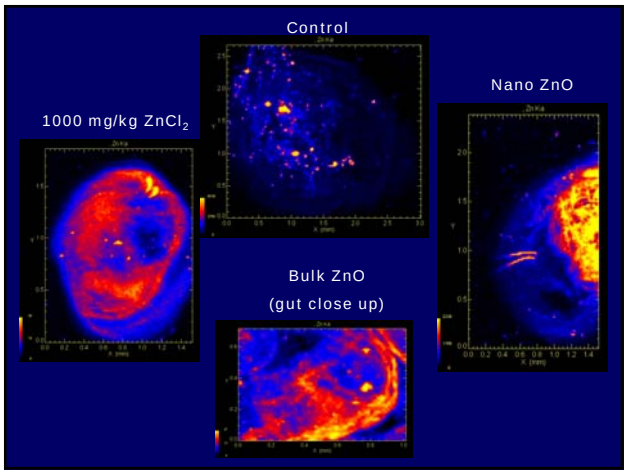
Initial experiments on trophic transfer of ZnO-np



Earthworm exposure to ZnO-np in artificial soil

- Bioaccumulation of Zn in *Eisenia fetida* after 14 days of exposure to artificial soil containing 1000 mg Kg^{-1} Zn
- No difference in Zn concentration between treatments





Summary and Major Conclusions

Characterization

- Size determination and surface chemistry is a critical issue
- TEM may not be the best method for size determination for small metal oxide nanomatierals
- Acetate controls 1-2 nm ZnO-np reactivity, passivates surface sites; not so for bulk (1.2 µm) intermediate for larger (~80nm) particles
- Removal of acetate leads to flocculation/ aggregation of 1-2 nm ZnO-np primary particles but promotes surface reactivity

Trophic transfer in bacterial-nematode model challenging

ZnO-np are bioavailable from soils as demonstrated in earthworm exposures

Bacteria

- No difference in growth rate between ZnO-np & Zn^{2+}(aq) for *C. necator* and *B. vietnamiensis* $PR1_{301}$
- Higher OAc utliisation rates with Zn^{2+}_{aq} compared to ZnO-np in *Cupriavidus necator*
- Evidence for Zn bioavailability from Zn ion, but not ZnO-np
- Cells with compromised membranes associated with ZnO-np compared to free ion
- Different mechanism(s) of toxicity ?

Nematodes

- ZnO-np-s LC_{50}/EC_{50} not significantly different from $Zn^{2+}_{(aq)}$ ---Behavior– 8-10 times and reproduction– 75-100 times more sensitive than lethality- ZnO-np-l display no toxicity in same conc. range
- Different mechanism (s) of toxicity?
- At [Zn]>100 $mg.L^{-1}$, 1-2 nm ZnO-np decreases Cu toxicity as compared to Zn^{2+}(aq) – 80 nm ZnO will be compared-Zn and ZnO-snp enhance mtl-2 expression
- No significant GFP was induced either in 100uM Cd or 500uM ZnO-np through PR1 exposure (trophic transfer).
- GFP mtl2 (and mtl1) expression is induced locally but is tissue specific (not nec. related to total Zn)

Manuscripts

H. Ma, P.M. Bertsch, T. C. Glenn, N.J. Kabengi, P. L. Williams. In press. 2009. Toxicity of manufactured zinc oxide nanoparticles in the nematode Caenorhabditis Elegans. Environmental Toxicology & Chemistry. DOI: 10.1897/08-262)

H. Ma, N.J. Kabengi, P.M. Bertsch, J.M. Unrine, T. C. Glenn, P. L. Williams. In Review. Phototoxicity of nanoparticulate ZnO under natural sunlight irradiation in the nematode Caenorhabditis Elegans. Environmental Science and Technology

B. A. Neely, D. W. Bearden, A. J. Sutter, N. J. Kabengi, P. M. Bertsch, and P. J. Morris. 2008. Toxicity of engineered ZnO-NP to *Burkholderia vietnamiensis* $PR1_{301}$: Comparison to Zn^{2+} and the affects of counter-ion utilization. *Environmental Toxicology and Chemistry* (In Review)

J. Unrine, P.M. Bertsch, and S. Hunyadi. 2008. Bioavailability, trophic transfer, and toxicity of manufactured metal and metal oxide nanoparticles in terrestrial environments. Pp. 343-364 *in* V. Grassian (editor) *Nanoscience and Nanotechnology*, John Wiley & Sons, Inc.

Manuscripts in preparation on:

The spatial distribution of Zn and metallothionein expression in *C. elegans* exposed to dissolved Zn^{2+} and ZnO nanoparticles

Toxicity of ZnO-NP and Aqueous Zn to the Soil bacterium *Cupriavidus necator.: A Protoemics approach.*

Bioavailability and Fates of CdSe and TiO_2 Nanoparticles in Eukaryotes and Bacteria

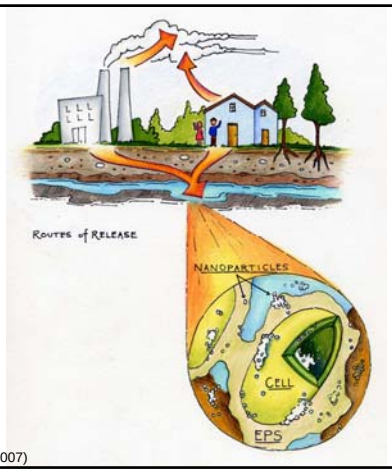
P. A. Holden
Bren School of Environ. Sci. & Mgmt., University of CA, Santa Barbara
J. L. Nadeau
Dept. Biomedical Engineering, McGill University
G. D. Stucky
Dept. Chem. & Biochem., Materials Research Laboratory, University of CA, Santa Barbara

UCSB UCCEIN Center for Environmental Implications of NanoTechnology

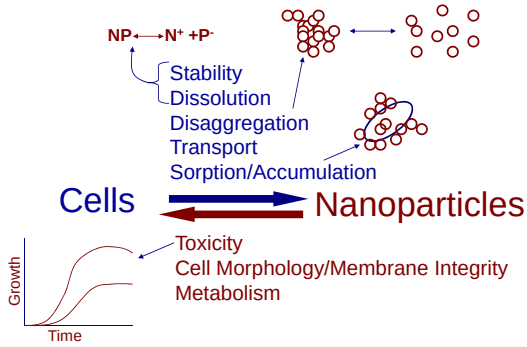
McGill

Main question:

How are NPs toxic to bacteria and eukaryotic cells?



(Allison Horst, 2007)



Factors Influencing Toxicity

UPTAKE
NP size
conjugates
ROS
stability

STABILITY
(extra / intracellular)
media & conditions
compartment

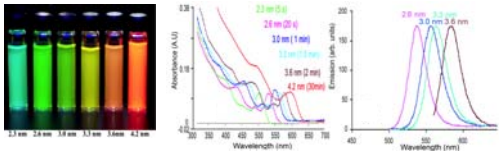
RESPONSE
glutathione
membranes
ROS scavengers

NP characteristics
core chemistry
coating
conjugates
morphology

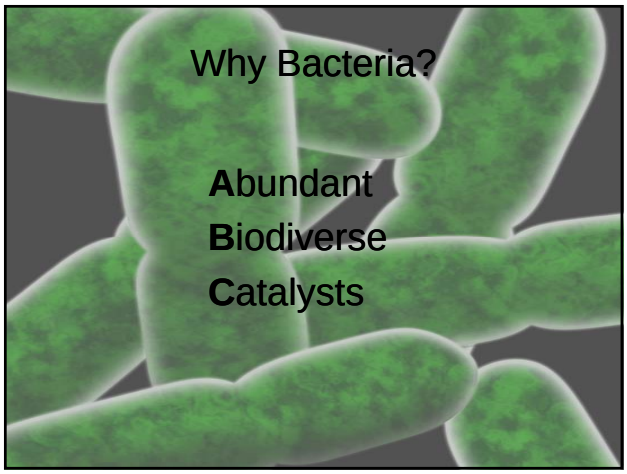
"designer" NPs

(directed synthesis)

CdSe Quantum Dots



Titanium Dioxide NPs

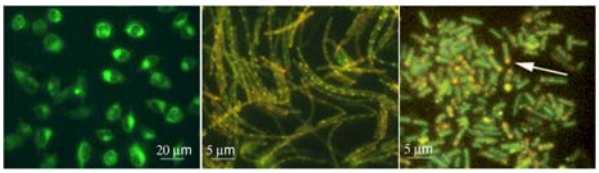


Report of 2 Subprojects

1. Effects and fates of Cd(II) vs. CdSe QDs in *P. aeruginosa.*

2. TiO_2 interactions w/ *P. putida*: aggregate stability.

1. CdSe QDs: Cell labeling



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 from Cd(II)

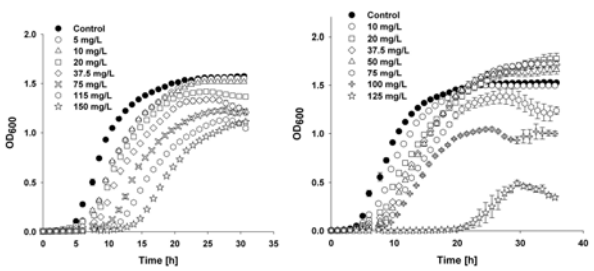
(Kloepfer et al., 2003; Kloepfer et al., 2005; Clarke et al., 2006)

Labeling-inspired questions:

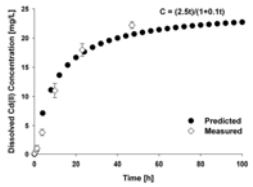
- Is light necessary?
- Are bare QDs internalized?
- Is external binding prerequisite?
- What are the quantitative fates of QDs?
- How are they toxic?

P. aeruginosa growth depends on dose.

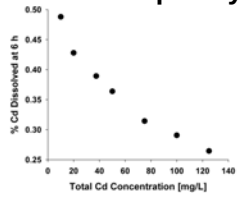
Cd(II) **QDs**



QDs dissolve quickly,

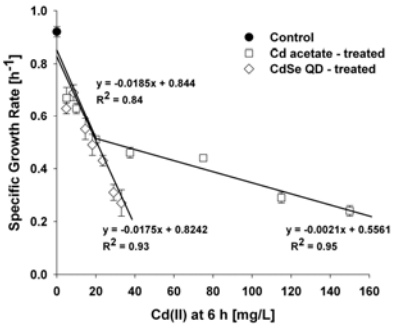


….but incompletely.

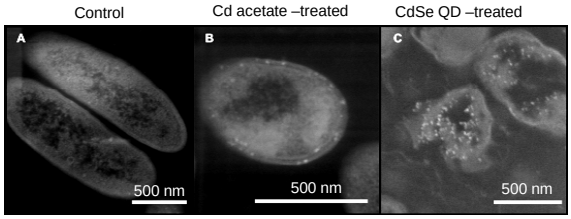


Dissolution was greater in sterile controls.

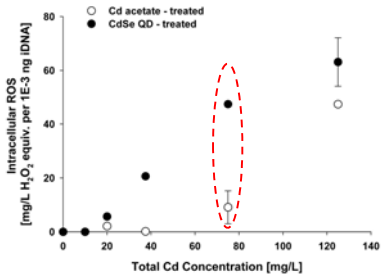
QDs add to Cd(II) toxicity, above a threshold



Above the threshold:
membrane damage in QD-grown cells.



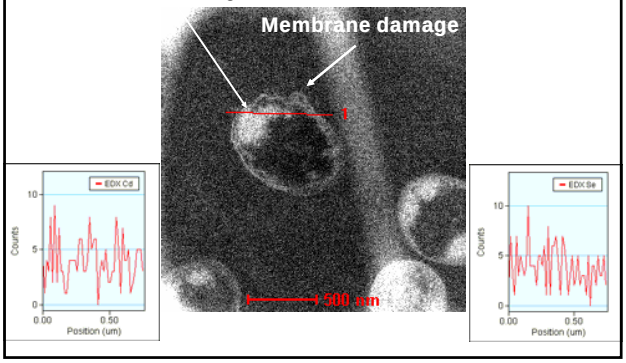
Above the threshold:
intracellular ROS in QD-grown cells.



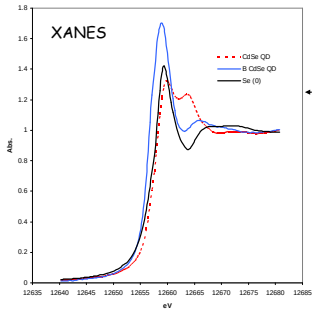
(Method: DCFH-DA to dichlorofluorescein, DCF).

Above the threshold: metals in QD-grown cells.

Cd, Se and CdSe rich-regions



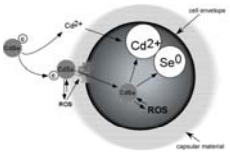
Se oxidation state: "tracer" for QD integrity



Cellular selenium appears to be mostly Se(0), with some organo-complexes

However:
→ STEM suggests intact QDs
→ XRD supports (wurtzite)

Metal / loid fates w/ cells

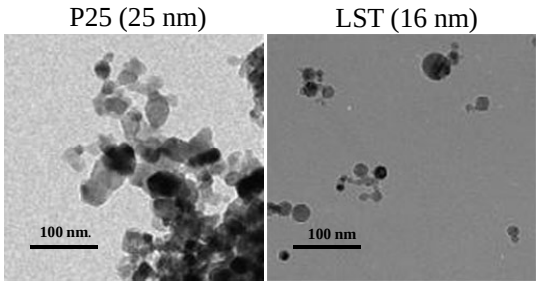


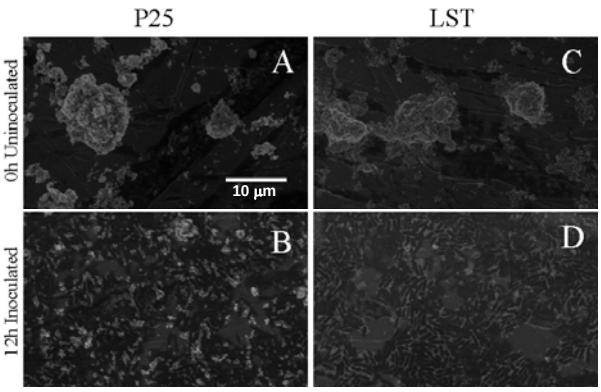
- 0.15 pg cadmium / cell (dissolved, mostly)
  - 4% of administered
  - 4600X enrichment
- 0.0083 pg QD-cadmium / cell
  - Based on 200,000 QDs / cell
  - 30X enrichment

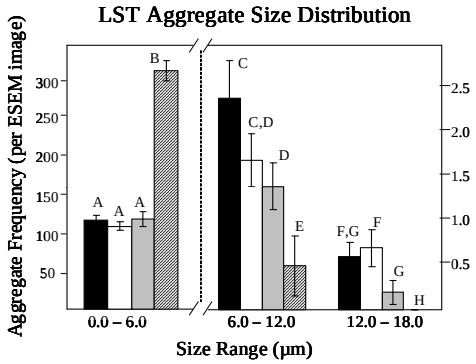
1. Summary

- QDs appear more toxic than Cd(II)
  - Above a threshold
  - Related to ROS
  - Sorption to membrane not a prerequisite
- *Pseudomonas* alters fate of QDs
  - Intracellular: QDs appear mostly broken down
  - Extracellular: QDs are relatively stabilized

2. Titanium Dioxide NPs





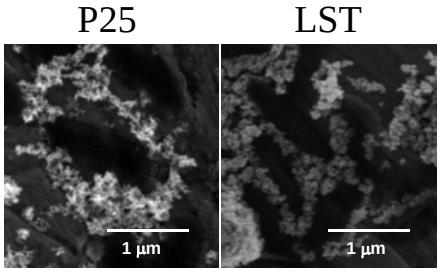


2. How do agglomerates disperse?

H1: Biosurfactant mediated

H2: Metabolism of chemical linkers

H3: Preferential binding to cells



Ongoing and future

- Mechanisms: what's behind the observations of bacteria and QDs?
- High Throughput Screening (HTS): where are there transferable paradigms?
- Scaling up: how are soil ecosystem processes and soil biota affected?

UCCEIN Center for Environmental Implications of NanoTechnology

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Acknowledgements

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  - John Priester
  - Peter Stoimenov
  - JP Zhang
  - Chris Ehrhardt
  - Randy Mielke / JPL
  - Sam Webb / SSRL
  - Allison Horst

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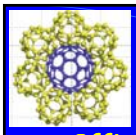
- Funding
  - U.S. EPA
  - U.S. DOE (DE-FG02-06ER64250)
  - UC Toxic Substances Research & Teaching, Nanotoxicology Lead Campus Program
  - NSF /EPA UC CEIN



Office of Research and Development (ORD) National Health and Environmental Effects Research Laboratory (NHEERL) Manufactured-Engineered Nanomaterial Health Effects Research Program

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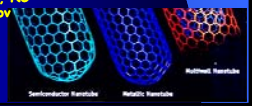
Interagency Environmental Nanotechnology Grantees Workshop
November 19 - 21, 2008
Tampa, FL



ORD, NHEERL Manufactured-Engineered Nanomaterial Health Effects Research Program

Feb. 2007

OECD

S63: Nanomaterials Testing

S67: Alternative Test Methods in Nanotoxicology

Oct. 2008

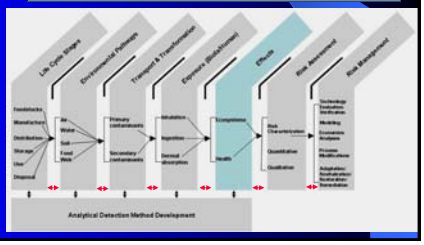
Draft Development

ORD, NHEERL Manufactured-Engineered Nanomaterial Health Effects Research Program

Oct. 2008

ORD Research Themes:

- Sources, Fate, Transport, and Exposure
- Human Health and Ecological Effects Research to Inform Risk Assessment and Tests Methods
- Risk Assessment Methods and Case Studies
- Preventing and Mitigating Risks



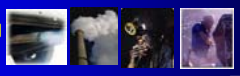
ORD, NHEERL Manufactured-Engineered Nanomaterial Health Effects Research Program

Categories of Nanoparticles

Anthropogenic

Natural; Biological

Environmental
Occupational



Engineered

Laboratory R&D

Manufactured

ORD, NHEERL Manufactured-Engineered Nanomaterial Health Effects Research Program

Research Implementation: "Nano" Health Effects Team

Draft Development
"Safety for Success"

Team's Vision: Research for the Responsible Development and Application of Nanomaterials Leading to a *Sustainable Technology*

Team's Long Term Goals: 1) determine the health effects of manufactured-engineered nanomaterials and their applications; 2) establish approaches/models/methods to quantify and predict these effects/risks.

Team's Composition: 10 projects with 15 investigators from each NHEERL Health Division with expertise in the following areas of toxicology: pulmonary, cardiovascular, neurological, developmental, mutagenesis, cancer, reproductive, and ocular.

ORD, NHEERL Manufactured-Engineered Nanomaterial Health Effects Research Program

"Multi-Tiered Approach" for Nanomaterials Health Effects Research

Tier 1
Physicochemical Characterization

Common Set of Well Characterized Manufactured-Engineered Nanomaterials

Collaborations
-ORD, NCCT "ToxCast"
-NTP

-Identify and Validate Alternative *In Vitro* Tests
-Predictive Toxicity

-Screen/Rank
-Design *In Vivo* Testing

Tier 2
In Vitro Toxicology
(Non-Cellular and Cellular Based Assays)

Tier 3
In Vivo Toxicology

ORD, NHEERL Manufactured-Engineered Nanomaterial Health Effects Research Program

Current List of Manufactured-Engineered Nanomaterials For Health Effects Research

NHEERL			EPA and Other Federal Offices	OECD NMs List	ORD NRS List of NMs
Nanomaterial	Health	Ecology			
SWCNT MWCNT	X X	X X	OSW; OPPTS; FDA	Yes	Yes
TiO_2	X	X	OSW; OW; OPPTS; OAR; NTP; NIOSH FDA	Yes	Yes
CeO_2	X		OTAQ; OAR; ORD/NCEA; NTP; FDA	Yes	Yes
Zero Valent Iron	X	X	OSWER; OW	Yes	Yes
Ag	X		OPP; OW,; CPSC; CSPC; FDA	Yes	Yes

ORD, NHEERL Manufactured-Engineered Nanomaterial Health Effects Research Program

Tier 1: Physicochemical Characterization

Nanomaterial	Commercial Source	Supplier Surface Area (m^2/gr)	Contractor Surface Area (m^2/gr)	Supplier % Purity	Contractor % Purity	Supplier Crystalline Form	Contractor Crystalline Form
TiO2 (25nm)	Evonik-Degussa P25	50+/-15	50.9	≥99.5	>99	anatase rutile	86% anatase 14% rutile
			52.9		99.9		86% anatase 14% rutile
TiO2 (30-40nm)	Nanostructured & Amorphous Materials Inc.	>30	22.2	95	99.9	rutile	86% anatase 14% rutile
TiO2 (10nm)	Alfa Aesar	100 - 130	118	99	98.8	anatase	anatase
		100 - 130	101	99	98.8	anatase	anatase
		100 - 130	273	99	97.3	anatase	anatase
TiO2 (32nm)	Alfa Aesar	45	49.8	99.9	97.9	anatase	85% anatase 15% rutile
		45	41.5	99.9	99.1	anatase	anatase
TiO2 (200-400nm)	Mknano	6.8	11.6	99.97	98.7	rutile	rutile
TiO2 (200nm)	Acros	not provided	6.99	not provided	99.9	anatase	anatase

ORD, NHEERL Manufactured-Engineered Nanomaterial Health Effects Research Program

Tier 2: In Vitro Toxicology

Non-Cellular Assays

Cellular Assays

Non-Cellular Assays: Biochemical Interactions - antioxidant depletion (GSH; Vit. C); protein binding; Surface Properties - reactivity (TBARS; ESR; DCFH); charge (zeta charge); aggregation (DLS; SEM; TEM).

Cellular Models: Pulmonary toxicity (airway epithelial cells; alveolar: macrophages and epithelial cells); Cardiovascular toxicity (cardiomyocytes; endothelial cells); Liver toxicity (HepG2 cells); Gastrointestinal toxicity (Caco-2; NCM460 cells); Neurotoxicity (microglia and neuronal cells; astrocytes); Ocular toxicity (lens and retinal pigment epithelial cells).

Cellular Endpoints: Growth; Cytotoxicity; Cellular Uptake; Oxidative Stress; Altered Function (phagocytosis; cytokine production; barrier permeability; etc.).

Strategic Information: 1) Ranking/Design - LC50 concentrations for cellular and non-cellular endpoints to prioritize and design *in vivo* testing. 2) Provide mechanistic and biokinetic data at the cellular level; 3) Provide an iterative model to identify alternative testing approaches.

ORD, NHEERL Manufactured-Engineered Nanomaterial Health Effects Research Program

Summary

1. ORD Nanotechnology Research Strategy has been developed to addresses the impact and research needs which nanotechnology has on the Agency (US EPA Nanotechnology White Paper, 2007).

2. To address some of the challenges associated with assessing the health effects of manufactured-engineered nanomaterials ORD's strategy incorporates:
   - --the National Academy of Sciences vision for "Toxicity Testing in the 21st Century";
   - --a multi-tired approach for the screening of Agency relevant nanomaterials to prioritize them for subsequent *in vivo* testing, assist in their design as well as establish collaborations (NCCT; EPA's 1st Nano Health Effects CRADA; NTP).

3. The multi-tiered approach will be employed in a comparative and iterative manner to identify *in vitro* assays that correlate with *in vivo* responses in order to identify and develop validated alternative toxicity testing methods for nanomaterials.

Engineered Nanomaterial Ecological Effects Research Within ORD's National Health and Environmental Effects Laboratory

Steve Diamond
USEPA/Mid-Continent Ecology Division

for

David R Mount . . . . . USEPA/ Mid-Continent Ecology Division
Christian Andersen . . USEPA / Western Ecology Division
Mark G. Johnson . . . . USEPA / Western Ecology Division
Paul Rygiewicz . . . . . USEPA / Western Ecology Division
David Olszyk . . . . . USEPA / Western Ecology Division
Robert Burgess . . . . . USEPA / Atlantic Ecology Division
Kay Ho . . . . . USEPA / Atlantic Ecology Division

Interagency Environmental Nanotechnology Grantees Workshop
Tampa, FL, November 19-21, 2008

United States Environmental Protection Agency

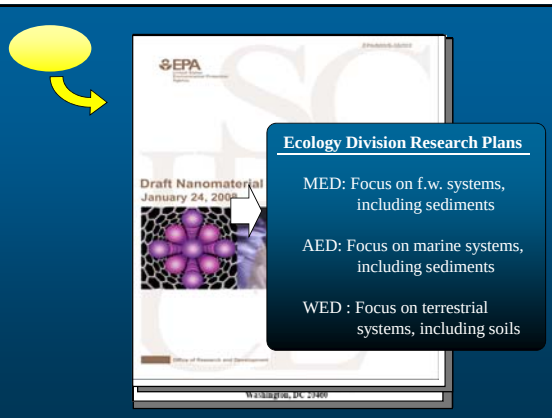
Office of Research and Development (ORD)

National Health and Environmental Effects Research Laboratory

Human Health Divisions

Ecology Divisions

Atlantic
Mid-Continent
Western
Gulf Breeze



Ecology Division Research Plans

MED: Focus on f.w. systems, including sediments

AED: Focus on marine systems, including sediments

WED : Focus on terrestrial systems, including soils

EPA Mission: Protect human health and the environment.

NHEERL mission: help the Agency evaluate the risks that nanomaterials pose to humans and ecosystems.

ECO-effects research themes

- **Evaluate current methods for assessing hazard**
- **Assess hazard for nanomaterials**
- **Identify nanomaterial characteristics that predict toxicity**
- **Identify mechanisms of action, ADME (accumulation, distribution, metabolism, and elimination)**
- **To incorporate knowledge of production volume and potential pathways of exposure, and to do so within a product life-cycle framework**

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Interagency Environmental Nanotechnology Grantees Workshop
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Test guideline reviews initiated by OECD:

Organization for Economic and Cooperative Development

↳ **Working Party for Manufactured Nanomaterials**

↳ **Steering Group 4: Test Guidelines**

↳ **Section 2: Biotic Effects (ecotoxicity tests)**

also:
Physical/Chemical Properties (Section 1)
Degradation and Accumulation (Section 3)
Health Effects (Section 4)

The Society of Environmental Toxicology and Chemistry (SETAC) North America 29th Annual Meeting, 5-9 November 2008, Tampa, FL, USA

Test guideline reviews initiated by EPA, Office of Prevention, Pesticides and Toxic Substances (OPPTS):

850-Series test guidelines: Ecotoxicology

~ 50 test Guidelines reviewed
(24 are harmonized and reviewed for OECD)

- aquatic, marine, sediment, soils, terrestrial direct contact
- vertebrates, invertebrates, plants, microbes
- acute and chronic exposure durations

Reviewers included all P.I.s for nanotechnology research at AED, MED, and WED

- also, ARCoE and USGS scientists

The Society of Environmental Toxicology and Chemistry (SETAC) North America 29th Annual Meeting, 5-9 November 2008, Tampa, FL, USA

OPPTS TG Reviews Summary:

Adequate aspects
- Toxicological principles
- Endpoints

Inadequate aspects
- Media preparation
- P/Chem properties of materials
- Quantification of exposure
- Exposure metrology

Inadequacies all related to particulate and fibrous nature of nanomaterials and the colloidal nature of exposure media

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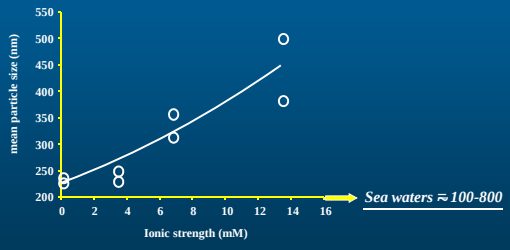
Particle Size Effects of Duration and Ionic Strength



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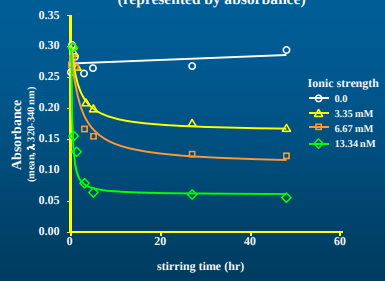
Change In Bulk Concentration (represented by absorbance)



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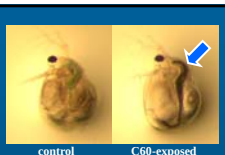

C60 ingestion by Ceriodaphnia
(C60 in digestive tract)

TiO2* ingestion by *Daphnia magna
(TiO_2 in digestive tract)

Degussa p-25
100 mg/L, stirred
~ 24 uptake
2-hr UV-A exposure
-all controls alive/mobile
-all UV-Exp. immobilized
VERY preliminary observation

Also:

- **We have initiated work with nano-Ag**
  - 22nm citrate-doped
  - produced by EPA NRMRL division (Thabet Tolymat)
  - will be used in fate studies
  - 48-hr LC50 (22-nm p.s.)
  - we have successfully imaged n-Ag using two-photon, scanning, confocal microscopy
- **We have obtained SW&MWCNT from Nikkiso Co., Japan to be used in OECD Sponsorship Program assays**

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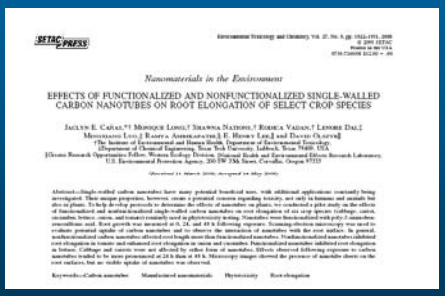


Nanomaterials in the Environment

EFFECTS OF FUNCTIONALIZED AND NONFUNCTIONALIZED SINGLE-WALLED CARBON NANOTUBES ON ROOT ELONGATION OF SELECT CROP SPECIES

NHEERL/Eco Nanotechnology Research

Additional efforts:

1) Involvement in OECD planning, review, and testing in collaborations related to the Nanomaterials Sponsorship Program.

2) Continued collaboration with South Carolina University, Oregon State Universities, ARCoE, USGS. Potential collaboration with newly-funded nano centers.

3) Providing technical support to EPA regulatory offices.

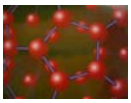
Interagency Environmental Nanotechnology Grantees Workshop
Tampa, FL, November 19-21, 2008

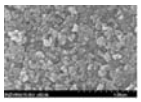
RESEARCH & DEVELOPMENT
Building a scientific foundation for sound environmental decisions

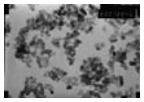
RICE

CBEN

Microbial Impacts of Engineered Nanoparticles



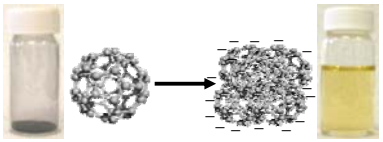




Shaily Mahendra,
Delina Y. Lyon, Dong Li,
Mark Wiesner and Pedro J. Alvarez

EPA Project R832534

nC_{60}: The Environmentally Relevant Fullerene



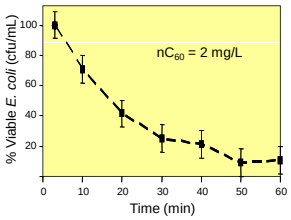
Solid or solution nC_{60}

- ✓ Highly stable water suspension
- ✓ Negatively charged surface
- ✓ Sizes range, typically 30-300 nm

Important form of C_{60} in the aqueous environment

nC_{60} is a Potent Antibacterial Agent

Ct (mg/l * min) for 99% kill:
- 0.03-0.05 for free chlorine
- ~ 100 for nC_{60}
- 95-180 for chloramines

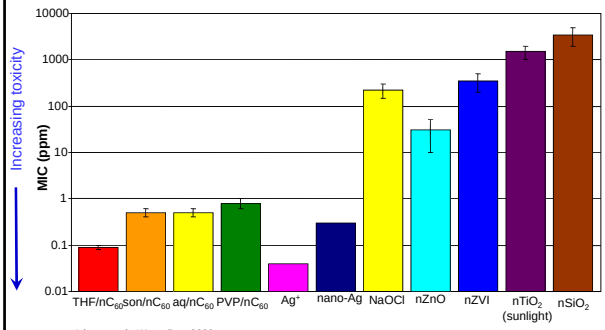


Bacteria	Description	MIC (mg/L)
Bacillus subtilis	Gram +, soil	0.001 - 0.025
Streptomyces albus	Gram +, soil	<0.05
Pseudomonas aeruginosa	Gram -, soil	0.05 - 0.066
Ralstonia pickettii	Gram -, pathogen	0.025 - 0.0375
Burkholderia cepacia	Gram -, pathogen	0.0125 - 0.025
Desulfovibrio desulfuricans	Gram -, anaerobe	0.1-0.2

Broad spectrum antibiotic

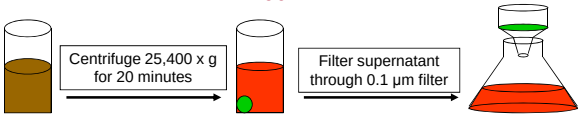
Lyon et al., *Water. Sci. Technol.*, 2008

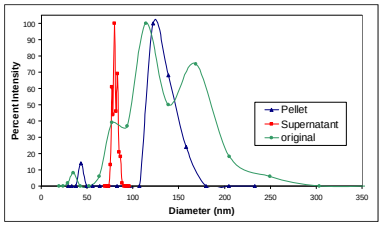
Bacterial Toxicity of Select Nanomaterials

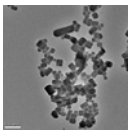


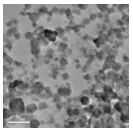
Adams et al., *Water Res*, 2006

Effect of nC_{60} Particle Size









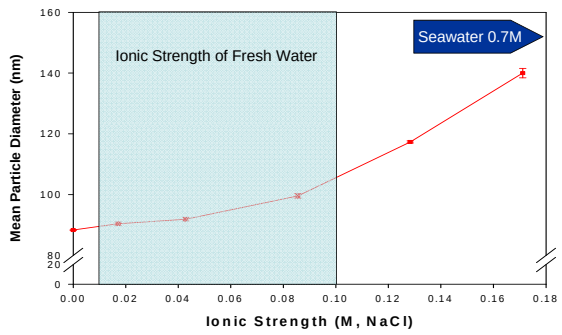
Lyon et al., *Environ. Sci. Technol.*, 2006

nC_{60} Particle Size vs. Toxicity

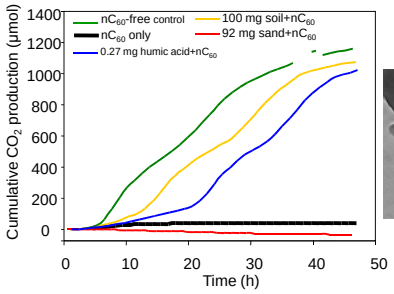
	B. subtilis MIC (mg/L)	Average Diameter (nm)	Surface Area:Volume
nC_{60}	0.75 - 1.0	100	0.06
>100 nm particles	7.5 - 10	110	0.055
	x100		x 2
<100 nm particles	0.01-0.1	50	0.12

Lyon et al., *Environ. Sci. Technol.*, 2006

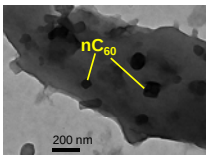
Salts Promote Aggregation, Decrease Toxicity



NOM reduces nC_{60} Bioavailability and Toxicity



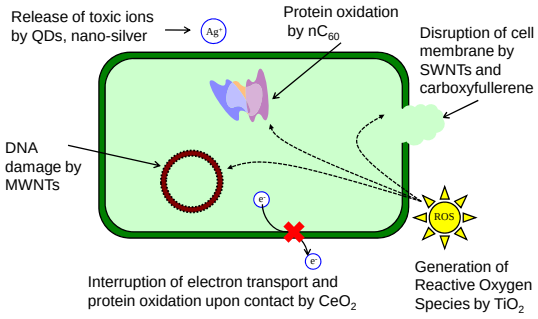
nC_{60} trapped by humic colloids



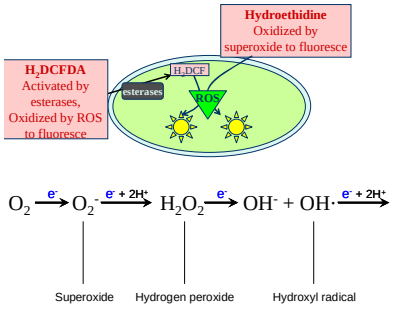
Humic acid concentrations as low as 0.05 mg/L eliminate toxicity

Li et al. *Environ. Toxicol. Chem.* 2008

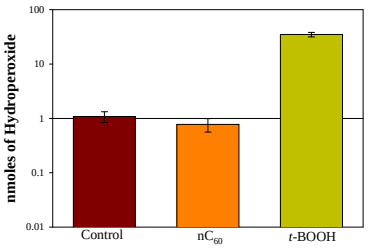
Bacterial Toxicity Mechanisms



Does nC_{60} Produce ROS in Bacteria?



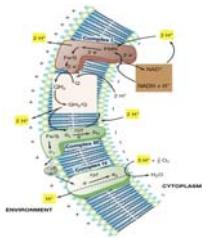
No Lipid Peroxidation due to ROS



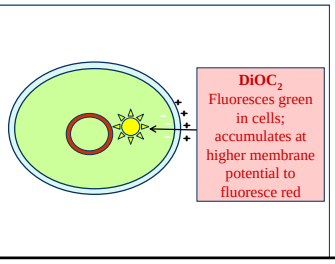
- Chromogenic assay based on hydroperoxide reaction with ferrous ions to make ferric ions
- no lipid perooxidation in nC_{60}-exposed samples (P=0.15)
- *Tert*-butylhydroperoxide is positive control

Lyon et al, *Nano. Lett.*, 2008.

Membrane Potential Collapse

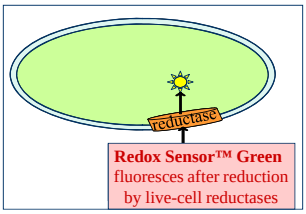


- Assay monitors $DiOC_2$
  - Red fluorescence indicates higher membrane potential
- Higher red/green ratio means higher membrane potential
- CCCP is an ionophore

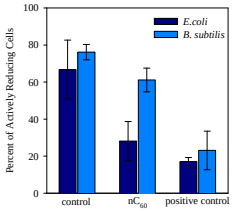


Lyon and Alvarez, *Environ. Sci. Technol.* 2008.

Changes in Reductase Activity



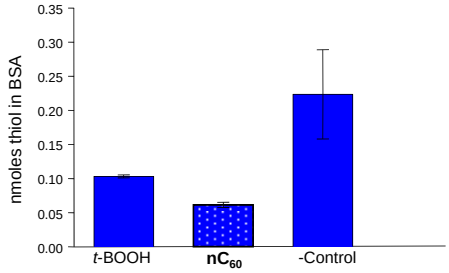
Healthy respiring cells fluoresce (redox-sensor green)



nC_{60} (ORP = +483mV) hinders normal oxidation-reduction activity, similar to sodium azide (+ control), which disrupts electron transport

Lyon and Alvarez, *Environ. Sci. Technol.* 2008.

Direct Protein Oxidation



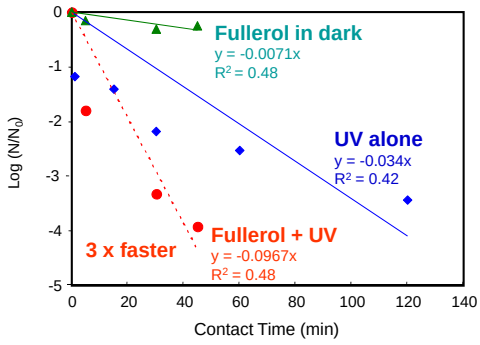
- Loss of thiol groups relative to control reflects oxidation
- *Tert*-butylhydroperoxide is positive (oxidizing) control
- Pure protein (bovine serum albumin) shows oxidation

Lyon et al, *Nano. Lett.*, 2008.

Potential Application: Enhancing UV Disinfection

- UV disinfection is increasingly used to inactivate cyst-forming protozoa such as *Giardia* and *Crystosporidium*.
- However, UV is relatively ineffective to treat viruses unless the contact time and energy output are significantly increa$ed

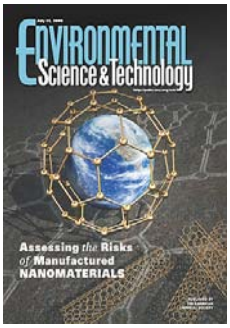
Virus inactivation by UV and Fullerol



Hotze et al. *Environ. Sci. Technol.* 2007

Conclusions and Significance

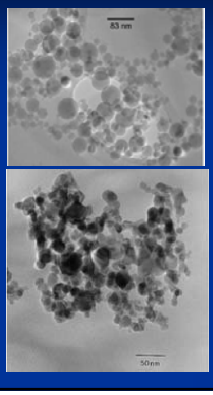
- **Ecotoxicology:** nC60, ZnO, TiO_2, and nZVI can be toxic to environmental bacteria, and possibly higher organisms.
- **Implications:** Biodiversity and food webs? biogeochemical cycling? mitigated by NOM and salinity.
- **Applications:** Water disinfection, biofouling control



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

Christopher Theodorakis
Southern Illinois University
George Cobb
Texas Tech University
Elizabeth Carraway
Clemson University

Metal Oxide Nanoparticles



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

Objectives

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

Hypothesis

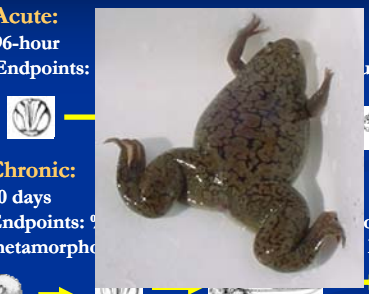
- Nanoparticle exposure will affect the survival, growth, development, egg hatchability, and metamorphosis of these organisms

Approach

Flow-through exposure, nanoparticle suspension in water

Xenopus laevis

Acute:
96-hour
Endpoints: urvival (LC50)



Chronic:
70 days
Endpoints: ons,
metamorph LOEC)

Acute Study: FETAX Assay

- ***Xenopus laevis* Definitive Test**
  - 3 replicates of 7 concentrations including a control (total exposures = 21)
    - 31.6, 10, 3.16, 1, 0.316 and 0.1 mg/L
    - Control: FETAX solution
      - FETAX solution: NaCl, $NaHCO_3$, $CaCl_2$, $CaSO_4{\cdot}2H_2O$, $MgSO_4$, and deionized or distilled water
  - 10 embryos per exposure

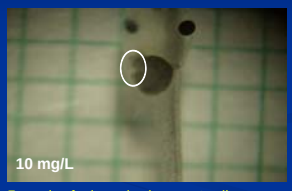
Acute Study Results

- Growth
  - Significant n total body length at 10mg/L
- Mortality
  - ***No mortality observed***
- Malformation
  - EC50 ~ 10 mg/L
- **Developmental Dose Determination**
  - EC15 ~ 1.9 mg/L
  - 2, 1, 0.5, 0.25, 0.125 and 0 mg/L

Acute ZnO Malformation Results

Typical Malformation Observed

Example of a regular, tight gut coil.



Example of a irregular, loose gut coil.

Majority of malformations were gut malformations.

Methods and Materials

- **ZnO Nanoparticles**
  - **Alfa Aesar: NanoTek®**
    - **40-100nm APS**
    - **Uses and properties**
      - **UV protection**
      - **Antimicrobial properties**
      - **Maintains a high level of transparency in coatings, polymers, caulks, adhesives and other resin systems.**
- **Solutions were made by sonicating ZnO nanoparticles in FETAX solution.**

Flow-Thru Design

- Water Chemistry
  - pH, DO, conductivity, ammonia, salinity
  - Every 48 hrs
- Zn Analysis
  - Before new solution
  - After new solution
    - ~24 hrs
- Tissue Analysis
  - End of study

Exposure Chamber

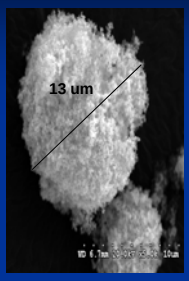
Zn Analysis

- Thermo AA Series Spectrometer
  - Flame Atomic Absorbance
- Solution
  - Add 150 uL of concentrated HNO_3 to 30 mL sample
- Tissue
  - Freeze Dry for a minimum of 24 hrs
  - Digest using EPA method 3050B

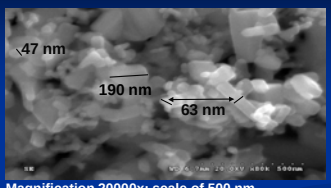
Electron Microscopy

- **Scanning Electron Microscope (SEM)**
  - **Hitachi S4300VP**
  - **Size determination of nanoparticles**
- **Nanoparticle Preparation for Imaging**
  - **Mount on SEM stub with conductive tape**
  - **Hummer V Sputter Coater**
    - **~5nm of gold-palladium alloy**

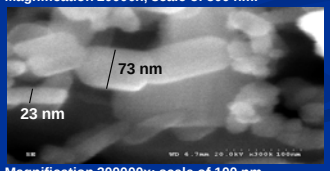
SEM



Magnification 5000x; scale of 10 um.



Magnification 20000x; scale of 500 nm.

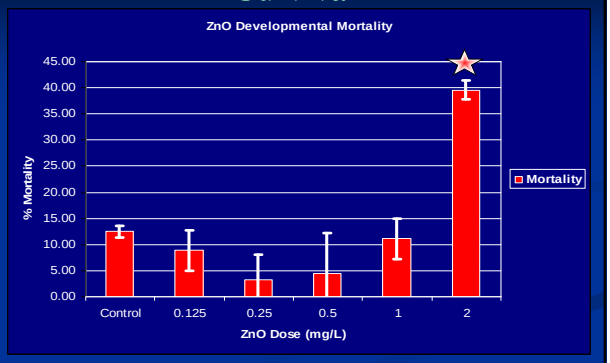


Magnification 300000x; scale of 100 nm.

Endpoints for Developmental Study

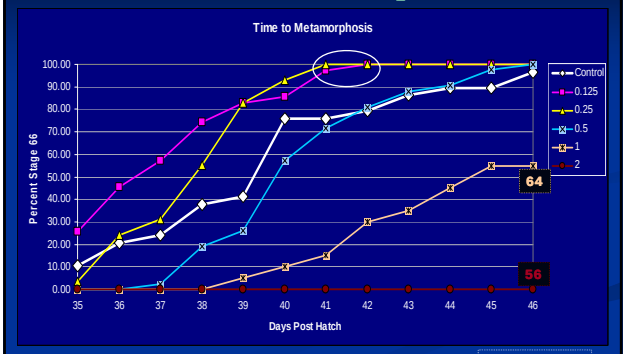
- Mortality
- Time to Metamorphosis
- Growth
  - SVL: Snout Vent Length
  - TBL: Total Body Length
  - HLL: Hind Limb Length
  - NF Stage

Survival



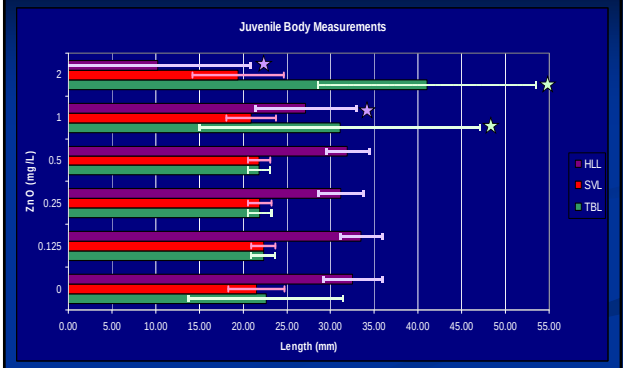
★: significant p-value<0.05 compared to all doses

Time to Metamorphosis



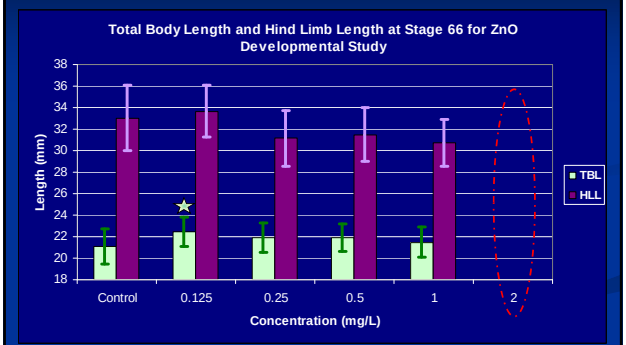
Average stage

Growth



★: significant p-value < 0.05 compared to control

Stage 66 Juveniles



★: significant p-value < 0.05 compared to control

Fathead minnow

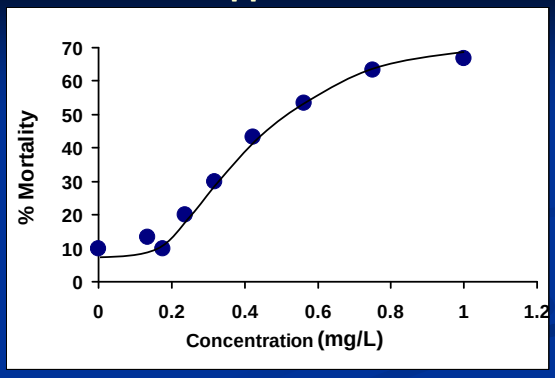
Acute:
96-h
Endpo[illegible]s: growth (EC50) , survival (L[illegible]50)

Early life stage:
28 days
Endpoint[illegible] growth, %[illegible]
(EC50, N[illegible])

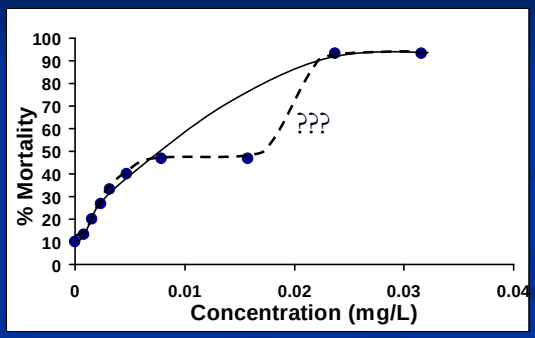
Methods

- Fathead minnow larvae (<24 hrs) were exposed to aquatios suspensions of nanoparticles
- Fish were kept in reconstituted fresh water
- Water temperature was maintained at 21° C, photoperiod 18:6 day:night
- Static renewal design: ½ of the test solution was changed daily
- Nanoparticles were purchased from Alfa Aesar, Nanophase, Inc., and SunNanosystems
- Fifteen larvae were maintained in 400 ml test solution in 600 ml beakers
- LC50s calculated using the Probit method

Copper Oxide



Metallic Copper



Iron Oxide

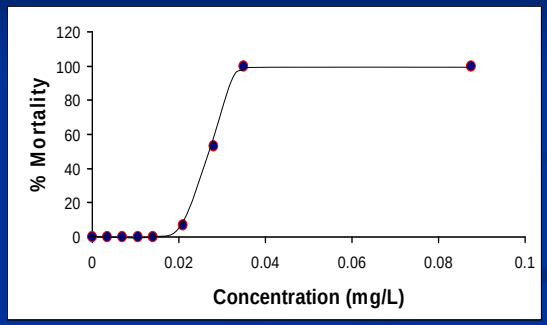


Table 1 – LC50 values for fathead minnows exposed to metal or metal oxide nanoparticles for 96 h.

Nanoparticle	LC50 (mg/L)	95 % Confidence Interval
CuO	0.662	0.492 - 0.866
Cu	0.009	0.006 - 0.013
Fe_2O_3	0.03*	-
TiO_2	>1000	NA
ZnO	>1000	NA

*Estimated value, not enough range in response to calculate LC50

Discussion - Xenopus

- Nanoparticle size
  - Individual particles varied greatly
    - 23-190nm
  - Aggregates
    - 2-15 μm
- Mortality
  - 2 ppm ZnO induced a significant increase in mortality
  - Chronic exposure resulted in a higher mortality rate

Discussion - Xenopus

- Growth
  - Total Body Length for Stage 66 juveniles
    - Low dose ZnO juveniles were significantly longer than controls (hormesis)
  - Stage progression was accelerated
    - Low dose ZnO tadpoles completed metamorphosis 5 days earlier than controls (hormesis)
  - Stage progression was inhibited
    - 58% stage 66 juveniles in 1 ppm ZnO
    - NO stage 66 juveniles in 2 ppm ZnO

Discussion – Fathead minnow

- Titanium dioxide and zinc oxide nanoparticles are non-toxic to fathead minnows in 96-h exposures.
- Copper oxide nanoparticles are highly toxic to fathead minnows
- Metallic copper and iron oxide nanoparticles are very highly toxic to fathead minnow larvae

Continuing Work

- Measurement of metal concetrations and nanoparticle size distributrions
- Determination of contribution of dissolved vs particulate metals to toxcitiy
- Comparison of toxicity of metal nanoparticles to dissolved ionic metals.
- Re-running LC50 with iron oxide to get more data points between 0.06 and 0.10
- Toxicity of Cu to Xenopus
- Chronic toxicity of Cu, CuO and Fe_2O_3 to fatheads

Acknowledgements

- Mike Wages
- Shawna Nations, Gabriele Chavez, Jamie Rotter, Zhi Mu
- Texas Tech Imaging Center
  - Mark Grimson
- EPA for funding
  - Star Grant USEPA Grant Number RD-83284201-0

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Single Conducting Polymer Nanowire Immunosensors

Ashok Mulchandani, Nosang V. Myung, Wilfred Chen and Marylynn V. Yates

University of California, Riverside, CA

EPA Review 08 Tampa, Fl. 20th Nov. 2008

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OUTLINE

- Introduction
- Importance of nanowire and conducting polymer
- Objective
- Approaches
  - In-situ electrochemical synthesis
  - Magnetic aligning of multisegmented nanowire
  - AC dielectrophoretic positioning and maskless anchoring
- Biological functionalization
- Protein sensing
- Summary
- Gas sensor
- Future work

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Affinity-based detection

- Health care
- Homeland security
- Environment monitoring
- Food safety & quality

- Antibodies
- Receptors
- Binding proteins
- Nucleic acid

- Advantages
  - High sensitivity
  - High selectivity
- Disadvantages
  - Label required
  - Not real-time
  - Indirect

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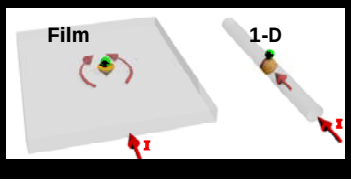
Major Advantages of 1-D Nanostructures as Sensing Materials

One-dimensional (1-D) nanostructures (e.g. nanowires, nanotubes…)
- **High surface area to volume ratio**
- **Integrable into microelectronics**
- **Higher sensitivity than conventional**



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Conducting polymers

- Exhibit electrical, electronic, magnetic and optical properties of metals or semiconductors while retaining the attractive mechanical properties and processing advantages
- Applied as conductometric, potentiometric, amperometric and voltammetric transducers and as active layers of FETs
- Can be synthesized electrochemically
- Benign conditions enable the direct deposition of conducting-polymer materials with embedded bioreceptors in one step
- Conductivity can be modulated over 15-orders of magnitude

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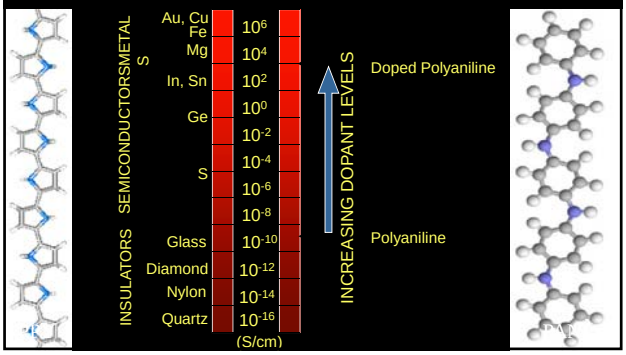
Conducting polymers



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Objective

- Develop new methods for cost-effective fabrication of single nanowire conducting polymer affinity-based sensor arrays for label-free, highly sensitive, selective, precise, and accurate detection of bioagents such as toxins, viruses and bacteria at point-of-use.

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Approaches

- In-situ fabrication of conducting polymer nanowires in e-beam lithography patterned nanochannels between pair of electrodes
- Magnetic aligning of template synthesized multi-segmented nanowire on prefabricated electrodes
- AC dielectrophoretic positioning and maskless assembly on prefabricated electrodes

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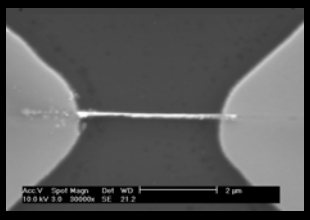
IN-SITU FABRICATION IN E-BEAM LITHOGRAPHY PATTERNED NANOCHANNELS BETWEEN ELECTRODES



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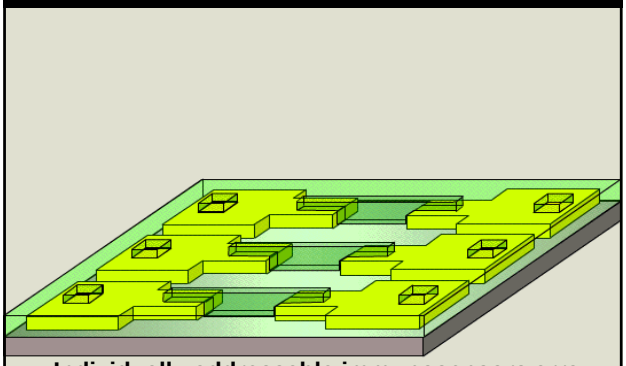

Individually-addressable immunosensors array fabrication

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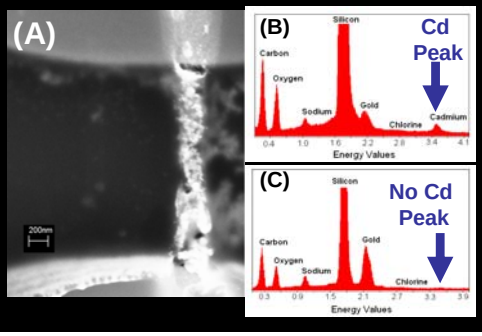
Avidin coated quantum dot entrapment in polypyrrole NW



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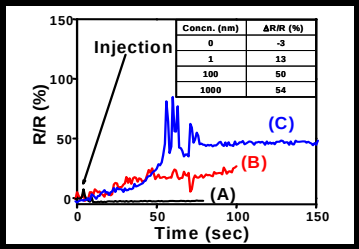
Nanowire bioaffinity sensor response



Electrical responses of an unmodified nanowire (A) to 100 nM biotin-DNA (single stranded) and avidin-embedded polypyrrole (200 nm) nanowires to 1 nM (B) and 100 nM (C) biotin-DNA. The responses were recorded on two separate polypyrrole-avidin nanowires.

Ramanathan et al., 2004, Nano Letters, 4, 1237-

Ramanathan et. al., 2005, J. Am. Chem. Soc. (JACS), 127, 496-497

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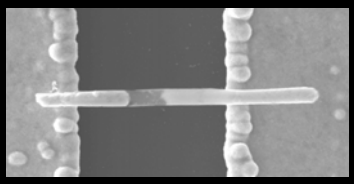
TEMPLATE-DIRECTED SYNTHESIS AND MAGNETIC ASSEMBLY OF MULTI-SEGMENTED NANOWIRE



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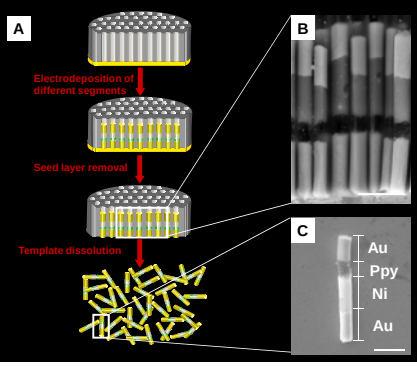
Electrodeposited Segmented Nanowires



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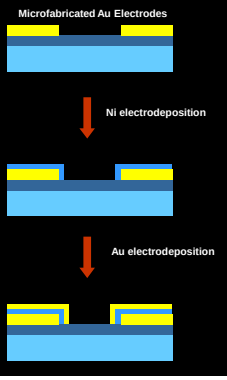
Electrode preparation

- Prefabricated gold microelectrodes were deposited with Ni
  - Electrolyte: 0.91 M $Ni(H_2NSO_3)_2$ + 0.10 M H_3BO_3 + 30 ppm HCl
  - Potential: -0.9 V
  - Deposition time: ~10 sec
- To avoid surface oxide formation of Nickel a thin layer of gold was electrodeposited
  - Electrolyte: Technic Au
  - Temp.: 60 ºC
  - Potential: -0.5 V
  - Deposition time: 1 min



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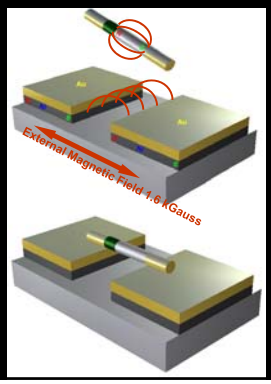
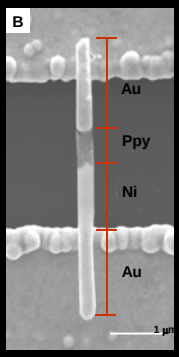
Magnetic aligning and assembly





Bangar et al., *Electroanalysis*, In Press

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Limitations

- Magnetic (Ni) segment integration required
- Multisegmented nanowire architecture results in mechanical weakness especially at the interfaces
- Low aspect ratio can potentially result in lower dynamic range
- Due to limitation of use of NaOH for template dissolution, over-oxidation of Ppy segment resulted in lower conductivity and possibly in lower sensing performance

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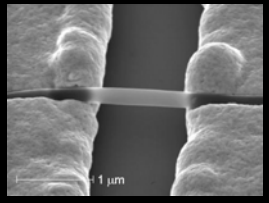
TEMPLATE SYNTHESIS FOLLOWED BY DIELECTROPHORETIC ALIGNMENT AND MASKELESS ELECRODEPOSITION ANCHORING



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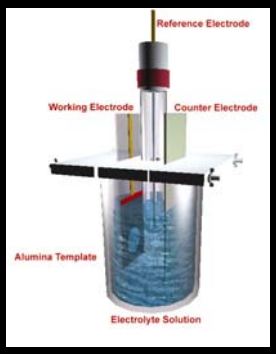
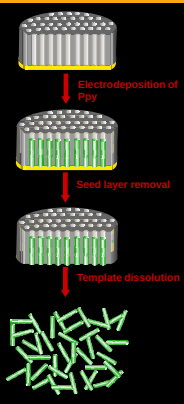
Template synthesis





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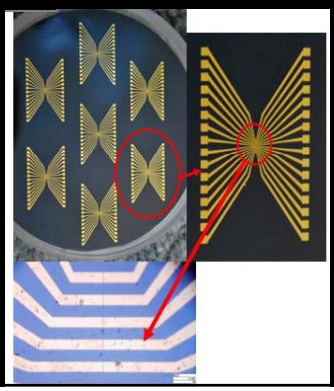
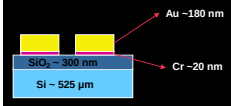
Electrode Design



Microfabricated Gold Electrodes

1. Si/SiO substrate
2. Cr adhesion Layer ~ 20 nm
3. Gold Layer ~ 180 nm



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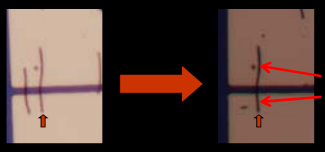
Contact Resistance

AC Dielectrophoretic Alignment: 5 MHz, 1 V p-to-p.



Device limitation: Modulation of contact resistance upon exposure to liquid medium.

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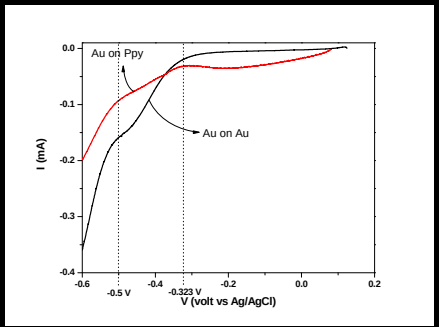
Maskless electrodeposition



Linear Sweep Voltammograms of gold (Au) on different surfaces (scan rate = 1 mV/s). Area = 0.5 cm². Electrolyte: Technic Au, pH = 7.0

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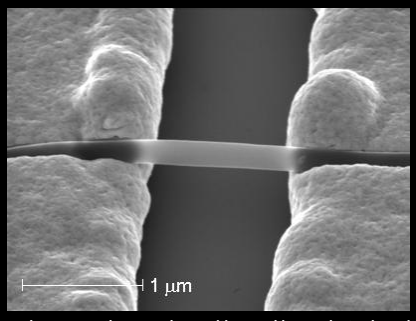
Maskless gold electrodeposition



Ppy Nanowire were then anchor with maskless electrodeposition, using Chronoamperometry method (E(V) : -0.5, Deposition time: 600 s, Gold Colloid Solution ($HAuCl_4$) was used as a electrolyte

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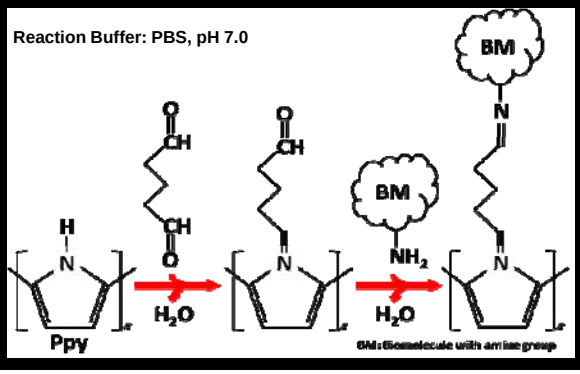
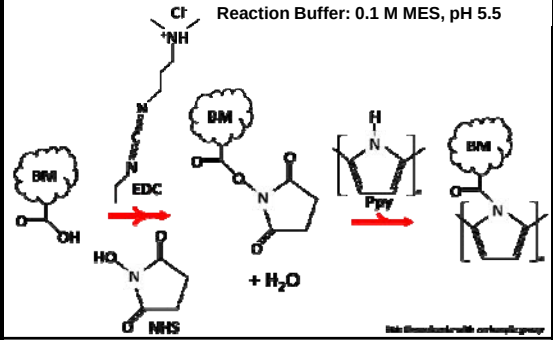
Glutaraldehyde functionalization

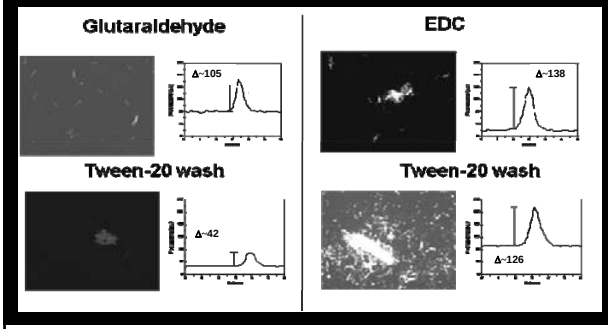
Reaction Buffer: PBS, pH 7.0



Carbodiimide functionalization

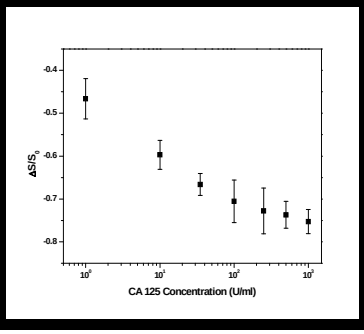


Glutaraldehyde vs. Carbodiimide



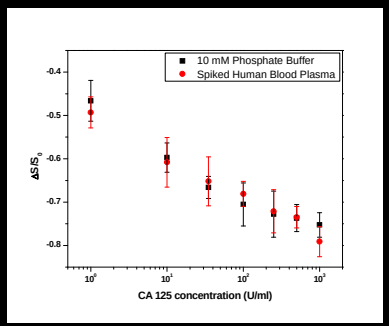
Fluorescence images and corresponding fluorescence line profile across single nanowire functionalized with BSA-FITC with respective covalent route.

Protein detection



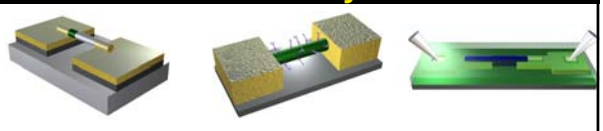
Calibration plot showing detection of CA 125 cancer antigen in 10 mM phosphate buffer using Anti-CA 125 antibody immobilized Ppy nanowire biosensor.

Selectivity



Calibration plots showing detection of CA 125 cancer antigen in PB and spiked human blood plasma using Anti-CA 125 antibody immobilized Ppy nanowire biosensor.

Summary



	Multi-segmented	Masklessly connected	In-Situ
Template	Alumina	Alumina	E-beam lithographic nano-channel
Polymer conductivity	~10^{-3} S/cm	~10^{0} S/cm	~10^{1} S/cm
Polymer-functionalization	Post-fabrication	Post-assembly	During or after fabrication
Sensing	Ammonia: LDL 100 ppm, poor recovery	CA 125: 45% conductance change at 1 U/ml. Dynamic range up to 1000 U/ml. No effect of other proteins	Avidin-Biotin interactions: LDL 1 nM of Biotin-ssDNA conjugate.
Multi-analyte sensing	Site specific deposition of pre-functionalized nanowires	Site-specific functionalization	Site-specific deposition into individual channel
Cost-benefits	Limited	Most cost-effective	Cost-benefits compromised due to use of e-beam lithography or FIB.

Carbon Nanotubes: Environmental Dispersion States, Transport, Fate, and Bioavailability

Elijah Petersen, Walter J. Weber, Jr. – University of Michigan
Collaborators
Jack Huang – University of Georgia, Griffin
Jussi Kukkonen, Jarkko Akkanen, and Kukka Tervonen – University of Joensuu, Finland
Dr. Denis O'Caroll – University of Western Ontario, Canada
Dr. Peter Landrum - NOAA

This research is funded by U.S. EPA - Science To Achieve Results (STAR) Program
Grant # RD833321

Graham Environmental Sustainability Institute – University of Michigan

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Presentation Outline

1. **Background**
2. **Carbon-14 Nanotube Synthesis**
3. **Uptake and Depuration Behaviors for *Lumbriculus variegatus***
4. **Uptake and Depuration Behaviors for *Daphnia Magna***
5. **Additional Results**

2

1. Background Overview

Ecological

- Transport
- Bioavailability/Bioaccumulation
- Biodegradation
- Toxicity

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1. Background:
Current Analytical Techniques and Limitations

1. Spectrofluorimetry
Drawbacks: Carbon Nanotube Bundles, Metallic Nanotubes
2. External Chemical Labeling
Drawbacks: Changes Nanotube Physicochemical Properties
3. Raman Spectroscopy
Drawbacks: Only single-walled carbon nanotubes, qualitative

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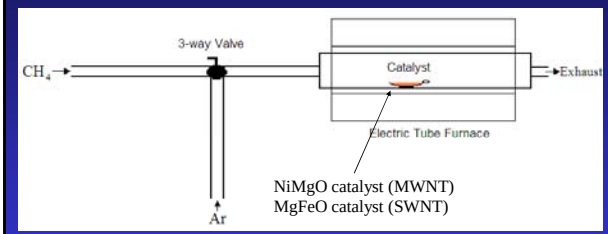
1. Background:
Advantages of C^{14} Nanotubes

1. Readily quantifiable
2. Can be used with all types of carbon nanotubes
3. Does not change the chemical or physical properties of the carbon nanotubes

5

2. Carbon-14 Nanotube Synthesis
Chemical Vapor Deposition



Petersen, E. J., Huang, Q. G., Weber, W. J., Jr., *Environmental Health Perspectives.* **2008**, 496-500.
Petersen, E. J., Huang, Q. G., Weber, W. J., Jr., *Environmental Science and Technology.* **2008**, 3090-3095.

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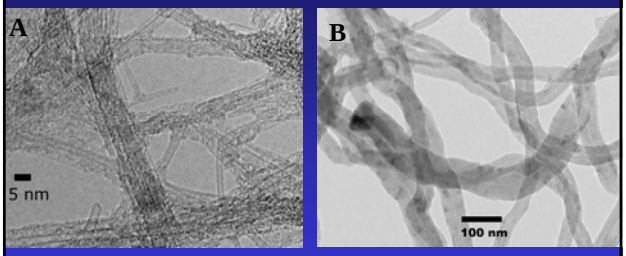
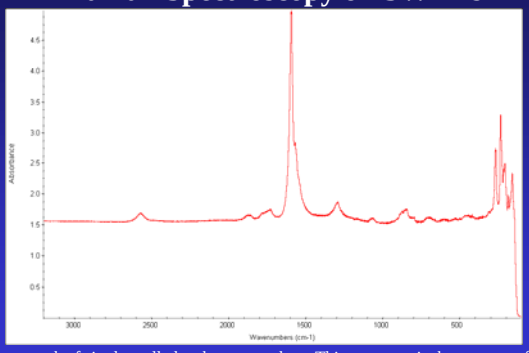
2. Carbon Nanotube Characterization: Transmission Electron Microscopy



Transmission electron micrographs of (A) single-walled at 250kx magnification and (B) multi-walled carbon nanotubes at 100kx magnification.

7

2. Carbon Nanotube Characterization: Raman Spectroscopy on SWNTs



Spectrograph of single-walled carbon nanotubes. This spectrum is the average of nine measurements

8

2. C^{14} Nanotube Synthesis: Summary of Results for HCl Purified Nanotubes

	SWNT	MWNT
Carbon Purity (Percentage)	92 ± 0.4	99 ± 1
Radioactivity (uCi/g)	1350 ± 30	122 ± 4
Detection Limit (nanograms)	34 ± 1	380 ± 10

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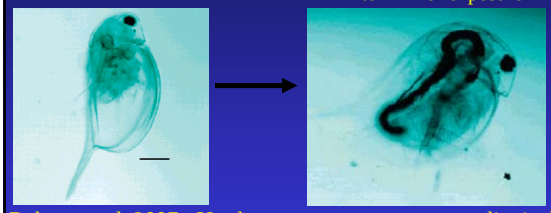
3. Uptake and Depuration Behaviors for *Lumbriculus variegatus* Aquatic Organism Uptake

After 1 hr of exposure



Roberts et al. 2007 - Used raman spectroscopy to qualitatively detect lysophophatidylcholine coated SWNTs in *daphnia magna*.

Roberts et al., *Environ. Sci Tech.* **2007;** 41(8) pp 3025 - 3029

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3. Uptake and Depuration Behaviors for *Lumbriculus variegatus*

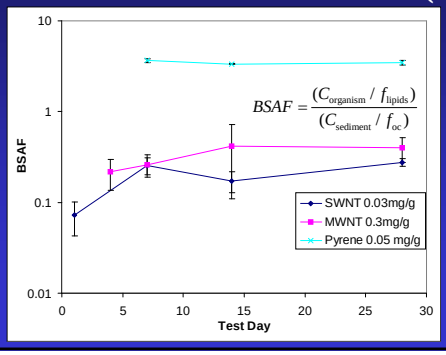
Lumbriculus variegatus has been

- used as a bioindicator for environmental pollution
- selected by the U.S. Environmental Protection Agency as the freshwater organism for assessing bioaccumulation
- commonly used in laboratory experiments for uptake of a broad range of compounds

Petersen, E. J., Huang, Q. G., Weber, W. J., Jr., *Environmental Health Perspectives.* **2008**, 496-500.

11

3. Uptake and Depuration Behaviors for *Lumbriculus variegatus* Biota-Sediment Accumulation Factors (BSAF)



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3. Uptake and Depuration Behaviors for *Lumbriculus variegatus*
BSAFs After 14d Exposure with Different Spiking Conditions

	Mean	Standard Deviation
Pyrene (0.05 mg/g)	3.353	0.050
MWNT #1 (0.37 mg/g)	0.418	0.308
MWNT #2 (0.37 mg/g)	0.506	0.092
MWNT (0.037 mg/g)	0.370	0.093
SWNT (0.03 mg/g)	0.174	0.045
SWNT (0.003 mg/g)	0.141	0.006
MWNT Sediment Only (0.37 mg/g)	0.035	0.015

These results indicate that the carbon nanotubes measured after the 6 hour depuration interval were in the gut of the organisms and not absorbed into the tissue.

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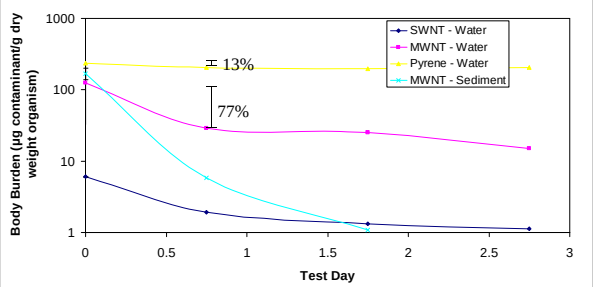
3. Uptake and Depuration Behaviors for *Lumbriculus variegatus*
Aquatic Worm Depuration



Water – indicates depuration in beakers with only water
Sediment – indicates depuration in beakers with sediment and water

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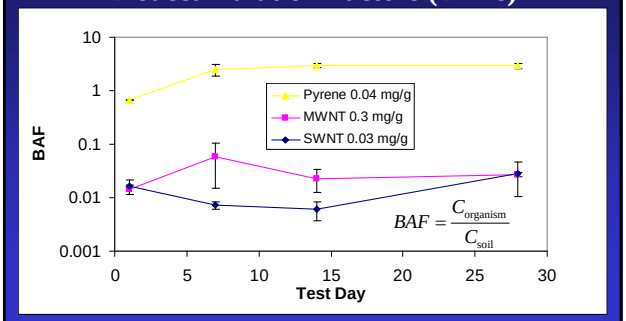
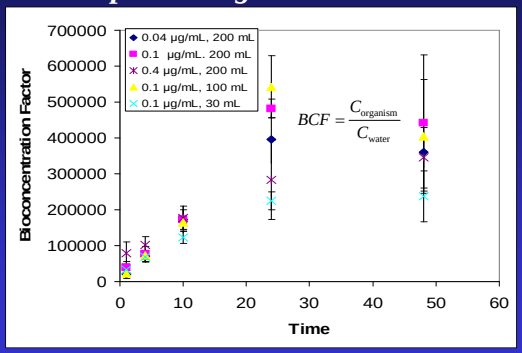
3. Uptake and Depuration Behaviors with *Eisenia foetida*
Bioaccumulation Factors (BAFs)



Petersen, E. J., Huang, Q. G., Weber, W. J., Jr., *Environmental Science and Technology*. **2008**, 3090-3095.

4. Uptake and Depuration Behaviors for *Daphnia Magna* - Accumulation



Petersen, E.J. et al. 2008. Submitted.

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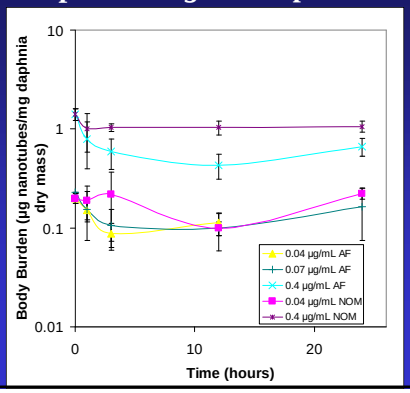
4. Uptake and Depuration Behaviors with *Daphnia Magna* - Depuration



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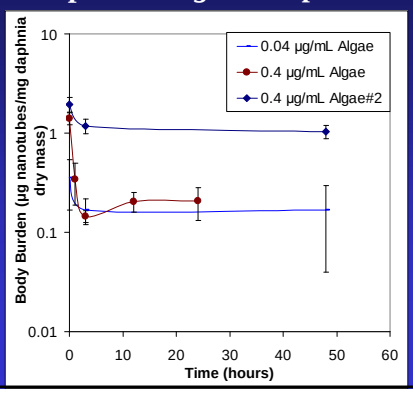
4. Uptake and Depuration Behaviors for *Daphnia Magna* - Depuration



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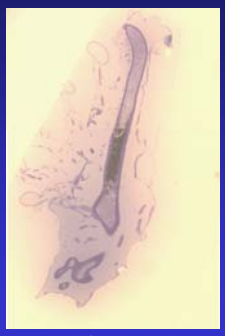
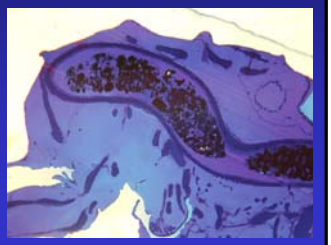
4. Uptake and Depuration Behaviors for *Daphnia Magna* - Light Microscope Pictures

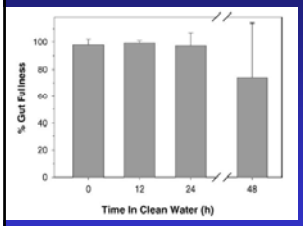


Exposed for 1 hr

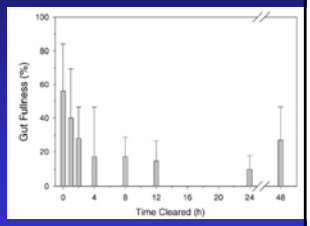


Exposed for 24 hours

4. Uptake and Depuration Behaviors for *Daphnia Magna* – Sediment Depuration



Sediment depuration in clean water



Sediment depuration in water with algae

Gillis et al. *Aquatic Toxicology*. 2005, 143-154.

5. Additional Results

1. Changing the hydrophobicity of multi-walled carbon nanotubes changes their octanol-water distribution behavior but does not impact accumulation by earthworms or aquatic worms.
   Petersen, E. J., Huang, Q., Weber, W. J., Jr. 2008. Relevance of K_{OW} Measurements to the Ecological Uptake of Carbon Nanotubes. Submitted.
2. Adding carbon nanotubes to soils affects the uptake of soil-borne pyrene by earthworms in a concentration-dependent manner. Low concentrations of nanotubes show no impact but higher concentrations decrease pyrene accumulation and act similarly black carbons.
   Petersen, et al. 2008. Influence of Carbon Nanotubes on Pyrene Bioaccumulation from Contaminated Soils by Earthworms. Submitted.
3. Polyethyleneimine (PEI) was covalently bonded to multi-walled carbon nanotubes to form nanotubes with positive, negative, or neutral surfaces charges, and the cellular toxicity of these nanotubes was tested.
   Shen, M., Wang, S.H., Shi X., Huang Q., Petersen, E.J., Pinto R.A., Baker, J.R., Jr., and Weber, W. J., Jr. 2008. Submitted.

5. Additional Results

4. We have developed a novel method to quantify fullerenes in ecological receptors and have found significant accumulation and limited depuration by *Daphnia magna*.
   Tervonen, K., Waissi, G., Petersen, E. J., Akkanen, J., Kukkonen J. V. K. 2008. Analysis of fullerene-C_{60} in *Daphnia magna* and kinetic measurements for its bioaccumulation and depuration. In Preparation.

Uptake and Depuration Behaviors for *Eisenia foetida* Gut Contents

The BAF for a non-bioaccumulating chemical was estimated to be 0.0315 ± 0.001.
Hartenstein, F.; Hartenstein, E.; Hartenstein, R., Gut Load and Transit-Time in the Earthworm Eisenia-Foetida. *Pedobiologia* **1981,** 22, (1), 5-20.

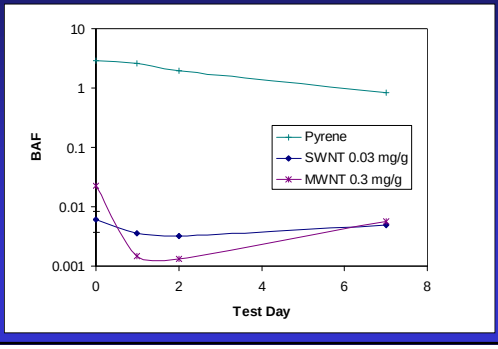
Similar results have also been obtained for *Eisenia andrei.*
Jager, T.; Fleuren, R.; Roelofs, W.; de Groot, A. C., Feeding activity of the earthworm Eisenia andrei in artificial soil. Soil Biol. Biochem. **2003,** 35, (2), 313-322.

Uptake and Depuration Behaviors for *Eisenia foetida* BAFs After 14d Exposure

	BAF
BAF for Non-Bioaccumulating Compound	0.0315
Pyrene Chelsea Soil (0.04 mg/g)	2.94 ± 0.25
Pyrene Ypsilanti Soil (0.04 mg/g)	14.0 ± 0.9
MWNT Chelsea Soil (0.3 mg/g)	0.023 ± 0.01
MWNT Chelsea Soil (0.03 mg/g)	0.016 ± 0.001
MWNT Ypsilanti Soil (0.3 mg/g)	0.014 ± 0.003
SWNT Chelsea Soil (0.03 mg/g)	0.0061 ± 0.002
SWNT Chelsea Soil (0.1 mg/g)	0.0078 ± 0.005
SWNT Ypsilanti Soil (0.03 mg/g)	0.022 ± 0.003

Weight Percent Organic Carbon Content of Chelsea Soil: 5.95%
Weight Percent Organic Carbon Content of Ypsilanti Soil: 1.14%

Uptake and Depuration Behaviors for *Eisenia foetida*
Depuration After 14d Exposure in Chelsea Soil



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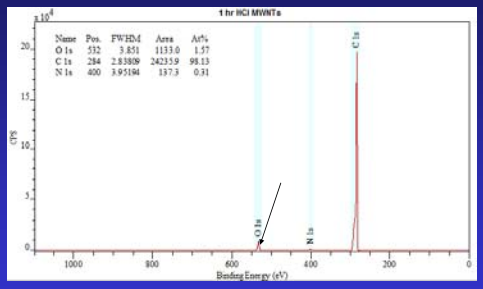
X-ray Photoelectron Spectroscopy (XPS)



1-hr HCl indicates purification with HCl for 1 hour

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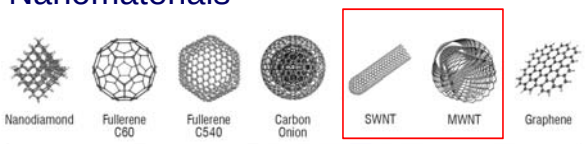
Aggregation and Deposition Behavior of Carbon Nanotubes (CNTs) in Aquatic Systems

Menachem Elimelech, Lisa Pfefferle, and Navid Saleh
Department of Chemical Engineering
Environmental Engineering Program
Yale University

Interagency Environmental Nanotechnology Grantees Workshop, Tampa, Florida, November 19-21, 2008

Yale

Engineered Carbon-Based Nanomaterials

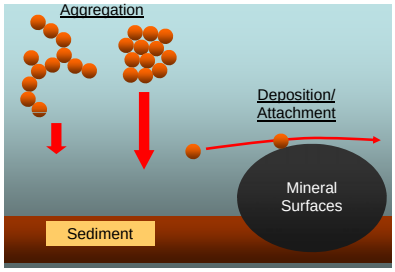


Mauter and Elimelech, *ES&T*, **42**, 2008, 5843-5859.

- Unique properties
- Exponential growth in production and applications
- Environmental and health impacts are not known

Yale

Aggregation and Deposition Behavior Determines Fate and Transport

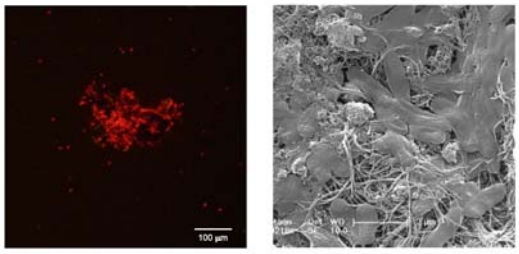


- Influences rate of settling and transport
- Influences reactivity and toxicity

Yale

Bacteria Attach to CNT Aggregates: Significant Cell Damage

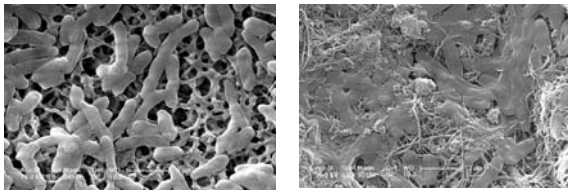
E. coli cells; single walled carbon nanotubes (SWNTs)



Kang, S., Pinault, M., Pfefferle, L.D. and Elimelech, M. "Single-Walled Carbon Nanotubes Exhibit Strong Antimicrobial Activity", *Langmuir,* **23**, 2007, 8670-8673

Yale

E. Coli Cell Membrane Damage in Contact with SWNT Aggregates

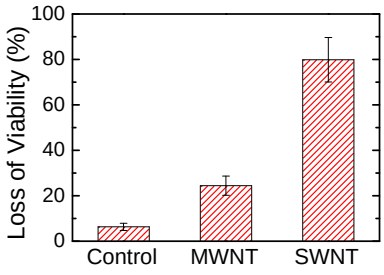


Control (without SWNT)

SWNT

Yale

SWNTs are Much More Toxic than MWNTs

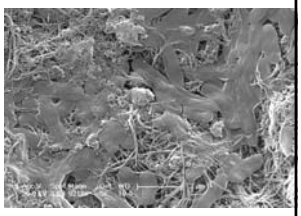


Kang, S., Herzberg, M., Rodrigues, D.F. and Elimelech, M. "Antibacterial Effects of Carbon Nanotubes: Size Does Matter!", *Langmuir*, **24**, 2008, 6409-6413

Yale

SWNTs are Much More Toxic than MWNTs

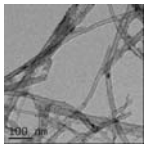
MWNTs



SWNTs

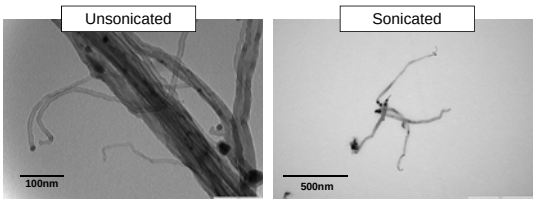
Yale

Aggregation Kinetics of Multiwalled Carbon Nanotubes (MWNTs)



Yale

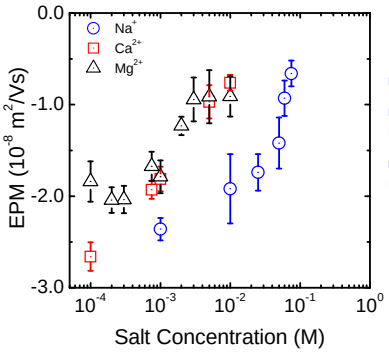
MWNT Sample



- Commercial MWNTs
- Long, bundled tubes
- Sonication debundled and shortened the tubes

Yale

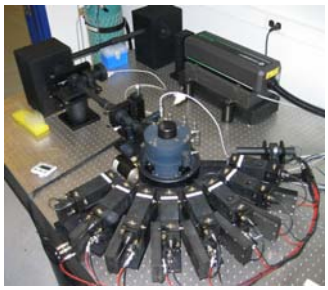
Electrokinetic Properties of MWNTs



- pH 6
- Higher salt conc., lower EPM
- Ca^{2+} and Mg^{2+} reduce EPM more than Na^+

Yale

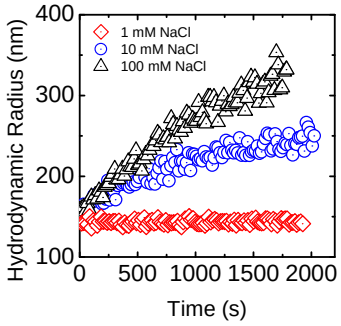
ALV Light Scattering Setup



- Dynamic light scattering to derive hydrodynamic radius
- YAG laser (532 nm)
- Scattered light intensity measured at 90° from incident beam

Yale

Attachment Efficiency

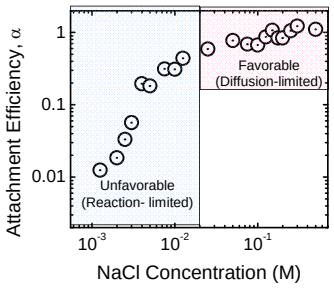


Attachment Efficiency:

$$\alpha = \frac{\left(\frac{dR_h(t)}{dt}\right)_{t\to 0}}{\left(\frac{dR_h(t)}{dt}\right)_{t\to 0,\, fav}}$$

Yale

Aggregation Kinetics with Monovalent Salt (NaCl)



pH 6

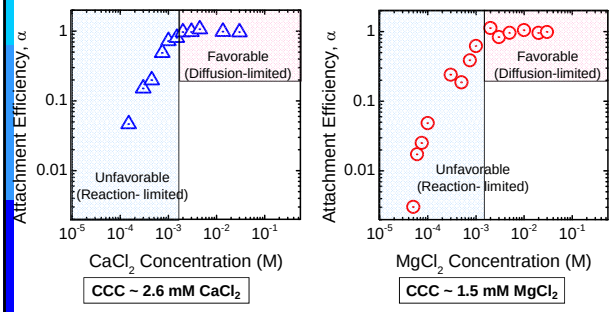
CCC ~ 25 mM NaCl

Saleh et al., *Environ. Sci. Technol.* **2008**, 42, 7963-7969

Yale

Aggregation Kinetics in Ca^{2+} and Mg^{2+}

pH 6



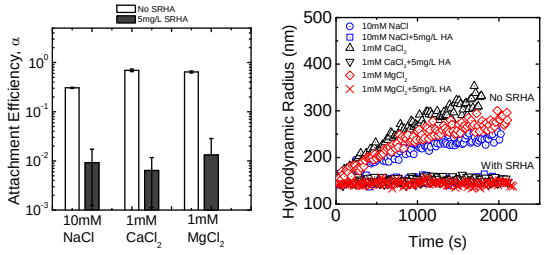
CCC ~ 2.6 mM $CaCl_2$

CCC ~ 1.5 mM $MgCl_2$

Saleh et al., *Environ. Sci. Technol.* **2008**, 42, 7963-7969

Yale

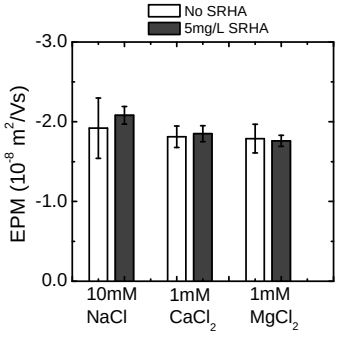
Effect of Natural Organic Matter: Suwannee River Humic Acid



- SRHA reduces aggregation rate considerably
- α values lowered 2-3 orders of magnitude by humic acid

Yale

Steric Interaction in Presence of Humic Acid



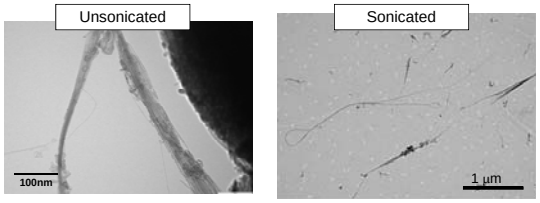
- EPM values do **not change** with humic acid
- Reduced aggregation rate due to **steric** repulsion

Yale

Deposition Behavior of Single-Walled Carbon Nanotubes (SWNTs)

Yale

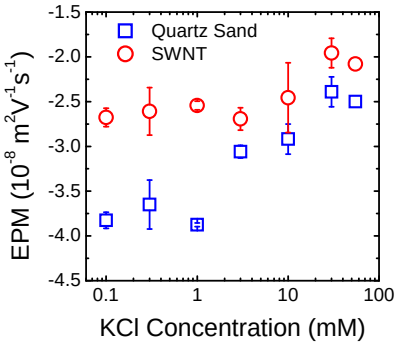
SWNT Sample



- Commercial SWNTs
- Long, bundled tubes
- Sonication debundled and shortened the tubes

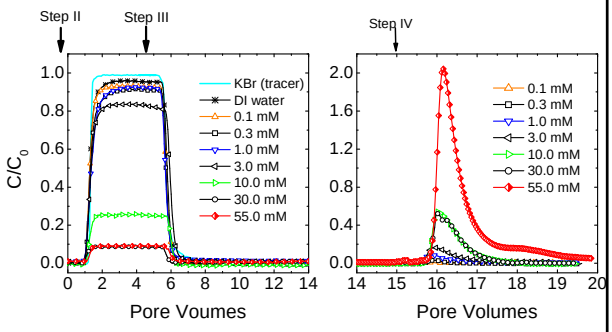
Yale

Electrokinetic Properties of SWNTs and Sand Grains



Yale

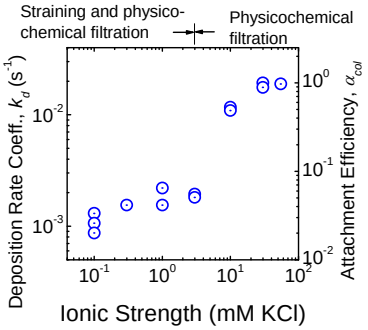
SWNT Breakthrough and Release



Jaisi et al., *Environ. Sci. Technol.* **2008,** 42 (22), 8317-8323

Yale

Deposition Mechanisms of SWNTs



Deposition Rate

$$k_d = -\frac{U}{fL}\ln\left(\frac{C}{C_0}\right)$$

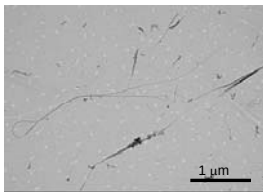
Attachment Efficiency

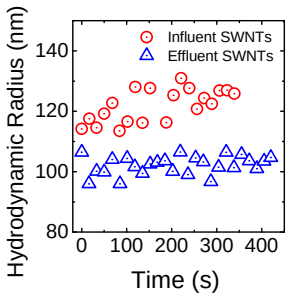
$$\alpha = \frac{k_d}{k_{d,fav}}$$

Jaisi et al., *Environ. Sci. Technol.* **2008,** 42 (22), 8317-8323

Yale

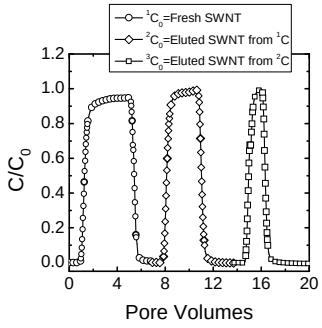
Long SWNTs Result in Straining





Yale

Subsequent Column Runs with a Single SWNT Sample (in DI Water)



Yale

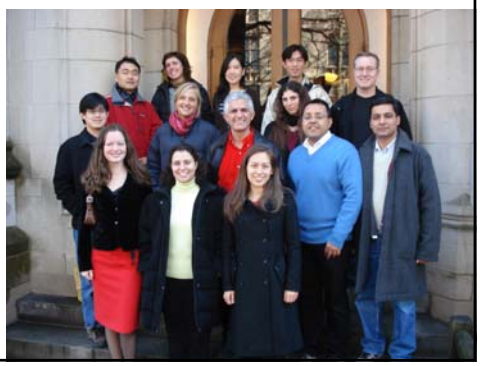
Concluding Remarks

- Eelectrostatic interactions control the aggregation behavior of CNTs
- Humic substances stabilize CNTs by electrosteric repulsion
- CNT transport in porous media is relatively limited because of straining

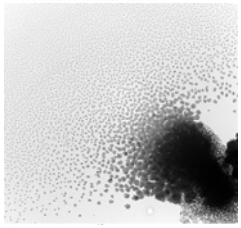
Yale

Acknowledgments

- National Science Foundation
- U.S. EPA



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



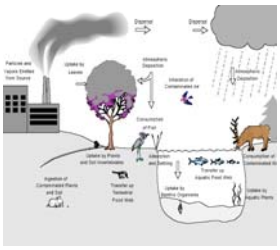
Peter J. Vikesland,
Linsey C. Marr,
Joerg R. Jinschek,
Laura K. Duncan,
Behnoush Yeganeh and
Xiaojun Chang

Funding:

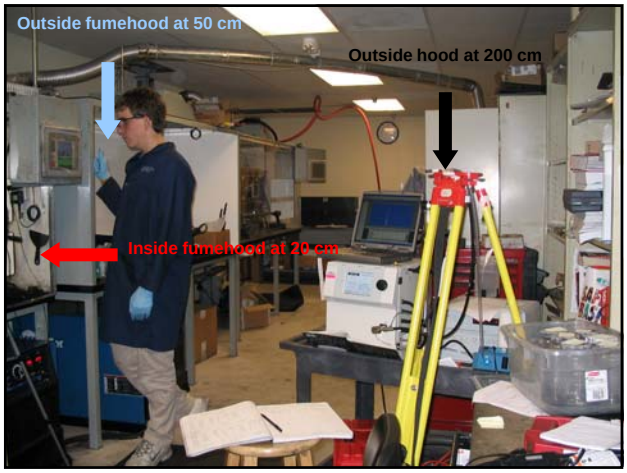
BES-0537117

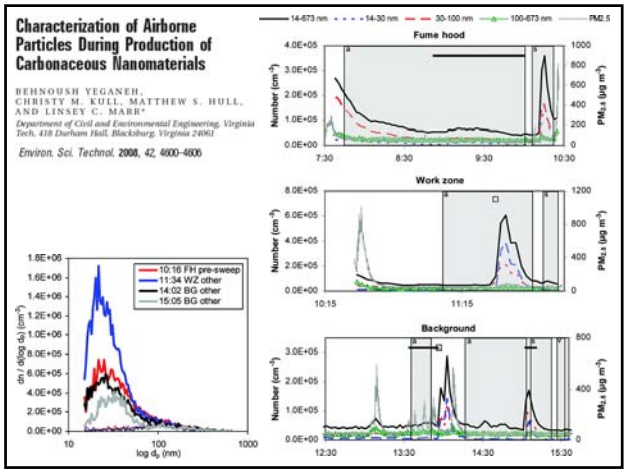
Research Questions

What is the potential for exposure to airborne nanomaterials during manufacturing?



How do atmospheric transformations of nanoparticles affect their fate in water and soil?





Research Questions

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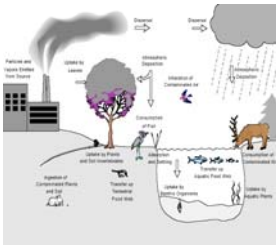
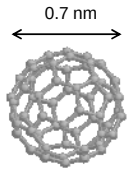
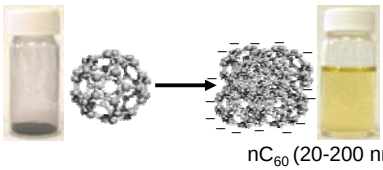


How do atmospheric transformations of nanoparticles affect their fate in water and soil?

C_{60} Fullerenes



Symmetry and conjugated π-bond system of C_{60} leads to unique properties
- –High reactivity to nucleophiles
- –Electron affinity (2.7 eV)
- –Photosensitization

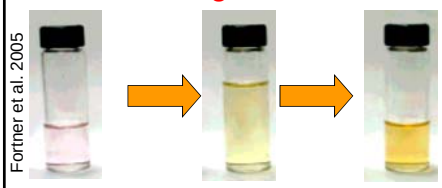


nC_{60} (20-200 nm)

Reported solubility in water is < 10^{-9} mg/L

Methods to produce nC_{60}

Solvent exchange



THF/nC_{60}

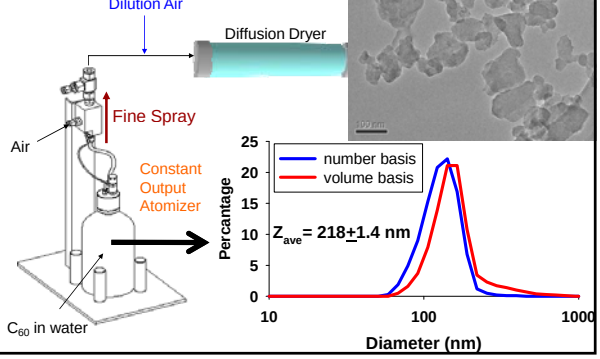
toluene/nC_{60}

TTA/nC_{60}

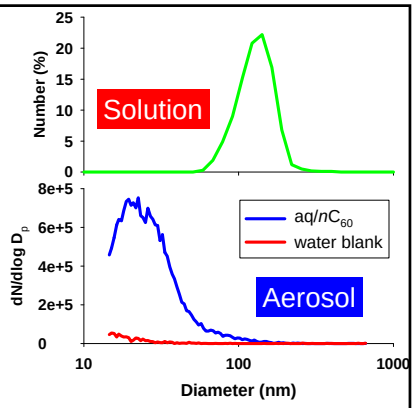
Extended stirring

aqu/nC_{60}

nC_{60} Aerosolization

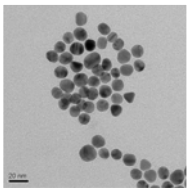


Upon aerosolization the mean particle size decreases substantially



Does this difference suggest something about the fundamental forces holding the nC_{60} aggregates together????

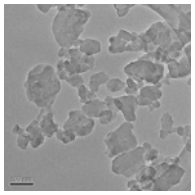
How representative of 'environmental' and 'physiological' systems are these nC_{60} suspensions?



Solvent exchange

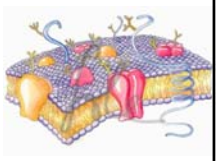
THF/nC_{60} retains solvent THF
Typically monodisperse
Form via recrystallization (bottom-up)

Extended stirring



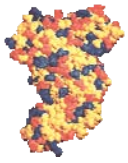
Heterodisperse
Forms via weathering (top-down)

Natural water and physiological fluid components



Electrolytes

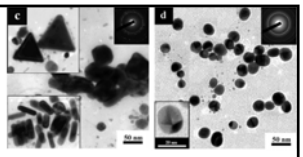
Organic macromolecules
- Proteins
- Lipids
- Carbohydrates
- Humic and fulvic acids



Low molecular weight organics
- Nucleic acids
- Amino acids
- ***Carboxylic acids***

Each of these components is expected to alter the mechanism(s) responsible for nC_{60} formation and stability…

Impacts of Organic Materials on nC_{60}

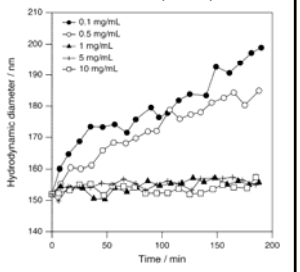


Xie et al. (ES&T)

Terashima and Nagao (*Chem Lett. (2007), 36, 302*)
Fulvic and humic acids increased apparent solubility of nC_{60} by 8x and 540x, respectively

Xie et al. (*Environ. Sci. Techol. (2008), 42, 2853*)
Fulvic and humic acids caused disaggregation of toluene/nC_{60} and THF/nC_{60}

Deguchi et al. (*Chem. Res. Toxicol. (2007), 20, 854*)
Human serum albumin stabilizes SON/nC_{60} aggregates and inhibits their aggregation



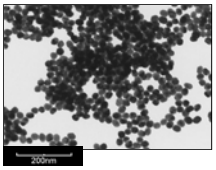
Deguchi et al. (Chem. Res. Toxicol)

Why carboxylic acids?

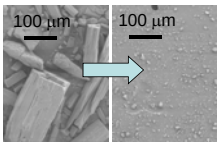
- nC_{60} aggregate size **decreases** in the presence of *natural organic matter* isolates (Duncan et al. 2008)

  w/o NOM Z_{avg} = 173 nm, PDI = 0.15
  w/ 1 mg/L Z_{avg} = 134 nm, PDI = 0.14

- Carboxylic acid groups are prevalent in many organic compounds
- Citrate is a well known stabilizer of many nanomaterials



Citrate stabilized gold nanoparticles



1) Pulverize C_{60}

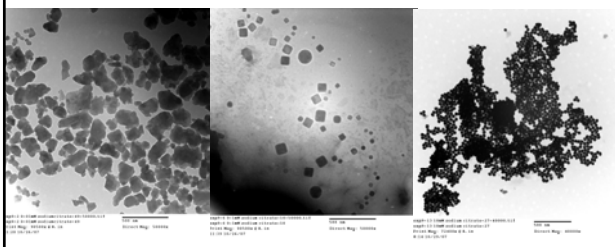
2) Mix C_{60} in citrate solutions

[Na_3Ct]= 0 0.1 1.0 10 mM

3) Settle nC_{60} for >24 hours

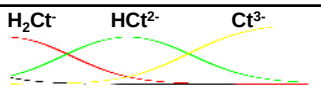
4) Characterize nC_{60}

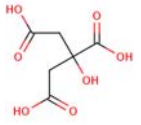
Effect of citrate on aggregate morphology



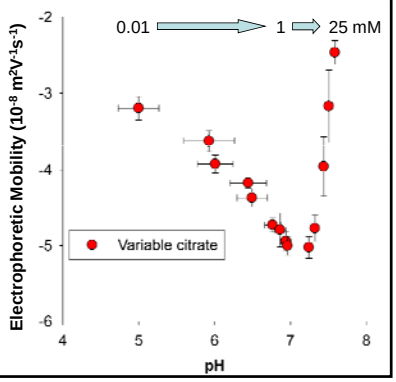
0.01 mM citrate | 0.1 mM citrate | 10 mM citrate

Citrate concentration alters solution pH



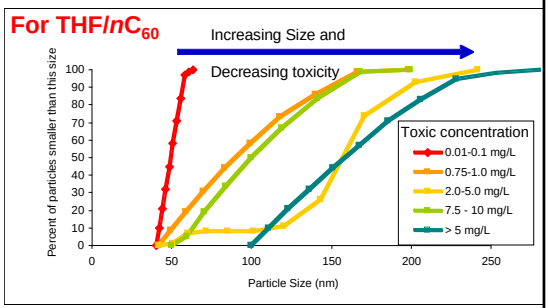


H_3Ct ⇕ pK_a = 3.13
H_2Ct^- ⇕ pK_a = 4.76
HCt^{2-} ⇕ pK_a = 6.39
Ct^{3-}



Should we worry about citrate mediated dissolution and reprecipitation?

POSSIBLY…



Source: P. Alvarez (Rice)

Questions?

Photochemical Fate of Manufactured Carbon Nanomaterials in the Aquatic Environment (Emphasis on C_{60})

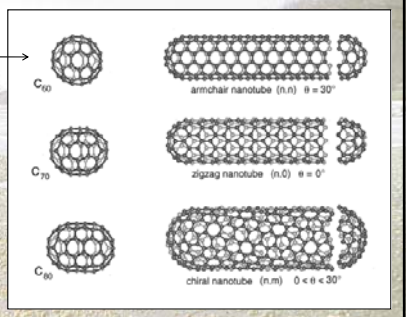
Chad T. Jafvert, Wen-Che Hou
Division of Environmental & Ecological Engineering
And School of Civil Engineering
Purdue University, West Lafayette, IN 47907

The research is funded by U.S. EPA - Science To Achieve Results (STAR) Program
Grant # 33334001

PURDUE UNIVERSITY

C_{60}

- Truncated icosahedron symmetry
- 20 hexagons and 12 pentagons, fully aromatic
- ~ 7 Å in diameter



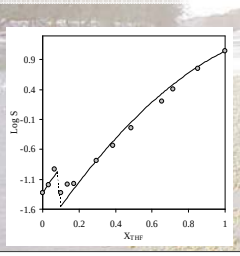
Previous Studies with C_{60} ‡

- "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" *(in preparation)*

‡Funded by NSF

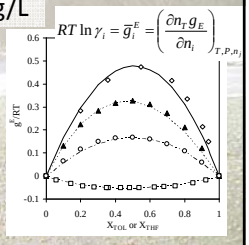
Previous Results

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



Solubility in THF-ACN mixtures

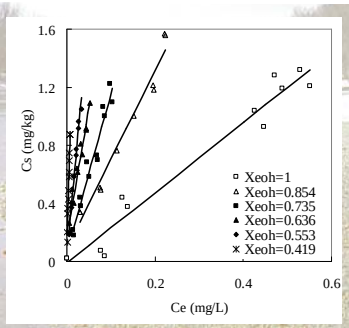
$$RT \ln \gamma_i = \bar{g}_i^E = \left(\frac{\partial n_T g_E}{\partial n_i}\right)_{T,P,n_j}$$



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}).

Sorption to Saturated Soil from ethanol-water solutions (Xeoh = ethanol mole fraction, soil = EPA15)

Tentative: $\log K_{om}{}^{o} = -\log S - \log \overline{V} - 0.62195$



Rationale for Current Study

- Carbon nanomaterials have many potential emerging uses. The potential widespread use will eventually lead to the appearance of carbon nano-materials in the aquatic environment.
- Although C_{60}'s aqueous solubility of is extremely low, C_{60} is known to form stable clusters (nC_{60}) in water (Deguchi et al., 2001).
- Photochemistry of aqueous clusters could be an important fate process.

(www.nanobama.com)

Current EPA-funded Study

Project period: May 2007 – April 2009

"Photochemical transformation of aqueous C_{60} clusters (nC_{60}) in sunlight"

(*Env. Sci. Technol.,* In press, 2008)

Parameters examined:

- Cluster size
- Preparation method
- pH – (3, 7, and 11 at μ = 19 mM)
- Humic substances
  (10 mg/L Suwannee River fulvic acid from IHSS)
- O_2 concentration

Photochemistry of C_{60} in Organic Solvents (Potential Aqueous Reactions of nC_{60})

$$C_{60} \xrightarrow{h\nu} {}^1C_{60} \xrightarrow{ISC} {}^3C_{60} \xrightarrow{k_q(O_2)} C_{60} + {}^1O_2$$

Arbogast et al., 1991

$${}^3C_{60} + \frac{n}{2}\,{}^1O_2 \longrightarrow C_{60}O_n$$

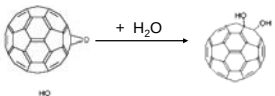
Juha et al., 1994

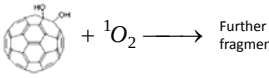
$$+ {}^1O_2 \xrightarrow{h\nu}$$

Further oxidation and fragmentation

Taylor et al., 1991

In water?

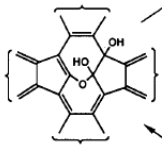




$$+ {}^1O_2 \longrightarrow$$

Further oxidation and fragmentation

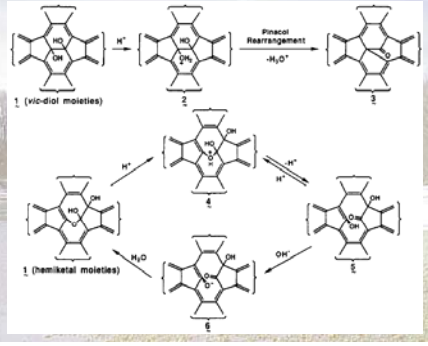
Potential products in the aqueous phase?



1 (hemiketal moieties)

- Polyhydroxlated C_{60} via acid (H_2SO_4 and HNO_3) reaction
- The product contains hemiketal moieties
- $C_{60}(OH)_{14\text{-}16}O_{7\text{-}8}$ as a hypothetical structure based on XPS curve fitting

Chiang et al., Multi-hydroxy additions onto C_{60} fullerene molecules. *J. Chem. Soc., Chem. Commun.* **1992**, 106, 1103-1105.



Fullerene hemiketal (RO-C-OH) aqueous chemistry

Chiang *et al.* Evidence of hemiketals incorporated in the structure of fullerols derived from aqueous acid chemistry. *J. Am. Chem. Soc.* **1993**, 115, 5453-5457.

In the absence of O_2

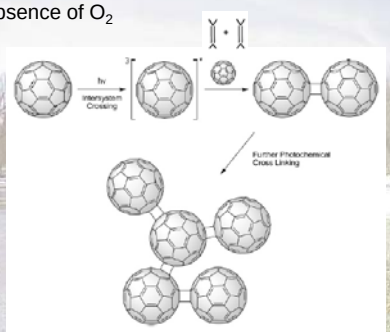


Photo-polymerization of C_{60} in absence of O_2 via the [2+2] cycloaddition.

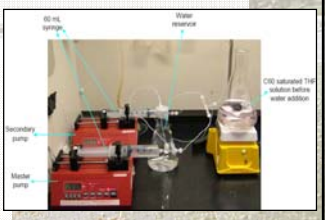
Giacalone *et al.* Fullerene polymers: Synthesis and properties. *Chem. Rev.* **2006**, 106, 5136-5190.
Rao *et al.* Photoinduced polymerization of solid C_{60} films. *Science* **1993**, 259, 955-957.

nC_{60} Preparation

- THF/nC_{60} - An equal volume of water added at 25 mL/min to C_{60}-saturated THF under mixing. Remove the THF on a rotary evaporator. (Smaller clusters prepared with a faster addition rate (1 L/min).
- Son/nC_{60} - sonicate a water-toluene mixture containing C_{60} until the toluene phase evaporates.

Analysis

- **nC_{60}**
  - Add 0.1 M $Mg(ClO_4)_2$ & extract w/ toluene (Fortner et al., 2005)
  - HPLC on a Cosmosil 5PBB Column
    Toluene as the mobile phase
    Detector at λ = 336 nm
- **nC_{60} size and morphology**
  - Dynamic light scattering (DLS)
  - TEM



Experimental Approach

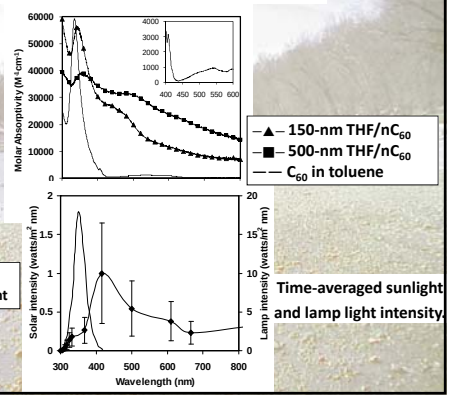
Irradiation

- Sunlight experiments were performed from 10 am to 5 pm on sunny or partly cloudy days on the roof of Civil Engineering building at Purdue (86° 55′ W, 40° 26′ N). The solar intensity data were obtained from a USDA UV-B station within 5 miles from where the irradiation occurred.
- Lamp light experiments were carried out in a merry-go-round photo-reactor with 8 24-W lamps (λ= 350 ± 50)

Sunlight irradiation

Merry-go-round photo-reactor

$$-\frac{dC}{dt} = \int_{\lambda=300\,nm}^{\lambda=?\,nm} \phi_\lambda \cdot I_{o,\lambda}\left(\frac{A}{V}\right)\cdot\left(1-10^{-\varepsilon_\lambda \cdot C \cdot l_{eff}}\right)\cdot d\lambda$$



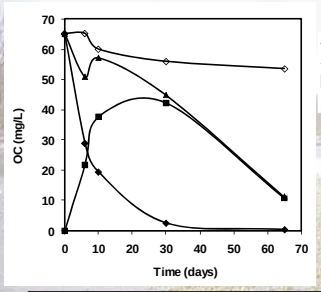
Time-averaged sunlight and lamp light intensity.

Results

Photo-transformation of 65 mg/L THF/ nC_{60} in the lamp light (λ=350 nm)

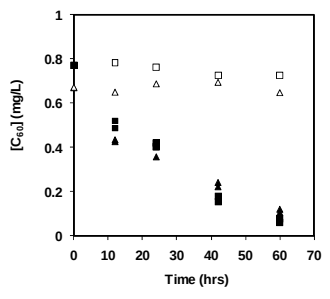
Irradiation time (day)	0	10	30	65
[nC_{60}] (mg/L)	65	19.5	2.6	0.47
Color				
TEM image*				
Mean diameter** (nm)	500	350	250	160
After Centrifugation***				

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



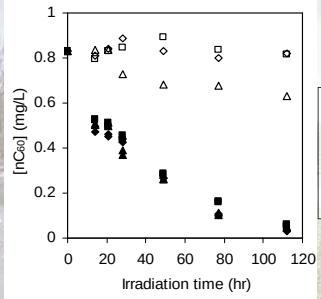
◊ nC_{60} dark controls
♦ nC_{60} irradiated samples
■ Aqueous phase OC
▲ Recovered OC (Soluble Carbon + C_{60})

Photo-transformation of 65 mg/L THF/nC_{60} in terms of organic carbon (OC) content in the lamp light.



Irradiated son/nC_{60} (▲)
Dark control of son/nC_{60} (Δ)
Irradiated THF/nC_{60} (■)
Dark control of THF/nC_{60}

Photo-transformation of THF/nC_{60} and son/nC_{60} under the mid-latitude solar exposure, May 13, 2008-June 6, 2008. Conditions: pH = 7, ionic strength = 19 mM.



Mean cluster diameter:
235 nm ± 8% at all pH values

Irradiated nC_{60} at pH=3 (▲)
Dark control of nC_{60} at pH=3 (Δ)
Irradiated nC_{60} at pH=7 (■)
Dark control of nC_{60} at pH=7 (□)
Irradiated nC_{60} at pH=11 (♦)
Dark control of nC_{60} at pH=11

Photo-transformation of THF/nC_{60} under the mid-latitude solar exposure in September-October, 2007, at pH=3, 7, and 11. Conditions: ionic strength = 19 mM.

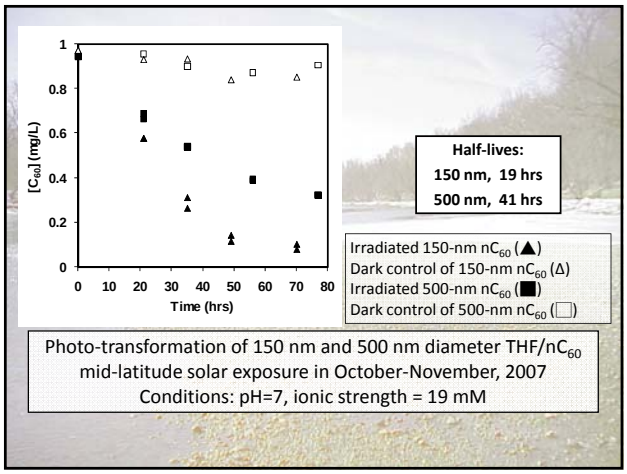


Photo-transformation of 150 nm and 500 nm diameter THF/nC_{60} mid-latitude solar exposure in October-November, 2007
Conditions: pH=7, ionic strength = 19 mM

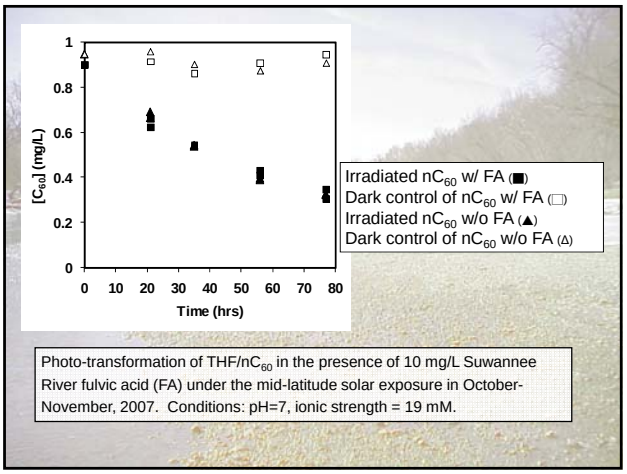


Photo-transformation of THF/nC_{60} in the presence of 10 mg/L Suwannee River fulvic acid (FA) under the mid-latitude solar exposure in October-November, 2007. Conditions: pH=7, ionic strength = 19 mM.

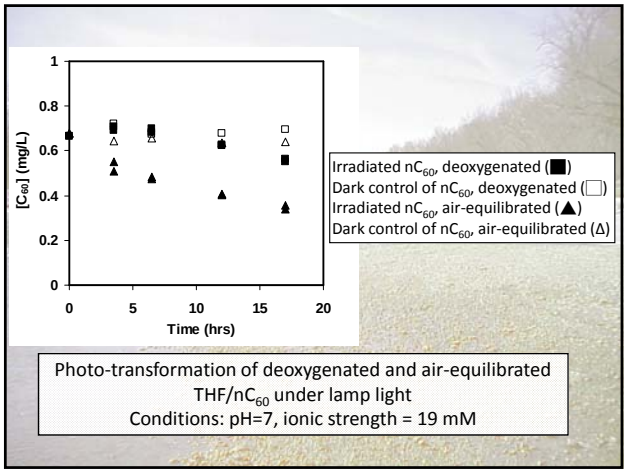
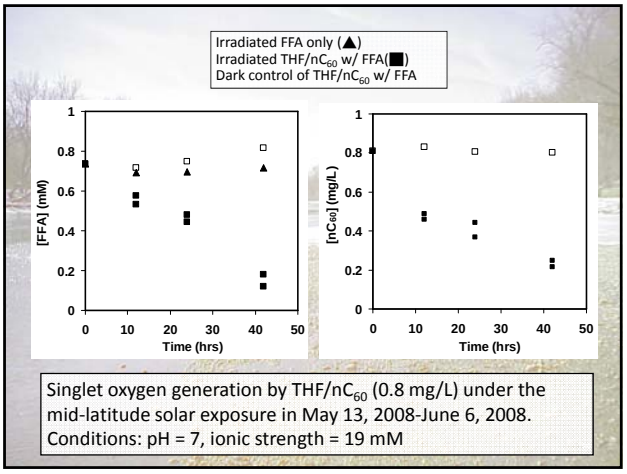


Photo-transformation of deoxygenated and air-equilibrated THF/nC_{60} under lamp light
Conditions: pH=7, ionic strength = 19 mM



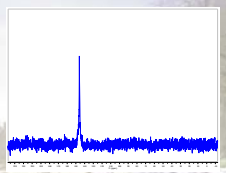
Singlet oxygen generation by THF/nC_{60} (0.8 mg/L) under the mid-latitude solar exposure in May 13, 2008-June 6, 2008.
Conditions: pH = 7, ionic strength = 19 mM

Summary

- Aqueous nC_{60} under lamp light (λ = 300-400 nm) resulted in losses of C_{60} and color, decrease in cluster size, "water-soluble" products.
- Loss occurred more rapidly with smaller clusters.
- pH, fulvic acid, & preparation method had minimal effect.
- The reaction rate was significantly reduced in deoxygenated samples, indicating O_2 plays a role.

Future work

- $^1O_2$ measurements
- Functional group-specific X-ray photoelectron spectroscopy (XPS)
- NMR analysis
- Head space CO_2 analysis
- Extend work to carbon nanotubes



NMR spectrum of 50 mg/L THF/nC_{60} (25% ^{13}C-enriched) in 20% (v/v) D_2O

Questions?

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USEPA STAR GRANTEE WORKSHOP
SEPTEMBER 5-7, 2007
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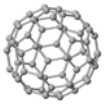
FATE AND TRANSFORMATION OF CARBON NANOMATERIALS IN WATER TREATMENT PROCESSES

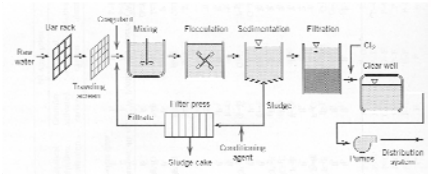
JAE-HONG KIM, PH.D.
ASSISTANT PROFESSOR

SCHOOL OF CIVIL AND ENVIRONMENTAL ENGINEERING
GEORGIA INSTITUTE OF TECHNOLOGY



TOPIC I

STABILITY OF CARBON NANOMATERIALS IN NATURAL WATERS AND REMOVAL BY CONVENTAIONAL WATER TREATMENT PROCESSES

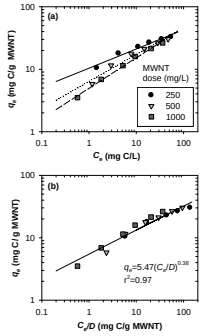


VISUAL EXAMINATION OF MWNT SOLUTIONS

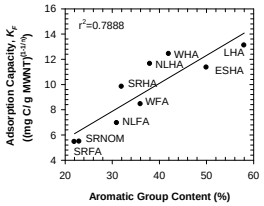
50 mg MWNT ADDED AND AGITATED **FOR ONE HOUR**

NOM-MWNT ISOTHERMS

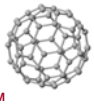
SUWANNEE RIVER NOM VS. MWNT



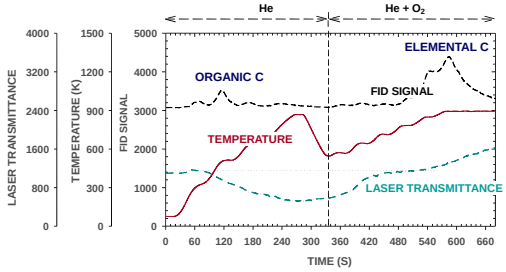
ADSORPTION CAPACITY APPEARS AS A FUNCTION OF NOM AROMATICITY



THERMAL OPTICAL TRANMITTANCE ANALYSIS



KEY CHALLENGE: QUANTIFY THE AMOUNT OF CNT ASSOCIATED WITH NOM



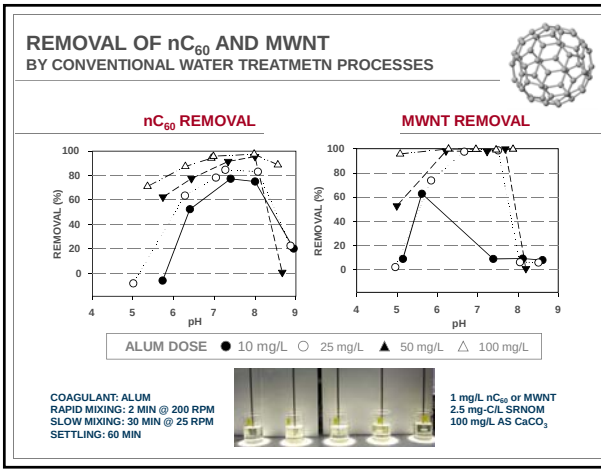
VARIOUS DISPOSAL SCENARIOS

STABILITY IN NOM SOLUTION DEPENDS ON THE TYPE AND PHASE OF FULLERENES

	PREPARATION METHODS			
	MIXING	SONICATION	SOLVENT EXCHANGE BY MIXING	SOLVENT EXCHANGE BY SONICATION
C_{60}	+	O	-	+
SWNT	O	+	O	O
MWNT	+	+	O	O

\+ NOM INCREASES DISPERSION
\- NOM DECREASES DISPERSION
O NO EFFECT

REMOVAL OF nC_{60} AND MWNT
BY CONVENTIONAL WATER TREATMETN PROCESSES



COAGULANT: ALUM
RAPID MIXING: 2 MIN @ 200 RPM
SLOW MIXING: 30 MIN @ 25 RPM
SETTLING: 60 MIN

1 mg/L nC_{60} or MWNT
2.5 mg-C/L SRNOM
100 mg/L AS $CaCO_3$

SUMMARY

NOM ENHANCES STABILIZATION OF CARBON NANOMATERIALS (C_{60}, SWNT, MWNT) IN NATURAL WATERS

ADSORPTIVE INTERACTION BETWEEN NOM AND NANOTUBES DEPENDS ON **WATER QUALITY PARAMETERS** (FOR EXAMPLE, pH AND IONIC STRENGTH) AND **NOM CHARACTERISTICS**

FULLERENES ARE EXPECTED TO BE **WELL REMOVED** BY WATER TREATMENT PROCESS

PUBLICATIONS

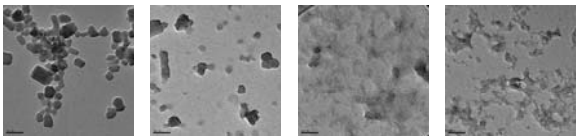
1. HYUNG, H.; FORTNER, J. D.; HUGHES, J. B.; KIM, J. H. (2007) "NATURAL ORGANIC MATTER STABILIZES CARBON NANOTUBES IN THE AQUEOUS PHASE." ***ENVIRONMENTAL SCIENCE & TECHNOLOGY***, 41, 179-184
2. HYUNG, H.; KIM, J. H. (2008). "NATURAL ORGANIC MATTER (NOM) ADSORPTION TO MULTI-WALLED CARBON NANOTUBES: EFFECT OF NOM CHARACTERISTICS AND WATER QUALITY PARAMETERS." ***ENVIRONMENTAL SCIENCE & TECHNOLOGY***, 42, 4416-4421
3. HYUNG, H.; KIM, J. H. (2009)."DISPERSION OF C_{60} IN NATURAL WATER AND REMOVAL BY CONVENTIONAL DRINKING WATER TREATMENT PROCESSES." (**IN PREPARATION**)

TOPIC II

CHEMICAL TRANSFORMATION
WATER STABLE C_{60} AGGREGATES

REACTION WITH O_3 IN THE AQUEOUS PHASE

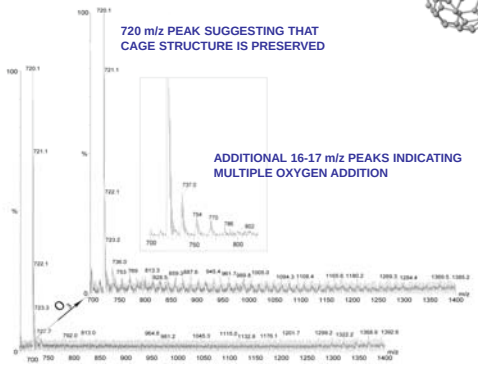
IN A SEMI-BATCH MODE



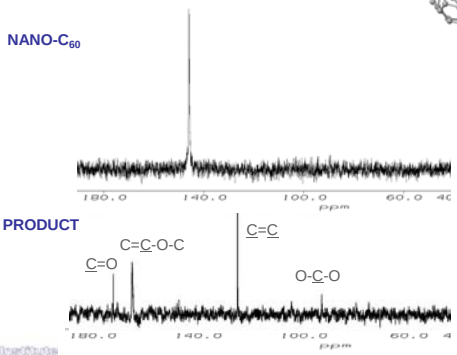
t = 0 min	t = 5 min	t = 15 min	t = 30-60 min
CT = 0 mg-min/L	CT = 6.6 mg-min/L	CT = 28.5 mg-min/L	CT = 74 - 150 mg-min/L
90 nm	**76 nm**	**40 nm**	**< 5 nm**

AGGREGATE SIZE DECREASES WITH REACTION TIME

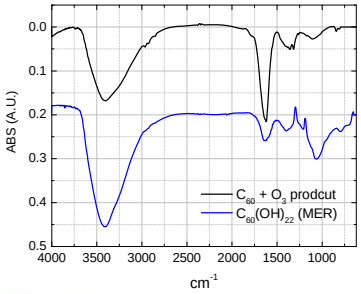
PRODUCT CHARACTERIZATION: MS



PRODUCT CHARACTERIZATION: ^{13}C NMR

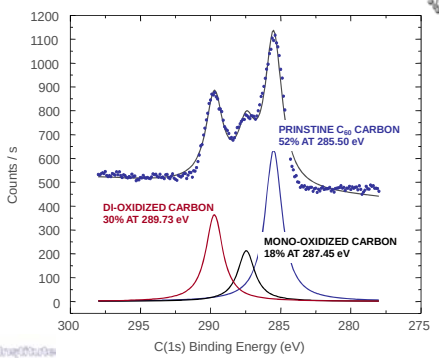


PRODUCT CHARACTERIZATION: ATR-FTIR



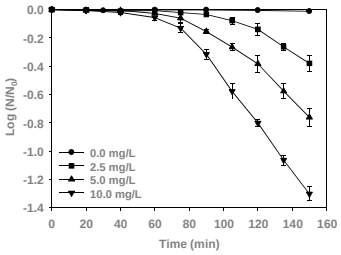
- 3300 cm^{-1} O–H
- 1765 cm^{-1} C=O
- 1630 cm^{-1} C=O, O-C-O
- 1360 cm^{-1} C-OH BENDING
- 1315 cm^{-1} C-O STRETCHING
- 1110 cm^{-1} C-O-C

PRODUCT CHARACTERIZATION: XPS



INTERACTION WITH E.COLI

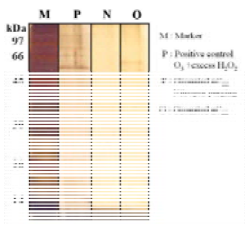
OZONATED C_{60} INACTIVATES *E.COLI* ONLY IN THE PRESENCE OF O_2 AND LIGHT



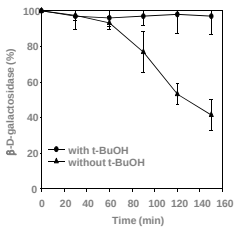
pH : 7.1 (10 MM OF PHOSPHATE BUFFER)
TEMPERATURE : 20 ± 1 ℃
LIGHT INTENSITY : 1.2 x 10^{-6} EINSTEIN/L.S
REACTOR : QUARTZ REACTOR (60 ML)
SAMPLE VOLUME : 30 ML
INITIAL CONCENTRATION OF *E. COLI* : 1.5 ~ 3.0 x 10^5 CFU/ML

MECHANISM OF *E.COLI* INACTIVATION

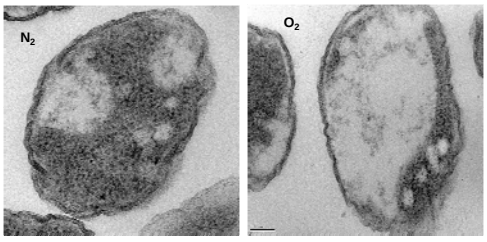
EXTRACTED PROTEIN ASSAY USING SDS-PAGE AFTER 1 LOG INACTIVATION



DEGRADATION OF INTRACELLULAR ENZYME

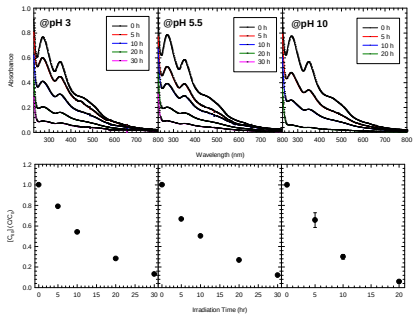


MECHANISM OF *E.COLI* INACTIVATION

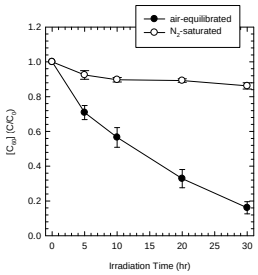


LITTLE SURFACE DISRUPTION
MORE DEGRADATION OF INTRACELLULAR COMPONENT
BY HYDROXYL RADICAL

PHOTODEGRADATION OF nC_{60}
BY UV_{254} IRRADIATION



EFFECT OF DISSOLVED OXYGEN



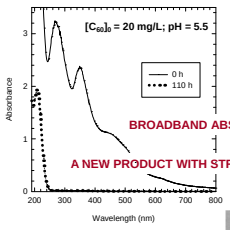
DRASTIC RETARDATION OF DEGRADATION KINETICS UNDER N_2-SATURATED CONDITION.

→ OXIDATIVE DEGRADATION PATHWAY

TOC WAS NOT CHANGED AFTER UV PHOTOLYSIS

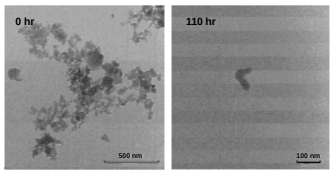
→ PHOTOCHEMICAL TRANSFORMATION, NOT MINERALIZATION

PRODUCT CHARACTERIZATION: UV AND TEM

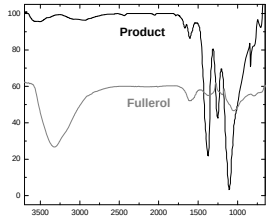


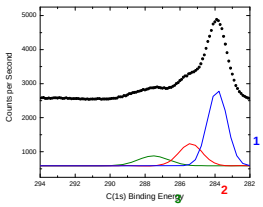
BROADBAND ABSORBANCE IN THE VISIBLE REGION DISAPPEARS

A NEW PRODUCT WITH STRONG ABSORPTION AT 210 nm FORMS.



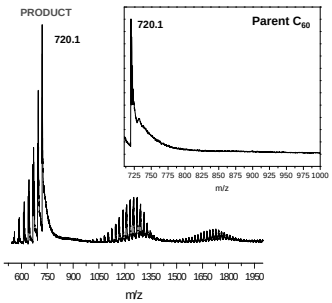
PRODUCT CHARACTERIZATION: FTIR AND XPS





Peak	Position	Area	% C(1s)	Carbon-ID
1	283.87	3112.2	64%	Underivatized C
2	285.39	1091.5	23%	Mono-oxidized C
3	287.52	638.39	13%	Di-oxidized C

PRODUCT CHARACTERIZATION: LDI-MS



TOXICITY OF UV PHOTOLYSIS PRODUCT
MIC TEST

The minimal inhibitory concentrations of parent nC_{60} and the UV photolysis products for *E.coli*

Concentration of C_{60} Cluster (or UV-treated Products) (mg/L)	UV Illumination Time (hr)					
	0	25	50	70	90	110
0	+	+	+	+	+	+
1	+	+	+	+	+	+
2	-	-	+	+	+	+
4	-	-	-	+	+	+
6	-	-	-	+	+	+
8	-	-	-	-	+	+
10	-	-	-	-	-	+

(+: microbial growth, -: no growth)

LONG-TERM EXPOSURE TO UVC (254 nm) RESULTS IN nC_{60} TOXICITY DECREASE.

RADICAL REACTIVITY OF nC_{60}

PULSE RADIOLYSIS
8-MeV TITAN BETA MODEL TBS-8/16-1S LINEAR ACCELERATOR
AT NOTRE DAME RADIATION LABORATORY

UNDER N_2-SATURATED CONDITION

$e_{aq}^- + A \longrightarrow A^{\bullet -}$

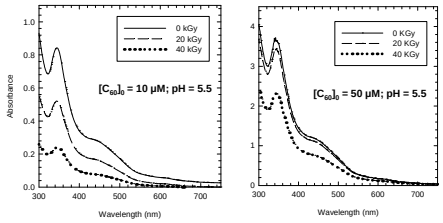
UNDER N_2O-SATURATED CONDITION

$e_{aq}^- + N_2O + H_2O \longrightarrow N_2 + OH^- + OH^\bullet$

GAMMA RADIOLYSIS
USING SHEPHERD® 109-86 COBALT 60 SOURCE
WITH A DOSE RATE OF 0.0722 kGy min-1

OH RADICAL-INDUCED OXIDATION OF nC_{60} IN WATER

γ-RADIOLYSIS (N_2O-SATURATED CONDITION)

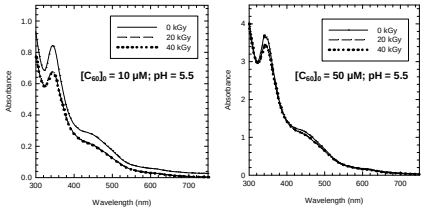


DOSES OF 20 AND 40 KGy CORRESPOND TO GENERATION OF 11 AND 22 mM OF OH RADICALS, RESPECTIVELY

⟶ EXCEPTIONAL STABILITY OF nC_{60} AGAINST OH RADICAL ATTACK

OH RADICAL-INDUCED REDUCTION OF nC_{60} IN WATER

γ-RADIOLYSIS (N_2-SATURATED CONDITION)



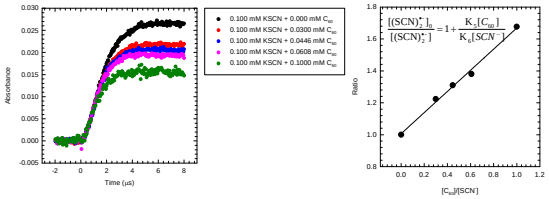
DOSES OF 20 AND 40 KGy CORRESPOND TO GENERATION OF 5.4 AND 10.8 mM OF HYDRATED ELECTRONS, RESPECTIVELY

⟶ RECALCITRANT AGAINST HYDRATED ELECTRON

REACTIVITY OF nC_{60} WITH OH RADICAL

$C_{60} + OH^\bullet \xrightarrow{k_5}$ *intermediate*

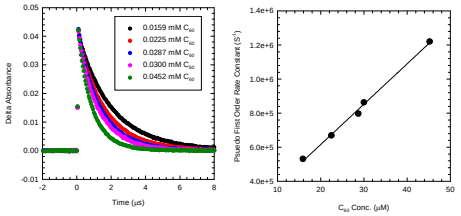
$SCN^- + OH^\bullet (+ SCN^-) \xrightarrow[1.05 \times 10^{10}\ M^{-1}s^{-1}]{k_5} OH^- + (SCN)_2^{\bullet -}$ (MONITORED AT 472 nm)



SECOND-ORDER RATE CONSTANT = 7.34 ± 0.31 x 10^9 $M^{-1}s^{-1}$.

REACTIVITY OF nC_{60} WITH HYDRATED ELECTRONS

DIRECTLY MONITORING OF THE DECAY KINETICS OF HYDRATED ELECTRON AT 700 nm IN THE PRESENCE OF nC_{60} AT DIFFERENT CONCENTRATIONS.

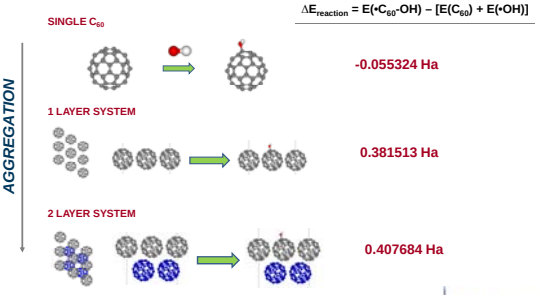


SECOND-ORDER RATE CONSTANT = 2.34 ± 0.02 x 10^{10} $M^{-1}s^{-1}$

STABILITY OF nC_{60}-OH RADICAL ADDUCT

DFT-CALCULATION OF $\Delta E_{reaction}$

$\Delta E_{reaction} = E(\bullet C_{60}\text{-}OH) - [E(C_{60}) + E(\bullet OH)]$



HARTREE = EQUAL TO THE ABSOLUTE VALUE OF THE ELECTRIC POTENTIAL ENERGY OF THE HYDROGEN ATOM IN ITS GROUND STATE

SUMMARY

OZONATION TRANSFORMS nC_{60} INTO **WATER SOLUBLE FULLERENE OXIDE** SPECIES

OZONATED C_{60} APPEARS **MORE TOXIC** THAN nC_{60}

IRRADIATION OF UV (254 nm) TRANSFORMS nC_{60} INTO **WATER SOLUBLE FULLERENE OXIDE** SPECIES

C_{60} PHOTOLYSIS PRODUCT APPEARS **LESS TOXIC** THAN nC_{60}

C_{60} IN THE AQUEOUS PHASE REACTS WITH HYDROXYL RADICAL AND HYDRATED ELECTRONS WITH RELATIVELY HIGH RATE CONSTANT RESULTING IN UNSTABLE PRODUCT

PUBLICATIONS

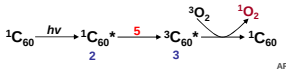
1. FORTNER, J. D.; KIM, D. I.; BOYD, A. M.; FALKNER, J. C.; MORAN, S.; COLVIN, V. L.; HUGHES, J. B.; KIM, J. H. (2007). "REACTION OF WATER STABLE C_{60} AGGREGATES WITH OZONE" ***ENVIRONMENTAL SCIENCE & TECHNOLOGY***, 41, 7497-7502
2. LEE, J.; CHO, M.; FORTNER, J. D.; HUGHES, J. B.; AND KIM, J. H. "UV PHOTOLYSIS OF C_{60} CLUSTERS IN THE AQUEOUS PHASE." (***IN PREPARATION***)
3. LEE, J.; SONG, W.; JANG, S. S.; FORTNER, J. D.; ALVAREZ, P. J.; COOPER, W. J.; KIM, J. H. "REACTION OF WATER STABLE C_{60} AGGREGATES WITH OH RADICAL AND HYDRATED ELECTRONS" (***IN PREPARATION***)
4. CHO, M.; FORTNER, J.D.; HUGHES, J.B.; KIM, J.H. "ESCHERICHIA COLI INACTIVATION BY WATER SOLUBLE OZONATED C_{60}: KINETICS AND MECHANISMS." (***IN PREPARATION***)

TOPIC III

PHOTOCHEMICAL ACTIVITY OF C_{60} IN THE AQUEOUS PHASE DURING UV IRRADIATION

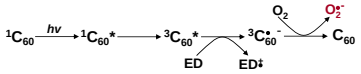
PHOTOACTIVITY OF C_{60}

C_{60} AS SINGLET OXYGEN PRECURSOR



ARBOGAST ET AL *J.PHY.CHEM.* (1991)

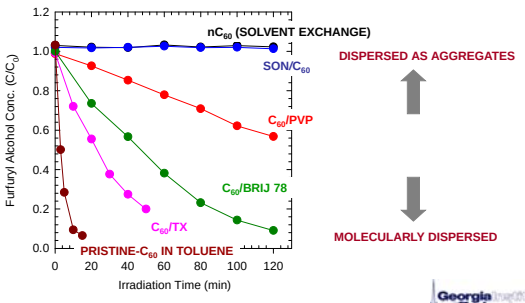
C_{60} AS SUPEROXIDE RADICAL ANION PRECURSOR



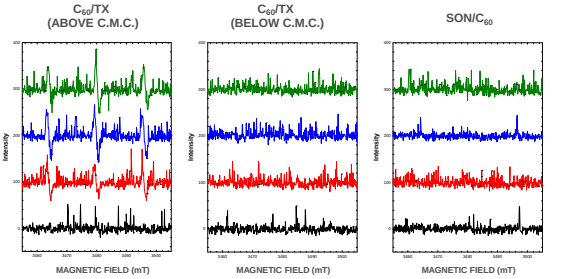
YAMAKOSHI ET AL *J.AM.CHEM.SOC.* (1991)

COMPARING PHOTOACTIVITY OF VARIOUS C_{60} SAMPLES

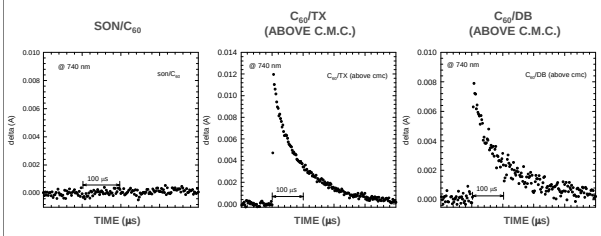
DISPERSION STATUS OF C_{60} IN THE AQUEOUS PHASE DETERMINES THE CAPABILITY OF C_{60} TO TRANSFER PHOTOENERGY TO OXYGEN



ELECTRON SPIN RESONANCE FOR DETECTION OF SINGLET OXYGEN

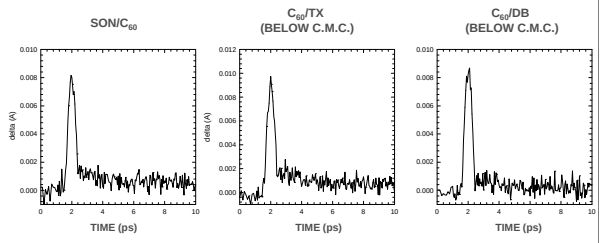


NANO-SECOND LASER FLASH PHOTOLYSIS FOR $^3C_{60}$* DECAY



SELF-QUENCHING/TRIPLET-TRIPLET ANNIHILATION ARE ENHANCED IN AGGREGATED C_{60}

FEMTO-SECOND LASER FLASH PHOTOLYSIS FOR $^3C_{60}$* DECAY



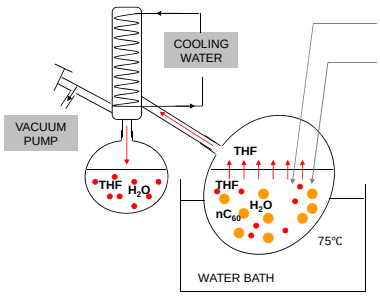
SELF-QUENCHING/TRIPLET-TRIPLET ANNIHILATION ARE ENHANCED IN AGGREGATED C_{60}

REPORTED TOXICITY OF nC_{60} AGGREGATES

ORGANISMS	nC_{60} AGGREGATES	INHIBITION CONCENTRATION	REFERENCE
B. subtilis	THF/nC_{60}	MIC: 0.08-0.10 mg/L	Lyon, D. Y, et al., ES&T, 2006
	Son/nC_{60}	MIC: 0.4-0.6 mg/L	
	AQUA/nC_{60}	MIC: 0.4-0.6 mg/L	
E. coli	THF/nC_{60}	Growth inhibited at 0.4mg/L	Fortner, J. D., et al., ES&T, 2005
Daphnia Magna	THF/nC_{60}	LD_{50}: 0.8mg/L	Oberdörster, E., et al., MER, 2006
	Water stirred/C_{60}	LD_{50}: >35mg/L	
Human dermal fibroblasts Human liver carcinoma cells Neuronal human astrocytes	THF/nC_{60}	LD_{50}: 2-50ppb	Sayes, C. M., Biomaterails, 2005
Human monocyte-derived macrophage	THF/nC_{60}	Observed absorption in the cytoplasm, lysosomes, and cell nuclei	Porter, A. E., et al., ES&T, 2007

POTENTIAL INTERFERENCE BY THF

THF/nC_{60} HAS BEEN CONSISTENTLY REPORTED TO BE MORE TOXIC THAN OTHER FORMS OF nC_{60}



RESIDUAL THF REPONSIBLE FOR TOXICOLOGICAL EFFECT?

OTHER FORMS OF THF DERIVATIVES POSSIBLE?

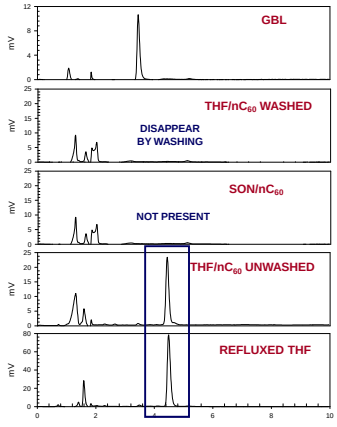
e.g., TOXICOLOGICAL EFFECT OF γ-BUTYROLACTONE (GBL) ON LARVAL ZEBRAFISH

HENRY, ENVIRON HEALTH PERSP., 2007

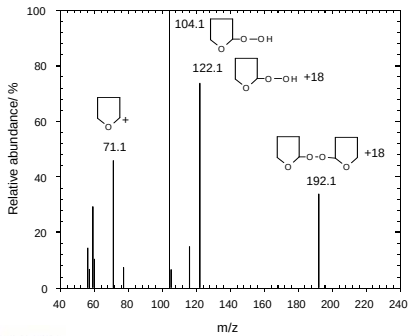
THF-PEROXIDE

KI TITRATION SUGGESTS THE PRESENCE OF **PEROXIDE**

THF/nC_{60} UNWASHED: **12 PPM**



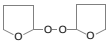
PRODUCT CHARACTERIZATION: LC/MSD



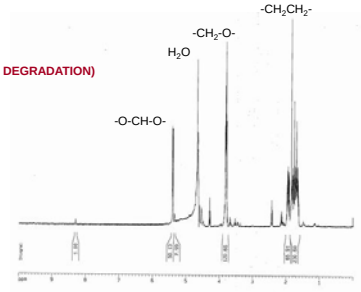
ELUENT: AMMONIUM ACETATE: 95%; METHANOL: 5%

PRODUCT CHARACTERIZATION: ^{1}H NMR

THF PEROXIDE COULD BE RESPONSIBLE FOR:



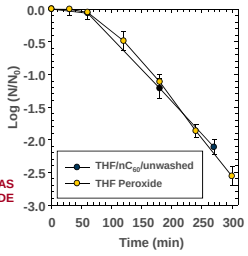
TOXICOLOGICAL EFFECTS
CHEMICAL REACTIVITIY (DYE DEGRADATION)
AGING OF THF/nC_{60}



MIC AND INACTIVATION KINETICS FOR *E.COLI*

nC_{60} Concentration (mg/L)	Number of repeated washing						
	1	2	3	4	5	8	12
0.03	–	–	+	+	+	+	+
0.10	–	–	–	–	+	+	+
0.25	–	–	–	–	–	+	+
0.50	–	–	–	–	–	+	+
1.00	–	–	–	–	–	–	+
2.00	–	–	–	–	–	–	–

MIC STRONGLY DEPENDED ON THE NUMBER OF REPEATED WASHING



***E. COLI* INACTIVATION WAS MOSTLY DUE TO THF PEROXIDE**

SUMMARY

STATUS OF C_{60} DISPERSION IN THE AQUEOUS PHASE AFFECTS ITS ABILITY TO TRANSFER ABSORBED PHOTOENERGY TO OXYGEN

C_{60} PRESENT IN WATER AS STABLE AGGREGATES **DOES NOT PRODUCE $^1O_2$ AND $O_2^{\bullet-}$** UNDER UV ILLUMINATION, IN CONTRAST TO PRISTINE C_{60}

WHEN C_{60} IS PRESENT AS AN AGGREGATE, **THE LIFETIME OF KEY INTERMEDIATE SPECIES** FOR ENERGY TRANSFER **IS DRASTICALLY REDUCED**, FUNDAMENTALLY BLOCKING THE ROS PRODUCTION MECHANISM

THF PEROXIDE FORMS DURING PREPRATION OF nC_{60} WHICH IS PARTIALLY RESPONSIBLE FOR THE REPORTED TOXICITY

PUBLICATIONS

1. LEE, J.; FORTNER, J. D.; HUGHES, J. B.; KIM, J. H. (2007). "PHOTOCHEMICAL PRODUCTION OF REACTIVE OXYGEN SPECIES BY C_{60} IN THE AQUEOUS PHASE DURING UV IRRADIATION." ***ENVIRONMENTAL SCIENCE & TECHNOLOGY***, 41, 2529-2535
2. LEE, J.; KIM, J. H. (2008). "EFFECT OF ENCAPSULATING AGENTS ON DISPERSION STATUS AND PHOTOCHEMICAL REACTIVITY OF C_{60} IN THE AQUEOUS PHASE." ***ENVIRONMENTAL SCIENCE & TECHNOLOGY***, 42, 1552-1557
3. LEE, J.; YAMAKOSHI Y.; HUGHES, J. B.; KIM, J. H. (2008). "MECHANISM OF C_{60} PHOTOCHEMISTRY IN THE AQUEOUS PHASE: FATE OF TRIPLET STATE AND RADICAL ANION AND PRODUCTION OF REACTIVE OXYGEN SPECIES." ***ENVIRONMENTAL SCIENCE & TECHNOLOGY***, 42, 3459-3464
4. ZHANG, B.; CHO, M.; FORTNER, J. D.; LEE, J.; HUANG, C. H.; HUGHES, J. B.; KIM, J. H. "DELINEATING OXIDATIVE PROCESSES OF AQUEOUS C_{60} PREPARATIONS: ROLE OF THF PEROXIDE." ***ENVIRONMENTAL SCIENCE & TECHNOLOGY*** (In Press)

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WILLIAM COOPER

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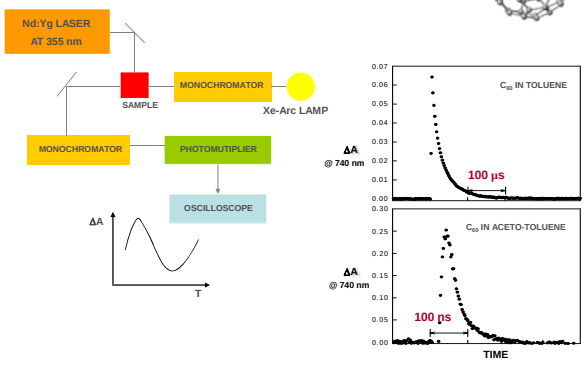
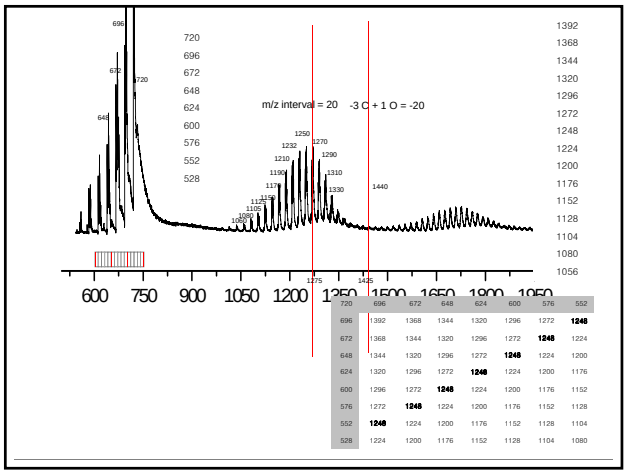
Grant # RD832526

CBEN
Center for Biological and Environmental Nanotechnology

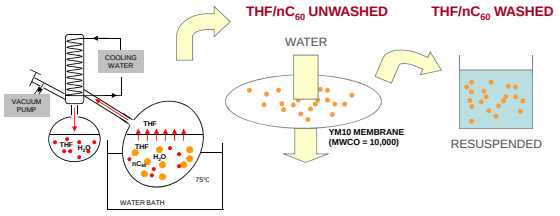
KOREA RESEARCH FOUNDATION

LASER FLASH PHOTOLYSIS FOR $^3C_{60}$* DECAY



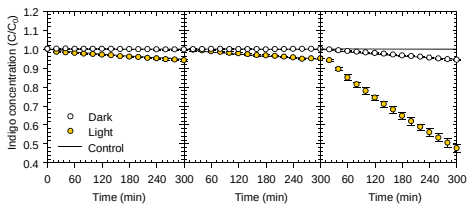


PREPARTION OF DIFFERENT nC_{60} SAMPLES



FORTNER ET AL., *ENVIRON. SCI. TECHNOL.* (2005)

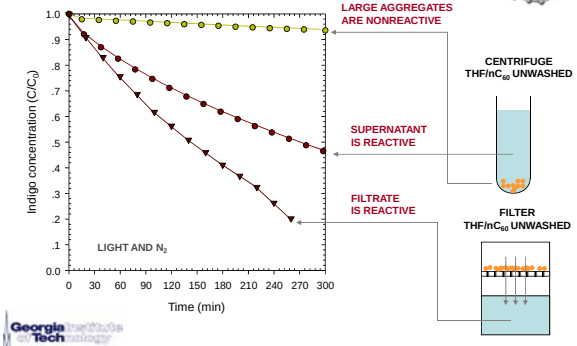
INDIGO DEGRADATION KINETICS WITH nC_{60}



UNWASHED THF/nC_{60} IS MORE REACTIVE

[nC_{60}] = 3 mg/L; [indigo] = 16 μM; pH = 7.0; temperature = 22°C. Control tests were performed without nC_{60}.

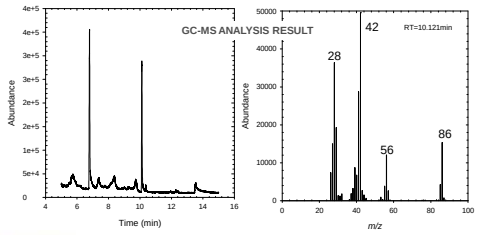
DEGRADATION OF INDIGO



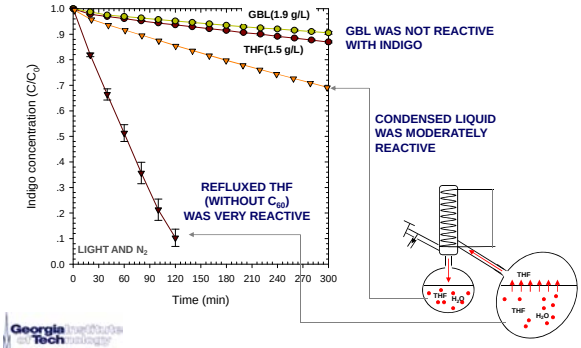
POTENTIAL THF DERIVATIVE

γ-BUTYROLACTONE (GBL)

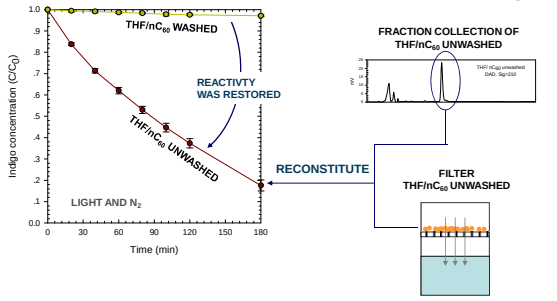
… WAS FOUND IN THF/nC_{60} UNWASHED



INDIGO DEGRADATION WITH GBL, THF AND REFLUXED THF



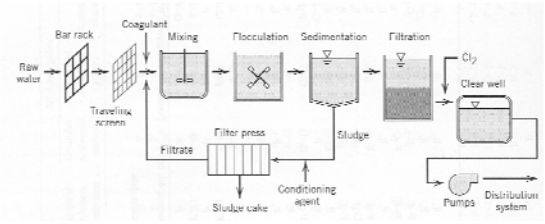
INDIGO DEGRADATION BY A FRACTION COLLECTED FROM HPLC



RECONSTITUTE

WATER TREATMENT PROCESS

BENCH-SCALE TESTS TO EVALUTE REMOVAL OF REPRESENTATIVE CARBON NANOMATERIALS (C_{60} AND MWNT) BY CONVENTIONAL WATER TREATMENT PROCESSES (COAGULATION/FLOCCULATION/SEDIMENTATION/FILTRATION)



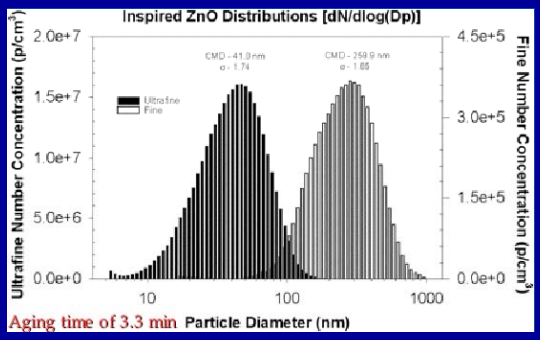
Role of Particle Agglomeration in Nanoparticle Toxicity

Terry Gordon, PhD
NYU School of Medicine

Study Hypothesis

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

Feasibility?



Beckett et al., Blue Journal, 2005

Objectives

- Measure the agglomeration rate of carbon +
  - Establish the agglomeration of freshly generated carbon nanoparticles at various distances (i.e., aging times) downstream from particle generation in a dynamic exposure system
- Identify whether agglomeration is affected by altering exposure conditions such as humidity and particle charge
- Compare the toxicity of singlet vs. agglomerated particles in mice exposed via the inhalation route
  - Expose mice to nanoparticles at different stages of particle agglomeration
- Are findings for carbon nanoparticles applicable to other nanoparticles?
  - Generate zinc and copper nanoparticles

Methods

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

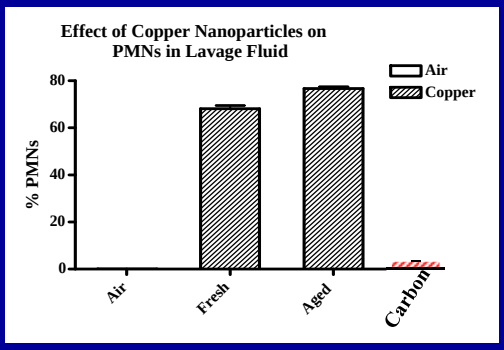
Data Presented Last 2 Years

- Fresh = 1.5 sec downstream (≈ 11 to 90 nm)
  vs.
  Aged = 3 minutes downstream (190 to 250 nm)
- Fresh vs. Aged carbon nanoparticles
  - Dose-response from 1 to 5 mg/m^3
- No difference in response with low or high humidity
- Particle charge had no effect
- Particle type had significant effect on results

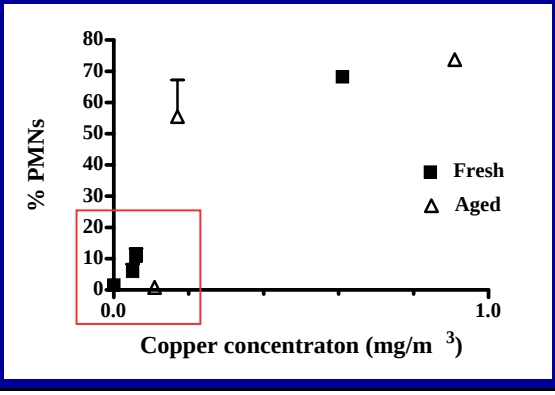
Effect of Other Nanoparticles?

- Copper
- Zinc

Copper Nanoparticles (0.8 mg/m^3)



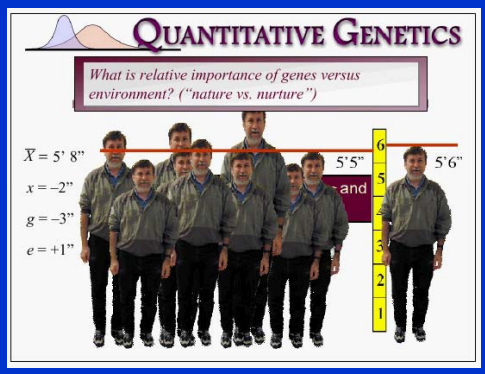
Fresh Copper Nanoparticles Effect on % PMNs

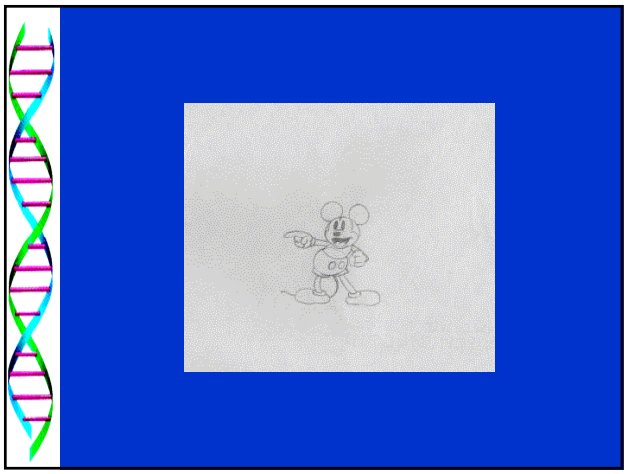


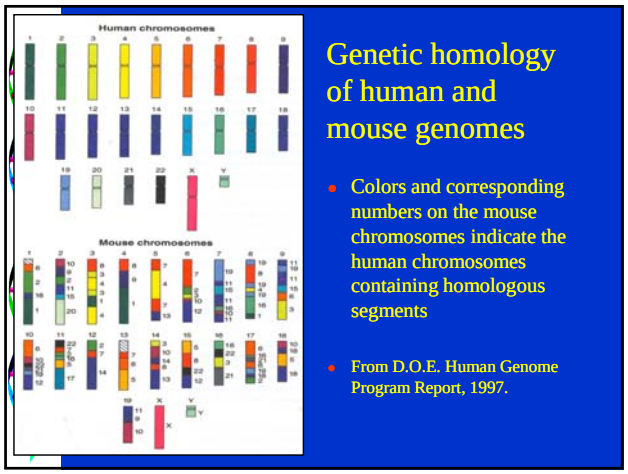
Copper and Zinc Effects

- Fresh Copper Nanoparticles Effect on Protein?
  - Same general dose-response as for PMNs
- Copper vs. Zinc Nanoparticles?
  - Similar dose-response curves (PMNs and protein) for both copper and zinc

Do All Humans Respond the Same?







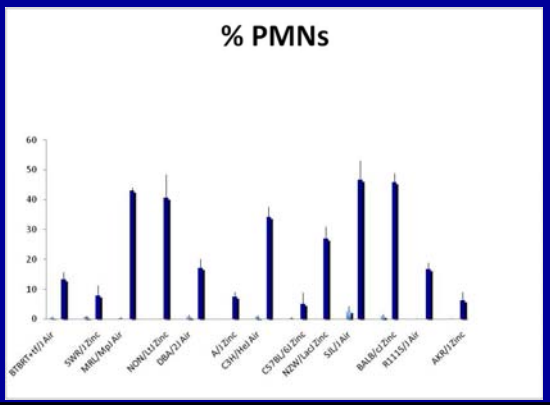
Genetic homology of human and mouse genomes

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

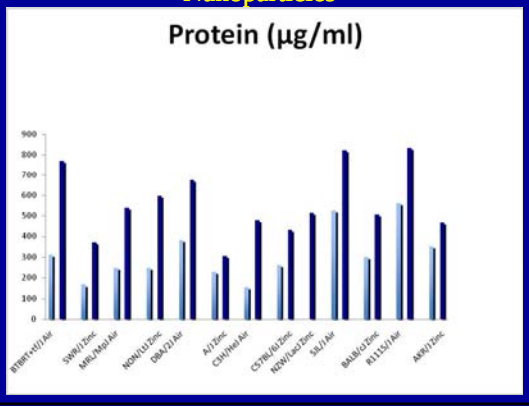
Strain Response

- 2 hr exposure to 0.6 to 0.8 mg/m^3 fresh zinc nanoparticles
- 13 inbred strains of mice
  - BALB/c
  - BTBR
  - MRL
  - SJL
  - AKR
  - NON
  - NZW
  - C3H/He
  - A/J
  - R111
  - C57BL/6
  - SWR
  - DBA

Young Adult Strain Response to Zinc Nanoparticles



Young Adult Strain Response to Zinc Nanoparticles



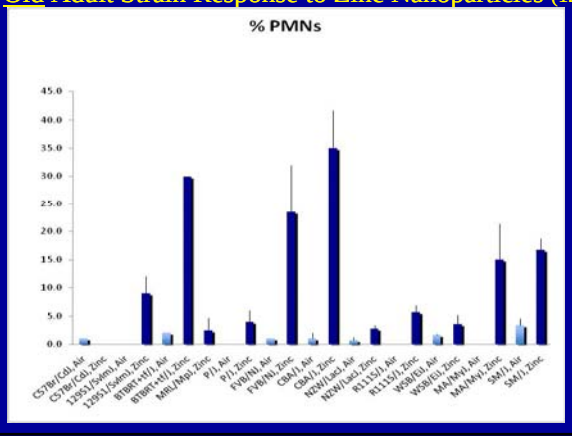
Conclusion

- Strain-dependent difference in response suggests genetic factors contribute to the response

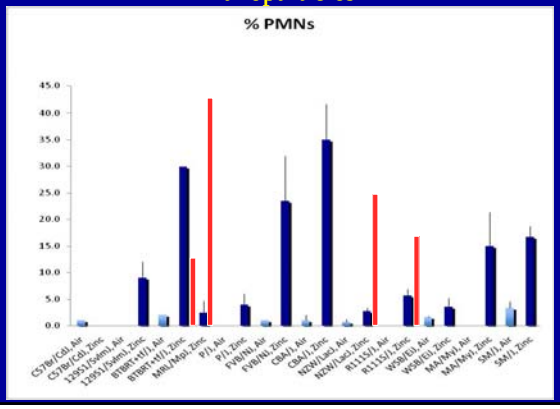
Age Effect?

- In many epidemiology studies, elderly and young (infants/children) are more susceptible to inhaled particles
- Would older mice be more responsive to inhaled nanoparticles?

Old Adult Strain Response to Zinc Nanoparticles (12



Young vs. Old Adult Strain Response to Zinc Nanoparticles



Conclusions

- Dose-response relationship between exposure to carbon and metal nanoparticles and lung inflammation/injury
  - Fresh >> Aged effects for carbon but less so for copper and zinc
- Humidity and charge had no effect on the toxicity of carbon nanoparticles

Conclusions (cont….)

- Copper and zinc nanoparticles
  - more toxic than carbon nanoparticles
  - Copper nanoparticles were somewhat more toxic than zinc nanoparticles
- Strain and age differences in response suggest that both genetic and age-related factors can influence the response to nanoparticles

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

Thanks to:

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ASSESSING THE ENVIRONMENTAL IMPACT OF NANOMATERIALS ON BIOTA AND ECOSYSTEM FUNCTIONS

Jean-Claude J. Bonzongo

Dept of Environmental Engineering Sciences, University of Florida, Gainesville, FL 32611-6450

Project Overall Hypothesis

Chemical elements used in the production of NM could lead to environmental dysfunctions due to:

(1)-The potential toxicity of these elements and their derivatives

(1)-The nanometer-size that make NM prone to bio-uptake/bioaccumulation

(1)-The large surface area which might lead NM to act as carriers/delivers of pollutants adsorbed onto them

Research Approach & Methods

TOXICOLOGY

Screening of NM for potential toxicity
(Carbon-based, metal, and metal oxide NM and quantum dots)

Effects on Ecosystem Functions

** Effect of NM on microbial-catalyzed chemical reactions (carbon cycle).*

Transport in porous media

** Soil column studies*

NMs as carrier/deliver of other pollutants

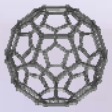
Toxicity and ToxicityMechanism(s)

Molecular modeling simulations

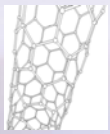
** permeation of NM into the cell*
** damage to the cell membrane by NM*

Lab investigation of toxicity and toxicity mechanisms

1. Toxicity and toxicity mechanisms of C60 and carbon nanotubes



C_{60}



Carbon nanotube (CNT)

Predicting Toxicity Mechanisms by use of Molecular Dynamics Simulations (MDS)

❑Task 1:

❖Understand the mechanisms of NM permeation of cell membranes

❑Task 2:

❖Assess the potential damages to the cell membrane and cell toxicity

Disruption of Cell Membranes and Toxicity

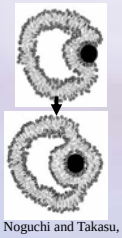
Physical Mechanisms

Morphological Changes

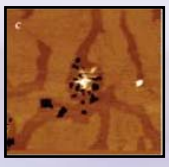
Formation of Holes

Chemical Mechanisms

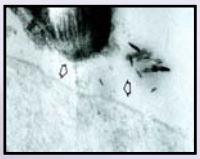
e.g. Lipid peroxidation



Noguchi and Takasu, *Biophys. J.* , 2002



Leroueil *et al., Acc. Chem. Res*, 2007

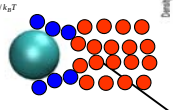


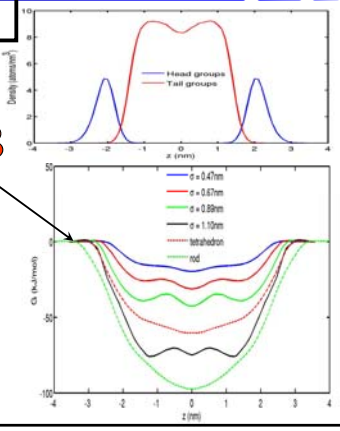
Panessa-Warren, *J. Phys. Condens. Matter*, 2006

Free Energy Profiles of Carbon-based NP

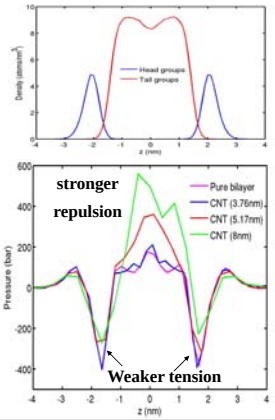
$$< F > (z_0) = \frac{dG(z_0)}{dz}$$

$$\langle \tau \rangle \propto \frac{\gamma}{k_B T} e^{-\Delta G / k_B T}$$



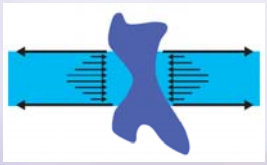


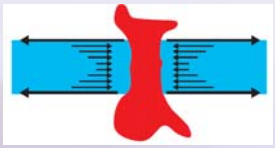
- ❖Negligible energy barrier for entry
- ❖Significant energy well in the bilayer center
- ❖Qualitative differences between spherical and non-spherical particles



Lateral Pressure Profiles Associated with CNTs

Cell Membrane and Potential CNT Toxicity





Carbon Nanotube Induced Change in Lateral Pressure Profile May Affect Membrane Proteins

Toxicity Screening Methods

- ***Ceriod*** …ute Toxicity Assay
- ***Selena*** …*ubcapiata*) Chronic Toxici…
- MetPLATE™

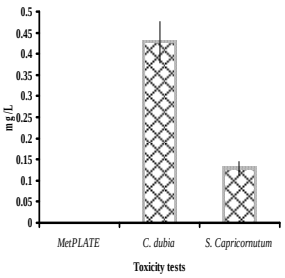
A short-term (48 hr) acute assay used to assess the toxicity of freshwater samples.

Sensitivity to toxicants, particularly metals. May equal or even surpass that of the 48-hr Ceriodap… acute tes…

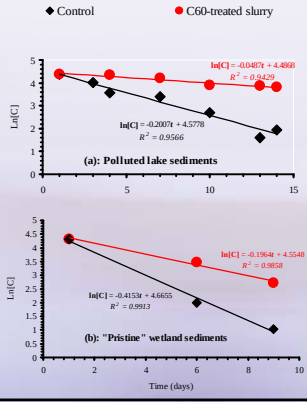
Based on the inhibition of the enzyme β-galactosidase by metals at toxic levels in a mutant strain of E. coli.

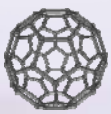
Toxicity of THF-C_{60} suspensions

Biotests	EC_{50} (ppm)
MetPLATE	-
C. dubia	0.43±0.11
S. capricornutum	0.13±0.05

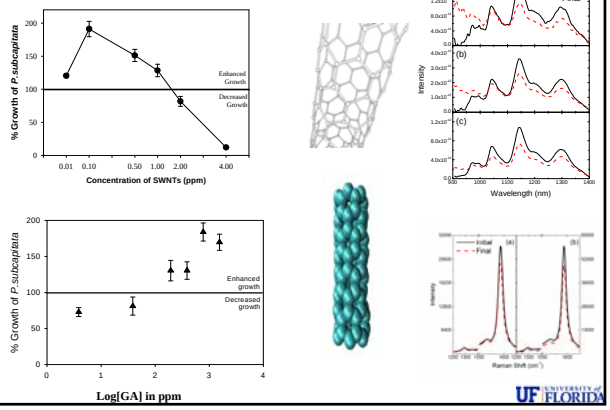


Effect of C_{60} on Microbial Degradation of Acetate in Sediment Slurries (THF-C_{60})



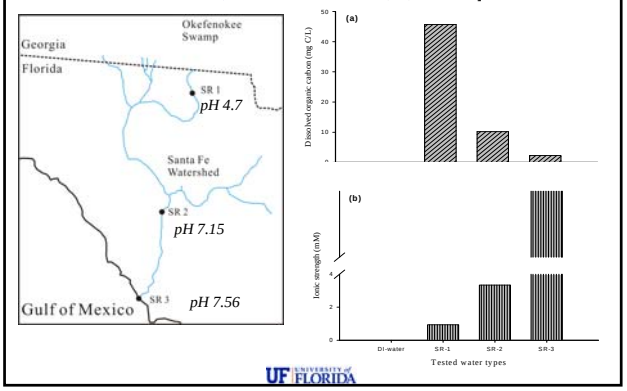


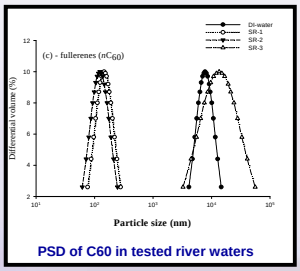
Biological Response of P. subcapitata to increasing concentrations of surfactant-suspended SWNTs



2. Natural River Water as Solvent for NM Suspensions: Effects of DOC, ionic strength, and pH

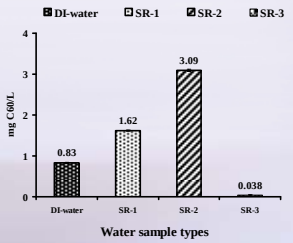
[see Gao et al., Environ. Sci. Technol. 2009, 43, 3322–3328]





PSD of C60 in tested river waters

Concentrations of Suspended C_{60} in River Waters of Different Chemical Compositions



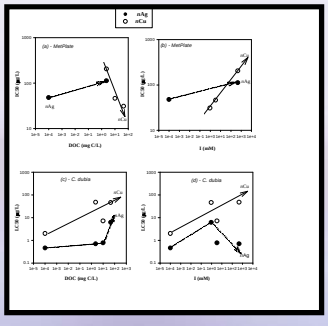
Toxicity results of C60 Suspended in River Water of Different Chemical Composition on Test Model organisms

***Ceriodaphnia dubia* assay: NO TOXICTY detected**

MetPLATE (*E. coli* based enzyme assay): NO TOXICITY detected

NANOMETALS

Toxicity of *n*Cu and *n*Ag as a function of river water DOC and Ionic Strength levels



Conclusions

1. **FACILITATED CARON-BASED NMs / ORGANISM INTERATIONS**

- **Easy penetration of the cell membrane**
- **Retention time within the membrane is a function of size and shape**
- **Carbon nanotube accumulation within cell membranes → change in lateral pressure profile**
  - → Important factor in activity of membrane proteins
  - → Longer CNTs cause larger change of pressure profile
- **Toxicity observed in lab experiments that favor cell-NM contact**

Conclusions (Cont'd)

2. TOXICITY OF CARBON-BASED NMs SUSPENDED IN NATURAL RIVER MATRICES

- **Solution chemistry vs hydrophobicity**
- **Toxicity reduction/elimination**
- **Significant differences between Carbon and metal based NMs with regard to aqueous suspension/solubility and toxicity**

Contributors/Acknowledgement

J. Gao, Ph.D. candidate (EES, UF)

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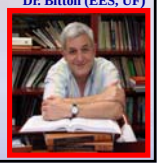
Y-M. Ban, Ph.D. candidate (CHE, UF)

Dr. Kopelevich (CHE, UF)

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

Dr. Bitton (EES, UF)



Long-Term Effects of Inhaled Nickel Nanoparticles on Progression of Atherosclerosis

November 20, 2008

Gi Soo Kang/ Dr. Lung Chi Chen
Dept. of Environmental Medicine
New York University School of Medicine

Inhaled NPs and their effects on the cardiovascular system

- Inhalation as a major route of exposure to NPs
- Well-established association between ambient particles and cardiovascular disease
- Strong potential to induce oxidative stress and inflammatory responses
  => major mechanisms for cardiovascular disease
- Possible direct interaction with cardiovascular tissue after translocation

Hypothesis

Inhaled NPs

Oxidative Stress ⇢ Inflammatory Responses

Progression of Atherosclerosis

Why Nickel?

- Commonly found in environment
- Widely used in industry
- Potential to generate oxidative stress
- Indications of potential cardiovascular effects by inhaled Ni

Nickel hydroxide ($Ni(OH)_2$)

widely used as a positive electrode in alkaline battery
⇒great interest in nanotechnology for various application

Few toxicological data

Study Design

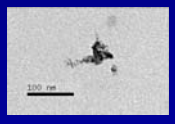
- Ni NPs: spark-generated (PALAS) from metallic Ni electrodes
- Dose: ~80 μg Ni/m^3 (PEL: $1mg/m^3$)
  for 5h/d, 5d/w, for either 1w or 5m
- Exposure route: whole body inhalation

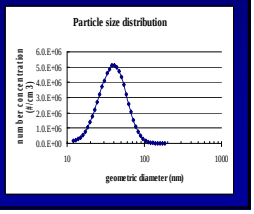
- 5m-old male *Apoe*$^{-/-}$ mice
  (N=6/group for 1w study,
  N=16/group for 5m study)

Control (n=16)	Ni NPs (n=16)	
N=8	N=8	→ mt-DNA damage, Gene expression
N=8	N=8	→ Elemental analysis, Histology

Particle Characterization

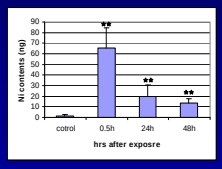
- Chemical composition
  - $Ni(OH)_2$, characterized by EDX, XPS and FTIR
- Size and concentration
  - primary particle diameter: ~5nm
  - count median diameter (CMD) of agglomerates: 37nm
  - number concentration: $2.3E+06/cm^3$
  - measured by TEM, AFM, DMA



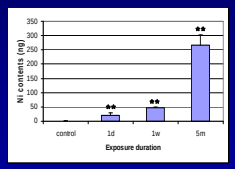


Deposition and Translocation

- Relatively rapid clearance from the lung
- Significant deposition/accumulation in the lung
- No significant accumulation in the blood



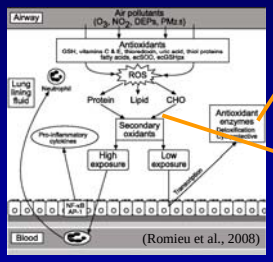
Total Ni contents in the lung at indicated hrs after 1d-exposure



Total Ni contents in the lung at 24h after the designated exposures

Oxidative Stress

Inhaled Ni NPs can induce oxidative stress not only in the lung but also in the cardiovascular system.

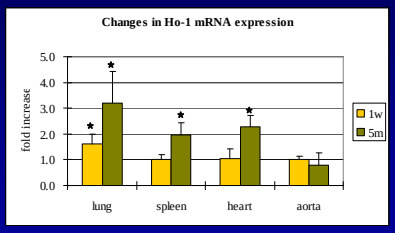


(Romieu et al., 2008)

- Ho-1 mRNA expression (lung, spleen, heart, aorta)
- mtDNA damage (aorta)

Oxidative Stress
1) Ho-1 mRNA expression

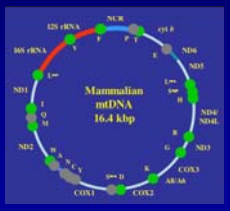
- Ho-1 (heme-oxygenase 1): sensitive marker for oxidative stress

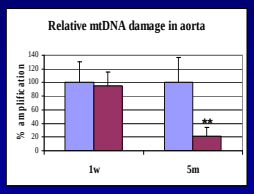


=> Indication of increased oxidative stress in various organs

Oxidative Stress
2) mtDNA damage

- mtDNA: highly susceptible to oxidative stress
- mtDNA damage in aorta: association with CVD
- Determined by semi-quantitative long PCR



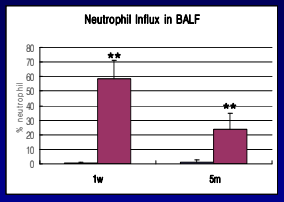


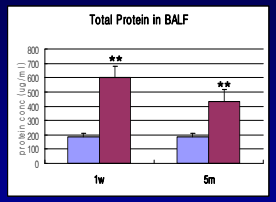
Inflammatory Responses

Inhaled Ni NPs can induce pulmonary and also systemic inflammatory responses.

- Pulmonary inflammation
  - bronchoalveolar lavage fluid (BALF) analyses
  - mRNA expression in the lung
  - histopathological analysis
- Systemic inflammation
  - mRNA expression in the spleen, heart, liver, aorta
  - inflammatory markers in serum

Pulmonary Inflammation
1) BALF analyses

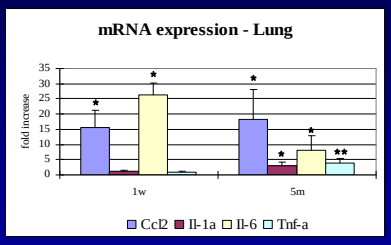




Control Nickel

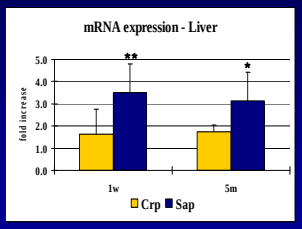
- Significant pulmonary inflammation at both time points
  => persisting effects by inhaled Ni NPs

Pulmonary Inflammation
2) mRNA expression



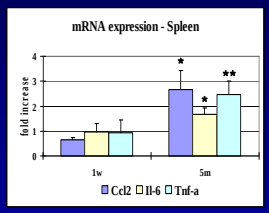
- Significant pulmonary inflammation at both time points
  => persisting effects by inhaled Ni NPs

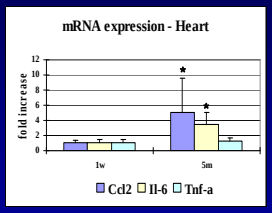
Systemic Inflammation
1) Acute phase proteins



Crp: C-reactive protein
Sap: Serum amyloid p component

Systemic Inflammation
2) Gene expression in extra-pulmonary organs

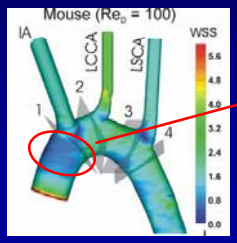




- Indication of systemic inflammation in the long-term

Atherosclerosis

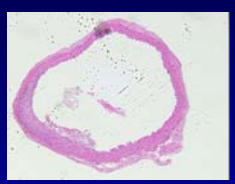
A long-term exposure to inhaled Ni NPs can enhance progression of atherosclerosis in a sensitive animal model.



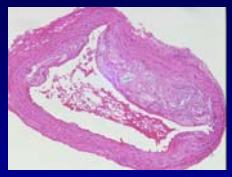
- Plaque quantification
  - H&E staining
- Atherogenesis
  - mRNA expression

http://www3.imperial.ac.uk/pls/portallive/docs/1/27647698.JPG

Atherosclerosis
1) Plaque formation on the aortic arch



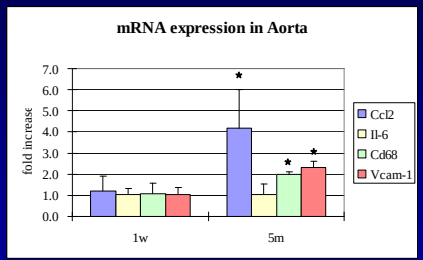
Control



Ni NPs

(n=6)	Control	Ni NPs
% Luminal Area	54.3 (±10.6)	41.1 (±6.4)*

Atherosclerosis
2) Gene expression



- Indication of macrophage infiltration, monocyte-adhesion

Supplemental Studies

Study 1 Particle effects?	Study 2 Dissolved Ni?
Same-size NPs of different chemical composition	Same-size NPs of $Ni(OH)_2$ vs $NiSO_4$

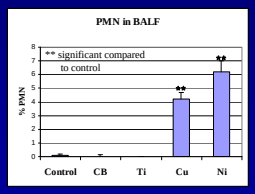
1d (4h) exposure to C57 mice

⬇

Endpoint
Pulmonary Inflammation

Ni NPs Toxicity: Particle Effects?

	Carbon	Ti	Cu	Ni
CMD (nm)	49.02	34.77	35.49	38.79
Geo. STD	1.54	1.66	1.44	1.49
# conc (#/cm3)	1.8E+07	2.5E+07	1.6E+07	1.8E+07
Total mass conc (ug/m3)	558	560	530	550

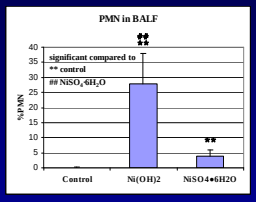


- Particle generated by the same method (PALAS spark-generator)
- Comparable in size, number and mass concentration of the particles
- Ni was most potent, followed by Cu

  => role of chemical composition

Ni NPs Toxicity: Dissolved Nickel?

	$Ni(OH)_2$	$NiSO_4 \cdot 6H_2O$
CMD (nm)	38.98	37.75
Geo. STD	1.47	1.87
# conc (#/cm3)	2.23E+07	3.94E+06
Total mass conc (ug/m3)	1200	3600
Nickel mass conc (ug/m3)	761	792



- $NiSO_4 \cdot 6H_2O$ particle generated from 0.15% solution using nebulizer
- Comparable in size and nickel mass concentration of the particles
- $Ni(OH)_2$ was significantly more potent.

Conclusion

- Inhaled Ni NPs, at occupationally realistic levels, can induce oxidative stress not only in the lung but also in the cardiovascular system.
- Inhaled Ni NPs can induce pulmonary and also systemic inflammatory responses.
- Long-term exposure to Ni NPs could exacerbate plaque formation in hyperlipidemic mice.
- Observed toxicity of $Ni(OH)_2$ NPs may not be explained solely by particle effects or dissolved Ni effect.

Significance

- The first sub-chronic inhalation study to investigate cardiovascular effects of NPs
- Exposure below the current occupational guidelines

⬇

To further investigate potential toxicity of $Ni(OH)_2$ NPs

To provide a database to establish size-specific regulations in occupational and environmental settings

Acknowledgement

- Dr. Lung Chi Chen
- Patricia Gillespie
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- Dr. Lu Chen (Columbia University – XPS analysis)

NIEHS National Institute of Environmental Health Sciences

This work is supported by a NIH grant (R01-ES015495)

Thank you !!!

Questions ???

2008 Interagency Environmental Nanotechnology Grantees Workshop, Nov 19-21 Tampa, FL

Aquatic Toxicity of Carbon-Based Nanomaterials at Sediment-Water Interfaces

(April 2007 – March, 2010)

Joseph Mwangi, Bin Hua, Hao Li, and Baolin Deng
University of Missouri, Columbia, MO

Chris Ingersoll and Ning Wang
USGS-Columbia Environmental Research Center
Columbia, MO

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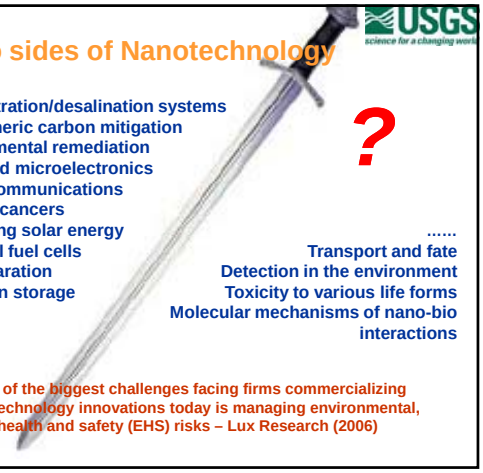
- James L. Kunz (USGS)
- Doug K. Hardesty (USGS)
- Eric L. Brunson (USGS)
- Jianzhong Zheng (Nanjing University)
- Electron Microscopy Core Facility (MU)

•Funding Sources
- Environmental Protection Agency STAR program (RD-833316)
  Drs. Nora Savage and Dr. Barbara Karn

Two sides of Nanotechnology



Water filtration/desalination systems
Atmospheric carbon mitigation
Environmental remediation
Advanced microelectronics
Global communications
Cure for cancers
Harvesting solar energy
Microbial fuel cells
Gas separation
Hydrogen storage
Sensors
……

?

…..
Transport and fate
Detection in the environment
Toxicity to various life forms
Molecular mechanisms of nano-bio interactions

One of the biggest challenges facing firms commercializing nanotechnology innovations today is managing environmental, health and safety (EHS) risks – Lux Research (2006)

Objectives

- ❑ Adapt a proper method for water and sediment toxicity testing 1-D nanomaterials (CNTs, SiC)
- ❑ Assess toxicity of representative 1-D nanomaterials in water or in sediment to representative sediment-dwelling organisms:
  - ❑ Amphipods (*Hyalella azteca)*
  - ❑ Midge (*Chironomus dilutus)*
  - ❑ Oligochaetes (*Lumbriculus variegatus)*
  - ❑ Freshwater mussels (*Villosa iris*)
- ❑ Identify factors controlling the toxicity towards the sediment-dwelling organisms.

1-D Nanomaterials used for toxicity testing

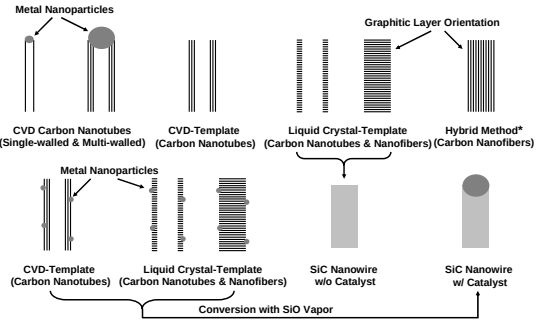
Single-walled CNT (SWCNTs; Shenzhen Nanotech Port, Inc. China):
Over 90% purity, average tube diameter of 2 nm, average tube length 5-15 µm, specific surface area > 400 m^2/g, < 2% ash, < 5% amorphous carbon.

Multi-walled CNT (MWCNTs, Shenzhen Nanotech Port Inc, China):
>95% purity, < 0.2% ash, < 3% amorphous carbon, tube diameters 10-20 nm, tube lengths 5-15 µm, specific surface area 40- 400 m2 per g.

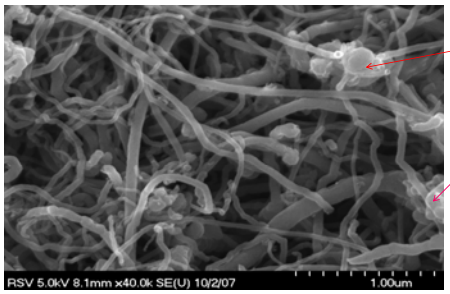
MWCNTs (Helix Material Solutions Inc., TX USA):
purity > 95%, total impurities < 0.2 %W, pH 6-7.

Silicon Carbide (SiC, Manufactured at the University of Missouri)
~40 – 200 nm diameter, 10-50 µm length

Nanomaterials with Well-Controlled Atomic and Meso Structures for Toxicity Testing



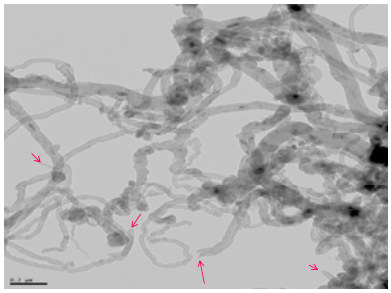
SEM micrograph of a MWCNTs sample



Deposits of impurities including metals at the end of the carbon nanotubes

Variable tube diameters and the rope like entangled morphology

Transmission Electron Microscopy of a MWCNTs

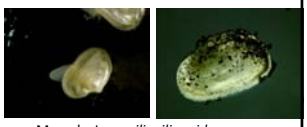


- Arrows show carbon nanotubes with open ends.
- Dark spots are metal clusters in the nanotubes

Image (x10,000) taken at 100kv with TEM FEI Quanta 600F

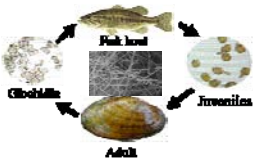
Testing organisms

Amphipod, *Hyalella azteca*



Mussels, *Lampsilis siliquoidea*

Midge *Chironomus dilutus*



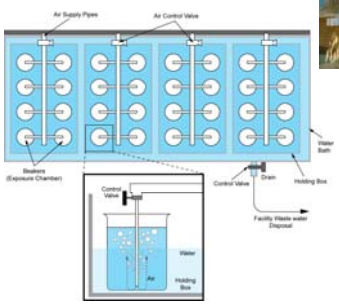
Oligochates
Lumbriculus variegates

Standard Operating Procedure for Handling

This Standard Operating Procedure (SOP) outlines procedures for the safe handling, storage and use of the nanomaterials in the laboratory and to avoid contaminating waste water. The SOP should be used in conjunction with the Material(s) Safety Data Sheets (MSDS) from the suppliers of the nanomaterials.

1. **Storage**
2. **Handling**
3. **Weighing and mixing with water or sediment**
4. **Replacement of water during an exposure**
5. **Decontamination of nanomaterial contaminated items**
6. **Disposal of material**

Test layout in diluter water bath



Test Conditions

(ASTM, 2007b, USEPA, 2000)

Test type:	Static renewal
Test Duration:	14 d
Test chamber:	300-ml beaker
Water volume:	200 ml
Water renewal:	100 ml on Monday, Wednesday, Friday
Feeding:	Monday, Wednesday, Friday.
Aeration:	air bubbling through mixture
Test water:	Hardness of 100 mg/L as $CaCO_3$
Test concentrations:	200 mg CNTs in 200 ml water
Mixing conditions:	Sonication and non sonication
Chemical residues:	dissolved metals in overlying water
Water quality:	DO, pH, conductivity, hardness, alkalinity, ammonia
Endpoints:	Survival and growth
Test acceptability:	(1) ≥80% survival in controls for amphipods and mussels; (2). ≥70% survival in control for midge; (3). 14-d biomass >0-d biomass for oligochaetes

Survival of amphipods, midge, mussels, and biomass of oligochaetes after 14 d exposure in as produced or modified carbon nanotubes in water only toxicity screening tests (Phase 1)

Sample	Treatment	Mean survival (%, SD)			Mean dry biomass (mg, SD)
		Amphipods	Midge	Mussels	Oligochaetes
1.	Control(MW)	88 (5)	80 (8)	98 (5)	16 (2)
	Non-sonicated	5 (10)	60 (8)	23 (17)	14 (1)
	Sonicated	3 (5)	43 (10)	43 (19)	13 (1)
2a.	Control(MW)	100 (0)	63 (15)	80 (28)	3.3 (2)
	Non-sonicated	8 (10)	55 (6)	35 (25)	0.8 (0.2)
	Sonicated	5 (10)	8 (10)	5 (5)	2.8.(1.3)
2b.	Control(MW)	100 (0)	75 (19)	97 (5)	3.7 (1)
	Non-sonicated	95 (6)	60 (14)	100 (0)	1 (0.3)
3.	Control (SW)	100 (0)	83 (5)	Not Tested	3.7 (0.8)
	Non-sonicated	20 (12)	0(0)	Not Tested	1.4 (0.5)
	Sonicated	0 (0)	10 (8)	Not Tested	2.8 (0.2)

1 – as produced MWCNTs from Helix material solutions, TX USA
2a- as produced MWCNTs from Shenzhen Nanotech Port, China
2b- nitric acid modified as produced MWCNTs from Shenzhen Nanotech Port, China
3. As produced SWCNTs from Shenzhen Nanotech Port, China

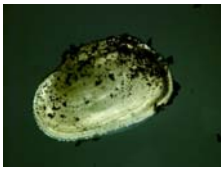
Midge *(Chironomus dilutus)*

6 d exposure in control treatment

6 d exposures in non-sonicated MWCNTs treatment

Oligochaetes (*Lumbriculus variegatus*)

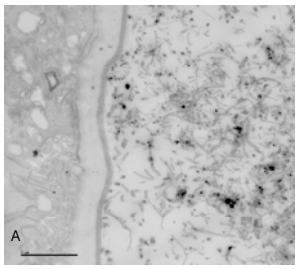
after 6 d exposures to MWCNTs treatment

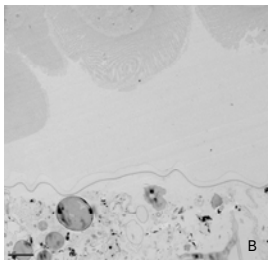


Rainbow mussels (*Villosa iris*) *after 14 d exposures to non-sonicated (top) and Sonicated (left) MWCNTs treatment*

TEM images of gut of midges

A) MWCNTs deposits in gut after 6 d exposures
B) Control with some food deposits only

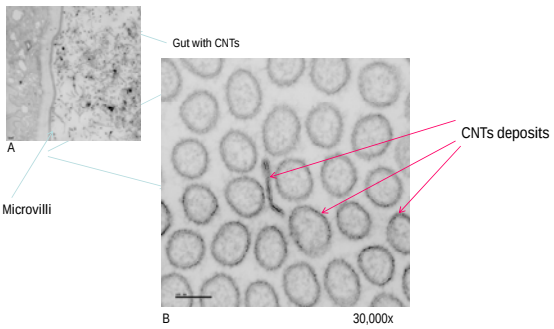




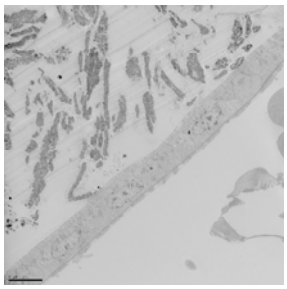
TEM images taken at 100 KV (JEOL JEM 1400, JEOL, Tokyo, Japan)

TEM images of midge

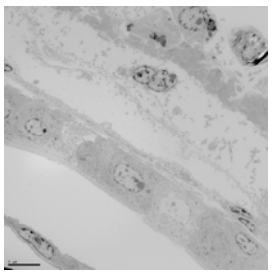
A) gut after 6-d exposures with MWCNTs deposits
B) CNTs trapped between and around some of the microvilli



Amphipods exposures

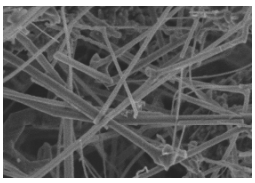


6-d exposures to SWCNTs



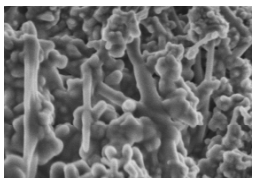
6-d in control

Scanning electron micrograph images of SiC nanowires



(a)

as-fabricated SiC nanowires (average diameter = 100 nm; length = 10-50 μm)



(b)

SiC nanowires after sonication

Table 1. Mean water quality characteristics ($n = 4$; Standard deviation are shown in parentheses) in SiC nanowires toxicity tests with four aquatic benthic invertebrate species

Test	Treatment of SiC nanowires	Organism	Duration	Test water	Dissolved oxygen mg/L	pH	Conductivity μS/cm	Alkalinity mg/L as $CaCO_3$	Hardness mg/L as $CaCO_3$
Water-only tests									
1	Non-sonicated	Amphipod	48 h	ASTM hard	8.6 (0.4)	8.5 (0.1)	581(29.7)	120 (0)	165 (7.1) Sonicated
	Amphipod	48 h	ASTM hard	8.9 (0.0)	8.8 (0.2)	731(231.9)	149 (38.2)	213(58.0)	
	Sonicated	Midge	96 h	ASTM hard	8.1 (1.3)	8.8 (0.1)	833 (347.9)	179 (72.1)	245 (106.1)
	Sonicated	Oligochaete	96 h	ASTM hard	8.1 (1.7)	8.7 (0.2)	846 (370.5)	171 (69.3)	253 (114.8)
	Sonicated	Mussel	96 h	ASTM hard	8.9*	8.6*	587*	122*	170*
2	Sonicated	Amphipod	48 h	ASTM hard	8.9 (0.1)	8.5 (0.1)	584 (33.9)	120 (0)	165 (7.1)
	Sonicated	Midge	96 h	ASTM hard	8.8 (0.2)	8.6 (0.1)	602 (58.5)	125 (7.1)	179 (0)
	Sonicated	Oligochaete	96 h	ASTM hard	8.9 (0.2)	8.4 (0.2)	587 (55.5)	120 (0)	165 (7.1)
	Sonicated	Mussel	96 h	ASTM hard	9.0 (0)	8.6 (0.1)	589 (41)	123 (2.8)	168 (2.8)
3	Sonicated	Amphipod	48 h	Diluted well 1	7.5 (0.1)	8.3 (0.2)	365 (23.3)	100 (17.7)	119 (8.8)
4	Sonicated	Amphipod	48 h	Diluted well 2	8.3 (0.7)	8.3 (0.4)	408 (8.5)	140 (26.9)	140(20.5)
Sediment tests									
1	Sonicated and mixed with control sediment	Amphipod	10 d	Diluted well 2	8.3 (0.4)	8.4 (0.4)	426 (33.2)	158 (14.1)	163(12.7)
2	Sonicated and applied on surface of control sediment	Amphipod	10 d	Diluted well 2	7.9 (0.1)	8.1 (0)	414 (16.3)	140 (12.0)	157(3.5)

*Water quality was only measured in control treatment at the beginning of the exposure.

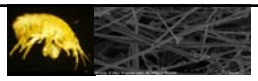


Table 2 Mean survival ($n = 4$, except in *test 2 with juvenile mussels with 3 replicates in treatment; Standard deviation are shown in parentheses) of four aquatic benthic invertebrate species in 48- or 96-h water-only exposure to SiC nanowires

Test	Treatment	Organism	Test Water	Survival (%, SD) Control	Survival (%, SD) Treatment
Water-only test					
1	Non-sonicated SiC	Amphipod	ASTM Hard	90 (8.2)	83 (7.0) ns
	Sonicated SiC	Amphipod	ASTM Hard	90 (11.5)	0 (0)*
	Sonicated SiC	Midge	ASTM Hard	83 (17.1)	75 (17.3) ns
	Sonicated SiC	Oligochaete	ASTM Hard	100 (0)	100 (0) ns
	Sonicated SiC	Mussel	ASTM Hard	30 (38.3)	30 (34.6)**
2	Sonicated SiC	Amphipod	ASTM Hard	98 (5.0)	15 (12.9)*
	Sonicated SiC	Midge	ASTM Hard	100 (0)	100 (0) ns
	Sonicated SiC	Oligochaete	ASTM Hard	100 (0)	100 (0) ns
	Sonicated SiC	Mussel	ASTM Hard	95 (10.0)	100 (0) ns
3	Sonicated SiC	Amphipod	Diluted Well 1	98 (5.0)	75 (9.6)*
4	Sonicated SiC	Amphipod	Diluted Well 2	98 (5.0)	48 (20.6)*

Sediment Test		Survival (%, SD) Control	Survival (%, SD) Treatment	Length (mm, SD) Control	Length (mm, SD) Treatment
1	Sonicated SiC mixed with control sediment	88 (9.6)	80 (8.2) ns	1.88 (0.22)	1.81 (0.21) ns
2	Sonicated SiC applied on surface of control sediment	93 (5.0)	80 (14.1) ns	1.97(0.22)	1.63(0.23)*

*= significantly different from the control ($p < 0.05$).
** = Test did not meet control acceptability requirement of 90% (ASTM 2007e).
ns = Not significantly different from the control.

Conclusions

Sonicated or non-sonicated as-produced single-walled and multi-walled CNTs are toxic to amphipods, midge, oligochates and mussels in water .

Sediment can reduce, but not totally eliminate, the toxicity of as-produced MWCNTs to amphipods.

Sonication significantly increases the toxicity of SiC nanowires to amphipods.

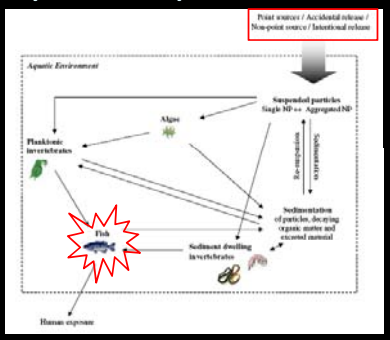
QUESTIONS?

Toxicity of Nanoparticles in an Environmentally Relevant Fish Model

Judi Blatt Nichols
Department of Environmental Medicine
New York University School of Medicine

November 20, 2008

Aquatic exposure routes



Baum 2008

Interactions between the environment and nanoparticles due to their physico-chemical properties may influence bio-availability and toxicity in aquatic organisms

- Particles:
  - Size
  - Density
  - Surface functional groups
  - Hydrophobicity
- Environment:
  - Water hardness
  - Salinity
  - Natural organic matter

Affect particle agglomeration and settling or suspension in water column

Why early-life stages of fish?

- Very sensitive to a wide range of environmental contaminants
- Easy to acquire large numbers allowing for robust statistical analysis
- Relatively inexpensive compared to mammals
- Treatments can mimic environmental conditions to determine likely occurrence in wild populations

Why Atlantic tomcod?

- Common fish found in Atlantic coastal estuaries from Hudson River to Labrador
- Wintertime spawners. Juveniles are dominant prey for predatory fish during summer months. Occupy critical node in food web - valuable indicator species
- Bottom dwellers with lipid-rich livers. Exposed to and accumulate extraordinary high levels of hydrophobic contaminants associated with sediments (i.e., dioxins, PCBs)
- Long embryonic developmental period (30+ days)
- Focal species for almost 20 years of research on toxic effects of contaminants on ecosystems in Dr. Wirgin's lab

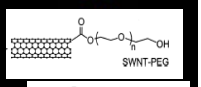
Hypothesis:

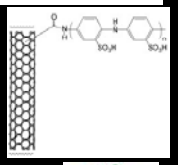
There are particle-type dependent differences in early life stage toxicity

- Embryonic exposure
  - Mortality
  - Hatching success and rate
  - Larval stage morphology
- Larval exposure
  - Mortality

Types of particles used:

- Fullerenes
- Functionalized single-wall nanotubes:
  - Polyethylene glycol (P7-SWNT)
  - m-polyaminobenzene sulfonic acid (P8-SWNT)
- Carbon black
- Metal nanoparticles:
  - Ag, Cu, Fe, Ni, Zn
- Manufactured nanoparticles: 3 atoms of metal (erbium, yttrium) within a C_{80} cage.
  - Soot - raw material
  - Mix – finished product
  - Sludge – leftover waste

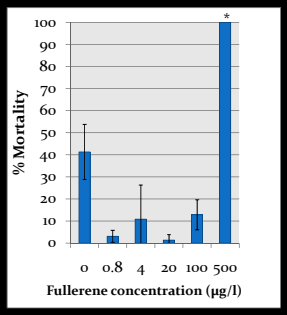


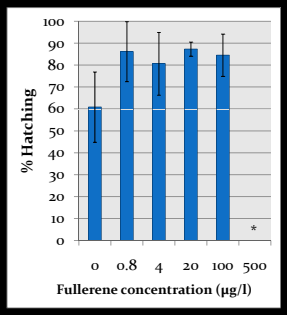


Experimental design:

- Tomcod production: 6 mating pairs from Shinnecock Bay, Long Island, NY used to produce embryos
- Stock suspensions of nanoparticles in 5 ppt sea water (except fullerene-DMSO), sonicated for 1 hr, graded dilutions prepared in 5 ppt sea water.
- Embryos exposed at 14 dpf, 30 embryos per replicate, 3 replicates per dose, 5 doses
- Static renewal design, every 48 hours, particle suspensions removed and replaced until embryos hatched or died (~1.5 months)
- Toxic endpoints evaluated:
  - Mortality
  - Hatching success
  - Time to hatch
  - Morphological abnormalities

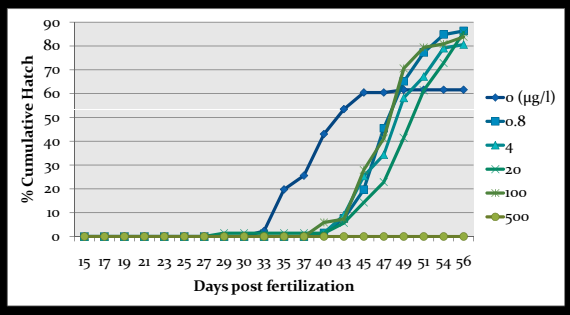
Mortality and hatching with fullerene exposure



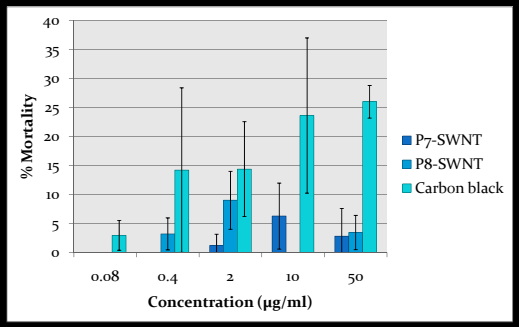


Mean ± SD, * p<0.05

Day of hatch is delayed with fullerene exposure

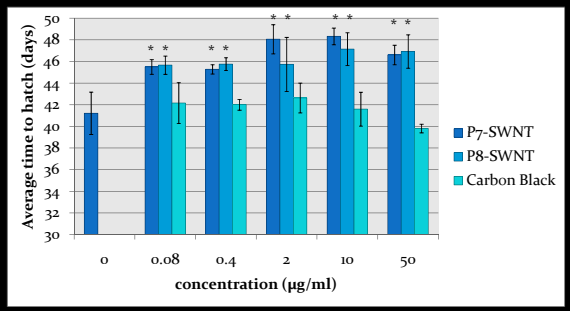


Mortality with SWNTs and Carbon Black



Mean ± SD

Time to hatch with SWNT and Carbon black



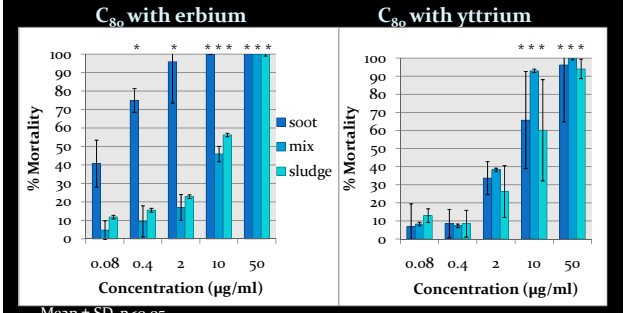
All particles and doses compared to same control, p<0.05

Mortality with metal nanoparticle exposure

particle conc. (µg/ml)	Ag	Cu	Fe	Ni	Zn
0	14.7 ± 7.7	13.8 ± 8.8	14.7 ± 7.7	13.8 ± 8.8	14.7 ± 7.7
0.016	10.9 ± 2.7	8.8 ± 4.1	2.8 ± 4.8	12.2 ± 4.3	7.9 ± 6.5
0.08	14.5 ± 5.5	4.0 ± 3.7	11.4 ± 1.5	7.1 ± 6.8	4.0 ± 4.0
0.4	17.5 ± 11.5	83.3 ±17.4*	8.4 ± 7.3	10.2 ± 2.0	6.5 ± 2.1
2	16.5 ± 2.3	100*	11.1 ± 1.0	7.5 ± 6.8	17.8 ± 2.4
10	7.2 ± 9.1	100*	100*	19.1 ± 3.0	100*

Mean ± SD, *p<0.05

Mortality with manufactured nanoparticle exposure



Mean ± SD, p<0.05

Conclusions:

- Fullerenes caused 100% mortality at 500 µg/l; hatching was delayed in all exposed doses.
- Functionalized SWNTs did not result in significantly more mortality to embryos than carbon black particles, although time to hatch was significantly delayed.
- For metal nanoparticles, Cu > Fe, Zn > Ag, Ni for mortality.
- Toxicity associated with erbium- and yttrium-containing particles for the mix, soot and sludge was dose dependent and statistically significant.

Future work:

- Determine if nanoparticle bioavailability and toxicity is influenced by aquatic media. Water samples will be collected from different estuaries and lakes varying in salinity and natural organic matter content and used to suspend particles in order to expose embryos and larvae.
- Characterize the particles used in 5 ppt sea water and the natural waters in terms of mean diameter and zeta potential.
- Expose a second species, *Fundulus heteroclitus* to a subset of particles to determine if the effects found in tomcod are replicated in other species.
- Use high-throughput microarrays to determine dose- and time-dependent changes in gene expression in tomcod and *Fundulus*.

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Ecotoxicology of Fullerenes (C_{60}) in Fish

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2. Ecotoxicology and Stress Biology Research Centre, University of Plymouth, Plymouth UK

Acknowledgments/Recognition

Ecotoxicology of Fullerenes (C_{60}) in Fish

Henry, Menn, Compton, Sayler:	Project P.I.s
June-Woo Park:	Postdoc
Shaun Ard:	PhD student
Tze Ping Heah:	Research Assist

This research is funded by U.S. EPA - Science To Achieve Results (STAR) Program
Grant # R833333

Project duration: 2007-2010

Project Objectives

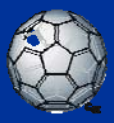
- Investigate physicochemical properties of aqueous C_{60} aggregates
  - Influence of dissolved organic material
- Investigate bioavailability of C_{60} in fish
  - Aqueous and dietary exposure
- Investigate the toxicity of C_{60} in fish
  - Zebrafish, channel catfish
    - Tissue accumulation and distribution of C_{60}
    - Changes in gene expression
    - Histopathology

Progress Report: Year 1

- **Changes in global gene expression in zebrafish exposed to aqueous C_{60}**
  - **Evaluation of vehicle effects**
  - **Aggregate characteristics**
  - **Toxicity**
- **Influence of C_{60} aggregates on bioavailability of other toxicants**
  - **Example: 17α-ethinylestradiol (EE2)**
- **Dietary exposure to C_{60}**
  - **Experiments with rainbow trout**

Background on C_{60}

- First manufactured carbon NP
- Nobel prize in Chemistry 1996
- Soccer ball shape
- Diameter ≈ 0.7 nm
- Partially delocalized π electrons



- Structure facilitates energy transfer
- Absorption of light
- Light energy transferred to form $^1O_2^*$
- Potential formation of free radicals
- Oxidative injury in organisms?

C_{60} in Consumer Products

(More than 100 fullerene patents)

Cosmetic products

- Radical Sponge (Vitamin C_{60} BioResearch, Tokyo)
- Zelens Fullerene C_{60} Day Cream (Zelens, London)

Toxicity?
Environmental fate?

Previous Research of C_{60} Toxicity

- Little or no toxicity found for C_{60}
  - C_{60} applied to mouse skin (Nelson et al 1993)
  - Mice IP administration of C_{60} (Moussa et al 1996)
  - Lung cell cultures and C_{60} (Baierl et al 1996)

Previous Research of C_{60} Toxicity

- Little or no toxicity found for C_{60}
  - C_{60} applied to mouse skin (Nelson et al 1993)
  - Mice IP administration of C_{60} (Moussa et al 1996)
  - Lung cell cultures and C_{60} (Baierl et al 1996)
- Toxicity reported in fish and *in vitro*
  - Oxidative injury in fish brains (Oberdörster 2004)
  - Toxicity in aquatic species (Oberdörster et al 2004)
  - Toxicity in human skin cell lines (Sayes et al 2004)

Challenges of Assessing Aquatic Toxicology of C_{60}

- **Water solubility (< 10^{-9} mg/L)**
- **Vehicle: Tetrahydrofuran (THF)**
  - **Dissolve C_{60} into THF**
  - **Add C_{60}-THF mixture to water**
  - **Evaporate off THF**
- **Vehicle effects?**

Assessment of THF Vehicle Effects

Henry, T.B., Menn, F., Fleming, J. T., Compton, R. Sayler, G. 2007. *Environmental Health Perspectives* 115:1059-1065.

Experimental treatments:

- Water control (synthetic soft water)
- C_{60}- water
- THF-C_{60} water
- THF- vehicle control

} 3 replicates per treatment

Larval zebrafish- age 72 hpf

Exposure duration 75 h

Exposure in 400 mL glass beakers

Endpoint: changes in gene expression

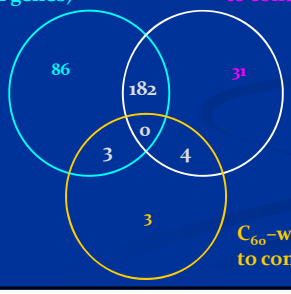
Affymetrix zebrafish array (≈15,000 gene transcripts)

Larval zebrafish (3-4 mm length)

Gene Expression Results: Affymetrix Array

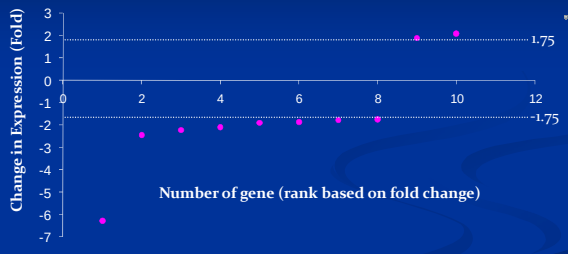
THF-C_{60} compared to control (271 genes)

THF-vehicle compared to control (217 genes)



C_{60}–water compared to control (10 genes)

Differentially Expressed Genes in C_{60}-water Relative to Control

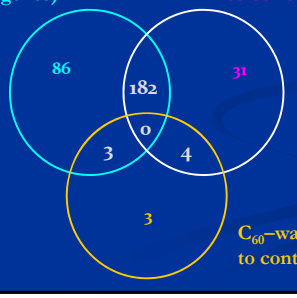


Conclusion: little or no effect of C_{60}–water on zebrafish gene expression

Gene Expression Results: Affymetrix Array

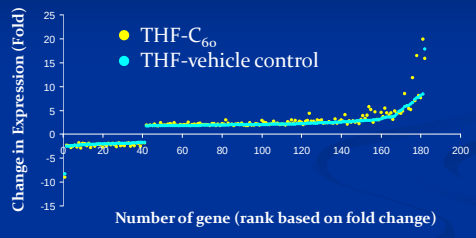
THF-C_{60} compared to control (271 genes)

THF-vehicle compared to control (217 genes)



C_{60}–water compared to control (10 genes)

Expression of Common Genes (182) in THF-Vehicle and THF-C_{60}



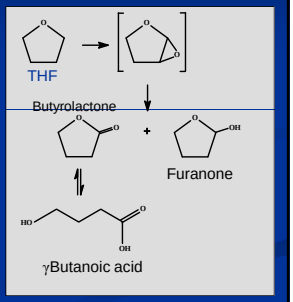
≈73% of genes have > change in expression in THF-C_{60} treatment

Common Genes of Interest in THF-C_{60} and THF-vehicle Compared to Control

Affymetrix Probe ID	THF-C_{60} Control fold	THF-water Control fold	Description/function
Dr.10624	7.00	7.32	Peroxidase activity
Dr.23788	5.39	6.05	Glutathione-S-transferase
Dr.9492	3.69	3.43	Oxidoreductase activity

What was Causing Toxicity of THF-C_{60}?

- THF not detected by GC-MS
- LC_{50} THF = 1.73%
- THF degradation products (low ppm)
- Biologically active
  - Butyrolactone
  - γButanoic acid
  - Furanone
- Butyrolactone tested
- LC_{50} butyrolactone = 47 mg/L



Effect of C_{60} Aggregates on Bioavailability of EE2

- Aqueous C_{60} stock: 666 mg/L in pure water (stirred, 4 months)
- Experimental treatments: 3 replicates

  1) 0 day
  - Solvent control (0.01% EtOH)
  - 17α-ehtinylestradiol (EE2) (1 ug/L)
  - C_{60} only: 16 mg/L, 40 mg/L, 65 mg/L
  - C_{60} (each concentration) + EE2 (1 ug/L)

  2) 28 day aged
  - Repeated exposure with aged solutions
  - Fresh EE2 solution (1 ug/L)

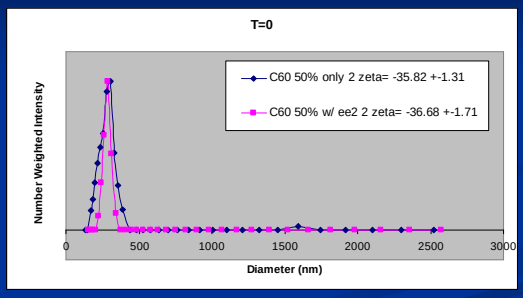
Effect of C_{60} Aggregates on Bioavailability of EE2

- Larval zebrafish (72 hpf) exposed for 75 hrs
- Endpoint: EE2 induced *Vtg* expression (qRT PCR)
  - EE2 synthetic estrogen
  - Vitellogenin genes (*Vtg*) induced by EE2
- C60 particle analyses: ZetaPALs
  - Evaluate aggregate size
  - Evaluate aggregate charge

C_{60} Aggregate Characteristics

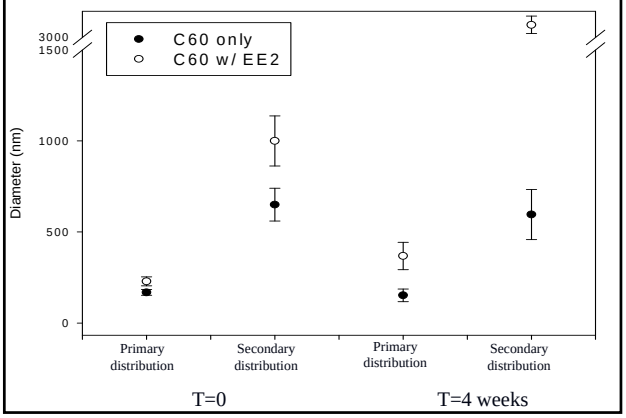
- Each treatment prepared stirred then solution allowed to settle for 1 hour
  - Sample collected from mid water
    - Particle size and charge assessed (ZetaPALs)
    - Total C_{60} determined by evaporation, toluene extraction, and UV-vis spectroscopy

C_{60} Aggregate Characteristics

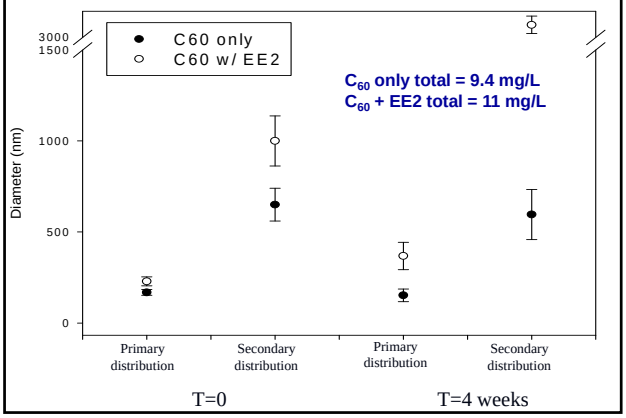


Most particles are near 200 nm diameter, few larger particles

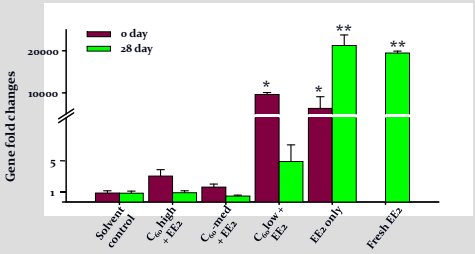
Aggregate Size Distributions for 40 mg/L C_{60} Treatments



Aggregate Size Distributions for 40 mg/L C_{60} Treatments



Vtg Expression Changes in C_{60} + EE2 Exposure



- EE2 bioavailability (assessed by *Vtg* expression) reduced by C_{60}
- EE2 became less bioavailable over time in presence of C_{60}

Conclusions

- Presence of EE2 altered characteristics of C_{60} aggregates
  - Zeta potential decreased, more tendency to aggregate
  - Particles were smaller; however, larger particles may have sedimented out of aqueous phase
- C_{60} reduced bioavailability of EE2 (reduced expression of *Vtg*)
  - Perhaps EE2 is absorbed within C_{60} aggregates
- Aging appeared to increase association of C_{60} with EE2 and reduced bioavailability of EE2

Development of Methods and Models for Nanoparticle Toxicity Screening:

Application to Fullerenes and Comparative Nanoscale Particles

Tian Xia, M.D. & Ph.D.
Andre Nel, M.D. & Ph.D. PI

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Department of Medicine
UCLA
Los Angeles, CA 90095

Two basic questions

Question 1: Are there any human diseases caused by nanomaterials?

Answer: No!

Question 2: Are there any human diseases caused by materials such as particles or fibers?

Answer: Yes, what can we learn from it?

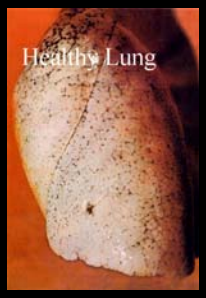
History of particle exposure

Lessons from Quartz (surface area, surface reactivity)

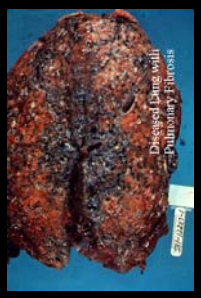
Quartz (Crystal SiO_2)

Widely used for electronics

Healthy Lung



Lung Fibrosis



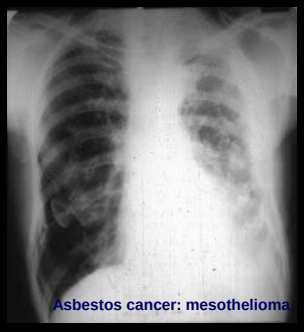
Lung fibrosis could be fatal (Seaton 1995), also cause inflammation, cell death, and cancer.

History of fiber exposure

Lessons from Asbestos (length, diameter, surface activity and durability)

Asbestos

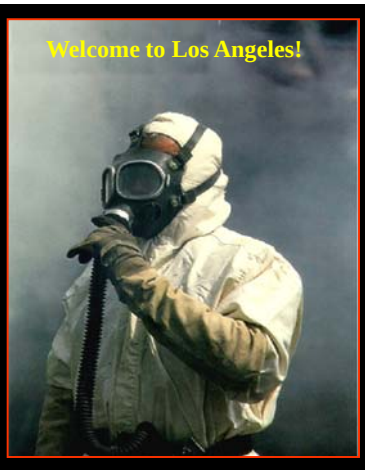
"miracle mineral" because of its soft and pliant properties, as well as its ability to withstand fire and heat.



Asbestos cancer: mesothelioma

Other lung disease: asbestosis

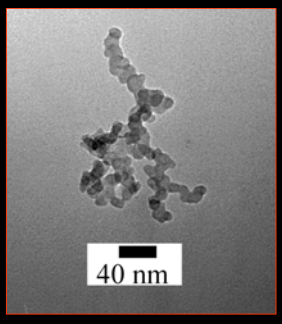
Welcome to Los Angeles!



Lessons from air pollution particle studies

Photochemical smog in Los Angeles

TEM of Ultrafine Particle

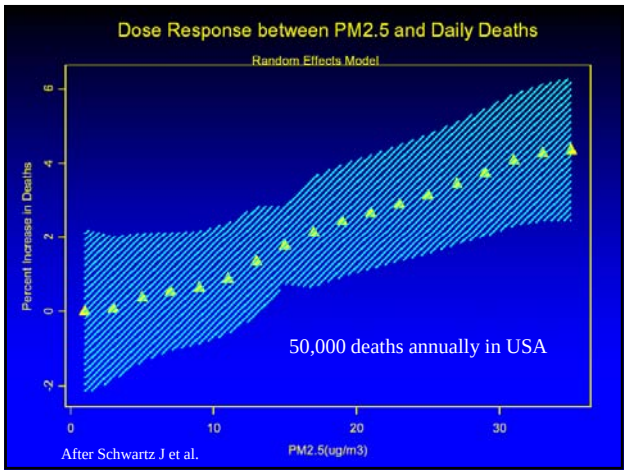


Main sources are emissions or condensation of vapors

Carbonaceous core coated with organic chemicals and metals

Not EPA regulated

Nel A. Biomolecular effects of particles. Science 2005.



Several lessons from history

- Oxidative stress plays a major role!
- Toxicity is related to particle physical characteristics

Quartz:

Freshly cut,

Defective surface,

Surface reactivity,

ROS

Asbestos:

Frustrated phagocytosis,

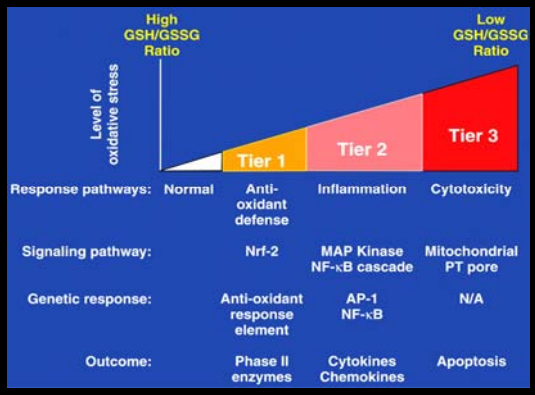
ROS

Air particles:

High organic chemicals and metal coating,

ROS

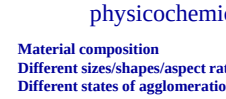
The Hierarchical Oxidative Stress Model

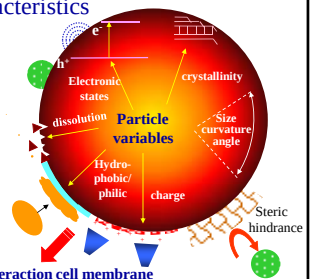


Nel et al. Science, 311, 622-627, 2006

This model has to consider a wide range of nanomaterial physicochemical characteristics

Material composition
Different sizes/shapes/aspect ratios
Different states of agglomeration
Different surface functional groups & catalytic activities
Different stabilities/bioavailabilities
etc etc





Contact/interaction cell membrane
Contact/interaction proteins
Contact/ interaction DNA
Cell uptake (endocytosis/phagocytosis etc)
Subcellular localization/organellar interactions
Mitochondrial functions/ATP production
Bio-accumulation/biopersistance
etc etc

Examples tested in our mammalian cell system:

Fullerenes:

Fullerol

Aqueous/nC_{60}

THF/nC60

Polystyrene NP:

Plain, 60 nm

Cationic, 60 nm

Anionic, 60 nm

Cationic, 600 nm

Metal oxides:

ZnO

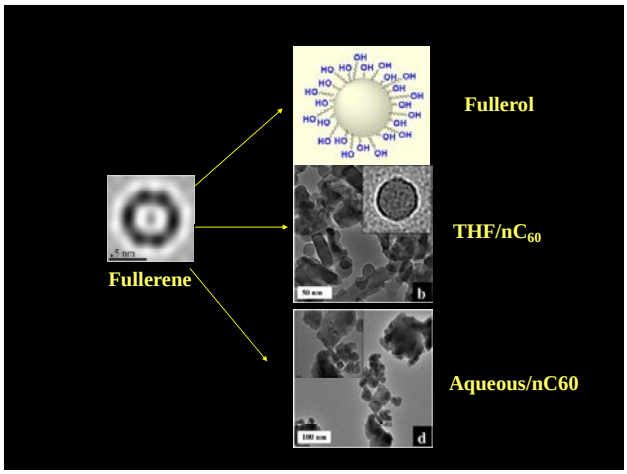
TiO_2

CeO_2

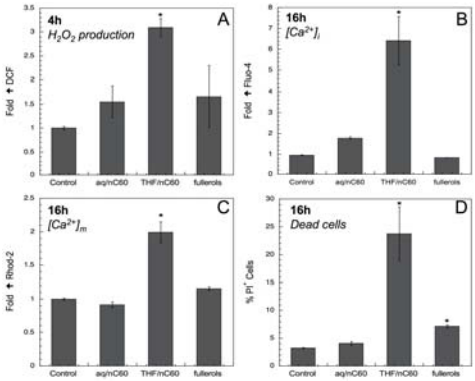
Methods:

Test oxidative stress markers in mammalian cell system

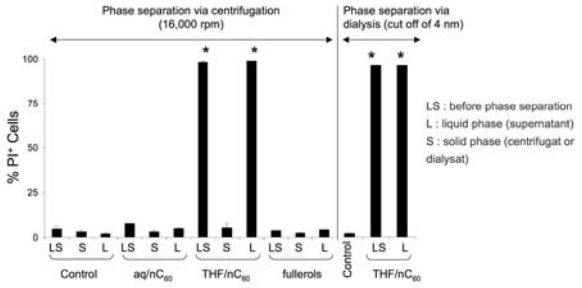
Extensive physicochemical characterization



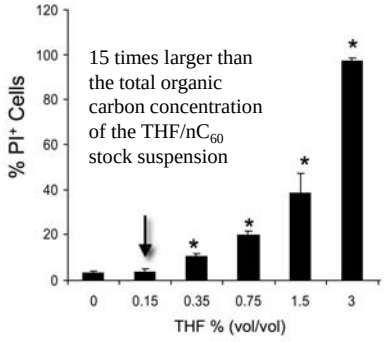
H_2O_2 production in cells (RAW 264.7) in presence of aq/nC_{60}, THF/nC_{60} and fullerols



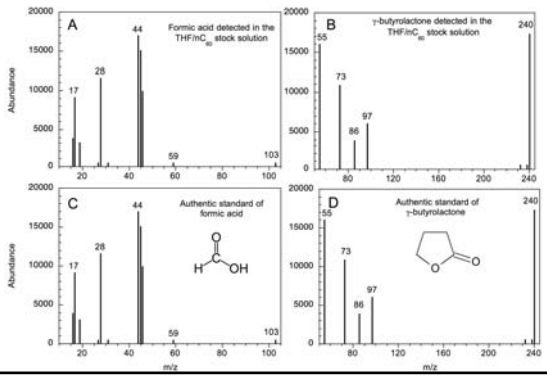
Comparative toxicity (PI) of the supernatant (liquid phase containing the dissolved residue from the synthesis) and the solid phase containing C_{60} aggregates.



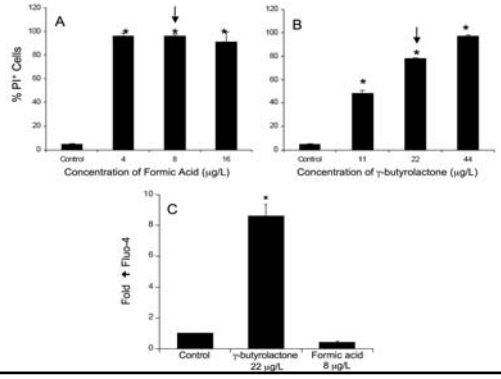
Toxicity (PI) of a pure THF solution towards RAW 264.7 cells.



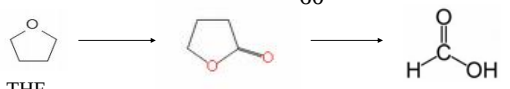
GC-MS spectrum of formic acid (A) and γ-butyrolactone (B) obtained from THF/nC_{60}. (148 ppm extracted in methanol). The spectrum obtained from authentic standard of formic acid (C) and γ-butyrolactone (D).

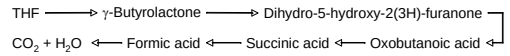


Dose dependant toxicity (PI uptake) of formic acid (A) and γ-butyrolactone formic acid (A) and γ-butyrolactone (B) in RAW 264.7 cells.



THF/nC_{60}





- The degradation product, **formic acid and γ-butyrolactone** can induce toxicity
- It is not the **THF** itself, only at high dose
- It is not clear whether fullerene speed up the degradation process.

TEM of nanoparticles

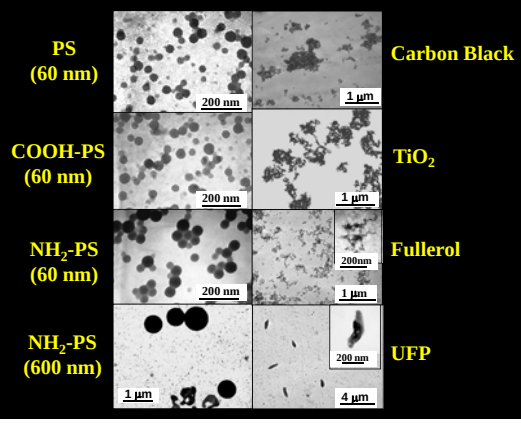
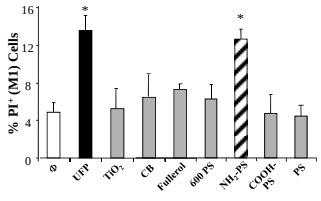


Table 1. Physical Characterization of Nanoparticles[a]

particle	av diameter (nm)	PDI	electrophoretic mobility U (μm cm/(V s))	zeta potential ζ (mV)	MATH (%)
		In Aqueous Media			
UFP	1034	1.0	−2.28	−29.1	8.2
PS	68	0.041	−2.85	−36.4	2.7
NH_2-$PS_{60\ nm}$	65	0.055	3.15	40.3	5.3
NH_2-$PS_{600\ nm}$	648	0.096	3.58	45.8	4.2
COOH-PS	56	0.063	−2.15	−27.6	0.0
TiO_2	364	0.466	−1.28	−16.4	1.6
carbon black	245	0.251	−4.26	−54.6	7.1
fullerol	218	0.388	−1.76	−22.6	0.6
		In Cell Culture Medium			
UFP	1778	0.379	−0.86	−11.0	
PS	90	0.200	−1.00	−12.7	
NH_2-$PS_{60\ nm}$	527	0.339	−0.87	−11.1	
NH_2-$PS_{600\ nm}$	1913	1.0	−0.96	−12.2	
COOH-PS	82	0.191	−0.85	−10.9	
TiO_2	175	0.877	−0.97	−12.4	
carbon black	154	0.278	−1.06	−13.5	
fullerol	106	0.700	−0.97	−12.4	

[a] The reported mean particle size (average diameter) is calculated based on an intensity weighted average; PDI = polydispersity index; MATH = microbial adhesion to hydrocarbon test.

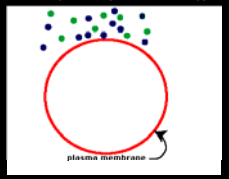
Cell toxicity to macrophages determined by PI uptake



Xia T, Nano Letters, 2006

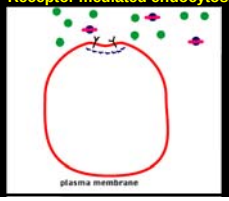
The role of cellular uptake mechanisms of nanoparticles

Pinocytosis (cell drinking)

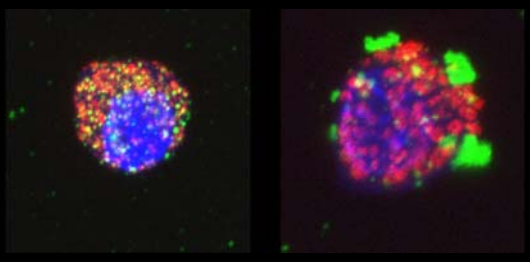


Phagocytosis (cell eating)

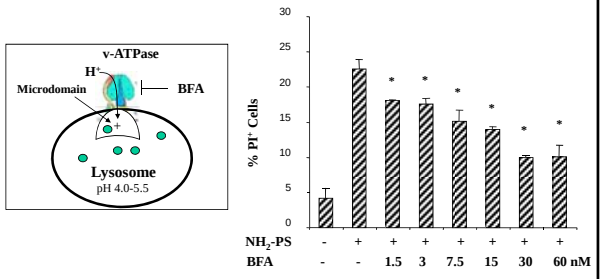
Receptor mediated endocytosis

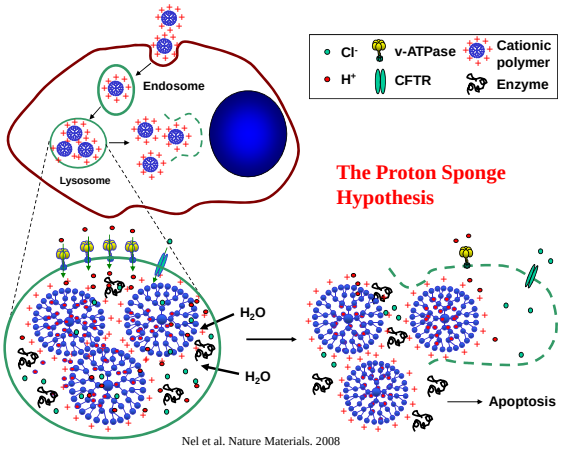


Macrophage cells take up cationic NH_2-PS nanoparticles



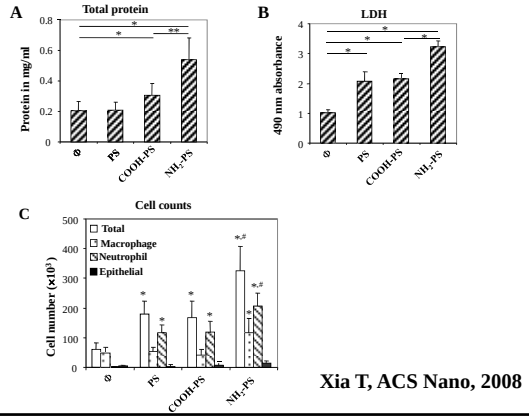
Bafilomycin A1 inhibit NH_2-PS induced cell death





Nel et al. Nature Materials. 2008

Toxicity induced by intratracheal injection of PS in mouse lung



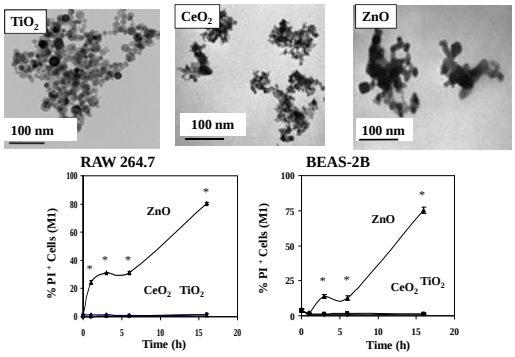
Xia T, ACS Nano, 2008

Very much like Ardystil syndrome

The paint workers in Spain and Algeria suffered from many complaints including nose bleeding, coughing, general disorders of the upper airway, and bronchial hyper-reactivity.

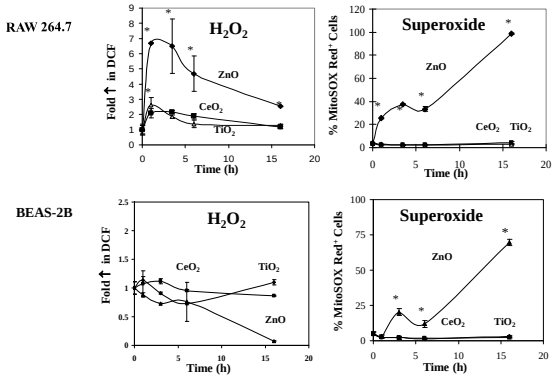
Epidemiological and toxicological studies have suggested Acramín, a polycationic paint component in the paint to be responsible for this disease.

Comparison of ZnO cytotoxicity with 2 other metal oxides



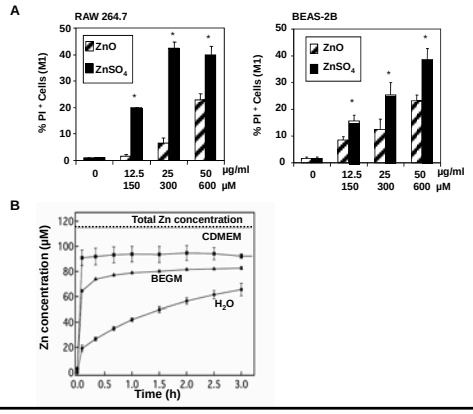
Xia et al. ACS Nano. 2008

Cellular ROS production by Flow cytometry



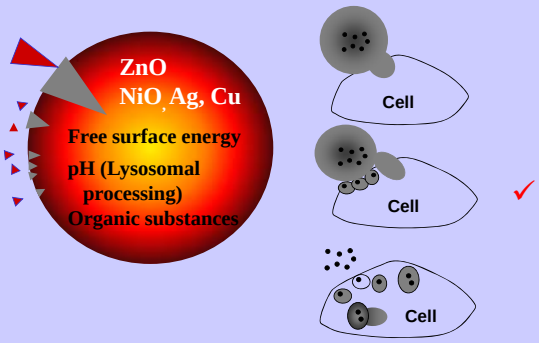
Xia et al. ACS Nano. 2008

ZnO has extensive dissolution in cell culture medium



California NANOSystems Institute

Injury through dissolution



Xia et al. ACS Nano. 2008

Metal Fume Fever

Welders exposed to ZnO, other metal oxides: Cu, Mg, Sn, or Cd

3-10 hrs post-exposure: flu-like illness,fever, general malaise, chills, dry cough, metallic taste, muscle aches, shortness of breath

TNFα levels elevated at 3 hr, IL-8 levels peaks at 8 hr, and IL-6 values peaks at 22 hr

Pathophysiology: marked increases in lung PMLs 20–24 hr after exposure

Resolves 24–48 hr after onset

Short-term tolerance: asymptomatic with repeated exposure

Use mechanisms of nanomaterial cytotoxicity to mitigate by adding safety design features

- 1, For toxicity, check the NP and the suspending solution
- 2. For fullerenes, be careful of the residual solvents; for carbon nanotubes, decrease the impurities and rigidity and/or functionalize the surface to increase solubility
- 3. For cationic particles, decrease the charge density or replacing cationic head groups with amphiphillic head groups
- 4. For ZnO, NiO, Ag, Cu, capping with surfactants, polymers or complexing ligands to decrease dissolution

Acknowledgements

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Joanne I. Yeh at Pittsburgh

Costas Sioutas at USC

Support:

R01 ES015498

Effects of Nanomaterials on Blood Coagulation

Interagency Environmental Nanotechnology Grantees Workshop

November 2008
Tampa, FL

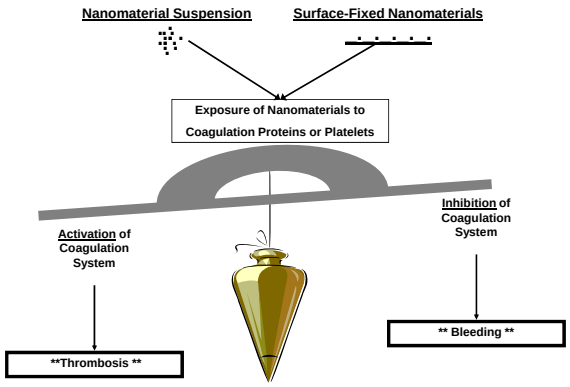
Peter L. Perrotta, MD
West Virginia University

Revised November 2008

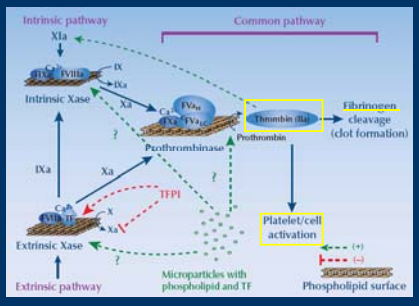
Nanomaterials & Coagulation Rationale for Toxicology Assessment

1) Common human diseases including myocardial infarction & stroke are related to clot formation (thrombosis)
2) These diseases are influenced by environmental factors, but not all risk factors are known
3) Nanomaterials entering workplace or home could have short and/or long-term effects on the blood coagulation system
4) Targets of nanoparticles related to toxicity are proteins (clotting proteins)

Blood Nanomaterial Interactions



Modern Coagulation Cascade



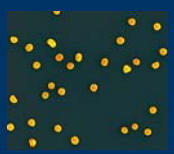
Nature Medicine 9, 991 - 992 (2003)

Issues in Blood Coagulation Testing

- Blood sampling: Limit activation of clotting proteins with blood drawing, limit protein degradation, etc.
- Plasma: More difficult to work with than serum
- Macro vs. nano testing: Adapt assays to small volumes
- Variability of assays: Higher than many other assays

Standardizing Coagulation Assays In Nanotoxicology Trials

- Few studies on coagulation
- Most studies on biomaterial interactions with surface fixed materials (prevent clotting at surface)
- No standardized assays for coagulation (Nanotechnology Characterization Lab)
- Initial studies on SWCNT in animal models and clotting systems difficult due to dispersion problems
- NIST Reference materials

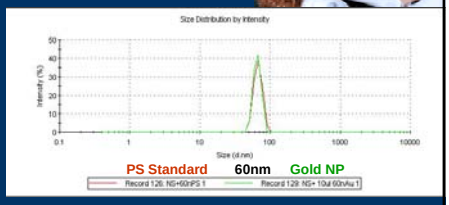


Citrate-stabilized gold NPs (10,30,60 nm) colloidal/H_2O suspension

http://ts.nist.gov/measurementservices/referencematerials

Particle Dispersion in Biological Systems

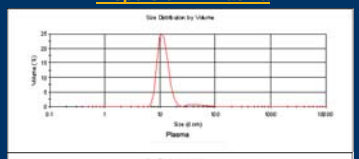
- Documenting dispersion before *in vitro* assays
  - AFM: Dry vs. wet
  - SEM, TEM, Cryo EM
  - Dynamic light scattering & zeta potential



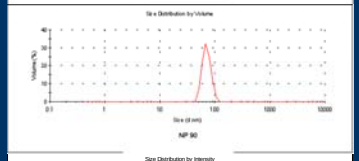
malvern.com/LabEng/products/zetasizer/

Dispersion in Plasma

Plasma
Albumin (14nm)
Fibrinogen

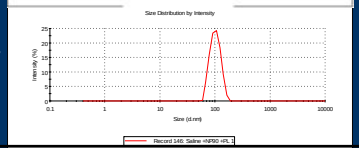


90 nm gold NP in physiologic buffer

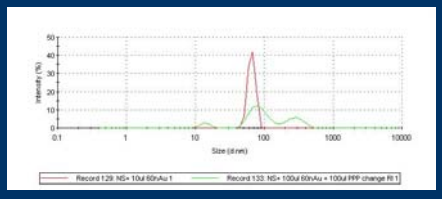


90 nm gold NP in dilute plasma

Shift in viscosity and refractive index of dispersant

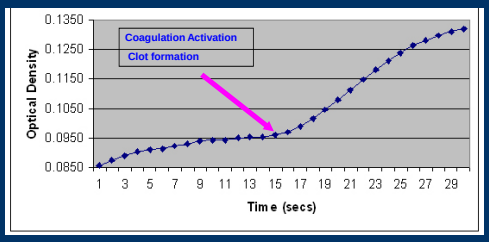


60 nM Au Nanoparticles



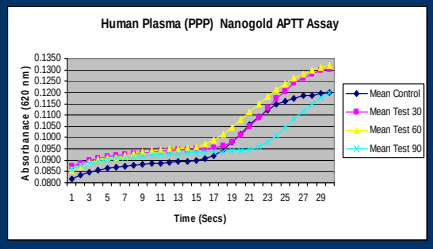
- **"Nanoparticle-protein corona":** NPs coated with proteins
- DLS limited with complex samples
- Appears useful for rapid documentation of particle size (with **uniform nanomaterials**), but technique requires refinement for other particle types

Global Clotting Times (aPTT)



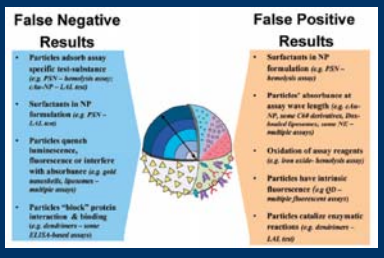
- Activated partial thromboplastin time (aPTT)
- Clotting time in seconds (max. rate of clot formation)
- "Intrinsic" system: misnomer
- **Contact activation** – biomaterials research

Effect of Au NPs on Clotting time



- Increased time to clot formation (with 90 nm)
- Decreased amplitude: Reduced amount of clot or clot stability
- Mechanisms: Interference with clot formation *in vitro* through interaction with clotting proteins?

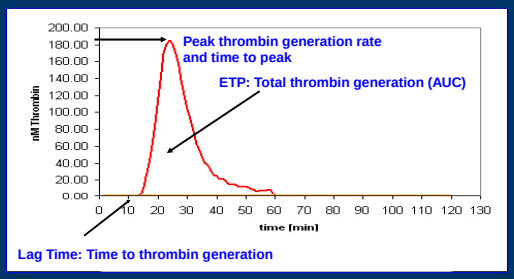
Standardizing Coagulation Assays



✓ Controls
✓ Standards
✓ Matrix effects

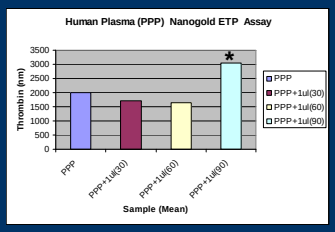
Dobrovolskaia et al. Molec Pharmaceutics, 2008

Endogenous Thrombin Potential (ETP)



- Clinical applications for determining who is at risk to form clots
- **Thrombin** is "bottom line" in clot formation by converting soluble fibrinogen to fibrin clots

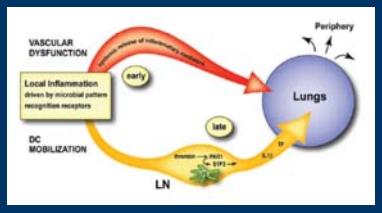
Nanoparticle Effect on Thrombin Generation



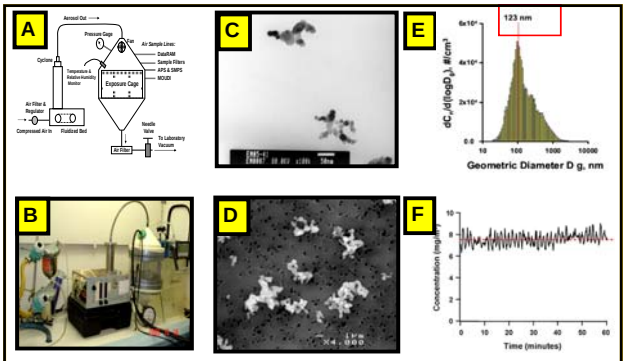
- Increased total thrombin generation (90 nm particle)
- **Nucleation effect *in vitro*?**: Particles provide surface for assembly of clotting factors to facilitate thrombin generation

Dissemination of coagulation & inflammatory mediators

- Wary translating *in vitro* findings to *in vivo* effects
- Particle exposures most likely through lungs
- Explore *in vivo* studies (animal inhalation models, *inflammation)

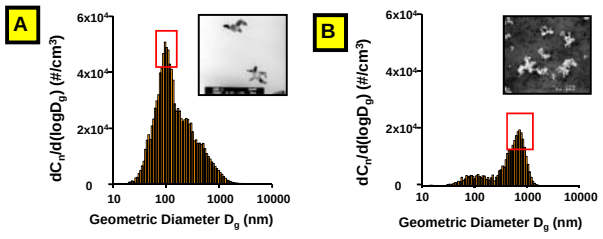


Proposed pathway for lymphatic dissemination of coagulation and inflammatory mediators in immune responses. Niessen et. al. Nature 2008



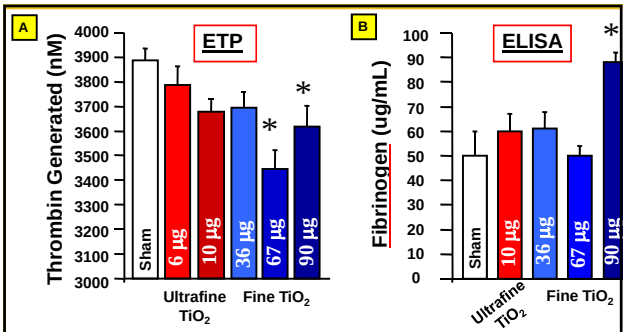
A) Inhalation exposure system schematic. **B)** Inhalation exposure system. **C)** TEM image of ultrafine TiO_2. **D)** SEM image of fine TiO_2. **E)** Ultrafine TiO_2 aerosol size distribution. **F)** Ultrafine TiO_2 aerosol generation.

Nurkiewicz et al. 2008



Particle size/distribution generated by the aerosol exposure system. **A)** Ultrafine TiO_2. **B)** Fine TiO_2.

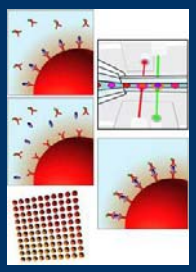
Nurkiewicz et al. 2008



A) *Endogenous Thrombin Potential* (ETP): The ability of rat plasma to generate thrombin measured using a fluorogenic thrombin substrate (Technothrombin TGA). ETP significantly lower than controls for rats exposed to the higher doses (67 and 90 µg fine Ti **B)** *Fibrinogen Levels*: Plasma fibrinogen levels were measured using an ELISA sensitiv rat fibrinogen. Fibrinogen levels were similar in all groups except in rats exposed to 90 µg TiO_2. *, P<0.05 vs. Sham-Control.

Luminex Technology

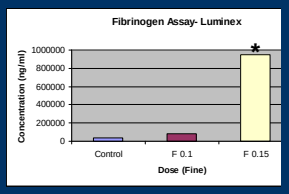
- Measure multiple analytes simultaneously in single reaction well (instead of multiple ELISAs)
- Capture analyte (ILs, cytokines, etc.) on microspheres distinguished by fluorescent intensity
- Add fluorescently labeled reporter tag
- Inject into instrument that can distinguish which microspheres (e.g. IL1 bead) and how much fluorescence is on the surface

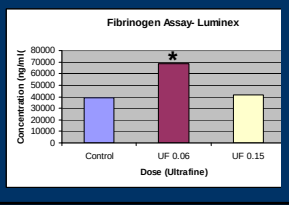


luminexcorp.com

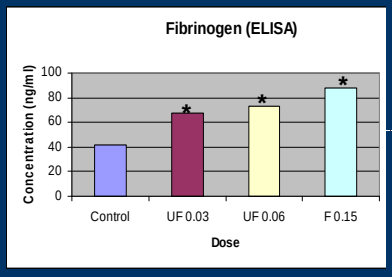
Fibrinogen by Luminex

- Rationale: Fibrinogen is independent risk factor for cardiovascular disease
- Findings: Variable increases in fibrinogen seen in most exposed rats
- Limited by variability of fibrinogen assays





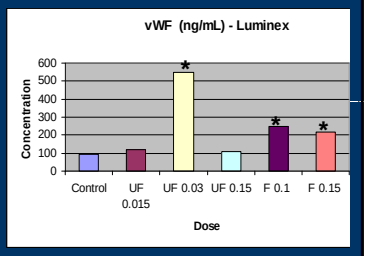
Confirm Findings with Increased Number of Exposures & Alternate Assay



Fibrinogen appears to acutely increase with short-term exposure to fine & ultrafine TiO_2

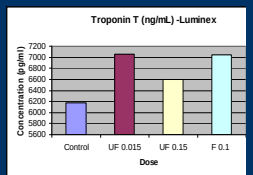
von Willebrand Factor (vWF)

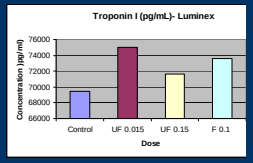
- Rationale: Risk factor for thrombotic events (not CV risk factor)
- Inflammatory marker or acute-phase reactant
- Findings: Variable increase in vWF with pulmonary TiO_2 exposures



Troponins

- Rationale: Marker of acute myocardial injury
- Finding: No significant differences between control & TiO_2 exposed animals
- Cannot extrapolate findings to human exposures

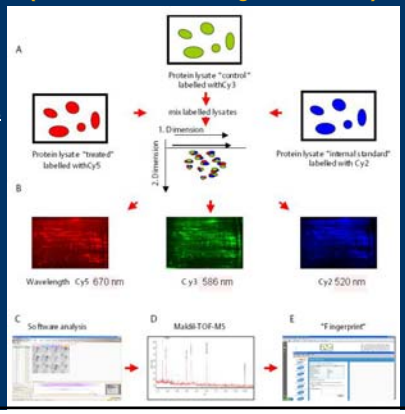




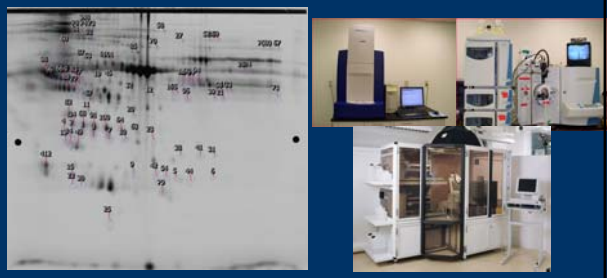
Global Protein Profiling
(DIGE: Differential in-gel extraction)

Rat Plasma Groups

1) Control
2) 0.15 mg/m³ UF TiO_2
3) 0.03 mg/m³ UF TiO_2
4) 0.10 mg/m³ fine TiO_2

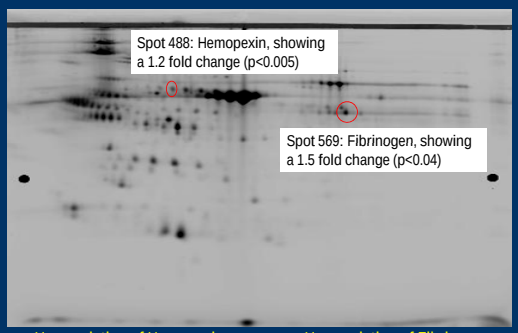


Image, compute, spot pick, mass spectrometry



- **428 distinct protein "spots"** identified by two-dimensional gel electrophoresis
- **72 spots were quantitatively different** between the test groups and controls by DIGE ($p < 0.05$)

Protein Changes Detected by DIGE



Up-regulation of Hemopexin Up-regulation of Fibrinogen

Significant differences in 45 distinct proteins by MALDI & LC/MS/MS

Coagulation Proteins (generally upregulated)

- Fibrinogen (α, β, γ chains): major clotting protein
- Plasminogen: degrades fibrin clots
- Antithrombin: anticoagulant
- Kininogen: absorbs to materials
- Other serine-protease inhibitors (serpins): control blood clotting proteins

Other Proteins of Interest

Inflammatory Proteins

- C-reactive protein: major inflammatory marker
- Complement C3: acute phase protein
- Complement C9: later phase complement system
- Pyrroline 5 carboxylate synthetase: stress protein
- Fetub: acute phase recovery protein

Miscellaneous Proteins

- Apolipoproteins (A1, E): lipid binding
- Desmoplakin: structural protein
- Angiotensinogen: increased by stress
- Ankyrin repeat domain
- Other poorly understood proteins not previously implicated in inflammatory responses

Proteomic Study Conclusions

- Exposure to fine and ultrafine TiO_2 through inhalation causes significant changes in the rat plasma proteome, many related to **coagulation** & **inflammation**
- These changes may be directly involved in the potential adverse effects of particle exposure, or may serve as markers (**biomarkers**) of toxicity
- Additional studies are needed to determine the specific protein "pathways" involved in the adverse health effects of small particle exposure (i.e. interactome)

How can human health be protected against hemostatic toxicity of nanomaterials?

- Minimize exposure in "zero-risk" society
- Identify synergistic risk factors for thrombotic disease
- Use model to predict potentially harmful effects of new and/or functionalized nanomaterials
- Decrease exposure through increasing aggregation & decreasing durability

❖ Develop biological sensors that can detect sub-clinical effects on hemostasis

"Every generalization is dangerous, especially this one"
Mark Twain

Nanotechnology Team

Nanoparticle characterization
- Nick Wu – Mech. Eng. WVU
- Darren Cairns – Mech Eng. WVU

Coagulation & Luminex
- Syamala Jagannathan –WVU Pathology
- Jeff Frisbee – CIRCS WVU

Nanomaterial Interactions
- Perena Gouma, Stony Brook University

Rat inhalation
- Tim Nurkiewicz, CIRCS, WVU
- Dale Porter, NIOSH
- Vince Castranova, NIOSH

Proteomics
- Linda Corum, WVU Pathology
- Steve Wolfe, WVU Pathology
- Andrew White, Univ. Charleston WV, INBRE student

Supported by Environmental Protection Agency (EPA #R832843)

Physical characteristics of nanoparticles affects interactions with aquatic organisms

Feswick, A.[1]; J. Griffitt[2]; J. Luo[1]; D. S. Barber[1]

1. Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL, USA.
2. Department of Coastal Sciences, University of Southern Mississippi, Ocean Springs, MS, USA.

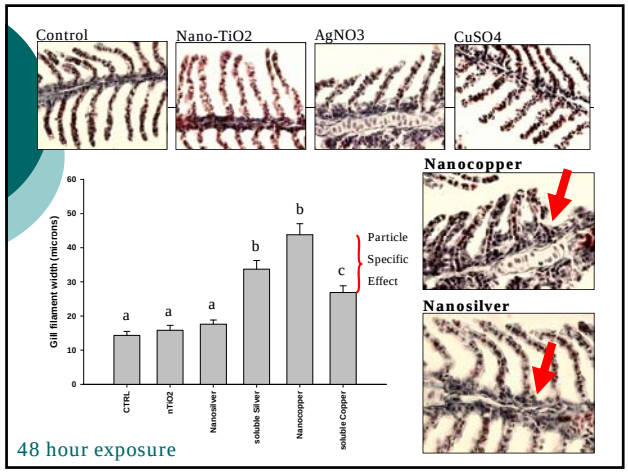
Importance of particle properties on toxicity

- Studies demonstrate that toxicity of nanoparticles can be affected by:
  - Size
  - Surface area
  - Hydrophobicity
  - Charge
- Developing an understanding of how these factors affect interactions with biological systems is critical to be able to predict toxicity

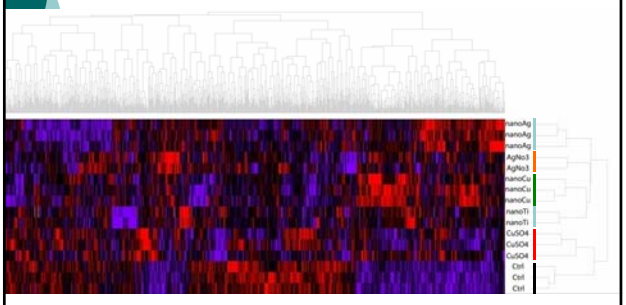
48 hour toxicity of metallic nanoparticles

	Nanoparticulate		Soluble	
	D. rerio	*D. pulex*	*D. rerio*	*D. pulex*
Nanocopper	**0.94 mg/L**	**60 ug/L**	0.13 mg/L	8.68 ug/L
Nanosilver	**7.1 mg/L**	**40 ug/L**	22.5 ug/L	0.85 ug/L
Nanoaluminum	> 10 mg/L	> 10 mg/L	7.92 mg/L	> 10 mg/L
Nano-TiO2	> 10 mg/L	> 10 mg/L	> 10 mg/L	> 10 mg/L
Nanonickel	> 10 mg/L	3.8 mg/L	> 10 mg/L	1.48 mg/L
Nanocobalt	> 10 mg/L	> 10 mg/L	> 10 mg/L	9.7 mg/L

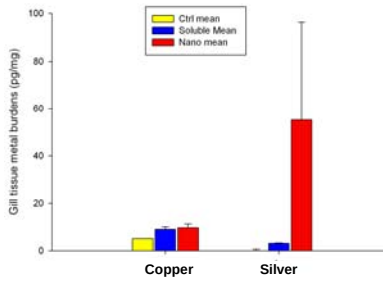
Griffitt et al., 2008



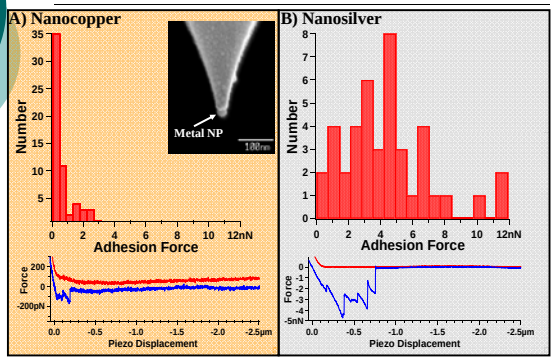
Gill transcriptional response varies with metal form and composition



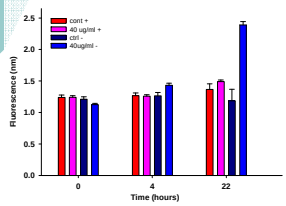
Gill metal content after exposure



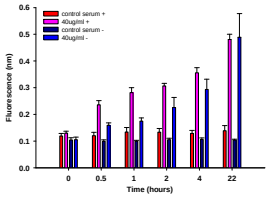
Nanosilver adheres to zebrafish gills



Uptake of two different dye-doped silica nanoparticles

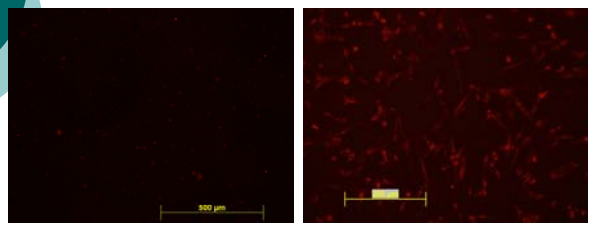


Rhodamine uptake (40ug/ml) in serum and serum-free media in RT-gill cells over time

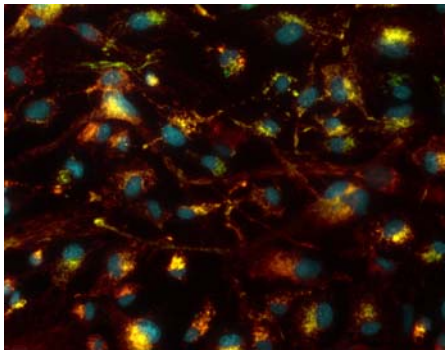


RuBpy Silica uptake (40ug/ml) in serum and serum-free media in RT-gill cells over time

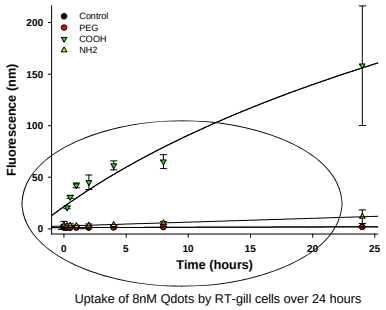
Uptake of Rubpy doped silica by gill cells



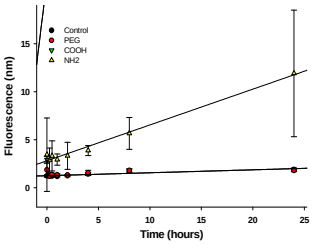
Intracellular localization of dye-doped silica particles



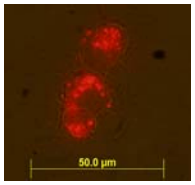
Uptake of Quantum dots by gill cells



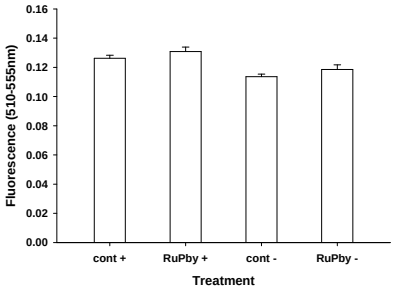
Uptake of 8nM Qdots by RT-gill cells over 24 hours



Uptake of 8nM Qdots by RT-gill cells over 24 hours

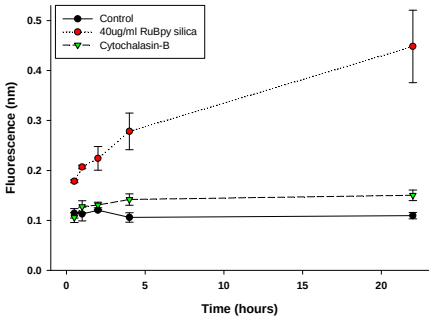


Uptake of RuBpy Silica requires active transport

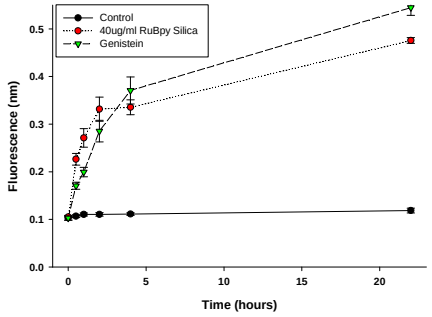


RuBpy Silica treated RT-gill cells (40ug/ml conc) at 4C for 1 hour

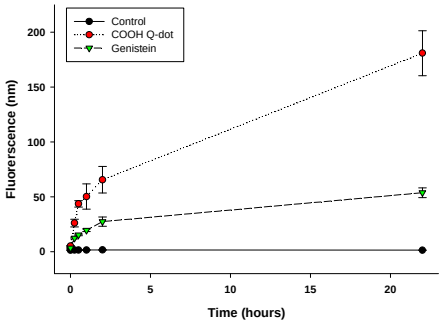
Uptake of nanoparticles by gills cells is mediated by endocytosis

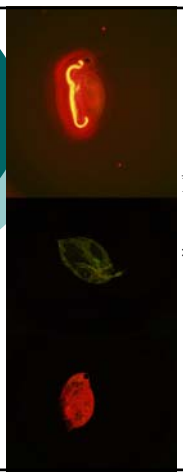


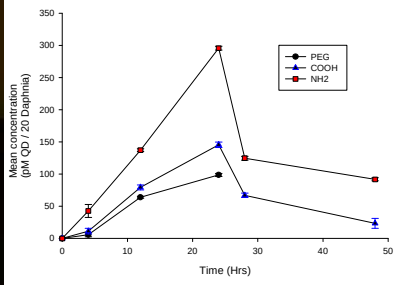
Genistein (caveolar-like inhibitor) does not reduce Rubpy silica uptake

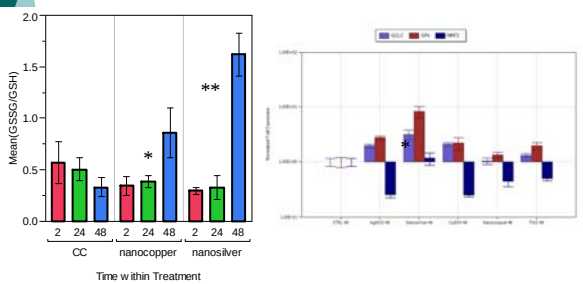


Genistein reduces COOH functionalized Q-dot uptake

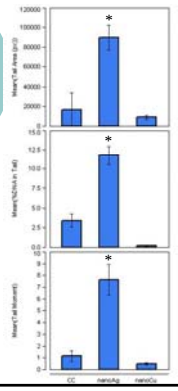




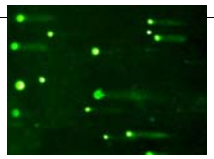




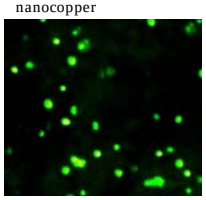
COMET assay



nanosilver



nanocopper



Conclusions

- Intact nanoparticles are taken up by gill cells and daphnia
- Physical properties of nanoparticles have significant impacts on their interaction with biological systems.
  - Charge is an important determinant of nanoparticle uptake
  - Effect of charge varies among models
- Mechanisms of particle uptake for particles with similar properties can differ
- Oxidative injury appears to play a role in nanosilver induced toxicity

Acknowledgements

- Dr. Joe Griffitt, April Feswick, Jing Luo
- Dr. Kevin Powers, Gil Brubaker
- Dr. David Julian

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  - National Science Foundation (BES054920)

Interagency Environmental Nanotechnology Grantees Workshop
November 19-21, 2008
Tampa, FL

Nanostructured Membranes for Filtration, Disinfection, and Remediation of Aqueous and Gaseous Systems

Grant Number: GR832372
8/1/05 – 7/31/08

Kevin Kit (PI),
Svetlana Zivanovic and
P. Michael Davidson

THE UNIVERSITY of TENNESSEE

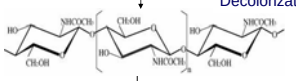
Objectives

- Develop electrospun nanofiber chitosan membranes to treat aqueous and gaseous environments by actions of filtration, disinfection, and metal binding
- Understand electrospinning process for chitosan in order to control membrane structure
- Investigate effect of membrane structure on filtration, disinfection, and metal binding
- Optimize performance/efficiency of chitosan membrane

Introduction - Chitosan

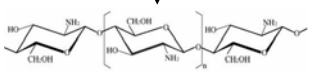
Chitosan is a carbohydrate polymer obtained from **Chitin** which is found in the shells of crustaceans, crab, shrimp etc

Decalcification in dilute HCl solution
Deproteination in dilute NaOH solution
Decolorization in sunshine or Oxalic acid



Chitin

Deactylation in conc. NaOH (40-50%)



Chitosan

Chitosan – Properties & Applications

- Insoluble in water, soluble only in aq. acids
- Amide group protonated at pH < 6.5
- **Anti-microbial properties**
- **Metal Binding Properties**
- **Non-toxic biodegradable natural polymer**

Biomedical

Dietary Supplement

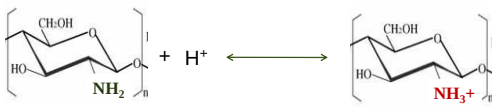
Chromatography & Waste water treatment

Food Packaging

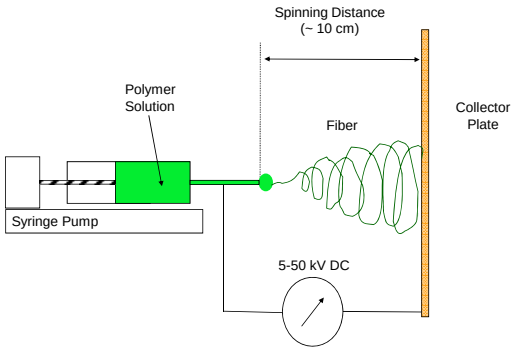
Chitosan Surface Properties

Surface properties of chitosan fibers is due to the protonated amine sites on fiber surface.

- Degree of protonation is a function of:
  - Degree of deacetylation
  - Solution pH
  - % Chitosan in fiber
  - Molecular weight
  - Crystallinity

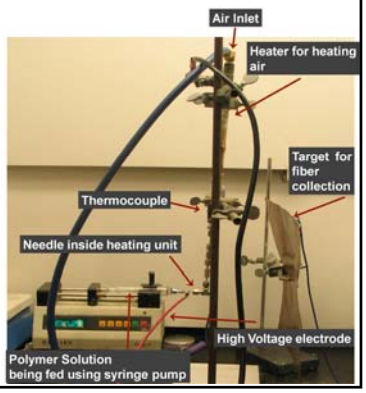


Electrospinning



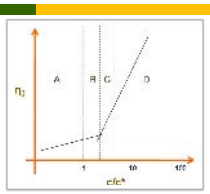
Experimental Set-Up

- Modified electrospinning set-up which allows us to heat solution while being ejected .
- Enables spinning of solutions at higher temperatures, by blowing hot air at different flowrates (25 ft^3/hr,75 ft^3/hr)
- Temperature controlled by variac



Electrospinning - Key Parameters

- **Polymer Solution**
  - Solution Viscosity/Entanglement density
    - Molecular Weight
    - Solution temperature
    - Concentration
      - Solubility
  - Surface tension
    - Polymer/Solvent
- Applied electric field
  - Voltage
  - Tip-target distance
- Solution flow-rate
- Solution conductivity



For electrospinning of PMMA

A. **(c/c*)< 1, dilute region, formation of droplets**
B. **1<(c/c*)<3, semi dilute un entangled region, formation of droplets along with few beaded fibers**
C. **3<(c/c*)<4, semidilute entangled region,formation of beaded fibers**
D. **(c/c*)>6, formation of uniform fibers without bead defects**

(Ref:P Gupta et.al, Polymer 2005, 46, 4799-4810)

Fabrication of Nanofibers - *Electrospinning*

Experimental Procedure

Polymers

- HMW Chitosan 80% DDA (Mv ~ 1400kDa) from Primex
- LMW Chitosan 83% DDA (Mw ~100 kda) from Sigma
- HMW PEO (900 kDa) from Scientific Polymer
- PAAm (5000) kDa from Scientific Polymer

+

Solvents

- aq.acetic acid

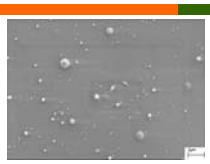
Electrospun @
- Polymer blend ratios
- Solution temperatures

i.e 25°C, 41°C,70 °C

Solution flow rate, spinning voltage, tip-target distance kept constant (0.08ml/min, 30kV,10cm)

Solutions prepared with optimum concentration of polymer dissolved in optimum strength of acid solvent so as to form beadless fiber mats

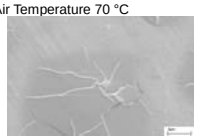
Electrospinning of Chitosan



1.4 wt % HMW Chitosan in 50 % Acetic Acid Solution
Air Flowrate 25 ft^3/hr
Air Temperature 70 °C

1.2 wt % HMW Chitosan + 1.5 wt% Urea in 90 % Acetic Acid Solution
Air Flowrate 25 ft^3/hr
Air Temperature 70 °C

5 wt % LMW Chitosan in 90 % Acetic Acid Solution
Spun at room temperature

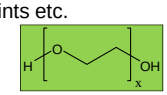


6 wt % hydrolyzed chitosan (Mv~20 kDa) in 90 % Acetic Acid Solution
Spun at room temperature

Electrospinning of Chitosan blends

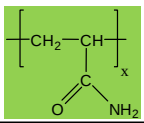
Chitosan/PEO blends

- PEO widely electrospun hydrophilic synthetic polymer
- Used as for non-fouling surfaces, packaging material for foods, binder and thickening agent for paints etc.



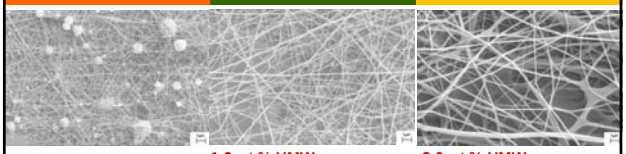
Chitosan/PAAm blends

- PAAm hydrophilic synthetic polymer, having amide groups like chitosan
- Used as a flocculent in waste water treatment as can bind heavy metal ions by forming coordination bonds
- Cationic polyacrylamide has been used for anti-microbial applications



Blend solutions prepared and fiber formation optimized to form, beadless fiber mats at highest chitosan content in blend solution

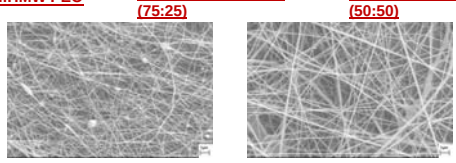
Chitosan/PEO - Effect of Blend ratios



1.33 wt % HMW Chitosan:HMW PEO (90:10)

1.6 wt % HMW Chitosan:HMW PEO (75:25)

2.0 wt % HMW Chitosan:HMW PEO (50:50)



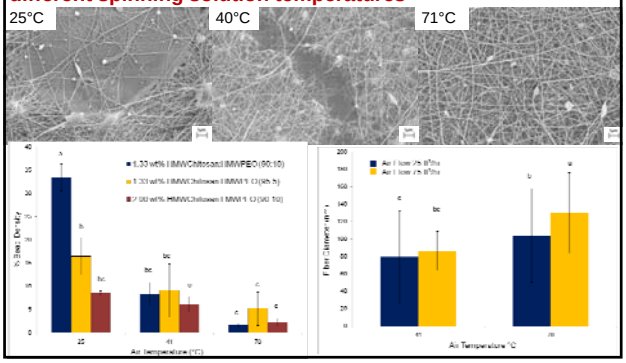
4.5 wt % LMW Chitosan:HMW PEO (90:10)

4.5 wt % LMW Chitosan:HMW PEO (75:25)

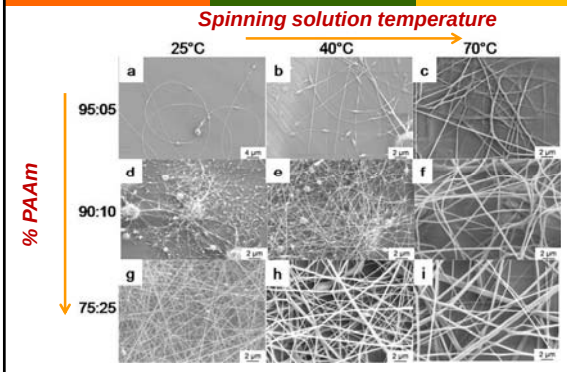
Chitosan/PEO blends - Spinning at Higher Temperatures

1.33 wt% HMW Chitosan:PEO (95:05) fibers obtained at different spinning solution temperatures

25°C 40°C 71°C

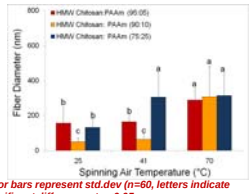


Chitosan/PAAm blends - Effect of Blend ratios and spinning temperature

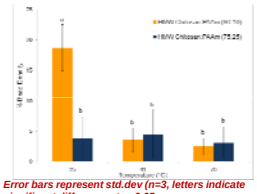


1.4 wt% HMW Chitosan:PAAm blend fibers obtained at different spinning solution temperatures, air flow rate 25ft³/hr

Chitosan/PAAm blends – FD and bead density



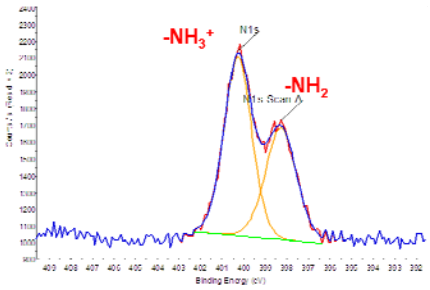
Error bars represent std.dev (n=60, letters indicate significant difference at p<0.05



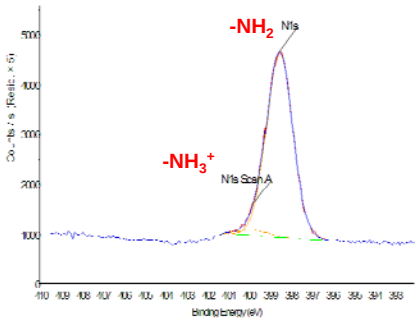
Error bars represent std.dev (n=3, letters indicate significant difference at p<0.05

Surface characterization of fibers - *XPS*

Pure 80 % DDA Chitosan



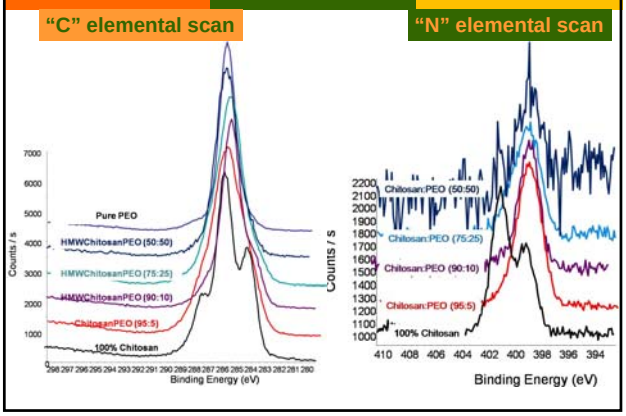
Pure PAAm



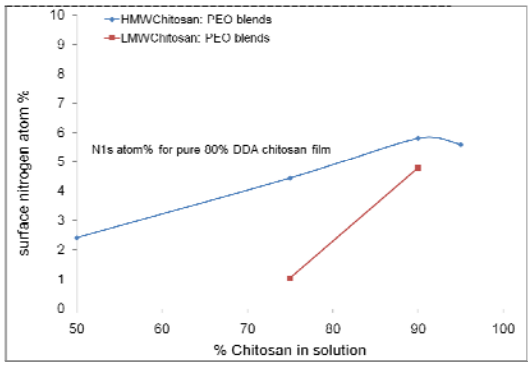
Surface Composition – Pure Polymers

Sample		Atom %					"C/N" ratio
		C1s	N1s	O1s	Cl2p	Al	
80% DDA HMW chitosan	theoretical	56.14	8.77	35.08			6.4
	from XPS (film)	61.11	5.6	28.18	5.11		10.92
Pure PEO	theoretical	66.67		33.33			∞
	from XPS (film)	66.77		32.39	0.11		∞
	from XPS (fiber)	96.26		3.74			∞
Pure PAAm	theoretical	60	20	20			3
	from XPS (film)	67.17	13.73	18.56	0.54		4.89
	from XPS (fiber)	61.24	12.48	22.68	0.45	3.14	4.91

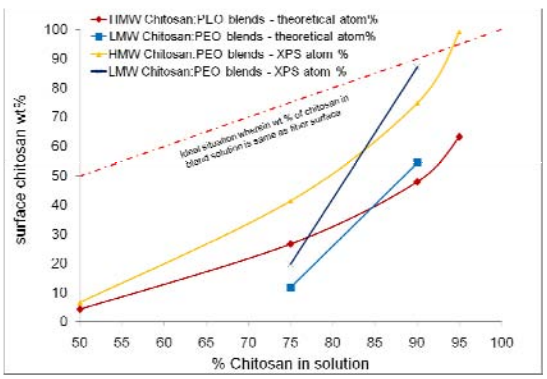
Chitosan/PEO blends



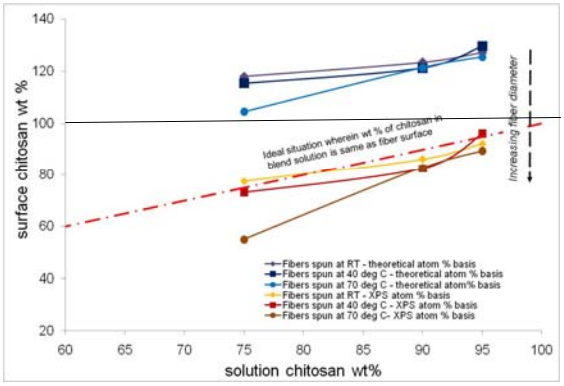
Effect of % chitosan in blend



Effect of % chitosan in blend



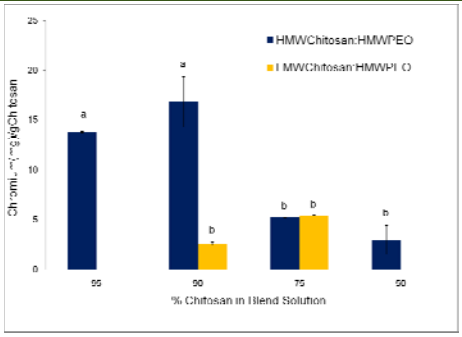
Effect of % chitosan in blend



Test Surface Properties - *Electrospun chitosan fibers*

Metal Binding- Chitosan/PEO blends

Effect of % chitosan in blend solution and chitosan molecular weight

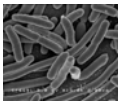


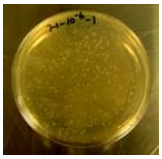
Error bars represent std.dev (n=3, letters indicate significant difference at p<0.05

Surface Properties – Antimicrobial

Electrospun fibers immersed in known concentration (9 log) of Escherichia E-coli K12 bacteria in phosphate buffer solution for 6 hours.

NH_3^+ binds with negative components of cell wall like lipids etc.





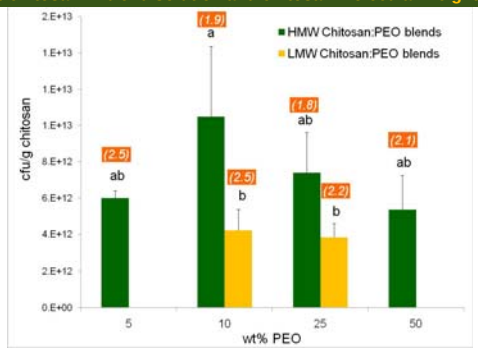
Survival rate of E-coli measured after 6 hours using pour-plate method using Trypticase Soy Agar (TSA) media

http://en.wikipedia.org/wiki/Escherichia_coli

2 log reduction is equivalent to 99% reduction in bacteria, 3 log is 99.9%

Anti-microbial – Chitosan/PEO blends

Effect of % chitosan in blend solution and chitosan molecular weight



Error bars represent std.dev (n=3, letters indicate significant difference at p<0.05, numbers in paranthesis is log reduction

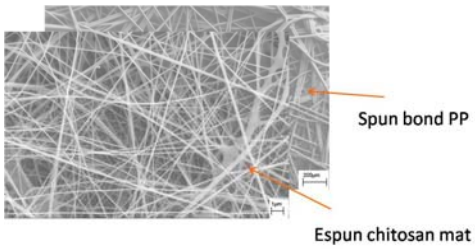
Anti-Microbial Chitosan/PAAm Blends

	Fiber Diameter (nm)	Log reduction (cfu/ml)	Std.Dev	cfu/g chitosan
1.4 wt% HMWChitosan:PAAm (75:25)@RT	132	3.11	0.35	2.61E13
1.4 wt% HMWChitosan:PAAm (75:25)@70 °C	328.03	3.17	0.19	2.47E13
1.4 wt% HMWChitosan:PAAm (90:10)@70°C	304.94	3.34	0.12	2.14E13
2.85 wt% LMWChitosan:PAAm (75:25)@RT	421.75	3.15	0.04	1.96E13

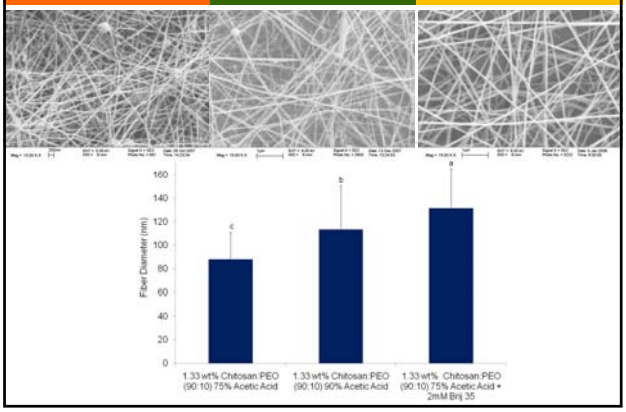
Fabrication and filtration performance - *Nanofibrous filter media*

Filtration

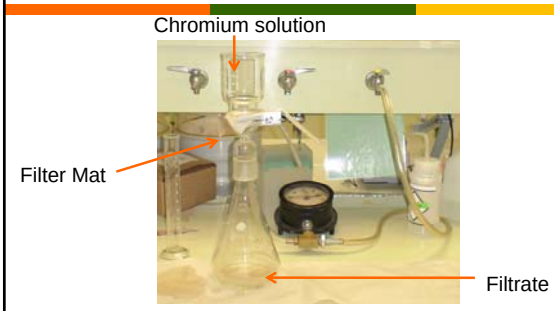
Fabrication of a composite filtration membrane by electrospinning chitosan blend fibers on spunbonded PP webs



Chitosan/PEO blends – Varying FD

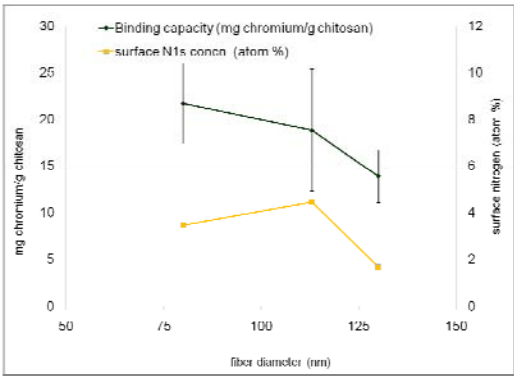


Filtration – Metal Binding

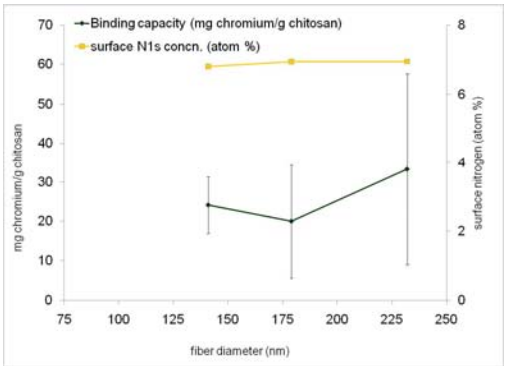


- 100 ml of 5 mg/l K_2CrO_4 solution passed through filter
- Cr(VI) reduction measured after 5 and 10 passes using UV-Vis
- Filtration time around 2 mins
- 1 mm Hg vacuum applied

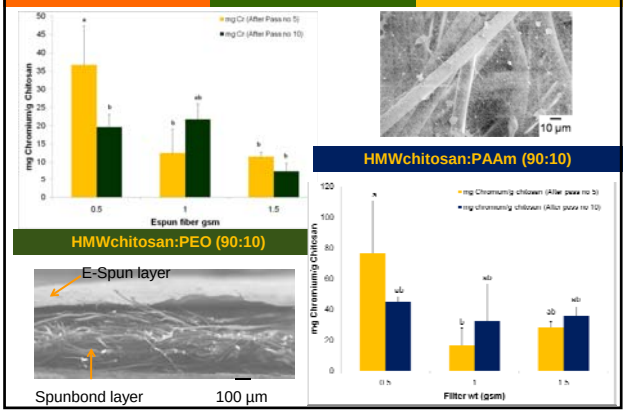
Effect of fiber diameter – chitosan/PEO



Effect of fiber diameter – chitosan/PAAm



Effect of gsm/basis weight



Surface structure & composition- After MB

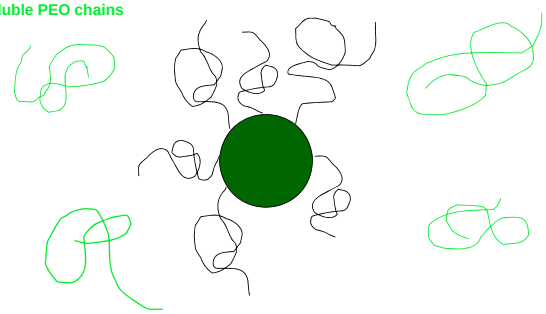
HMWchitosan:PEO (90:10)

HMWchitosan:PAAm (90:10)

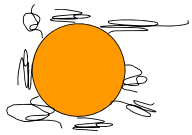
Surface structure & composition- After MB

tethered chitosan chains

soluble PEO chains



Surface structure & composition- After MB

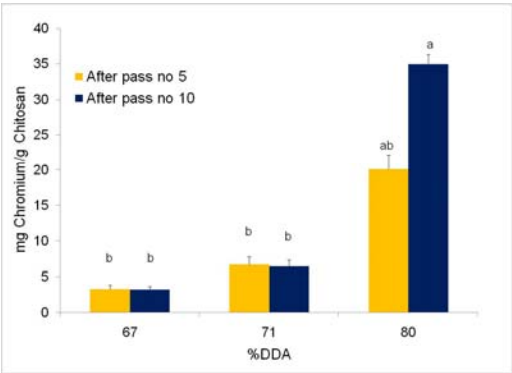


Effect of film formation

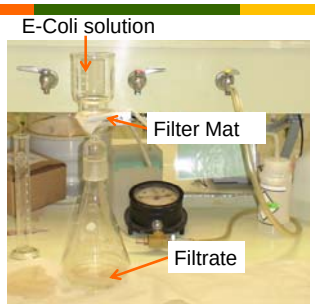
0.5 gsm *1.0 gsm* *1.5 gsm*

Formation of film layer could affect liquid flow through the fiber membrane possibly forming channels and affecting the wettability of the entire mat with increased gsm

Effect of % DDA

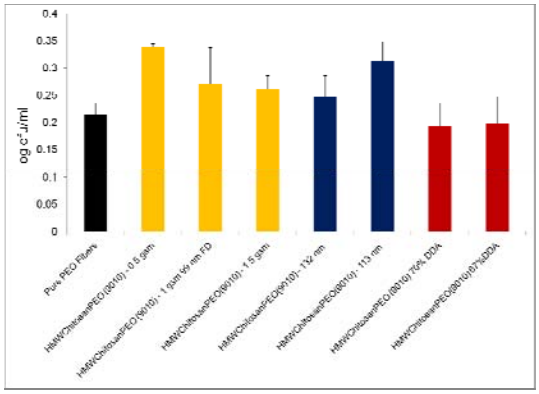


Filtration – Antimicrobial

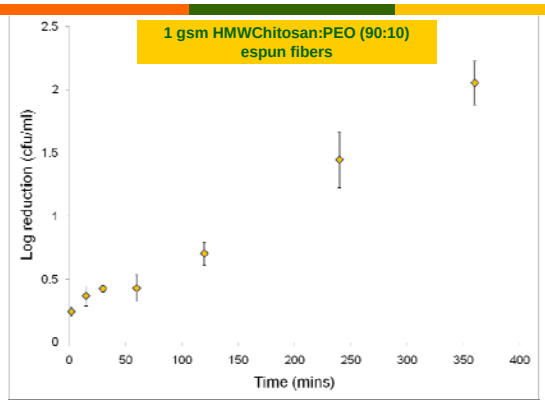


- 100 ml of 4 log E-coli solution passed through filter
- E-coli reduction measured after 1 pass using pour plate method
- Filtration time around 2 mins
- 1 mm Hg vacuum applied

Chitosan/PEO Filter - Antimicrobial

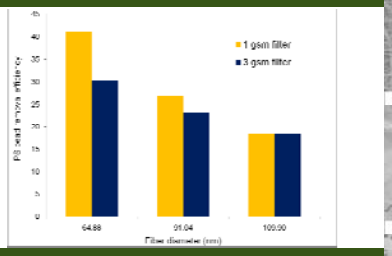


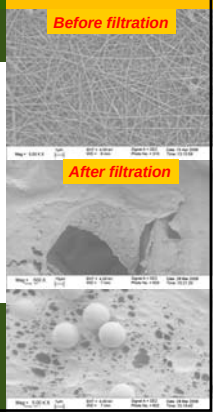
Chitosan/PEO Filter - Antimicrobial



PS beads filtration efficiency

Passed 10 ml of 200 ppm, 3µm diameter PS bead suspension through filter



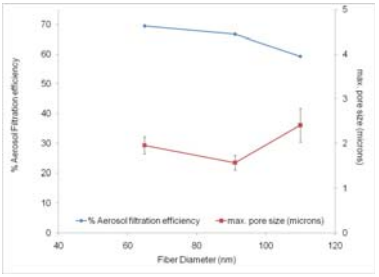


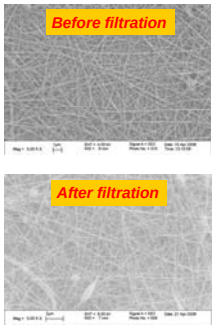
Electrospun layer damaged by vacuum applied during testing,therefore testing w/o vacuum

- 1 gsm filter mat ~ 50% removal efficiency
- 3 gsm filter mat ~ 70% removal efficienc

Aerosol Filtration Efficiency

Passed 31.9 liter/min of 0.075 µm diameter NaCl aerosol particles beads through 1 gsm espun filter using TSI 8130 filter tester





Conclusions

- Demonstrated ability to form beadless chitosan based nanofibers of controllable size and chitosan content
  - ***Chitosan/PEO blends – 95% chitosan in blend (FD 80 – 315 nm)***
  - ***Chitosan/PAAm blends – 90% chitosan in blend spun @ 70°C (FD 130 – 350 nm)***
  - ***Heating polymer solution helps expand processing window (% chitosan & fiber diameter)***
- Developed a model to predict Cr(VI) binding properties of chitosan nanofibers
  - ***For fiber diameter < 400 nm binding capacity decreased exponentially***
  - ***For 50<FD<200 nm % chitosan in blend and chitosan DDA influences binding capacity***

Conclusions

- Detailed surface analysis of fiber surface (XPS):
  - ***With decreasing % chitosan in blend solution surface chitosan wt% decreased non-linearly***
  - ***Nitrogen content decreases with increased fiber diameter and decreasing chitosan % DDA***
- Chitosan based nanofibers highly effective for:
  - ***HMWchitosan:PEO (90:10) blend fiber showed 16 mg chromium/g chitosan binding capacity compared to 0.44 mg chromium/g chitosan for a 93 µm thick film of same blend ratio***
  - ***Chitosan blend nanofibers show a 2-3 log reduction in E-coli K-12 with fiber mass 5 times less than blend films with similar anti-microbial properties***

Conclusions

- Nanofibrous filter media made using chitosan nanofibers showed:
  - ***0.5 gsm chitosan:PEO (90:10) nanofibrous filter media showed 35 mg chromium/g chitosan binding capacity***
  - ***After binding expts formation of film rich in chitosan seen on filter media***
  - ***Poor anti-microbial properties under dynamic testing***
- PS beads and aerosol filtration efficiencies increased with decreasing fiber size and increasing fiber gsm
  - ***Desired filtration efficiency can be achieved by optimizing electrospinning process parameters to control fiber size and porosity of filter media***

Acknowledgements

- Prof. Jochen Weiss
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- Prof. Roberto Benson
- Dr. Harry Meyer
- Dr. Peter Tsai
- Keyur Desai
- Christina Kriegel
- Jiajie Li
- Doug Fielden

Center for Life Cycle Analysis

COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK

Comparative Life Cycle Analysis of Nano and Bulk Materials in Photovoltaic Energy Generation

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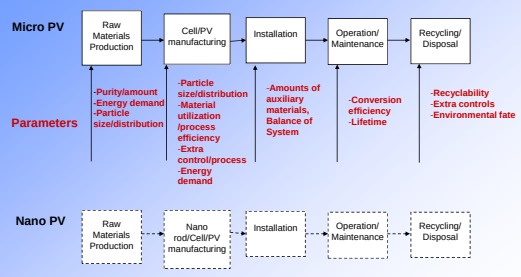
Grant # DE-FG36-08GO88101

Project Objectives

- Assess the life cycle mass and energy inventories of two main candidate nanomaterials for thin-film photovoltaic applications
- Make comparisons with the materials and solar cell structures that may replace, based on process-data
- Investigate the applicability of the results to other nanomaterial-based thin-film technologies

Comparative Life-cycle Analysis Framework



PV Paradigms for Comparison

- micro-crystalline CdTe vs. nanoscale CdTe *(long-term research -3rd generation PV technology*
  - *Vapor Deposition vs. Solution Growth techniques*
- amorphous Si vs. nano-crystalline Si *(technology near commercialization)*
  - *Vapor Deposition techniques*

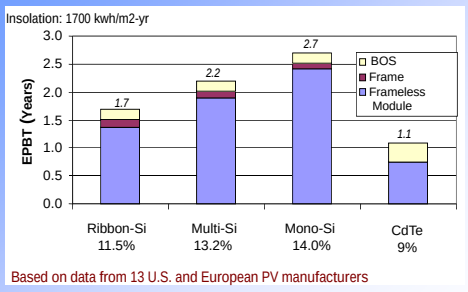
Current CdTe PV

Vapor Transport Deposition from 99.999% pure CdTe and CdS

First Solar, Perrysburg, Ohio

Module Efficiency = 10.5%

Energy Payback Times (EPBT)



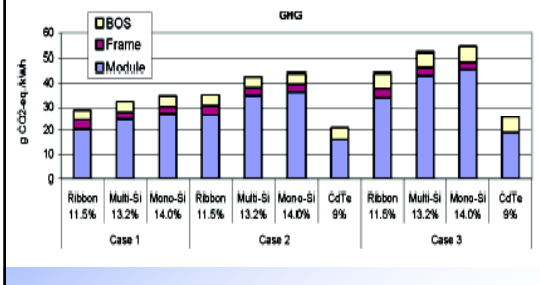
Based on data from 13 U.S. and European PV manufacturers

Collaborative work with U. Utrecht & Energy Research Center Netherlands
IEA PV Task 12

Fthenakis et al., Environmental Science and Technology *42, 2168-74, 2008*

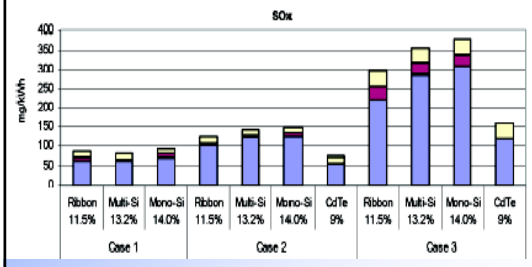
Life Cycle GHG Emissions –European and U.S. Cases

Insolation: 1700 kwh/m2-yr



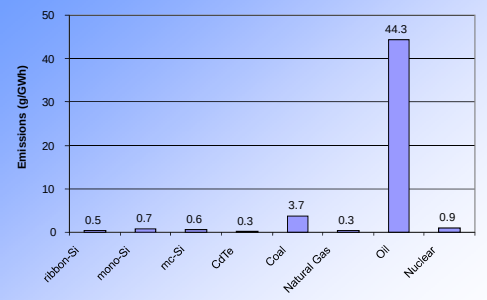
Fthenakis et al., Environmental Science and Technology ,*42, 2168-74, 2008*

Life Cycle SO2 Emissions –European and U.S. Cases



Fthenakis et al., Environmental Science and Technology *42, 2168-74, 2008*

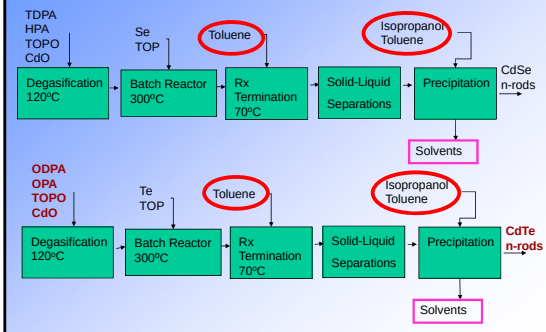
Life-Cycle Cd Emissions from Electricity Use



Fthenakis et al., Environmental Science and Technology *42, 2168-74, 2008*

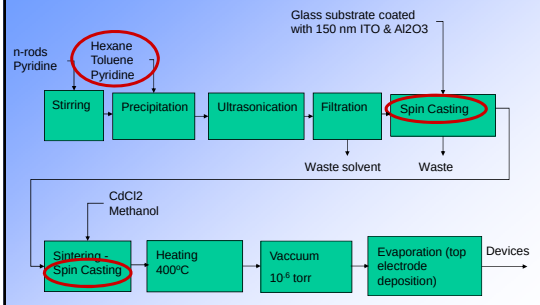
Nano CdTe PV Process

1. Synthesis of CdSe and CdTe nanorods



Nano CdTe PV Process

2. Device Fabrication



Preliminary Mass Balance

- Material Utilization in Nano-rods Synthesis (CdO and Te/Se used per mass of nano-rods produced):
  - Synthesis of CdTe rods: 77%
  - Synthesis of CdSe rods: 73%
- Material Utilization in Device Fabrication is very low: <1%

Mass of CdO, Te and Se used per m2	1.5	kg/m2
Total mass of materials used per m2	610	kg/m2

(glass substrate excluded)

EHS Implications

Solvent	LD_{50} (rat) (mg/kg)
Hexane	28700
Isopropanol	5045
Toluene	5000
Pyridine	891

- Hexane is classified as HAP by the EPA
  - It can probably be replaced by heptane
- Pyridine is an animal carcinogen
  - Its replacement is difficult

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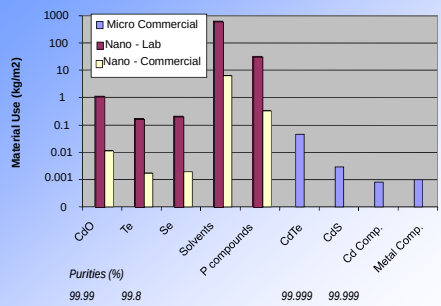
Material Use: Lab vs. Commercial scales



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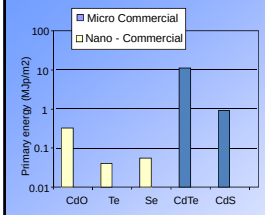
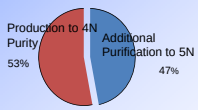
Effect of Material Purity on Energy Use

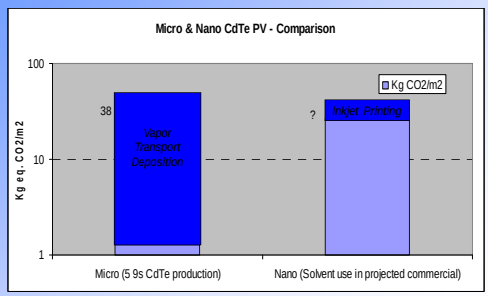




Energy Breakdown to produce 5N (99.999%) Te

15

Major CO2 emissions in CdTe manufacturing



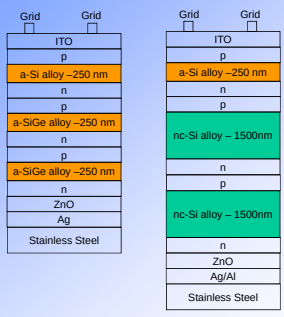
16

2nd Paradigm: amorphous-Si PV modules

United Solar, Auburn Hills, MI

17

Comparison of amorphous- and nanostructured- silicon PV

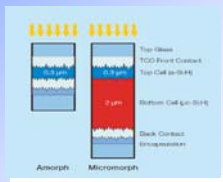


18

Comparison of amorphous- and nanostructured- silicon PV

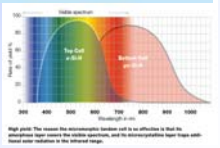
Add or replace layer (s) with nc-Si in a-Si module

- Typically top layer a-Si (200-300 nm) and middle or bottom layers nc-Si (1000-2000 nm)
- Change in deposition process (Increase H2 dilution & deposition times)



Life cycle implications:

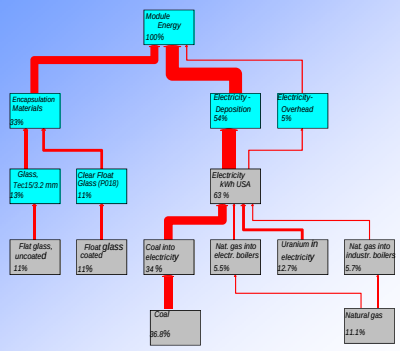
- Improved spectral response (thus efficiency)
- Increase energy and (upstream) material requirements
- Increase GHG emissions from module production



http://www.oerlikon.com/ecomaXL/index.php?site=SOLAR_EN_download_newsletter

19

Typical thin-film PV energy breakdown



20

Comparisons a-Si with nano-c-Si options

Module types	a-Si	Tandem a-Si/nano-c-Si
Thickness 1st layer a-Si (nm)	300	300
Thickness 2nd layer nc-Si (nm)	NA	1350
Deposition rate a-Si (nm/s)	0.3	0.3
Deposition rate nc-Si (nm/s)	NA	0.5
Reactor cleaning cycles	1	2
Silane input (g/m2)	2.8	12.6
Hydrogen input (g/m2)	17	213

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Amorphous vs. 'Micromorph' Si Cells Comparisons a-Si with nano-c-Si options

Cell Types	a-Si	Tandem a-Si/nano-c-Si
Small area cell efficiency (%)	13	15.4
Module efficiency (%)	7.6	8.7
Energy Ratio EPBT (yr)	2.3	2.7
CO2 emissions (kg CO_2/m^2)	59	74
Total GHG emissions* (kg $CO_{2eq.}$ /m^2)	94	141

* A cleaning cycle with SF6 = 116 g/m2 SF6

(Assuming 99% SF6 abatement efficiency)

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Amorphous vs. 'Micromorph' Si Cells Comparisons a-Si with nano-c-Si options

Forecast for 2013-2015

Cell Types	a-Si	Tandem a-Si/nano-c-Si
Module efficiency (%)	9	12
Energy Ratio EPBT (yr)	2	2
CO2 emissions (kg CO_2/m^2)	20	20
Total GHG emissions* (kg $CO_{2eq.}$ /m^2)	31	38

* A cleaning cycle with SF6 = 116 g/m2 SF6

(Assuming 99% SF6 abatement efficiency)

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What did we learn?

- We can project the mass and energy flows in future nanotechnology-enabled PV, guided by changes in material utilization, purity, deposition rates, film thickness and electric conversion efficiency.
- Solution grown nanostructured CdTe solar cells requires more extrinsic materials than micro-CdTe solar cells, but less volume and lower purity semiconductor precursors.
- Plasma-enhanced CVD of nc-Si requires materials for reactor cleaning that are GHG.
- Adding nc-Si layers to a-Si solar cells increases energy and GHG emissions that can be counterbalanced by cell efficiency increases.

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Next Steps

- Detailed investigation of solvent use & recycling efficiency
- Detailed investigation of energy use in solution-grown materials & in inkjet printing
- Investigation of CIGS PV production by inkjet printing
- Investigation of nanoparticle inks replacing screen-printed silver-glass-frit pastes for Si cell contact metallization

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Acknowledgment

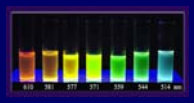
- Ilan Gur, UC-Berkeley
- Sergio Pacca, U. Michigan
- Ulrich Kroll, Oerlikon Solar-Lab
- Christophe Ballif, Institute of MicroTechnology
- Andrea Feltrin, Institute of MicroTechnology

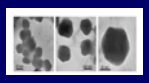
26

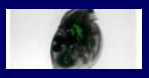
Life Cycle of Nanostructured Materials

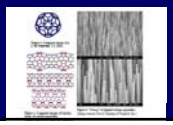
Thomas L. Theis
Hatice Sengul
Institute for Environmental Science and Policy

Siddhartha Ghosh
Department of Electrical and Computer Engineering
University of Illinois at Chicago



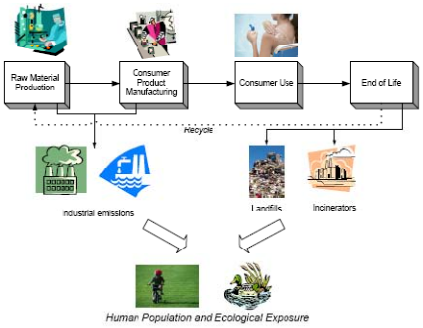






EPA Tampa 21 Nov 2008

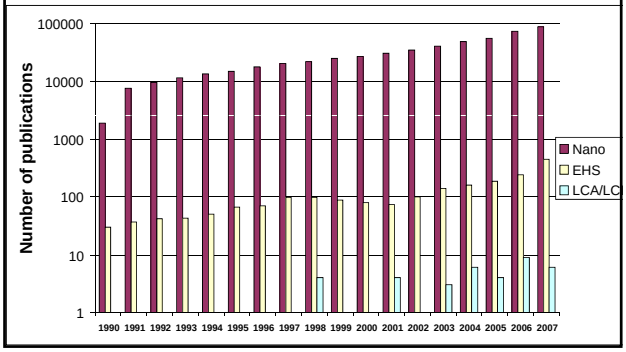
Why Life Cycle?



Why nano?

- Small amounts can have large effects
- Different physical properties as size decreases
- High specific surface areas
- Function can often be "tuned" by altering composition, size, shape, temperature, pressure
- Rich basis for new designs and applications
- Projected to generate $1.1 trillion in economic activity by 2016 (NNI, 2001)
- Production rates >10^5 tonnes/yr by 2020 (Royal Society 2004)
- An "enabling" technology with implications for energy, manufacturing, electronics, transportation, healthcare, pharmaceuticals, environmental control and purification, sensors and national security, chemical processing, and sustainable development

Nano-based publications



Nanomanufacturing

Definition: The fabrication of nanostructures, or the use of nano-based methods to manufacture a product

Two types: "Top-down" and "Bottom-up" (*Royal Society*, 2004)

Journal of Industrial Ecology 12(3):329-359

Top-down

Etching/milling

- **Etching**
- Wet etching (chemical etching)
- Dry etching
- reactive ion etching
- plasma Etching
- sputtering
- **Milling**
- Mechanical milling
- Mechanical alloying
- Cryomilling
- Mechanochemical bonding

Electrospinning

Lithography

•Conventional lithography
•Photolithography
•E-beam lithography

Next-generation lithography
Immersion lithography
Lithography with lower wavelengths than photolithography
Extreme ultraviolet (soft X-ray) lithography
X-ray lithography
Lithography with particles
e-beam lithography
Focused ion-beam lithography
Nanoimprint lithography
Soft lithography

Bottom-up

Vapor-phase deposition

- Vapor phase epitaxy
- Metal organic chemical vapor deposition
- Molecular beam epitaxy
- Plasma enhanced chemical vapor deposition
- Sputtering
- Evaporation

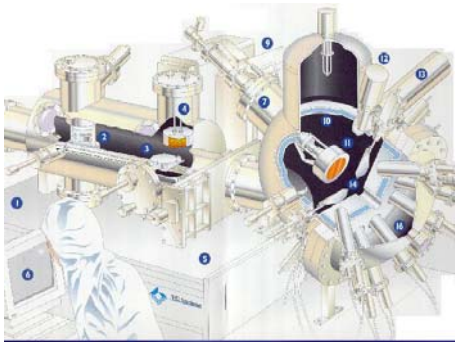
Nanoparticle synthesis

- Evaporation
- Laser ablation
- Flame synthesis
- Arc discharge

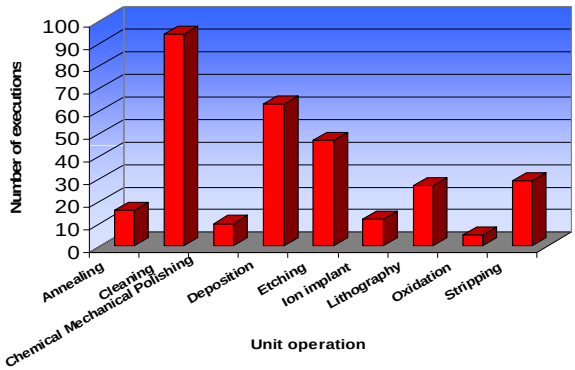
Liquid phase

- Precipitation
- Sol-gel
- Solvothermal synthesis
- Sonochemical synthesis
- Microwave irradiation
- Reverse micelle

Molecular beam epitaxy



Numbers of Mfg steps per wafer



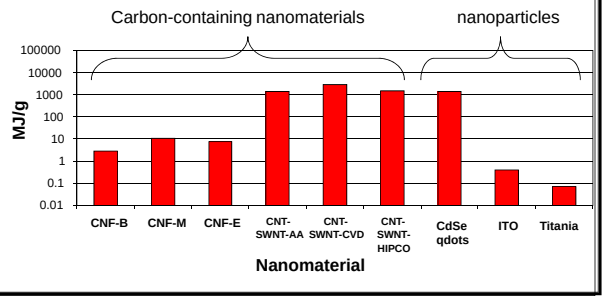
Sources of nanomanufacturing impacts

- **Strict purity requirements and less tolerance for contamination during processing than more conventional manufacturing processes (up to "nine nines").**
- **Low process yields or material efficiencies**
- **Repeated processing, postprocessing, or reprocessing steps of a single product or batch during manufacturing**
- **Use of toxic/basic/acidic chemicals and organic solvents** (eg. As, Ga, In, Cd, Zn, Sn, Sb, Hg, solvents, chlorinated and perfluorinated compounds, etc.)

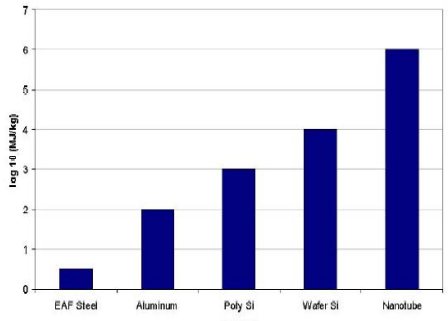
Sources of nanomanufacturing impacts

- **Need for moderate to high vacuum and other specialized environments such as high heat or cryogenic processing**
- **Use of or generation of greenhouse gases (directly or through energy consumption)**
- **High water consumption**
- **Chemical exposure potential in the workplace and through technological/natural disasters**

Cumulative energy requirement of nanomaterials



Energy requirements of materials

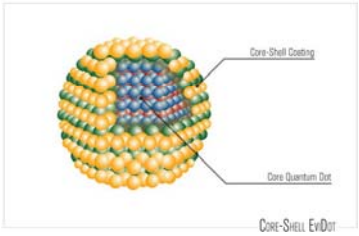


Gutowski et al. A Thermodynamic Characterization of Manufacturing Processes IEEE (2007)

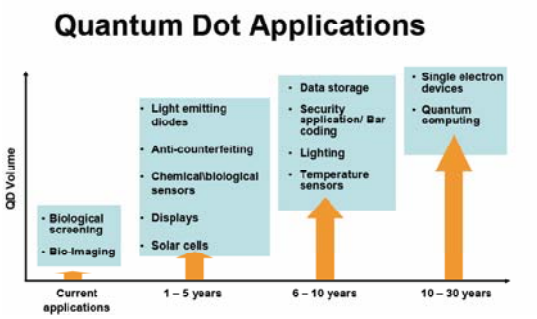
Some semiconductor materials (>600)

- Elemental: Si, Ge
- III-V binary: AlAs, GaAs, BN, GaN
- III-V ternary: $Al_xGa_{1-x}As$, AlInAs, InAsSb
- III-V quaternary: AlGaAsP, InGaAsN
- II-VI binary: CdSe, CdS, CdTe, ZnO, HgTe
- IV-VI binary: PbSe, PbS, PbTe, SnS
- II-V compound: Cd_3P_2, Cd_3As_2, Zn_3Sb_2
- Other: In_2O_3:SnO_2 (ITO)
- Organic: Anthracene, polymers
- Magnetic: GaMnAs

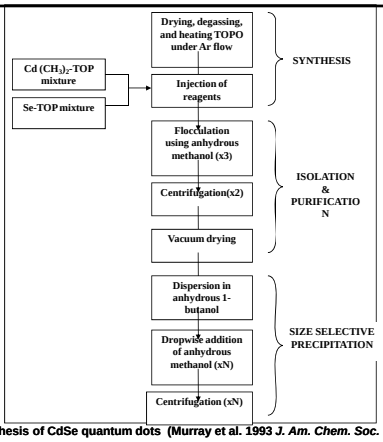
Quantum dots



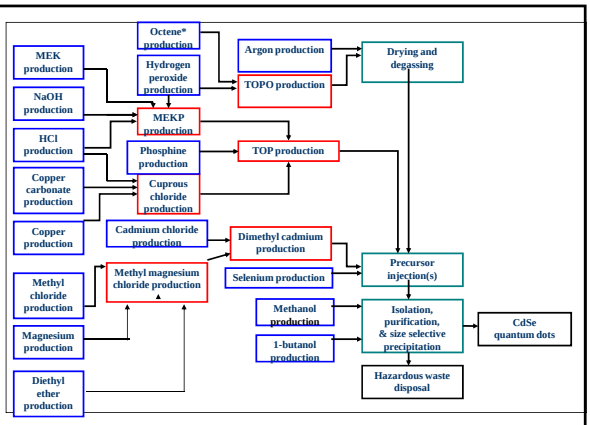
Quantum Dot Applications



O'Brien, P. "Making and Using Nanoparticles the Marketplace Potential", 2006 Expert Lecture Series Leaders in Nanotechnology, The Manchester Materials Science Centre.

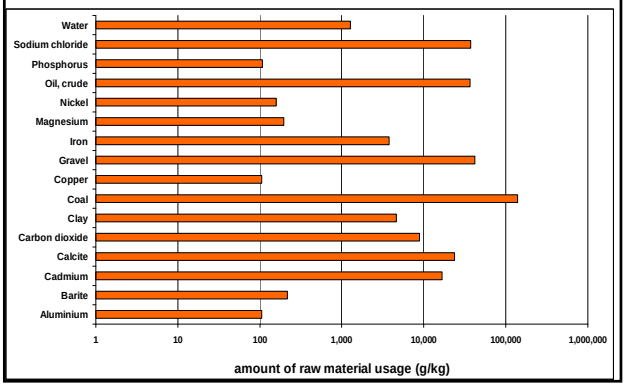


Liquid phase synthesis of CdSe quantum dots (Murray et al. 1993 *J. Am. Chem. Soc.* 1993, 115, 8706-8715)

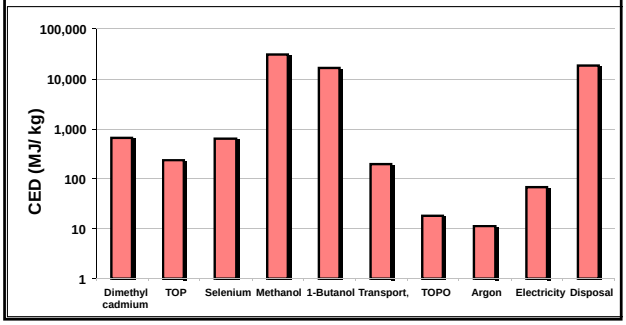


Material flows for the synthesis of CdSe quantum dots using sol-gel.

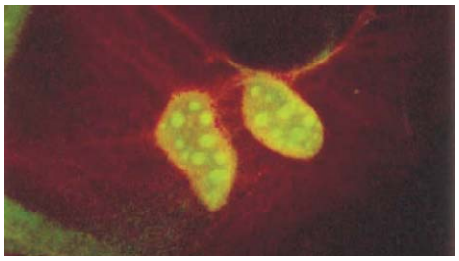
Raw material use for CdSe qdots



Cumulative energy demand CdSe q-dots

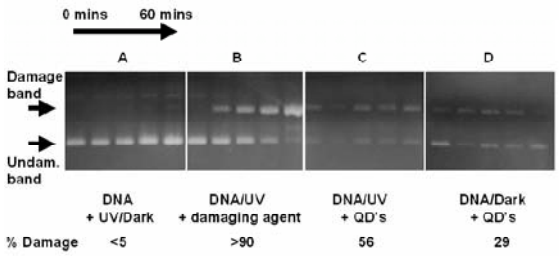


Fibroblast embedded with CdSe QD



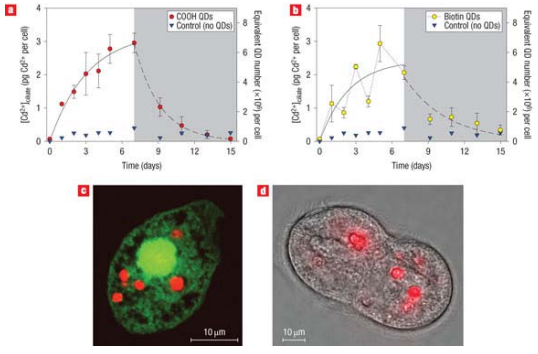
(Wang et al. 2005. Bioapplications of Nanosemiconductors. Materials Today 8(5): 20-31)

DNA damage of CdSe



Green, M. and E. Howman
"Semiconductor quantum dots and free radical induced DNA nicking"
Chem. Commun., 2005, 121 - 123

Uptake and depuration of QDs by *T. pyriformis*



Holbrook, RD et al. "Trophic transfer of nanoparticles in a simplified invertebrate food web" NATURE NANOTECHNOLOGY 3(6): 352-355 (2008)

Aquatic reactions

Solubility: $A_xB_{y\,(s)} \rightarrow A^{+y} + B^{-x}$
$(\log K_{s0} = \log [A^{+y}] + \log [B^{-x}])$

Protolysis: $HB_{(aq)} \rightarrow H^{+} + B^{-}$
$(pH = -\log K_a + \log [B^{-}]/[HB])$

Oxidation half-cell: $B^{-x} \rightarrow B^{(-x+1)} + e^{-}$
$(p\varepsilon = -\log K_o + \log [B^{(-x+1)}]/[B^{-x}])$

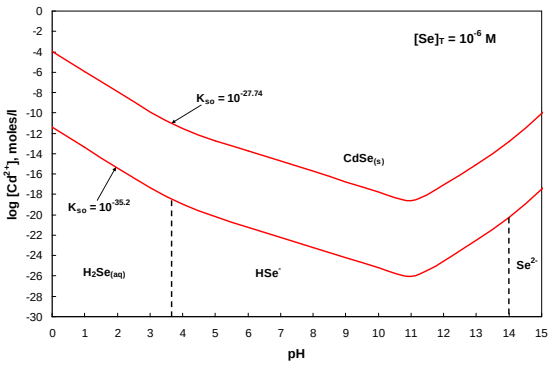
Aqueous solubility search

Sulfides, most oxides: abundant

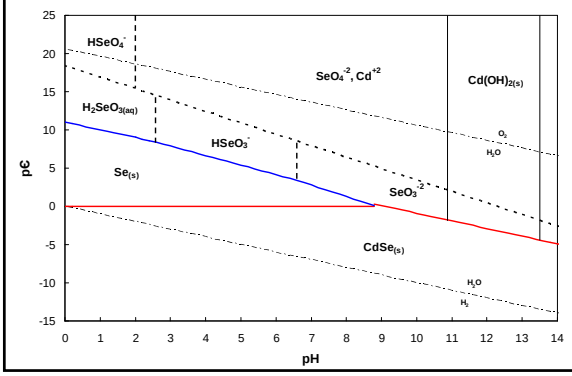
Binary selenides, tellurides: some

Nitrides, phosphides, arsenides, stibnides, tertiary, quaternary, doped, magnetic: none

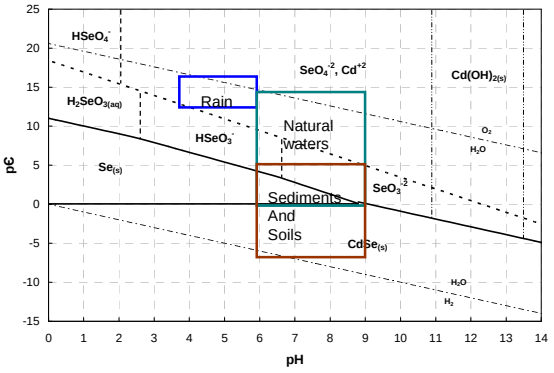
Solubility of CdSe in water



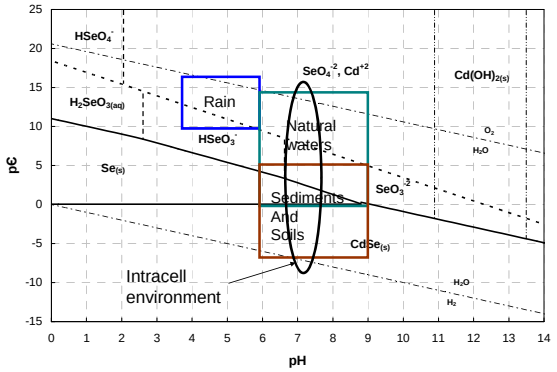
pε-pH diagram for CdSe



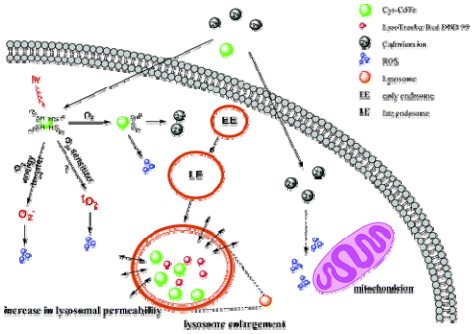
CdSe in aquatic environments



CdSe in aquatic environments



Mechanism of toxicity



Schematic representation of the mechanistic pathways implicated in the cytotoxicity of CdTe QDs in live cells, highlighting the salient changes in cellular morphology, the chemical species involved, and the chemical reactions that can lead to ROS and free Cd2+ ion release (Cho et al. (2007).

Other considerations…

- Since many semiconductors are comprised of electron poor and electron rich components, solubility results for CdSe may be generally true for many compounds
- But favorable thermodynamics doesn't always mean fast reaction rates
- Kinetics depend on many factors: temperature, ionic strength, presents of catalysts (or inhibitors), *particle size*, external oxidizing agents, light, etc.
- And, the impact of nanostructured materials on human and ecosystem function will depend on other systemic factors (loading, exposure, interdependence of components, mode of toxicity or uptake…)

Concluding remarks

- The ability to make and control very small structured materials has very large implications for human health, comfort and convenience, and economic well-being
- In comparison to basic nanoscience and the fabricaton of nanostructures, our understanding of environmental and life cycle behaviors of nanomanufacturing, nanomaterials, and nano-containing products exhibit exceptional lags
- Even so, it is clear that there will be a suite of significant waste management problems

Evaluating the Impacts of Nanomanufacturing via Thermodynamic and Life Cycle Analysis

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

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

Interagency Workshop on the Environmental Implications of Nanotechnology

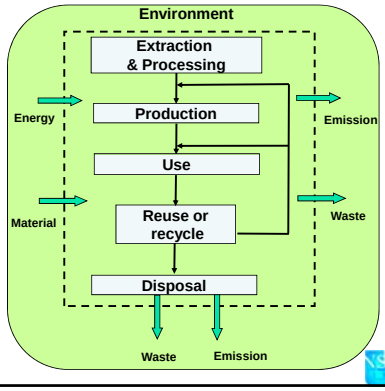
November 20-21, 2008, Tampa, Florida

Motivation

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

Life Cycle Analysis

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



Challenges in LCA of Nanotechnology

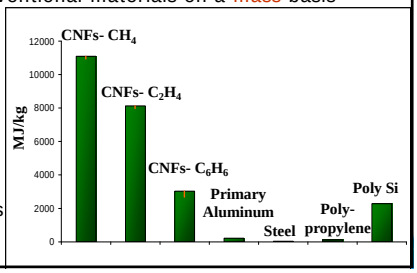
- Inventory for nanomanufacturing is not available
- Impact of engineered nanomaterials on humans and ecosystems is only partially known
- Predicting life cycle processes and activities is difficult since the technology is still in its infancy

Objectives

- Life Cycle Evaluation of Nanoproducts & Processes
  - Establish Life Cycle Inventory modules for Nanomaterials
  - Carbon Nanofibers
  - Polymer Nanocomposites Products
  - Titanium Dioxide nanoparticles
- Develop methods to identify opportunities for improving the life cycle
- Explore predictive model for LCA and impact assessment
  - Relationship between life cycle inputs and impact
  - Relationship between properties of nanoparticles and their impact

LCA of Carbon Nanofibers

- Extraordinarily high tensile strength
  - Tensile strength-12000 MPa, *10 times that of Steel*
  - Increases mechanical and impact strength of polyolefins
- Life cycle energy consumption is at least 100 times larger than conventional materials on a mass basis
- Greenness of CNF nano-products will depend on quantity used and resulting benefit
- Polymer nanocomposites



Khanna, Bakshi, Lee, *J. Ind. Ecol*, 2008

Polymer Nanocomposites


Housing


Aerospace


Automotive

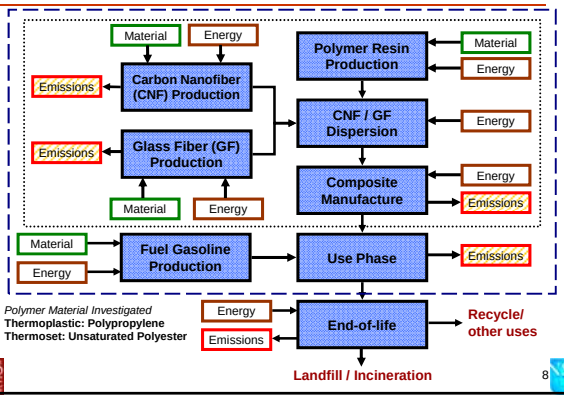
Polymer Nanocomposites


Sporting goods

Packaging

- Attractive features of Polymer Nanocomposites
  - Enhanced mechanical properties
  - High strength to weight ratios
  - Other functionalities- electrical conductivity

Life Cycle- Polymer Nanocomposites



Polymer Material Investigated
Thermoplastic: Polypropylene
Thermoset: Unsaturated Polyester

Functional Unit & Theory

- Functional Unit- Different material components with equal stiffness or strength
- For equal stiffness design, the material property to be maximized is:

$$\frac{E^{1/3}}{\rho} = \text{ASHBY'S MATERIAL INDEX}$$

- Structural elements perform a physical function
- Performance is defined as:

  p= f(F, G, M) where p=mass, volume, cost, etc.

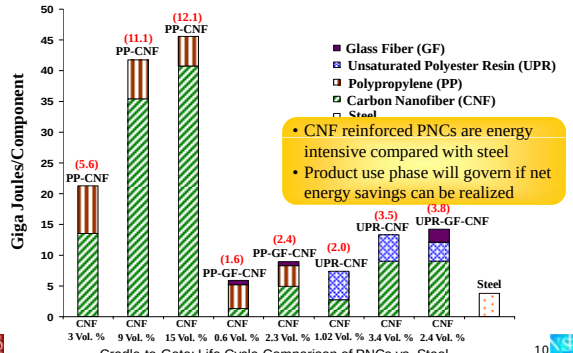
  F= functional requirements; G= geometric requirements

  M= material requirements; $p = f_1(F).f_2(G).f_3(M)$

Optimum Design- selection of material and geometry that maximize or minimize 'p' according to desirability

Ashby, M., *Matls. Selection in Mech. Design*: Pergamon Press

Energy Analysis for Equal Stiffness



- CNF reinforced PNCs are energy intensive compared with steel
- Product use phase will govern if net energy savings can be realized

Cradle-to-Gate: Life Cycle Comparison of PNCs vs. Steel

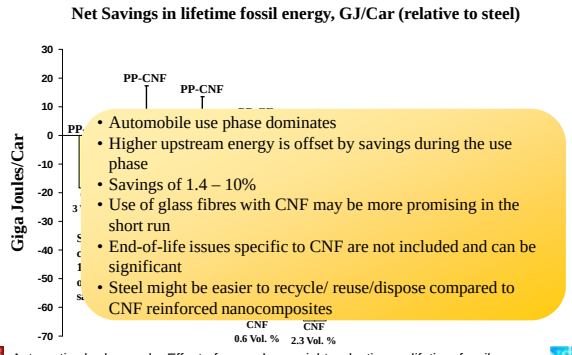
Auto Panel Case Study – Assumptions

- Midsize Car (3300 lbs) with polymer nanocomposite body panels vs. steel body panels
- Car lifetime: 150,000 Vehicle miles traveled (VMT)
- Constant fuel economy over the vehicle life time
- Body panels constitute 10% of the vehicle weight
- Sedan equivalent for fuel economy calculation

$$\text{mpg}_2 = \text{mpg}_1\left(\frac{W_1}{W_2}\right)^{0.72}$$

- Steel mix – 30 (virgin): 70 (recycled)
- Energy requirements for nanoparticle dispersion are ignored

Savings in Life Cycle Fossil Energy



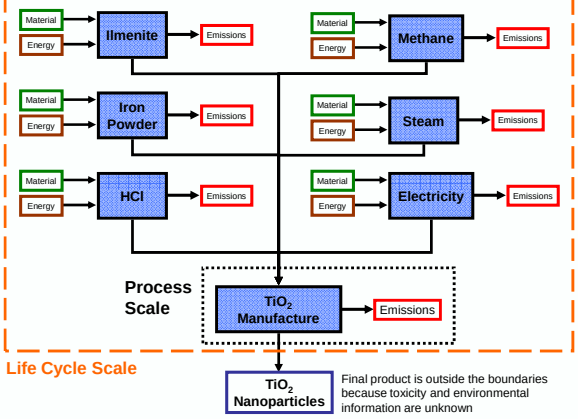
- Automobile use phase dominates
- Higher upstream energy is offset by savings during the use phase
- Savings of 1.4 – 10%
- Use of glass fibres with CNF may be more promising in the short run
- End-of-life issues specific to CNF are not included and can be significant
- Steel might be easier to recycle/ reuse/dispose compared to CNF reinforced nanocomposites

Automotive body panels- Effect of secondary weight reduction on lifetime fossil energy savings

LCA of TiO_2 Nanoparticles

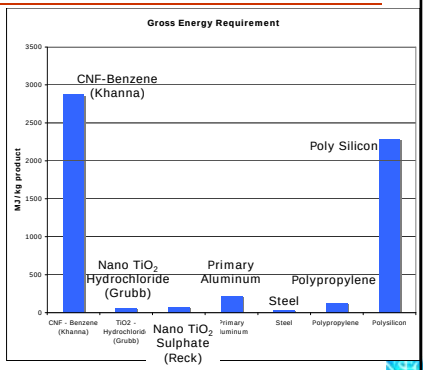
- Altair hydrochloride process
  - Ilmenite feed
  - Tailored for nanoparticle production
  - Near complete recycle of HCl
  - Claims of energy savings
  - Currently at the pilot stage (10,000 kg/yr)
- Life cycle inventory is needed
- Opportunity to identify improvements at early stages of development
- Some applications of nano Titania
  - Sun screens and cosmetics
  - Photocatalysts, etc.

LCA Boundary



Life Cycle Energy Consumption

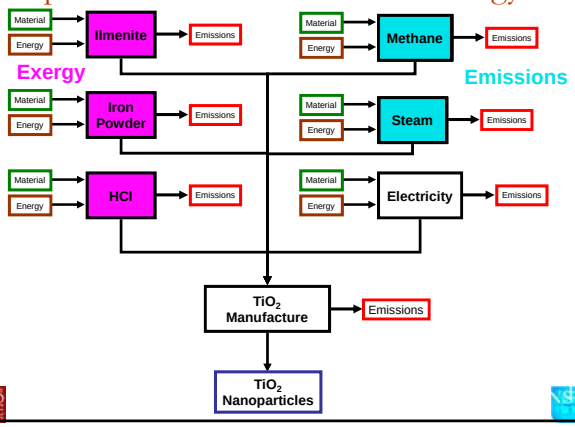
- Nano TiO2 consumes much less energy per ton than CNF
- However, total quantity of nano TiO2 used globally may be much larger



Identifying Improvement Opportunities

- LCA does have an improvement analysis step
  - Focus on modifications to reduce emissions with largest impact
  - Often receives little attention
- Consumption of resources has not received adequate attention in LCA
- This work explores the use of thermodynamic methods for identifying improvement opportunities
  - Energy and Exergy analysis

Improvements – Emissions vs. Exergy



Summary

- Developed life cycle inventories for polymer nanocomposites and nano TiO_2
- LCA of polymer nanocomposites for automotive use
  - 4-10% life cycle energy savings, mainly due to fuel savings in use phase
- LCA of nano TiO_2
  - Significantly less energy use and impact as compared to carbon nanofibres
- Completed life cycle exergy analysis of nano TiO_2
  - Complements emissions based LCA
  - Identifies improvement opportunities

Future Work

- Focus on other nanoproducts based on CNF or nano TiO_2
- Explore statistical relation between resource use and impact for predictive LCA
- Risk analysis

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

NC STATE UNIVERSITY

Impact of Physicochemical Properties on Skin Absorption of Manufactured Nanomaterials

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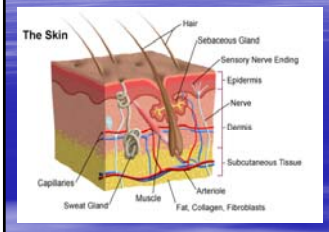
Project Significance

- Skin is the largest organ protecting our body from exogenous toxins and particulates.
- Skin confronts nanomaterials from occupational and environmental exposures.
- Hundreds of consumer products are already on the market. Sunscreens made of nanomaterials (TiO_2, ZnO) show superior UV protection performance. Fullerenes is used as radical sponge for facial moisturizer, anti-aging and anti-oxidant additives in skin care products
- Skin absorption of nanomaterials is critical in safety evaluation and risk assessment of the nanomaterials.

Skin Exposure



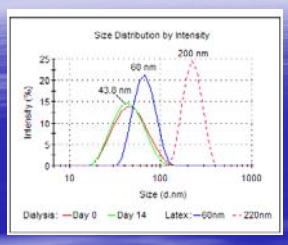
Stratum corneum (uppermost layer of skin, ca. 15 μm) is the primary barrier for small molecules or particulates.

- What happens if skin is exposed to nanoparticles?
- Which factors affect their absorption?
- How the physicochemical properties of the nanomaterials dictate their skin permeability ?
- Could a predictive model be established via structure-permeability relationship?

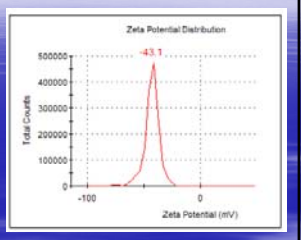
Impact of Physicochemical Properties on Skin Absorption of Manufactured Nanomaterials

- The objective of this project is to establish a structure-permeability relationship for skin absorption of manufactured nanomaterials for safety evaluation and risk assessment.
- Four dominant physicochemical properties (particle size, surface charge, hydrophobicity and solvent effects) in skin absorption will be studied.
- Fullerene and its derivatives will be used as model nanomaterials.

nC_{60} Characterization

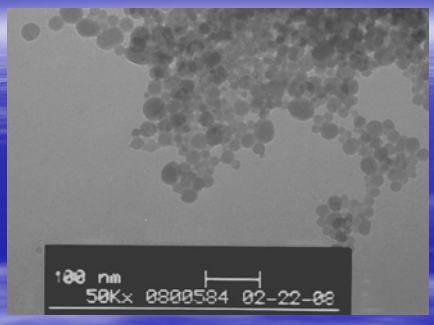


Dynamic size distribution of nC_{60} nanoparticles



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 TFA while provide nC60 concentration in water 100 times higher than the TFA 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.

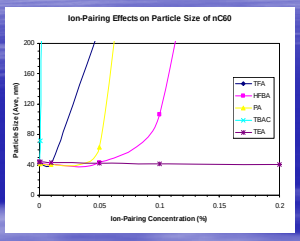


Transmission electron microscopy of nC_{60}

Ion-Pairing Effects on Skin Absorption of Charged Nanomaterials

- Most nanoparticles in aqueous solutions are charged colloidal particles.
- *It is hypothesized that an IP agent can neutralize the charges on nanoparticles, while not destabilizing the nanoparticles; so that the neutralized nanoparticles could penetrate into the SC* (knowing the fact that charged chemicals are difficult to permeated through SC).
- The effects of 5 IP agents on skin absorption of nC_{60} will be studied with three techniques:
  - Diffusion cell experiment,
  - Tape-stripping method in vitro
  - and in vivo Tape-stripping method.

Impact of nC_{60} Colloidal Stability on Skin Absorption



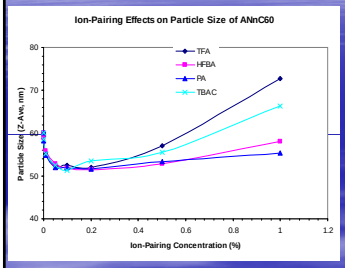
Ion-pairing effects on particle size of nC_{60} in aqueous solutions

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.

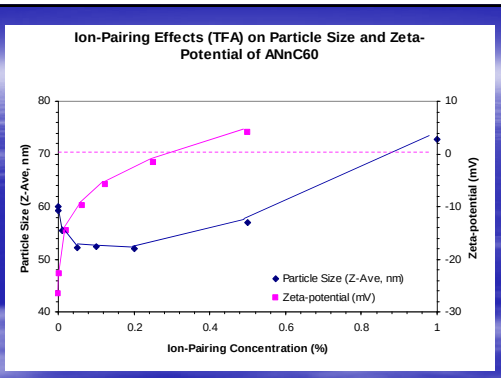
3 anions: TFA (trifluoroacetic acid), HFBA (heptafluorobutyric acid), and PA (phosphoric acid); 2 cations: TBAC (tetrabutylammonium chloride), TEA (triethylamine)

A SuperWrap Polymer for nC60 Colloidal Stability (IP Effects on Colloidal Stable Nanoparticles)

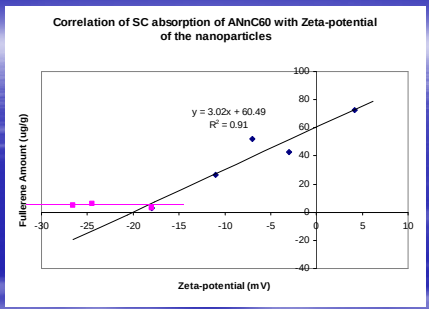


Ion-pairing effects on particle size of $ANnC_{60}$

- We have modified an industrial available dispersion agent (insoluble in water) to a water soluble polymer.
- When mixed with the polymer solution, nC_{60} particle size increased about 10 nm.
- The polymer wrapped nC_{60} nanoparticles ($ANnC_{60}$) will not aggregate in a strong electrolyte (e.g., 2M KCl), extreme pH (1 to 13), any ion-pairing agent.
- Ion-pairing effects on the skin absorption of $ANnC_{60}$ will be studied.



Ion-pairing effects (TFA) on particle size and Zeta-potential of $ANnC_{60}$



Correlation of SC absorption/adsorption of $ANnC_{60}$ with Zeta-potential of the nanoparticles

SC absorption of $ANnC_{60}$ was measured by submerging SC in a nanoparticle solution containing different concentrations of ion-pairing agent and equilibrated at 37ºC for 24 hrs. Then the SC was separated from the solution, washed, dried with paper and digested for quantitative analysis.

Diffusion Experiments

Bronaugh flow-through diffusion cell

- Widely used to measure skin permeability of chemicals, drugs.
- Measures the concentration in the receptor solution to calculate the flux through the skin.
- High-throughput.
- In vitro, time-limit 8 hr or 24 hr

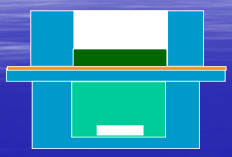
Flow-through diffusion experiments were conducted

- No fullerene was detected in the receptor solutions.
- The detection limit of our HPLC analysis method was 1 ng/mL C_{60}/nC_{60} in media.

Possible reasons:

- The diffusion rate of the nanomaterials within SC was very slow;
- Not enough time to reach the receptor solution in 8 hr or 24 hr.
- Reservoir effects of the SC.

In Vitro Tape-Stripping Method



Franz static diffusion cell

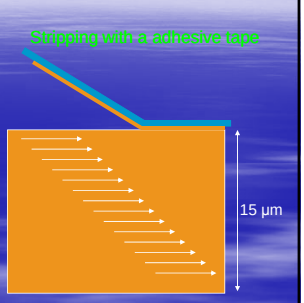


Illustration of the stratum corneum and tape-stripping method

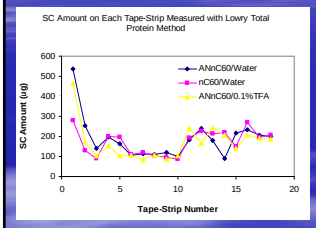
- Larger dose area for tape-stripping analysis
- Directly measures the quantity of nanomaterials penetrated into SC.
- Kinetics information could be obtained from the depth distribution of nanomaterials

In Vivo Tape-Stripping Method

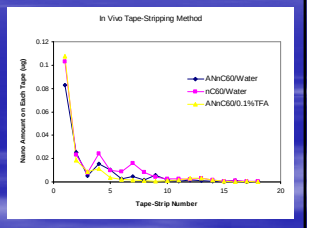
- Larger dose area for tape-stripping analysis
- Directly measures the quantity of nanomaterials absorbed into SC
- Kinetics information could be obtained from the depth distribution of nanomaterials
- No time-limit for study occupational exposure (weeks, or months)
- The morphology of pig skin is similar to human skin

Data from In Vivo Tape-Stripping Method

(4-day multiple doses to simulate occupational exposures)



SC amount on each tape-strip measured with Lowry total protein method



Nanomaterial amount on each tape-strip (doses were made daily for 4 days)

Skin Permeability from Tape-Stripping Data

Nanomaterial transport through the SC generally is assumed to follow Fick's second law of diffusion through a simple, homogeneous membrane (Crank 1975):

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial^2 x}$$

Under the boundary conditions of the experiments performed (at $x = 0$, $C=Cx=0=KCv$, $t\geq 0$; at $0 < x < L$, $C= 0$, $t= 0$; and at $x=L$, $C= 0$, $t \geq 0$), the concentration profile of the nanomaterial, i.e., C as a function of position x and time t, is given by the well known solution to Fick's second law:

$$\frac{C}{KC_v^o} = 1 - \frac{x}{L} - \frac{2}{\pi}\sum_{n=1}^{\infty}\frac{1}{n}\exp\left(-\frac{Dn^2\pi^2 t}{L^2}\right)\sin\left(\frac{n\pi x}{L}\right)$$

Skin permeability (kp);

$k_p = KD/L$

The original data from tape-stripping method are (m_n-n, t) and ($m_{sc,n}$-n, t). The concentration of nanomaterial on *nth* strip (C_n);

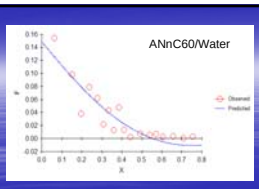
$$C_n = \frac{m_n \rho_{sc}}{m_{sc,n}}$$

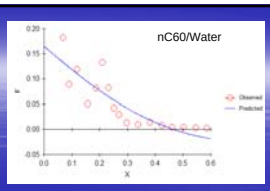
The location of C_n as a function of depth in the SC (x_n);

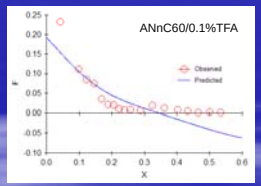
$$x_n = \frac{1}{A\rho_{sc}}\left\{\frac{m_{sc,n}}{2} + \sum_{i=1}^{n-1} m_{sc,i}\right\}$$

The thickness of the SC (L_{sc});

$$L = \frac{1}{A\rho_{sc}}\sum_{n=1}^{n=N} m_{sc,n} = \frac{M_{sc}}{A\rho_{sc}}$$

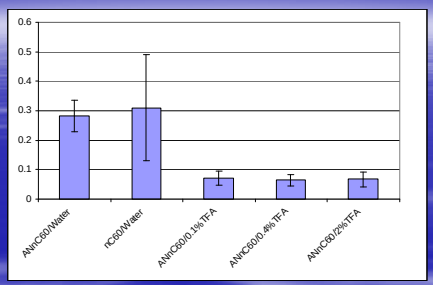






Dose	K	K-CV%	A	A-CV%
ANnC60/Water	0.148	9.61	0.0057	14.22
nC60/Water	0.164	12.35	0.0041	25.6
ANnC60/0.1% TFA	0.193	17.1	0.0013	37.88

Regression analysis of the tape-stripping data (in vivo)



Fullerenes detected in the skin after 4 day multiple exposures in vivo

The animals (n =3) were dosed (500µL) daily for 4 days, then tape-stripping was performed on the 5th day. The biopsies were collected after 26 tape-strips.

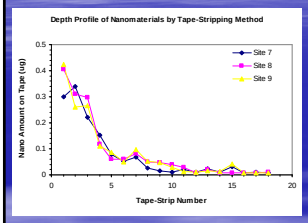
Summary for Ion-Pairing Effects

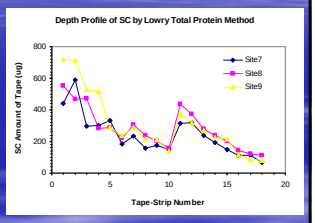
- Nanoparticles in aqueous solutions can be classified into "0mV Zeta-potential" stable or unstable nanomaterials.
- Ion-pairing agents cause the "0mV Zeta-potential" unstable nanoparticles to aggregate (e.g., nC60). Thus ion-paring agents will not aid in their skin penetration.
- Ion-pairing agents can be used to control the surface charge of "0mV Zeta" stable nanoparticles (e.g., ANnC60). The SC absorption (in vitro) is linearly correlated with Zeta-potential after a transition point.
- Skin permeation of nanomaterials is a slow process. No nanomaterial was detected in the receptor solutions in 8-hr or 24-hr diffusion experiments.
- Nanomaterials could be absorbed though the skin from aqueous solutions in long term exposures.
- Tape-stripping methods can be used to study the absorption kinetics of the slow skin permeation of nanomaterials.

Solvent Effects on Skin Absorption of Carbon Nanomaterials

- Solvents are among the most commonly used chemicals in workplaces. Many kinds of solvents will be used in manufacturing, processing, application and handling of nanomaterials.
- *It is hypothesized that skin absorption of nanomaterials is altered significantly by the solvent effects.*
- The solvent effects on the skin absorption of fullerene nanomaterials will be studied in 6 industrial solvents (toluene, cyclohexane, chloroform, ethanol, acetone and propylene glycol) using the diffusion, tape-stripping and in vivo methods.
- The skin permeability and partition coefficient of the nanomaterials between SC and solvents (log*K*sc/s) will be measured, which can be used for safety evaluation and risk assessment of the nanomaterials in the solvents.

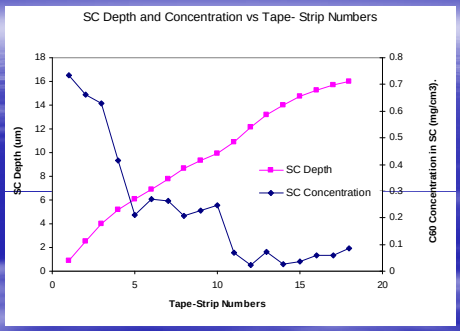
Data from In Vivo Tape-Strip after C_{60}/T Dose



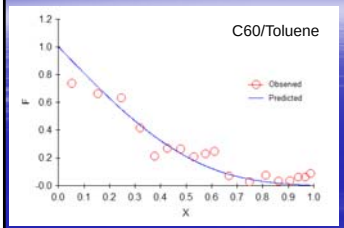


Nanomaterial amount on each tape-strip (m_n-n, t)

SC amount on each tape-strip measured with Lowry total protein method ($m_{sc,n}$- n, t)



SC thickness (L) and depth profile of nano concentration (C_n)



K	*K*-StdError	*K*-CV%
0.855	0.056	6.55
A	*A*-StdError	*A*-CV%
0.0135	0.0018	13.82

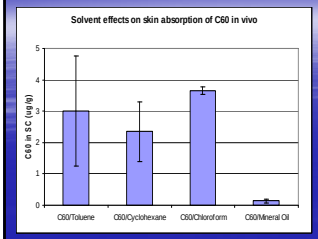
SC thickness (L): 16.2 um

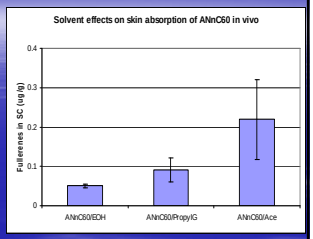
Partition coefficient (K): 0.855

Skin permeability (k_p) 0.185 um/hr

Regression analysis of tape-stripping data

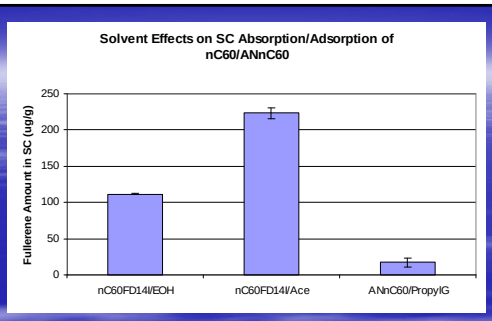
The animals were dosed for 2hr, then tape-stripping was performed on the animals 8-hr after dose. The skin was tape-stripped for 26 times. After 10th strip, two strips were combined into one digestion solution for quantitative analysis.





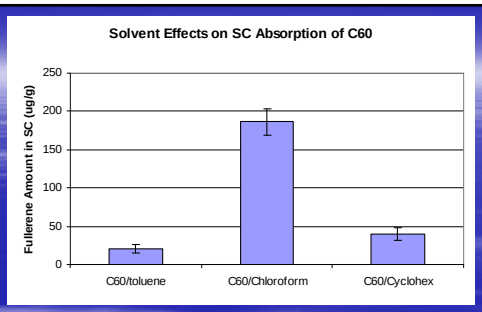
Solvent effects on skin absorption of fullerene nanomaterials after 4-day multiple exposure in vivo

The animals (n =3) were dosed daily for 4 days, then tape-stripping was performed on the animals under anesthesia within 1hr. The skin tissue biopsies were collected after 26 tape-strips.



Solvent effects on SC absorption/adsorption of nC_{60}/$ANnC_{60}$ in vitro

SC was weighed into a given nanoparticle solution of solvent. The SC was submerged in the nanoparticle solution and equilibrated at 37ºC for 24 hrs. Then the SC was separated from the solution and washed vigorously in a flow-through washing tube with deionized water. Finally, the SC was dried with paper and digested for quantitative analysis.



Solvent effects on SC absorption of C_{60} in vitro

SC was weighed into a given nanoparticle solution of solvent. The SC was submerged in the nanoparticle solution and equilibrated at 37ºC for 24 hrs. Then the SC was separated from the solution and washed vigorously in a flow-through washing tube with deionized water. Finally, the SC was dried with paper and digested for quantitative analysis.

Summary for Solvent Effects

- Fullerenes exist as molecular C_{60} or nC_{60} in different solvents which affect their skin absorption mechanism.
- nC_{60}/$ANnC_{60}$ were readily absorbed into the SC in vitro/in vivo; acetone gives higher adsorption comparing to ethanol and propylene glycol.
- C60 was readily absorbed into SC in vitro/in vivo; chloroform gives higher absorption compared to toluene and cyclohexane.
- Tape-stripping methods can be used to study solvent effects on skin absorption of nanomaterials and to provide partition coefficients and skin permeability for predictive model development.

Acknowledgements

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

This research is funded by U.S. EPA - Science To Achieve Results (STAR) Program
Grant # RD-83332801

THANK YOU

Safety/toxicity assessment of ceria (a model engineered NP) to the brain

The research team

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  - Department of Plant and Soil Sciences, U KY
- Michael T. Tseng
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The research team - continued

- Uschi M. Graham
  - Center for Applied Energy Research, U KY
- Rukhsana Sultana & D. Allan Butterfield
  - Department of Chemistry, U KY & (DAB) Center of Membrane Sciences, U KY
- Peng Wu & Eric A. Grulke
  - Chemical & Materials Engineering Department, U KY

Objective of this research

- It is known that some physico-chemical properties of engineered nanomaterials (ENMs) can influence their fate (ADME), including distribution across the blood-brain barrier (BBB).
- But the affects of various physico-chemical properties on the entry of ENMs into the BBB and brain cells and their beneficial and/or hazardous effects are not well studied:
  - Size
  - Shape
  - Surface chemistry
- Objective: Characterize the biodistribution and effects of nanoscale ceria that had entered blood.

Rationale for selection of material to be studied

- Ceria (CAS Reg #1306-38-3; CeO_2, cerium dioxide, cerium oxide) was selected because:
  - it is an insoluble metal oxide that can be readily observed in tissue (electron microscopy, elemental analysis), making it a useful tracer.
  - it is redox reactive.
  - it is available and can be manufactured in many sizes and shapes in the nanoscale range (up to 100 nm).
  - it can be functionalized (surface chemistry altered).
  - it has current commercial applications (catalyst and abrasive).
  - it has been reported to be cytotoxic as well as neuroprotective, representing the controversy about nanoscale materials.

Ceria ENM studied in our initial work

- A 5% dispersion of ceria ENMs in water (Aldrich cat #639648, produced by NanoProducts, Corp.) characterized by laser light scattering (Brookhaven 90Plus Particle Size Analyzer).
  - After 6 min probe sonication @ 50 W nearly 100% of the ENM were ~ 30 (range 21 to 39) nm (94% of the surface area; 77% of the volume), by multimodal size distribution analysis.
  - The remaining volume was ~ 90 to 200 nm.
  - Primary size ~ 3 to 5 nm (by high resolution transmission electron microscopy [HR-TEM])
  - Surface area was ~ 13 m^2/g.
  - Osmotic strength was 28 mOsm.

High resolution transmission electron microscopy (HRTEM) showed the material to be individual ceria crystals as part of a ceria nanocomposite

Search for an iso-osmotic vehicle for this ceria ENM

- The effects of saline and 10% sucrose on ceria ENM agglomeration were assessed by their addition and repeated particle size determination.
  - Saline caused agglomeration.
    - After 5 min: particles were 260 to 430 nm.
    - After 40 min: ~ 98% 300 to 480 nm and 2% 2960 to 3320 nm.
  - 10% sucrose caused agglomeration.
    - Within 1 hour ~89% were 110-140 nm and ~11% 350-441 nm.
- Problem: How to administer a ceria ENM dispersion i.v. to rats and avoid significant erythrocyte lysis?

Studies to predict *in vivo* agglomeration

- Freshly drawn whole rat blood was incubated with ceria ENM (0.14, 0.7 and 3.56 mg ceria/ml) for 1 hr, allowed to clot, fixed in formalin, and processed for high resolution transmission electron microscopy, scanning TEM, and energy-dispersive x-ray spectroscopy (HRTEM/STEM/EDS).
- Agglomerated ceria was seen in the extracellular space between erythrocytes. EDS verified the presence of cerium in the agglomeration.

Distribution and brain effects of intravenously administered ceria

- Objective: Assess the ability of ceria ENM to enter the BBB and brain cells, compared to peripheral organs, and to produce neuroprotection or neurotoxicity.
- Rationale for i.v. administration: Absorption of an ENM by any route will introduce it into systemic circulation, from which it may distribute to the brain.

Methods

- Un-anesthetized male Fisher 344 rats, implanted with two venous cannulae (femoral vein access, terminating in the vena cava) were infused i.v. with:
  - 0, 50, 250 or 750 mg ceria/kg in water.
  - concurrent equal volume and rate of infusion of 1.8% saline in a 2nd cannula.
- Blood was repeatedly drawn from some rats up to 4 hr for Ce analysis by inductively coupled plasma atomic emission spectroscopy & mass spectrometry (ICP-AES/ICP-MS).
- Rats were terminated either 1 or 20 hr after completion of the infusion.

Methods - continued

- Five minutes before termination the rat was anesthetized and given Na fluorescein (334 D_a) and an Evans blue (EB)-albumin complex (~ 68,400 D_a) in saline i.v. as BBB integrity markers.

Methods - continued

- After termination samples were obtained of:
  - brain, liver, spleen and blood to determine Ce by ICP-AES/ICP-MS.
  - brain, liver, spleen, and kidney for histological assessment and EM localization of ceria.
  - brain to determine fluorescein and EB.
  - brain to determine oxidative stress markers:
    - protein-bound 4-hydroxy-2-nonenal (HNE)
    - 3-nitrotyrosine (3-NT)
    - protein carbonyls

Results – Clinical toxicity

- Clinical toxicity was only seen in rats receiving 750 mg ceria/kg:
  - slight tachypnea
  - dyspnea
  - abnormal behavior

Results – Ce was rapidly cleared from blood after completion of i.v. ceria infusion

- The half-life of cerium clearance after termination of ceria infusion was well under 1 hr.
- Cerium concentration in plasma was much less than whole blood, but this was an artifact of centrifugation to generate the plasma.

Results - Intracellular ceria was seen in the spleen red pulp

- The ceria was seen as agglomerates.
- No histopathology was observed.

Results - Intracellular ceria was seen in the liver

- Ceria agglomerations were seen in Kupffer cells and hepatocytes.
- Cellular degeneration was observed in some hepatocytes.

Ceria induced Kupffer cell activation

- An increase of the number of Kupffer cells was seen as a function of ceria dose and time.

Results - Intracellular ceria ENM was seen in the kidney

- Ceria agglomerates (verified by EDS) were seen in the vascular space and in mesangial cells of rats terminated 20 hr after ceria infusion.
- Abnormal tubular epithelial proteinacious accumulation was observed in rats terminated 20 hr after ceria infusion.

Results -There was a near absence of ceria ENM in the brain

- Ceria was seen in the vascular lumen in the brain but only occasionally seen in astrocytes or neurons.
- No visual evidence of BBB breakdown was seen.

Results - Tissue Ce concentration was ceria dose-dependent

- Very similar distribution of cerium was seen 1 and 20 hr after completion of the ceria infusion.
- Ceria concentration in the spleen was slightly greater than in the liver, which was greater than in the brain and serum by 2 to 3 orders of magnitude.

Results – No great changes in oxidative stress indicators were seen in the brain

- 1 hr after ceria infusion there were no significant changes in protein-bound 4-hydroxy-2-nonenal, 3-nitrotyrosine, or protein carbonyls
- 20 hr after ceria infusion HNE increased in the hippocampus and protein carbonyls decreased in the cerebellum

Results – There was a small increase in blood-brain barrier permeability 20, but not 1, hr after ceria infusion

- Brain fluorescein and Evans blue were not significantly changed 1 hr after ceria infusion.
- Brain fluorescein was elevated 20 hr after ceria infusion.
- But there was considerable variability in the results, especially with Evans blue.

Relating these ceria doses to its use as a diesel fuel additive

- This ~ 30 nm ceria ENM nanocomposite was quite non-toxic when introduced i.v.
  - The 50, 250 and 750 mg ceria/kg i.v. doses in these ~ 0.3 kg rats would equal all of the 5 ppm ceria in 3, 15 and 45 liters of diesel fuel.

Conclusions

- Ceria was rapidly cleared from the blood by peripheral reticuloendothelial tissues.
- Much less ceria entered the BBB cells or the brain.
- Ceria ENM agglomerates *in vivo*.
- This ceria induced mild oxidative stress and stress response in the brain.
- This ceria provides an inert core ENM enabling the study of the effects of size, shape and surface chemistry on biodistribution, biotransformation and neurotoxic or neuroprotective potential.

Internalization and Fate of Individual Manufactured Nanomaterial Within Living Cells

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http://biomarkers.pnl.gov/staff/orr.asp

Rationale

1) The cellular interactions and intracellular fate of nanomaterials dictate the cellular response and ultimately the level of toxicity.

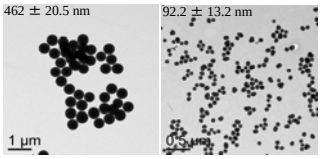
If we understand mechanisms that underlie the cellular interactions and internalization pathways of well-defined nanoparticles we could delineate relationships between particle properties, cellular response, and mechanisms of toxicity or biocompatibility.

2) Nanomaterials are likely to be presented to cells in vivo as individual particles or small nanoscale aggregates (<100 nm).

If we study one particle at a time we are more likely to delineate mechanisms that occur *in vivo*.

Manufactured amorphous silica nanoparticles are used extensively in a wide range of industrial applications

The wide use of synthetic amorphous silica results, in part, from the relative ease of controlling their size and purity.



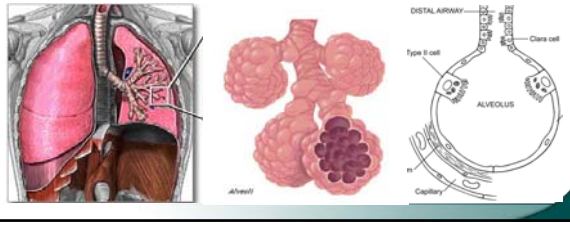
Surface charge: Unmodified (ζ= -40 mV)
Surface aminated (ζ= +20 mV)

Alveolar type II epithelial cells are important target:

Air born particles ranging from 5 nm to 1 µm that enter the respiratory tract are likely to be deposited in the alveolar region.

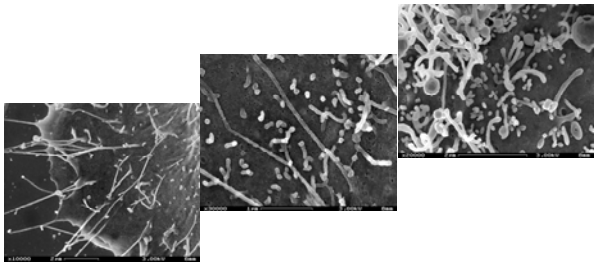
Type II cells play critical roles in the function of the alveoli by secreting pulmonary surfactants, and by differentiating into type I epithelial cells when these are damaged.

Importantly, type II cells participate in the immune response to certain particles and pathogens by releasing chemokines.

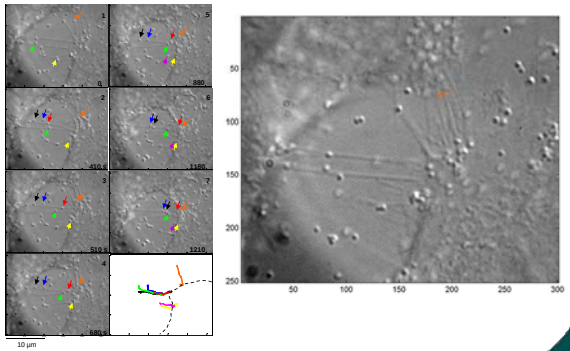


Alveolar type II epithelial cells carry apical microvilli:

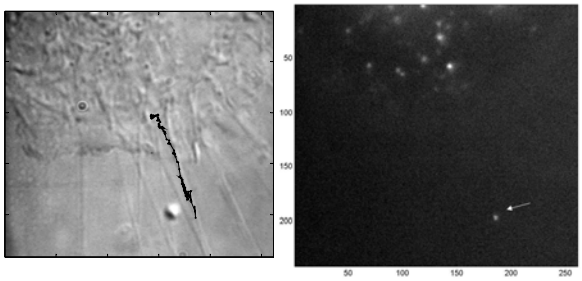
C10: a Non-tumorigenic cell line, derived from a normal lung of an adult mouse and preserves its phenotype, including lamellar bodies and surface microvilli:



Positively charged 500 nm particles are propelled along microvilli in a retrograde motion, unraveling the coupling of the particle with the intracellular environment across the cell membrane:



Positively charged 100 nm particles travel along microvilli in a more complex, anterograde and retrograde motions:



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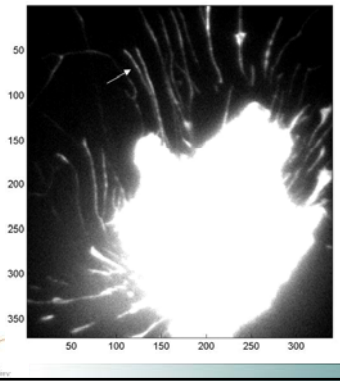
The retrograde motion of the particles and the retrograde flow of actin clusters depend on the integrity of actin filaments:



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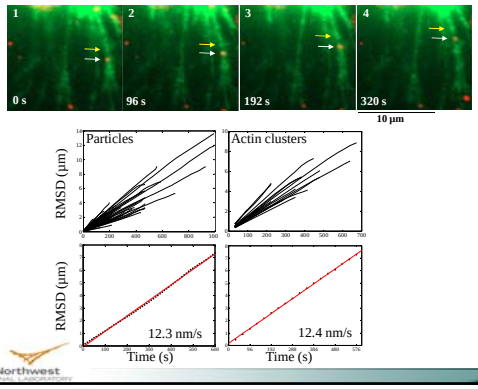
The retrograde motion of the particles and the retrograde flow of actin clusters occur at the same rate:



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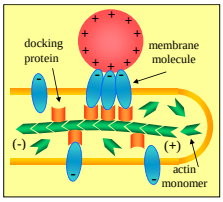
The retrograde motion unravels charge-dependent coupling of the particles with the intracellular environment across the cell membrane:



Positively charged particles bind a negatively charged transmembrane molecule that, in turn, interacts directly or indirectly with the actin filaments within microvilli.

As actin monomers are added to the distal tip of the filaments, a retrograde motion is generated, leading to the retrograde motion of the membrane molecule and its bound particle.

Orr et al., *ACS Nano*, 2007 1(5):463-475.

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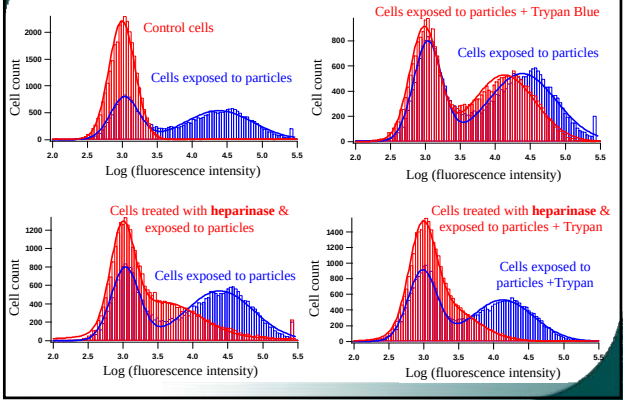
Heparan sulfate proteoglycans play a critical role in the attachment and internalization of positively charged 500 nm particles:



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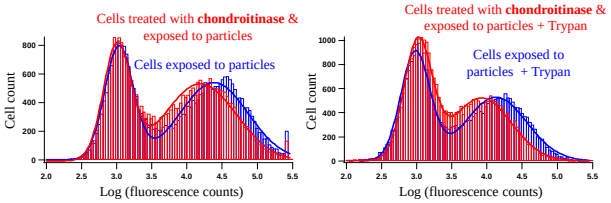
Chondroitin sulfate proteoglycans play a smaller role in the attachment and internalization of positively charged 500 particles:



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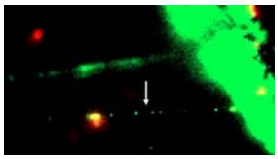
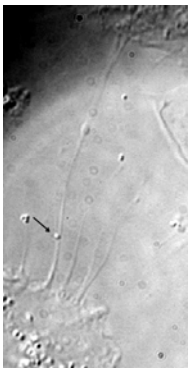
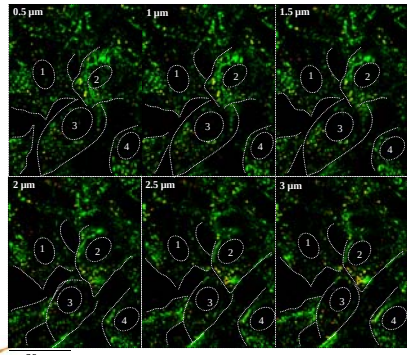
Syndecan-1, a trans membrane heparan sulfate proteoglycan, engages positively charged 500 nm particles in the movement along microvilli:



Syndecan-1 is green
Particles are red

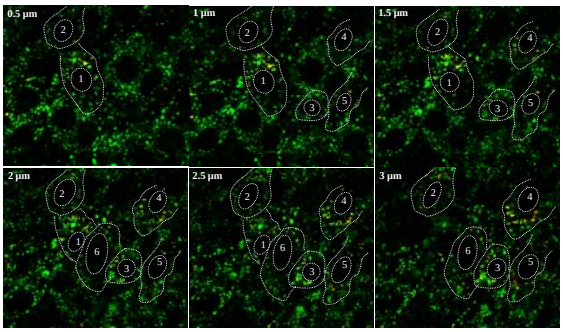


Syndecan-1 and positively charged 500 nm particles are co-localized in small and discrete cellular structures:



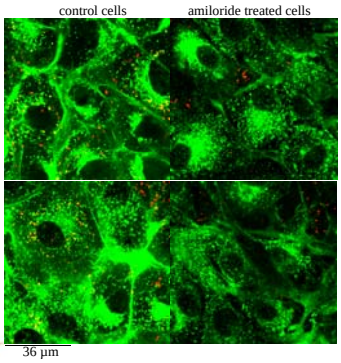
Syndecan-1: green
Particles: red

Positively charged 500 nm particles are also co-localized with 70 kD dextran, a tracer for macropinocytosis:

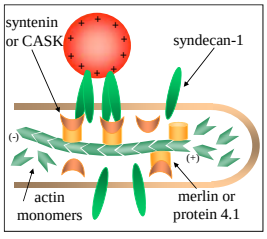


Dextran: green
Particles: red

The internalization of the particles is blocked by amiloride, an inhibitor of macropinocytosis:



Syndecan-1mediates the initial interactions of the particles at the cell surface, their coupling with actin filaments across the cell membrane, and their subsequent internalization via macropinocytosis.



Orr et al., *Toxicology and Applied Pharmacology*, 2009 236, 210–220.

Summary

A new retrograde pathway is described, unraveling the coupling of positively charged submicro- and nanoscale inorganic particle with the intracellular environment across the cell membrane.

This pathway brings a new mechanism by which positive surface charge supports particle recruitment, and potential subsequent toxicity, in polarized epithelial cells bearing microvilli.

Heparan sulfate proteoglycans are identified as critical players in the attachment and internalization of positively charged submicrmeter inorganic particles.

Syndecan-1, a transmembrane haparan sulfate proteoglycan, is found to mediate the cellular interactions and fate of the particles and therefore govern their cellular response.

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Joel Pounds

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Methodology Development for Manufactured Nanomaterial Bioaccumulation Test

PIs: Yongsheng Chen, Qiang Hu, Milton Sommerfeld, Yung Chang, John Crittenden, and C.P. Huang*

Arizona State University

*University of Delaware

Nov. 21, 2008

Outlines

- Assess toxicity of manufactured nanomaterials in several aquatic model organisms
- Determine bioconcentration of manufactured nanomaterials in aquatic organisms
- Evaluate biomagnification of manufactured nanomaterials in food chain

Test Nanomaterials

Particles	Particle Size	Purity (%)
C60	< 200 nm	99.5
SWCNTs	D < 2 nm L = 5 – 15 µm	CNTs > 90 SWCNTs > 60
MWCNTs	D = 10 – 20 nm L = 5 – 15 µm	> 98.0
nZnO	20 nm	> 99.6
$nTiO_2$	≤ 20 nm	> 99.5
nAl_2O_3	80 nm	> 99.9

Model Organisms

- Algae
- Daphnia
- Zebrafish Embryos and Zebrafish

Reasons:

1. They are at the lower level of the food chain;
2. Toxicity indicators and their genetic database have been well-established

Toxicity of Nanoparticles on Green Algae

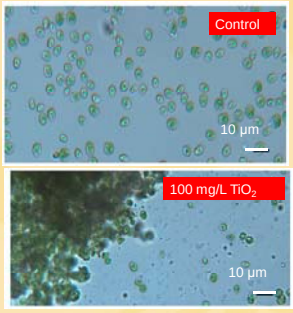
NPs	Regression Equation	Correlation Coefficient	EC_{50} (mg/L)
nZnO Suspension	y = 38.862x + 49.194	R^2 = 0.9542	1.049±0.565
C_{60} Suspension	y = 26.42x + 20.456	R^2 = 0.8988	13.122±4.182
$nTiO_2$ Suspension	y = 39.902x + 2.7719	R^2 = 0.9275	15.262±6.968
MWCNTs Suspension	y = 38.468x + 4.3117	R^2 = 0.9964	15.488±7.108
SWCNTs Suspension	y = 27.978x + 12.097	R^2 = 0.8434	22.633±9.605
nAl_2O_3 Suspension	y = 14.204x - 10.044	R^2 = 0.5471	>1000

High toxicity

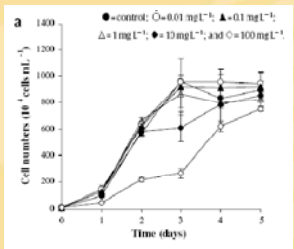
Low toxicity

Zhu X., L. Zhu, Y. Chen, Y. Lang
J. Nanoparticle Research. 2008 DOI 10.1007/s11051-008-9426-8

Green Algae Aggregation and Growth Inhibition



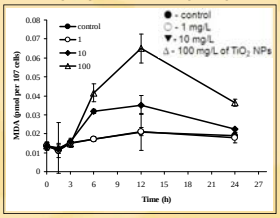
Algae growth inhibition



J. Wang, X. Zhang, Y. Chen, et al. Chemosphere, 73 (**2008**): 1121-1128

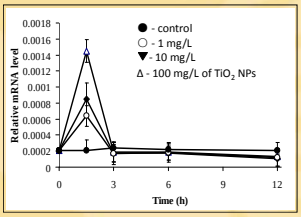
Lipid Peroxidation and Gene Expressions

Lipid peroxidation (MDA)



For the first 12 h, A dose-dependent increase in the maximum malondialdehyde (MDA) content was clearly indicative of cellular lipid peroxidation induced by TiO_2 NPs.

Gene expressions: *Catalase*



After 1.5 h treatment, the maximum transcripts of *catalase* occurred; However, the catalase gene expression up-regulation was transient.

J. Wang, X. Zhang, Y. Chen, et al. Chemosphere, 73 (**2008**): 1121-1128

Toxicity of NPs on *Daphnia magna*

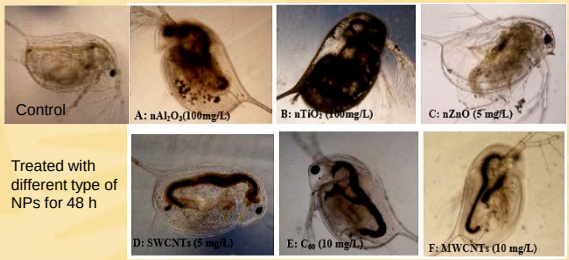
High toxicity

Material (particle size)	EC_{50} (mg/L)	95% CI	LC_{50} (mg/L)	95% CI
nZnO (20 nm)	0.62	0.41-0.81	1.51	1.12-2.11
SWCNTs (<2nm)	1.31	0.82-1.99	2.43	1.64-3.55
C_{60} (<200nm)	9.34	7.76-11.26	10.52	8.66-12.76
MWCNTs (10-20nm)	8.72	6.28-12.13	22.75	15.68-34.39
$nTiO_2$ (< 20nm)	35.31	25.63-48.99	143.39	106.47-202.82
nAl_2O_3 (80 nm)	114.36	111.23-191.10	162.39	124.33-214.80

Low toxicity
(48 h)

Zhu X., L. Zhu, Y. Chen, Y. Lang
J. Nanoparticle Research. 2008 DOI 10.1007/s11051-008-9426-8

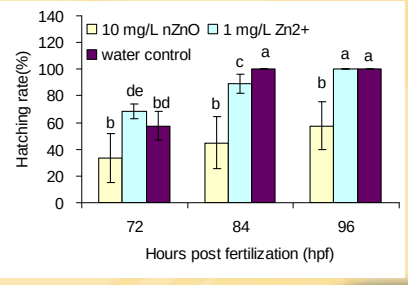
The Morphology of *Daphnia magna*



Treated with different type of NPs for 48 h

Zhu X., L. Zhu, Y. Chen, Y. Lang
J. Nanoparticle Research. 2008 DOI 10.1007/s11051-008-9426-8

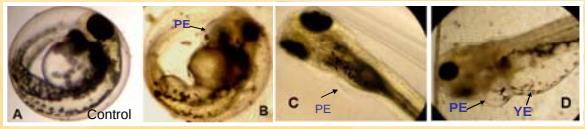
Impacts on Zebrafish Embryo Hatching



At 10 mg/L nZnO concentration, the released Zn^{2+} is around 1 mg/L (20 nm filter was used)

Toxicity of ZnO NPs on Zebrafish Embryo Hatching

One did Hatch also display some abnormality

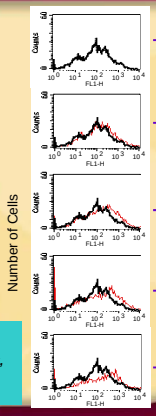


***Pericardial edema* (PE) and *yolk sac edema* (YE) induced by aggregates of ZnO nanoparticle**

ROS Assessment

Fluorogenic dye was used to reveal the ROS. Specifically, The embryos from 4-day treatment of NPs and Zn^{2+} were prepared into single cell suspension, and then incubated with fluorogenic dye, DCFDA, The cells were then analyzed by flow cytometry.

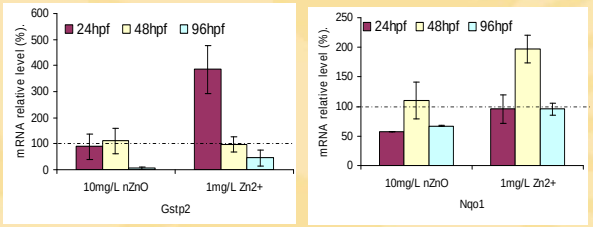
The data indicates that the nZnO NPs, not Zn^{2+} cause higher level intracellular ROS, which may contribute to the developmental toxicity



Control	PC=90
1mg/L Zn^{2+}	PC=77
1 mg/L nZnO	PC=257
10 mg/L nZnO	PC=429
100 mg/L nZnO	PC=609

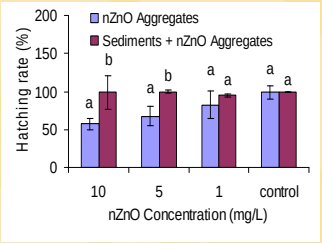
Quantitative RT-PCR for Oxidant-Associated Genes

Given the higher level of ROS in nZnO-treated groups, we expect to see increased expression of two genes (gstp2, Nqo1) coded for anti-oxidant enzyme.

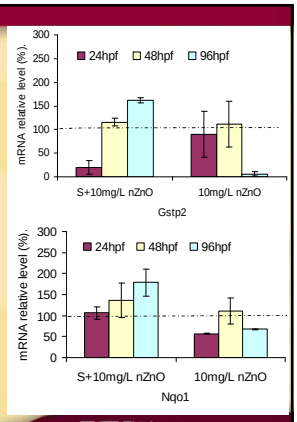


It is possible that nZnO-treated groups fail to up-regulate their anti-oxidant genes, which may explain the higher level of ROS shown in the previous slide.

Sediments Impacts on Toxicity of Zebrafish Embryos



Sediment could be a mitigating agent to reduce the toxicity caused by nZnO NPs.



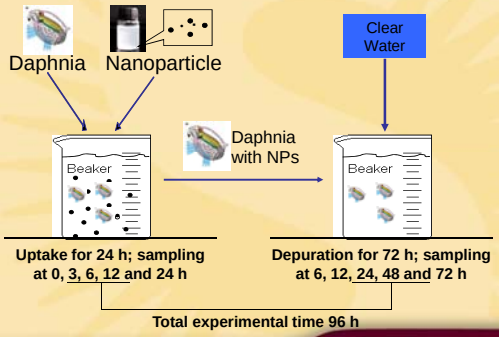
Environmental science & technology in preparation

Summary Remarks

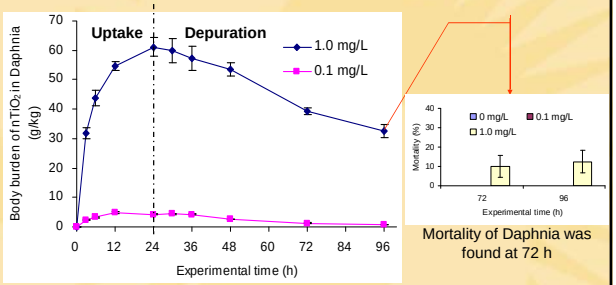
From the general toxicity tests:

- The toxicity rank order of carbon-based NPs is: SWCNTs > C_{60} > MWCNTs; metal oxide NPs is: nZnO > $nTiO_2$ > nAl_2O_3.
- nZnO caused oxidative stress on aquatic organisms
  - Toxicity is not solely caused by Zn^{2+}.
  - Toxicity is correlated with a higher level of ROS .
  - Toxicity appears to be inversely correlated with the expression of two anti-oxidant genes.
- Sediment could reverse the toxicity induced by the ZnO NPs.

Scheme for Experiments on $nTiO_2$ Bioconcentration by Daphnia



Uptake and Depuration of $nTiO_2$ in *Daphnia magna*



Mortality of Daphnia was found at 72 h

Bioconcentration Factors (BCFs) for $nTiO_2$ in *Daphnia magna*

$$\frac{1}{C} = \left(\frac{K_M}{C_{sat}}\right)\frac{1}{t} + \left(\frac{1}{C_{sat}}\right)$$

Michaelis–Menten kinetics

Dose (mg/L)	Exact dose[a] (mg/L)	Whole body concentration(dw) (g/kg)	BCFs (l/kg)	K_M=$t_{u0.5}$ (h)	$t_{u0.9}$ (h)	$t_{d0.5}$ (h)	$t_{d0.9}$ (h)
0.10	0.08	4.52[b]	56,562.50	3.87	34.84	26.76	88.90
1.0	0.517	61.09	118,062.84	3.72	33.51	74.52	247.59

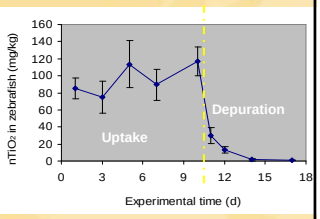
a. Exact concentration of $nTiO_2$ in culture medium after exposure for 24 hours
b. Average of the concentrations at 12 and 24 hours

Biomagnification Tests by Feeding Daphnia to Zebrafish

Daphnia (8-10 days old): exposed to 0.1 mg/L $nTiO_2$ for 24 hours

Fed two times each day (about 8% wet weight daily ratio)

Zebrafish (Danio rerio) (5-8 months old)



Biomagnification factor (BMF) = 0.0259. From this preliminary data, it can be speculated that there is **no biomagnification of $nTiO_2$ from Daphnia to zebrafish**.

Future works

- Determine the bioaccumulation behavior of NPs under different exposure conditions, such as static, semi-static and flow-through system.
- Determine the distribution (or fate) of NPs in different parts of exposure system, including water, organism body and the excretion, based on the mass balance profile or using a stable isotopic tracer approach.
- Long-term experiments on biomagnification and toxicity (e.g. in reproductive system) will be conducted.

Achievements

Journal articles related to this project

1. Sun H., Zhang X., Chen, Y., et al. Enhanced accumulation of arsenate in Carp in the presence of titanium dioxide nanoparticles. *Water, Air & Soil Pollution.* 2007, (178):245–254.
2. Zhang X., Sun H., Chen Y., et al. Enhanced bioaccumulation of Cd in carp in the presence of titanium dioxide nanoparticles. *Chemosphere* 2007, (67):160–166.
3. Zhu X., Zhu L., Lang Y., Chen Y. Oxidative stress and growth inhibition in the freshwater fish *Carassius auratus* induced by chronic exposure to sublethal fullerene aggregates. *Environmental Toxicology and Chemistry*, 2008, 27(9): 1979-1985.
4. Wang J., Zhang X., Chen Y., Sommerfeld M., Hu Q. Toxicity assessment of manufactured nanomaterials using the unicellular green alga *Chlamydomonas reinhardtii*. *Chemosphere*, 2008, 73: 1121–1128.
5. Zhu X., Zhu L., Chen Y., Tian S. Acute Toxicities of Six Manufactured Nanomaterials Water Suspensions on Daphnia magna. *Journal of nanoparticle research*, 2009. Article in press

Presentations

6. Zhang X., Chen Y., Sun H., Crittenden J. Adsorption/Desorption of Cd by titanium dioxide nanoparticles and sediment particles as well as their facilitated bioaccumulation of Cd into Carp. NSTI (Nano Science and Technology Institute,) Nanotech 2007 Conference, Santa Clara, California, USA. May 20-24, 2007
7. Zhu X., Zhang X., Chang Y., Chen Y. Toxicity of ZnO nanoparticle sedimentation on the embryo development of zebrafish (*Danio rerio*). NSTI (Nano Science and Technology Institute) Nanotech 2008 Conference, Boston, Massachusetts, USA. June 1-5, 2008.
8. Zhang W., Zhu X., Zhang X., Chang Y., Chen Y., Rittman B., Crittenden J. Potential toxicity of nanomaterials and their removal. International Environmental Nanotechnology conference. Chicago, Michigan, October, 2008. (Oral presentation)

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1) **People from my group:**
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Xiaoshan Zhu, Post-docs
Xuezhi Zhang, Post-docs
Jiangxin Wang, Post-docs

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PIs: Yongsheng Chen, Yung Chang, Qiang Hu, Milton Sommerfeld, John Crittenden, and C.P. Huang from U of Delaware

Nanoparticle Fate Project – Progress Report

David Y. H. Pui, Nick Stanley, and T.H. Kuehn
University of Minnesota; Minneapolis, Minnesota

C. Asbach, T. Kuhlbusch, H. Fissan – Institute of Energy and Environmental Technology (IUTA); Duisburg, Germany

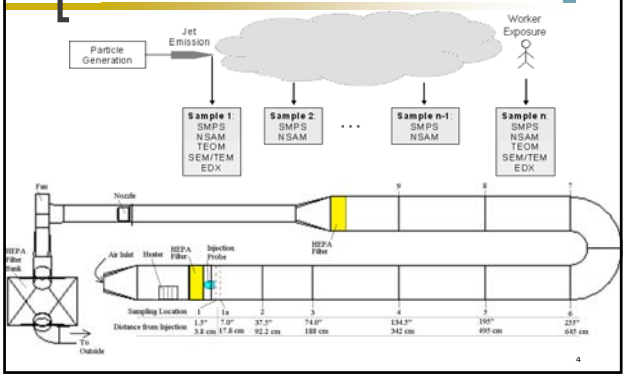
Presentation Outline

- Objectives and Background
- Filtration Study
- Wind Tunnel Testing
  - Particle Dispersion
  - Velocity Profiles
- Burner Particle Characterization
- Conclusions and Future Work

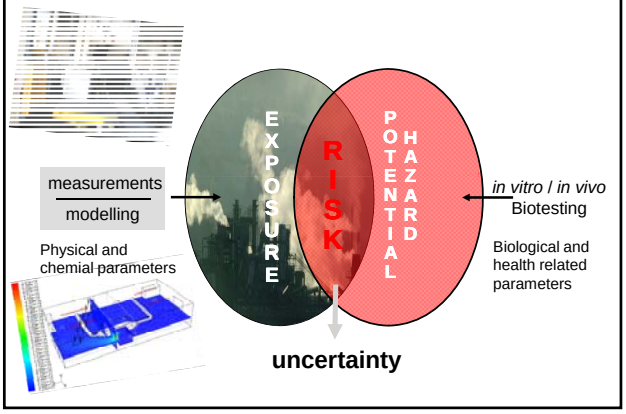
Overall Project Objectives

- Measure and model the fate of nanoparticles as they are emitted through a leak from a nanoparticle production process into a workplace environment.
- Observe changes in particle and aerosol properties, such as number and surface area concentrations, morphology, and chemical composition.

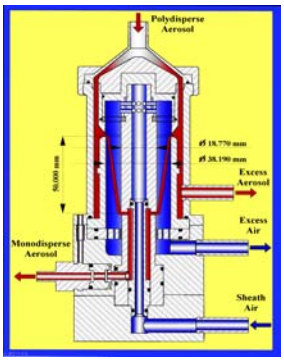
Background and Setup



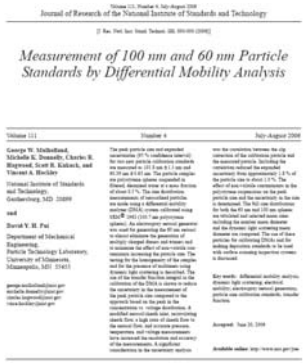
Risk



DMA and SMPS



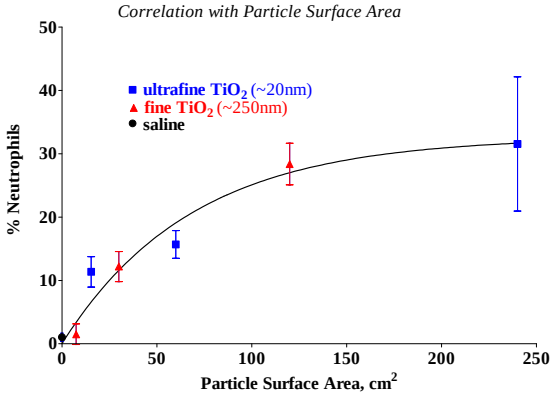
New NIST Nanoparticle Standards: 60 nm and 100 nm SRM


Journal of Research of the National Institute of Standards and Technology

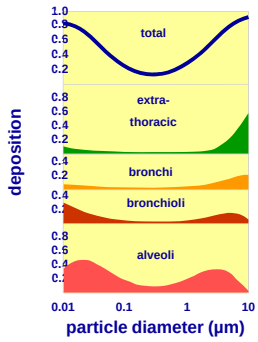
Measurement of 100 nm and 60 nm Particle Standards by Differential Mobility Analysis

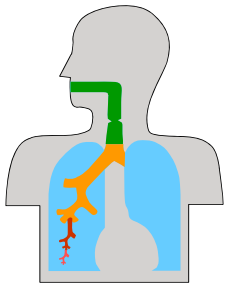
Percent of Neutrophils in BAL 24 hrs after Instillation of TiO_2 in Rats

Correlation with Particle Surface Area



Particle Deposition in Healthy Adult Subjects

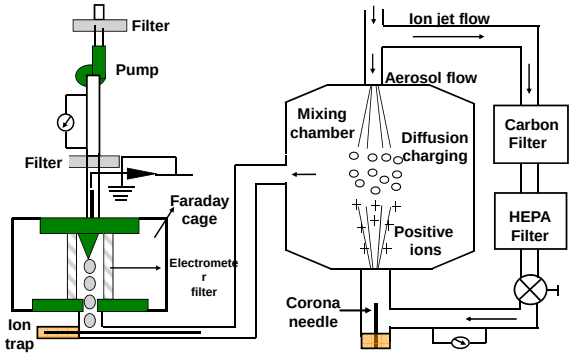




particle density: 1 g cm^{-3}
respiratory flow rate: 300 cm^3 s^{-1}
breathing at rest cycle period : 5 s

ICRP 66 (1994); MPPDep (2000)

Electrical Aerosol Detector

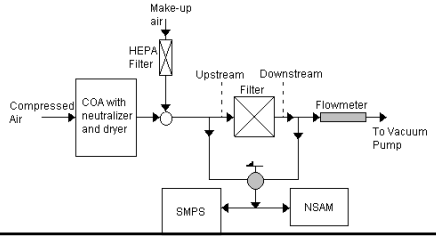


Nanoparticle Surface Area Monitor Model 3550

AEROTRAK™ 9000 Nanoparticle Aerosol Monitor

Filtration Study (FILTRATION, submitted 8/08)

- Objectives: To determine compatibility of instruments and measure overall filter efficiency based on number, SA, and size distributions.
- Aerosol: DOS - 10 ppm and 1 ppm (spherical particles)
- Flow Rate at Flowmeter: 20 L/min and 10 L/min
- Constant Output Atomizer (COA) Pressure: 25-30 psi
- COA Flow Rate: 5 L/min



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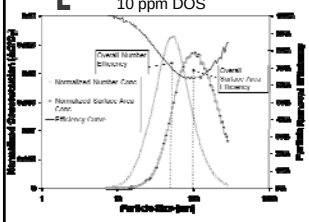
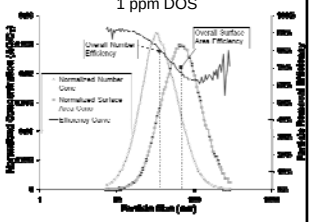
Filtration Study (FILTRATION, submitted 8/08)

10 ppm DOS



1 ppm DOS

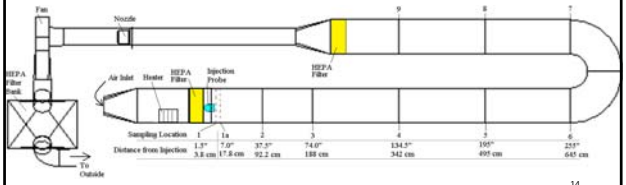


- NSAM and SMPS correlate very well (max discrepancy ~10%)
- When concerned with an aerosol mainly composed of nanoparticles, the surface-area filter efficiency represents:
  - A more health relevant filter evaluation.
  - A better characterization of the filter.

13

Particle Dispersion Study - Setup

- Flow rates: 200 & 500 cfm (Face Velocities: 0.25 & 0.64 m/s)
- 6-Jet Atomizer (TSI Model 9306A) with 0.1% KCl solution at 12 l/min flow rate
- Same sampling probe for SMPS and NSAM
- Simultaneous measurements



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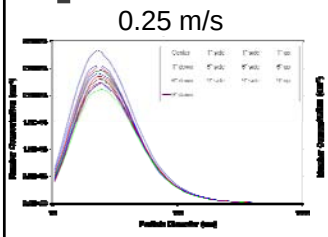
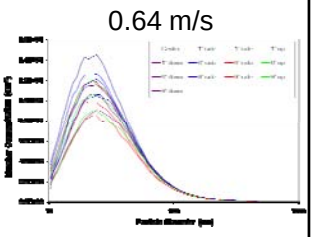
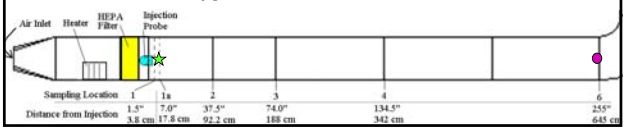
NSAM/SMPS Dispersion Study

0.25 m/s



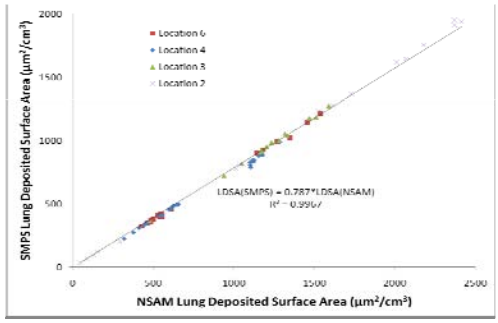
0.64 m/s





NSAM/SMPS Dispersion Study

- LDSA(SMPS) = 0.787*LDSA(NSAM)



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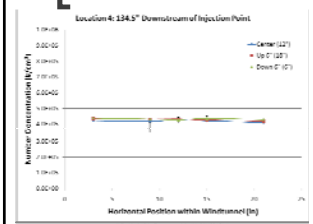
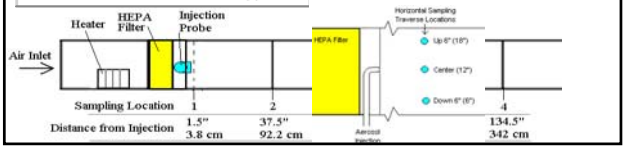
CPC Dispersion Study (0.25 m/s)

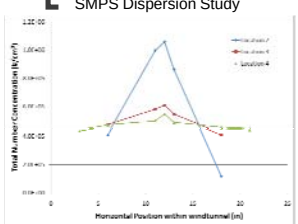


- CPC used to determine amount of dispersion within wind tunnel.
- Flow is uniformly dispersed 342 cm downstream at 0.25 m/s face velocity.

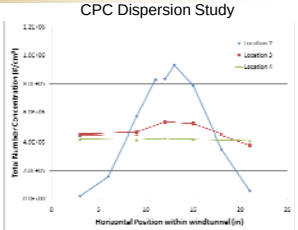


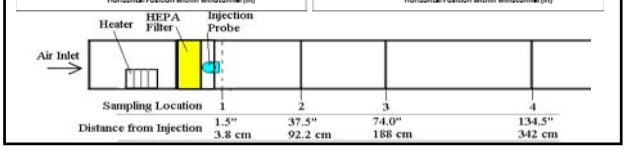
Dispersion Study Comparison

SMPS Dispersion Study

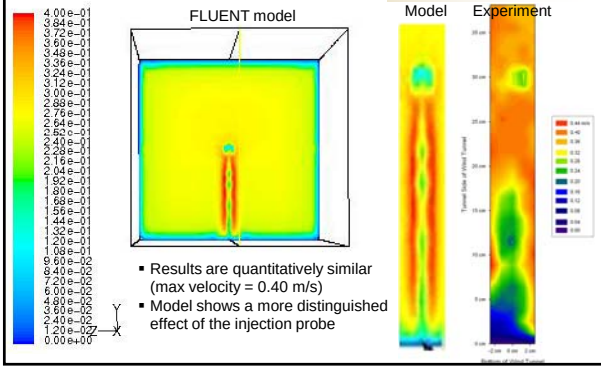


CPC Dispersion Study





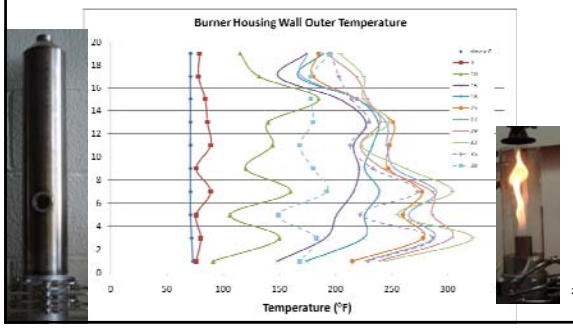
Flow Profile at 20 cm Downstream



- Results are quantitatively similar (max velocity = 0.40 m/s)
- Model shows a more distinguished effect of the injection probe

Burner Housing Temperature

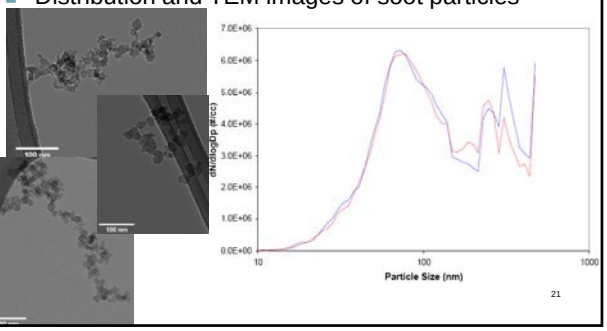
- Air-Fuel ratio: 28 (62% excess air)



20

Burner Particle Characterization

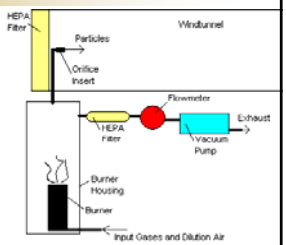
- Distribution and TEM images of soot particles



21

Burner Particle Injection System

- Diffusion burner will produce TiO_2, SiO_2, or Carbon Black (soot) nanoparticles.
- Use short and narrow tube to limit residence time.
- HEPA Filter ensures only injected particles will be the sample aerosol.



- Different orifice insert diameters will be used to simulate different leak sizes.
- Assume the pressure inside of pipe/reactor is not affected by the leak.

22

Nanoparticle Fate Future Work

- Experimentally and numerically investigate fate of nanoparticles upon release into wind tunnel, using burner setup.
  - Use burner with housing to produce each test aerosol (TiO_2, SiO_2, and soot).
  - Conduct burner produced aerosol tests in wind tunnel with injection system.
- Develop dilution set to possibly be used at sampling location.
- Study the effect of background particles on nanoparticle fate.
- Numerically model the fate of nanoparticles at IUTA for a more complete understanding of the coagulation and dispersion processes with high spatial resolution.

23

Acknowledgements

- **The Nanoparticle Fate project is sponsored by NSF (NSF G2006-Star-F2 Fate and Transport)**

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Center for Environmental HEALTH SCIENCES

The University of Montana-Missoula

Fun with Carbon and TiO_2 Nanoparticles

Andrij Holian, Raymond Hamilton, Nick Wu[2], Dale Porter[3], Krishnan Sriram[3] and Mary Buford
The University of Montana
Department of Biomedical and Pharmaceutical Sciences
Center for Environmental Health Sciences
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NIH-ES015497
NSF-CBET-0834233

Summary of Dispersion Studies

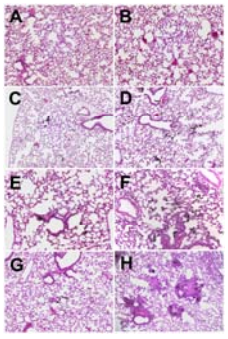
- CNP toxicity may be dependent on size, size distribution,aggregation, shape, surface chemistry, surface area and surface charge
- All of these properties could be affected by suspension media
- Can not predict optimal media for any one particle since chemistry will be a factor
- Variations in literature can in part be explained by sources and dispersion
- Overall: presence of lipids or proteins resulted in smaller aggregates

Buford MC et al Particle Fibre Toxicol 2007

Balb/c mouse lung histology (H&E) following instillation of 250 µg SWNT (SES)

A-F 24 hrs, G&H 7 days
A, C, E, G PBS
B, D, F, H 100% FCS
A&B vehicle control
100x except E&F 200x

More effective dispersion resulted in more distinct areas of inflammation

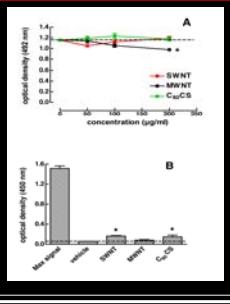


Hamilton RF et al., J Nanotox 2007

Cytotoxicity of CNP: AM from Balb/c

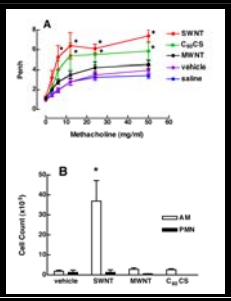
- A) MTS cell viability/ proliferation assay @48 hr (N=10)
  - Only MWNT had effects at high conc, no effects at 4 or 24 hr
  - BMDM observed proliferation
- B) TUNEL assay for apoptosis @24 hr (N=5)
  - 200 µg of each

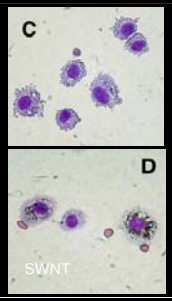
* P < 0.05



Hamilton RF et al., J Nanotox 2007

Intranasal Instillation of CNP (150 µg: 24 hrs)





Hamilton RF et al., J Nanotox 2007

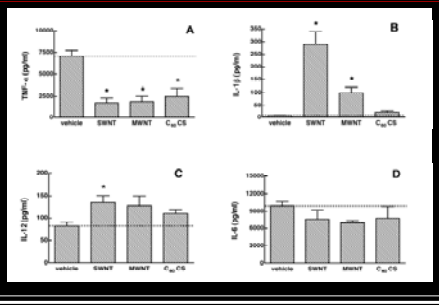
N=8, *P<0.05

APC Assay

- 1X10^5 macrophages C57Bl/6 or Balb/c
  - 1hr @37° C mixing with particles
  - 3hr in 96-well plate with OVA (10 mg/ml)
- 4x10^5 CD4+ T cells OT-II or DO11.10
  - Supernatants and/or T cells collected at 48hr
- Mac supernatants collected at 24hr
- Supernatants frozen until assayed by ELISA or Luminex

APC-TC co-culture

CNP (200 µg/ml) effects on AM cytokines in presence of OVA antigen stimulation (24 hrs)

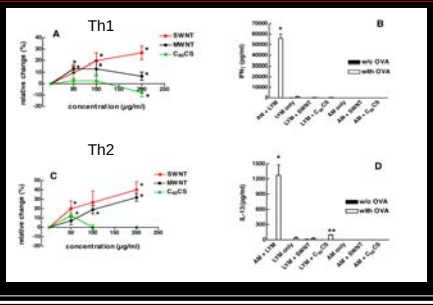


No effect w/o OVA

Hamilton RF et al., J Nanotox 2007

N=4, *P<0.05

Effect of CNP on APC activity of Balb/c AM

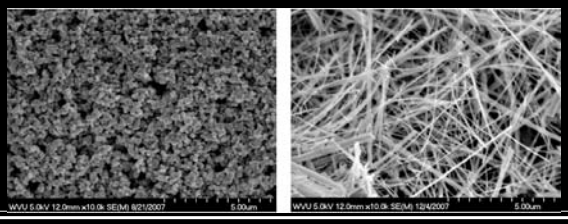


Hamilton RF et al., J Nanotox 2007

N=4, *P<0.05

SEM of TiO_2 Nanospheres and Nanowires

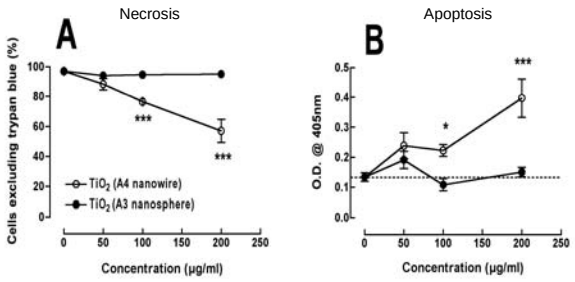
- Nanoparticles generated by Dr. Nick Wu
- Anatase crystal structure



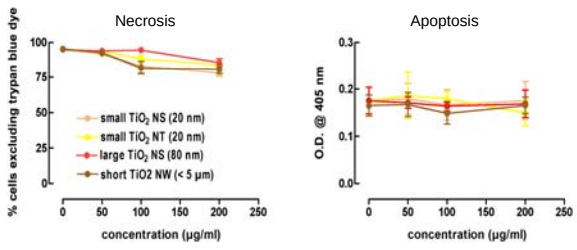
80 nm

80 nm X 22 microns

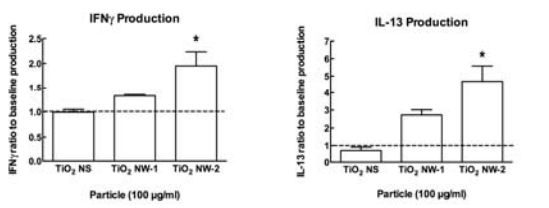
Toxicity of TiO_2 Nanowires (4 hr) C57B/6 AM



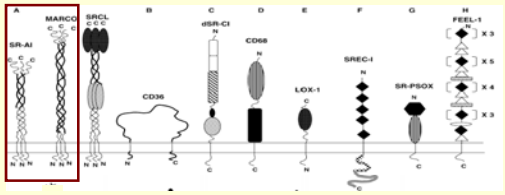
Comparison of Various TiO_2 NP



Effect of TiO_2 NP on APC activity



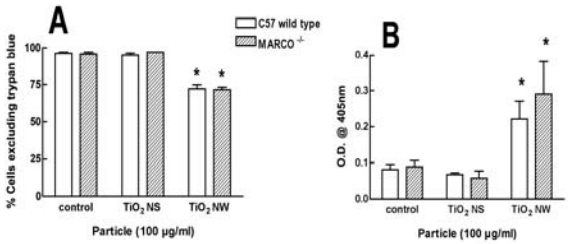
Role of scavenger receptors



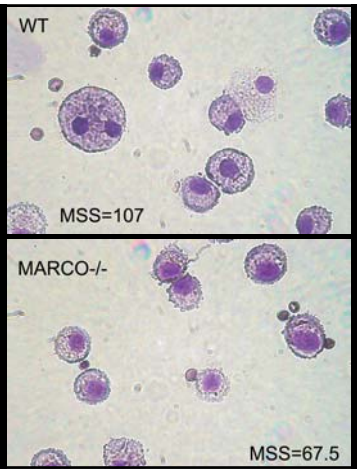
- Scavenger receptors (SR) - eight classes A-H
- SR-A family- SRAI, SRAII, SRAIII , SRCL, SCARA5 and MARCO
- SRA (I/II) and MARCO are implicated in binding of environmental particles (negatively charged) and subsequent signaling
- MARCO primary SR in murine models (Hamilton RF et al J Biol Chem 2006) and SRCR region primary binding site (Thakur SA et al Toxicol Sci 2008)

Murphy, J. E. et. al, Atherosclerosis, 2005

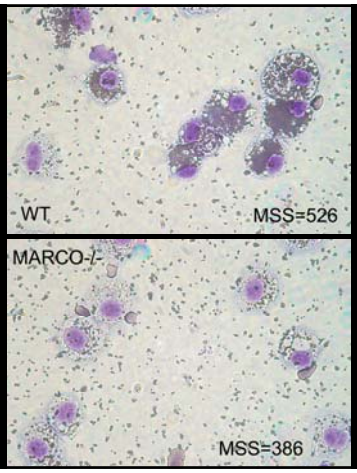
Role of MARCO in TiO_2 NW Toxicity



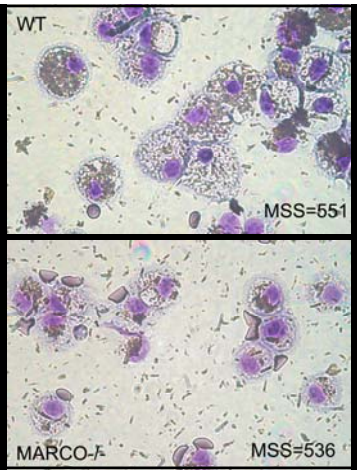
Control



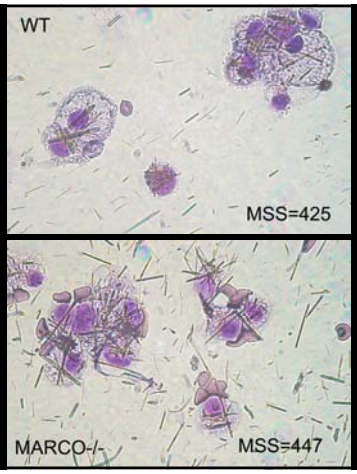
Nanospheres

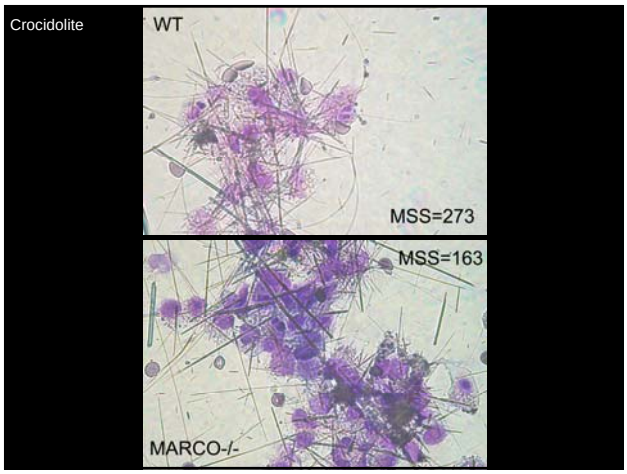


Short Nanotubes

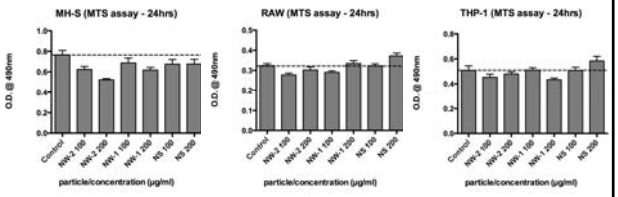


Nanowires





Toxicity of NP in Macrophage Cell Lines?



None of the macrophage cell lines expressed MARCO
Conducting 2D-Gel/MS analysis of membrane proteins

Membrane oxidation by TiO_2 NP

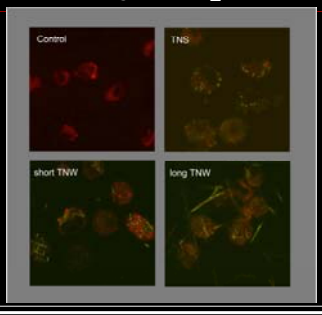
AM incubated with BODIPY 581/591

Nonpolar and electrically neutral, inserts into membrane

Shifts from red to green fluorescence up peroxidation

All forms of TiO2 NP were effective

Therefore, peroxidation not central to toxicity



Summary

- Carbon nanoparticle toxicity difficult to predict from conventional in vitro assays
- Dispersion medium affects outcome for CNP
- Shape of TiO_2 NP important determinant of toxicity
  - Long NW > Short NW >> Nanospheres (In vivo identical)
- MARCO important receptor for NP
- MARCO not involved in long NW toxicity
- RedOx probably not involved in mechanism of NW toxicity
- No unique changes in intracellular ROS

Biological Fate & Electron Microscopy Detection of NPs During Wastewater Treatment

Paul Westerhoff
Bruce Rittmann
Terry Alford
Ayla Kiser, Yifei Wang, Troy Benn

November 2008

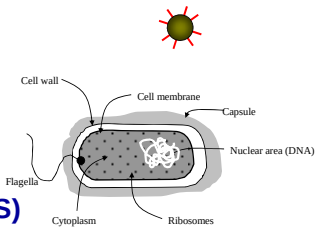
Project Goal

Goal: to quantify interactions between manufactured NPs and WW biosolids:

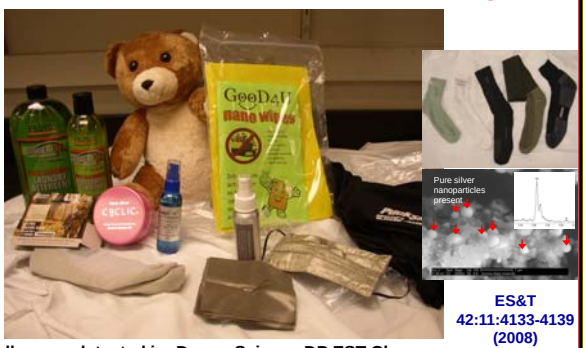
- Develop mechanistic models for NP removal in WWTPs
- We hypothesize that dense bacterial populations at WWTPs should effectively remove NPs from sewage, concentrate NPs into biosolids and/or possibly biotransform NPs.
- The relatively low NP concentrations in sewage should have negligible impact on the WWTPs biological activity or performance.

What is Wastewater Biomass

- Active bacteria
- Inert or residual biomass
- Extracellular polymeric substances (EPS)
- Protozoa and other higher life forms
- Mineral



Nanomaterial Release from Commercial Products into Sewage



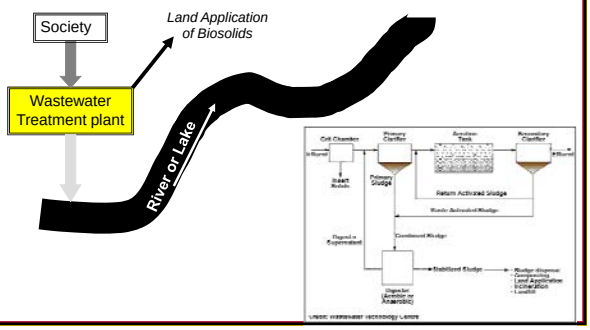
Fullerenes detected in: Derma Science DP EST Charge Plus by Dr. Products

ES&T 42:11:4133-4139 (2008)

Initial Screening for nano-Ag products

Products	Average Mass of Sample (g)	Average Mass Silver per Mass Product [ug-Ag/g-product]	% Silver
*Cyclic Soap Pink Cleansing Bar	0.54	0.401	<0.01
*Silver Ion Generator Spray Applicator	0.02	111000	11.1
*Silver Nano Wipes	0.05	210	0.021
*Bio Safe Face Kit inside	0.03	18.14	<0.01
*Bio Safe Face Kit outside	0.01	189000	18.9
*Benny the Bear Memory Foam Plush Bear Fur	0.07	4.97	<0.01
*Benny the Bear Memory Foam Plush Bear Foam	0.21	39.37	<0.01
PuckSkin Shirt	0.05	36	<0.01
PuckSkin Fabric	0.13	41.88	<0.01
*Bio Safe Hand Kit	0.01	180000	18

Other NMs released from Commercial Products may be present in sewage



Activated Sludge Wastewater Treatment Process

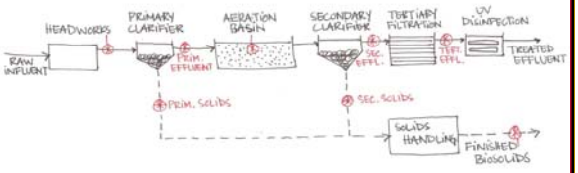
Consider TiO_2 which is already in widespread use in products

- TiO_2 particulates (200 nm) are used in foods and have no toxicological or adverse health effects (Lomer et al., 2000)
- TiO_2 is insoluble and chemically inert
- TiO_2 level range in foods is up to 0.782% (<1 to >200 mg TiO_2 per portion size):
  - Marshmallows, salad dressing, white chocolate, candies, non-dairy creamer, icing
- Average daily intake of TiO_2 estimated at 5.4 mg/day (Ministry of Agriculture, Fisheries and Food, 1993)

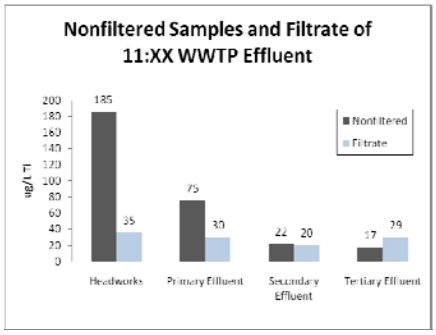
Where does all the TiO_2 in these products used in society end up?

TiO_2 may be a good SENTINEL nanomaterial

Wastewater Treatment Plant Sampling of liquids & biosolids (Mesa, Arizona)

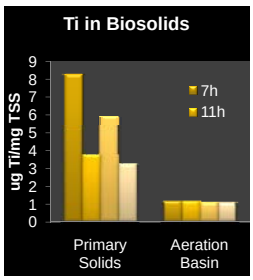


Titanium removal in full-scale Particulate removal dominates over removal of < 0.7 um titanium

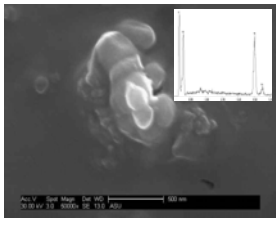


Most Titanium ends up in biosolids

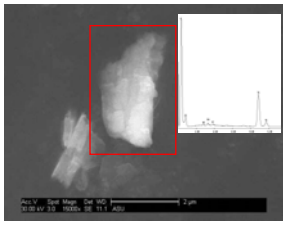
WWTP ID	Metals in biosolids (ug/mg TSS)		
	Ag ($x10^{-3}$)	Ti	Fe
A	12	2.04	36.5
B	11.7	2.94	69.5
C	36.4	3.27	19.3
D	3.6+/-1.4	1.78+/-0.02	4.9
E	38	2.42	35.0
F	14.7	2.72	16.8
G	31	6.39	130



TiO_2 in biosolids



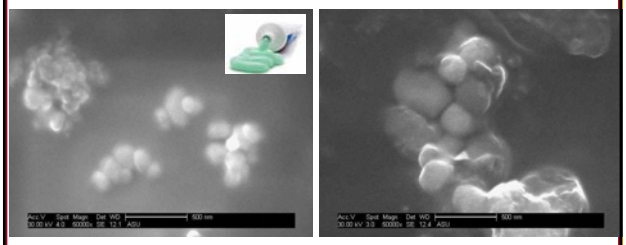
Nano-Scale TiO_2



Micro-Scale TiO_2

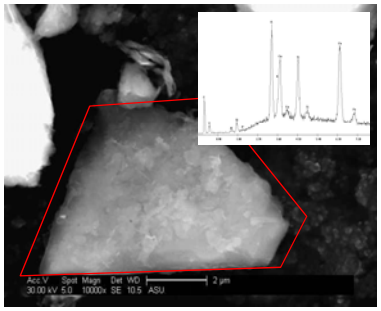
Oxidized away biosolids in organics with hydrogen peroxide H_2O_2 does not affect titanium dioxide

TiO_2 in commercial products are similar to TiO_2 extracted from biosolids



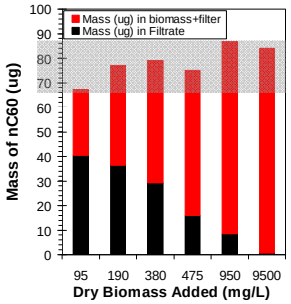
TiO_2 in Toothpaste TiO_2 in Biosolids

Titanium in soil particles are not pure TiO_2



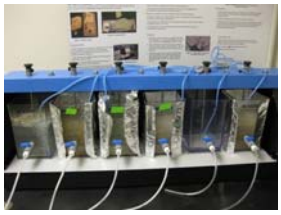
nC_{60} Fullerene and Biosolids

- **Full-scale WWTP Survey:**
  - Biosolids contain < 50 µg- C_{60}/g-dry biosolids
  - Liquid effluent contains < 700 ng-C_{60}/L
- C60 partitions to wastewater biosolids in laboratory tests
- Similar experiments conducted for other NPs with / without NOM

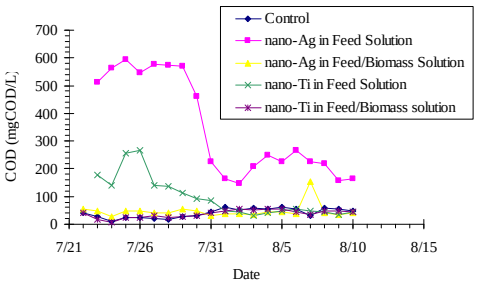


Sequencing Batch Reactors

- Experiments with heterotrophs (ongoing with nitrifiers)
- SBR operation:
  - Aerate for 8 hours
  - Settle for 2 hour
  - Manage HRT 2x/day
  - Manage SRT 1x/6 day
- 3 reactors for HombiKat TiO2
- 3 reactors for carboxylated nano-Ag
- NPs fully characterized
- Feed solution contained salts, glutamic acid and glucose
- Measure fate of NPs & performance of reactor



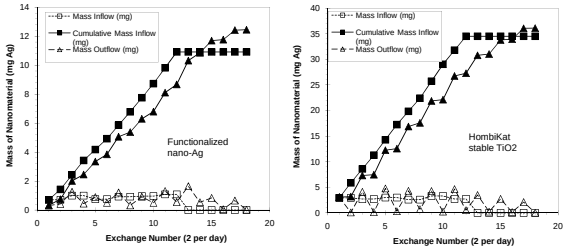
COD Removal in each SBR Reactor



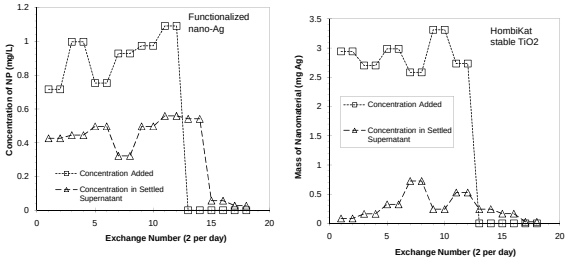
Reactors containing biomass & NPs showed no loss of performance due to NPs

COD removal occurred even in NP controls (not initially seeded with biomass)

Mass Balances During Experiments



Functionalized NPs Poorly Removed



Summary of Key Points

1. Nanomaterials are present in commercial products and will be released into sewage systems
2. Biosorption of engineered NMs onto wastewater biomass will occur
3. Nano-Ag & TiO2 had no effect on heterotrophic activity in Sequencing Batch Reactors
4. 100% removal of engineered NMs will never occur
5. Functionalized NMs are removed less well than metal oxides
6. Engineered NMs will be present in wastewater effluents at < 100 µg/L levels – but this constitutes ~ 10^8 NM/mL that will join the >10^{10} #/mL of natural nanomaterials already in our rivers
7. TiO_2 may serve as a *SENTINEL* NM in the environment that indicates where other NMs will eventually occur.

Genomics-based determination of nanoparticle toxicity: structure-function analysis

Alan T. Bakalinsky, Oregon State University

collaborators:
Qilin Li, Rice University
Jim Hutchison, University of Oregon

Interagency Environmental Nanotechnology Grantee Workshop

21 Nov 08 Tampa, FL

Overall project goals

- Discover genes that mediate toxicity as a first step towards elucidating mechanisms of action
- Correlate toxicity with physical/chemical structure

Genetic approach:

makes no assumptions about mechanisms

Principle:

- A mutant with greater sensitivity or resistance to a nanomaterial is likely to be mutated in a gene relevant to the biological response to the material
- Identifying the mutated genes can identify processes central to toxicity

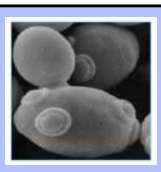
The mutant screen:

- Choose model organism
- Choose toxicity endpoint
- Determine wild-type response
- Screen for mutants with altered response
- Identify mutated genes
- Rationalize how gene loss leads to altered response

How can gene loss lead to resistance?

- Impaired uptake
- Lack of activation
- Improper localization

The yeast model



Because so many cellular functions are shared across vast taxonomic distances, what is true in *Saccharomyces cerevisiae* is often true in other species.

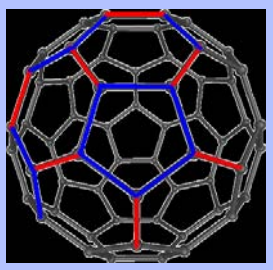
Best understood eukaryote, experimentally tractable

>80% of its 6,000 genes characterized

>31% have human homologs

Comprehensive “deletion libraries” available

Is nC_{60} toxic?

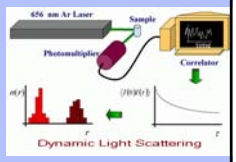


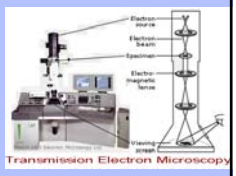
Endpoint / nC_{60} conc.	Organism	nC_{60} prep/size, zeta p	Reference
Oxidative damage in brain tissue **0.5 ppm**	Juvenile largemouth bass	THF/30-100 nm	Oberdörster et al., 2004
DNA damage **2.2 ppb** for aq; **4.2 ppb** for EtOH	Human lymphocytes	Aq/178 nm, -13.5 mV EtOH/122 nm, -31.6 mV	Dhawan et al., 2006
Mortality **1 ppm**	*Daphnia magna* (crustacean)	Aq	Oberdörster et al., 2006
No mortality **0.5 ppm or 1 ppm**	Fathead minnow or Medaka	Aqu	Oberdörster et al., 2006
No effect in many tests **1 ppm**	Soil microbial community	THF/85 nm	Tong et al., 2007
Mortality **200 ppb**	Embryonic zebrafish	DMSO/300-1100 nm	Usenko et al., 2007
No mortality (post-wash) **24 ppm**	*D. magna*	THF/192 nm, -31.1 mV Aq/448, -17.8 mV	Spohn et al., 2007

Endpoint/nC_{60} conc.	Organism	nC_{60} prep/size	Reference
Growth inhibition at **0.4 ppm** in low P min med **No growth inhibition** in LB at **2.5 ppm**	*E. coli* and *B. subtilis*	THF	Fortner et al., 2005
Growth inhibition at **0.4 ppm** in low P min med & at **2.5 ppm** in min. med + air. **No growth inhibition** in LB at **2.5 ppm** or in min. med without air	*E. coli* and *B. subtilis*	THF	Lyon et al., 2005
Growth inhibition in low P min. med **8-10 ppb** for THF; **0.1-1 ppm for** Toluene, Aqu, PVP	*B. subtilis*	THF/39 Toluene/ ~2 Aq/75 PVP/~2	Lyon et al., 2006

Characterization Methods

- Particle size
  - Dynamic Light Scattering (DLS)
    - Zetasizer Nano ZS (Malvern Instruments)
- Surface zeta potential
  - Electrophoretic measurement
    - ZetaPALS (Brookhaven Instruments)
- Morphology
  - Transmission Electron Microscopy (TEM)
    - JEOL-2010 TEM
- C_{60} concentration
  - UV absorbance
    - Shimadzu UV-2550 spectrophotometer.
  - Total organic carbon (TOC)
    - Shimadzu TOC-V_{CSH}





Organisms and assays

Organisms:

Yeast: *Saccharomcyes cerevisiae* BY4742

Gram negative bacterium: *E. coli* DH5alpha

Toxicity assays:

Cell survival, cell yield

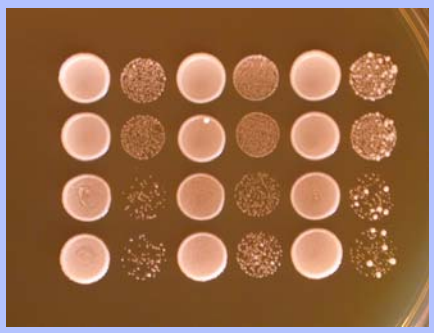
3 types of nC_{60} preparations (3 lots each):

Tol/nC_{60}

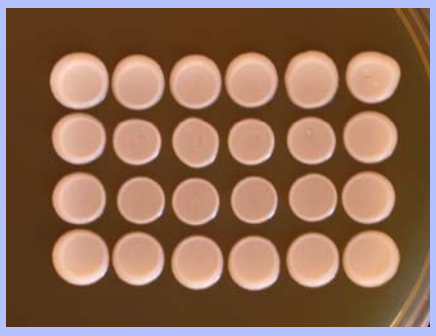
THF/nC_{60}

Aq/nC_{60}

Expectation



control test



Yeast survival assay

Inoculum grown 24 h at 30˚ at 200 rpm in YNB, washed 2X in water, resuspended in water and diluted 10-, 100-, or 1,000-fold into 100 or 250 µl aliquots of water with or without 30 ppm nC_{60} in triplicate.

Cells plated on YEPD in duplicate after 24 h incubation at 30˚ at 200 rpm.

E. coli survival assay

Inoculum grown 24 h at 37˚ at 200 rpm in reduced phosphate MD, washed 2X in 0.9% saline, resuspended in 0.9% saline and diluted 10-, 100-, or 1,000-fold into 100 or 250 µl aliquots of 0.9% saline with or without 30 ppm nC_{60}, in triplicate.

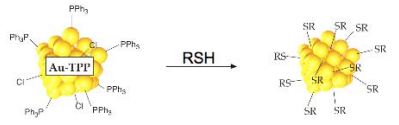
Cells plated on LB in duplicate after 24 h incubation at 37˚ at 200 rpm.

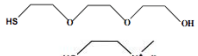
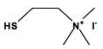
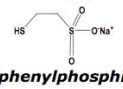
nC_{60} study: conclusions

- nC_{60} did not inhibit growth of either *E. coli* or yeast in minimal media as assessed by final cell yields.
- nC_{60} generally had no impact on survival of yeast in water over 24 h when $\geq 10^5$ cells/ml were treated. Survival decreased modestly when fewer cells were exposed.
- nC_{60} reduced survival of *E. coli* significantly over 24 h in 0.9% saline, particularly at low cell concentration ($<10^6$ cells/ml).
- No obvious correlations between size or zeta potential and cell survival.

Gold Nanoparticles

0.8 nm
11 Au Atoms
10 ligands



Charge	-SR	Ligand name
Neutral:		2,2-mercaptoethoxyethoxyethanol (MEEE)
Cationic:		N,N,N trimethylammoniumethanethiol (TMAT)
Anionic:		2-mercaptoethanesulfonate (MES)

From gold triphenylphosphine (AuTPP) nanoparticles

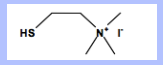
AuNPs synthesized in J. Hutchinson laboratory, University of Oregon. Slide adapted courtesy of R. Tanguay.

TMAT Analogs

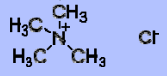
The toxicity of several compounds with similar structure to the Au-TMAT functional group was assessed.

No reduction in survival was observed at functional group concentrations 2-3X higher than that of the primary Au-TMAT particle.

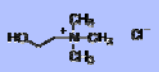
TMAT



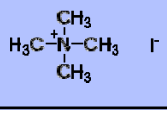
Tetramethylammonium Chloride



Choline Chloride



Tetramethylammonium Iodide



http://www.sigmaaldrich.com

Screen for Au-TMAT-resistant mutants

- 4,800 mutants screened in pools for survival
- 250* putative positive clones isolated
- 42 confirmed in initial re-test
- 12 confirmed in replicated re-test
- 5 candidate clones sequenced
- 4 genes identified: GYL1, DDR48, YMR155w and YGR207c

*To date, 218 of these 250 have been re-tested

- *GYL1*

  GTPase-activating protein, involved in ER-Golgi vesicle trafficking, exocytosis, autophagy, ortholog of human *RAB6A*, a RAS oncogene family member
- DDR48

  DNA damage-responsive protein, has GTPase, ATPase activity, no human orthologs
- YMR155w

  uncharacterized protein, no human orthologs
- YGR207c

  uncharacterized protein, no human orthologs

- Yeast *gyl1Δ/+* and YMR155wΔ/+

  Heterozygotes exhibit similar drug sensitivities as assessed by reduced growth fitness in rich medium.

  Hillenmeyer et al., (2008) Science 320:362

Gold NP study: conclusions

- None of the three Au NPs reduced yeast cell yields in minimal medium.
- The positively-charged Au-TMAT reduced yeast survival more than the negatively-charged or neutral Au derivatives.
- The reduction in cell survival was reproducible with the number of cells killed being proportional to mass of AuNP.
- An entire yeast deletion library (~4,800 mutants) was screened for resistance to Au-TMAT.
- *GYL1*, *DDR48*, YMR155w and YGR207c cause susceptibility.
- Additional resistant mutants have yet to be identified.

A hypothesis

Observations/known phenomena:

1. Stationary phase cells are sensitive to Au-TMAT--growing cells are not.
2. Autophagy is a normal and essential response to nutritional starvation in stationary phase cells.
3. Autophagy involves the turnover of cytoplasm, proteins, organelles by engulfment within specialized vesicles that fuse with the vacuole (lysosome).
4. *GYL1* plays a role in autophagy.
5. A *gyl1Δ* mutant is relatively resistant to Au-TMAT.

Hypothesis:

Au-TMAT is toxic because it interferes with a *GYL1*-dependent step in autophagy.

Acknowledgements

- ***Bakalinsky laboratory, Oregon State University:***
- **Mark Smith**
- **Alex Hadduck**
- **Vihangi Hindagolla**
- **Matthew Boenzli**
- ***Li laboratory, Rice University:***
- **Bin Xie**
- **M. Alexandra Bacalao**
- **Allison Harris**
- **James Winkler**
- **Steven Xu**
- ***Hutchison laboratory, University of Oregon***
- **John Miller**

Funding: EPA-STAR R833325

Role of Surface Chemistry in the Toxicology of Manufactured Nanoparticles

Prabir K. Dutta
The Ohio State University

Goal: Evaluating how surface structure of particles influences their toxicity

- Aluminosilicates
- C particles

Asbestos-related lung diseases

Mineral	*Composition*	*Toxicity*
Crocidolite	$Na_2Fe_2^{III}(Fe^{II}, Mg)_3Si_8O_{22}(OH)_2$	carcinogenic
Amosite	$(Fe^{II}, Mg)_7Si_8O_{22}(OH)_2$	carcinogenic
Chrysotile	$Mg_3(Si_2O_5)(OH)_4$	carcinogenic?
Erionite	$NaK_2MgCa_{1.5}(Al_8Si_{28}O_{72})$	**carcinogenic**
Mordenite	$Na_8(Al_8Si_{40}O_{96})$	**benign**

Erionite Toxicity –why?

Disease

Morphology **Durability** **H_2O_2, O_2^-** **OH^***

- Surface structure
- Surface reactivity
- Develop Biological Correlations

Hypothesis for toxicity : Hydroxyl Radical

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^* + OH^-$$

Need Fe(II) and H_2O_2

H_2O_2 from phagocytosis

Asbestos: Source of both H_2O_2 and Fe(II, III) species.

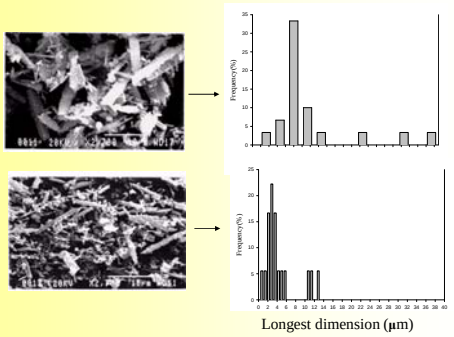
Erionite: Source of H_2O_2 but no Fe?

Erionite (zeolite): Ion exchanging material

$$(Erionite)^-M^+ + Fe(III) \rightarrow (Erionite)^-Fe(III) + M^+$$

- Acquisition of iron in the lung
- All zeolites should be toxic-this is not the case
- Mordenite is not toxic

Zeolite fibers-mordenite and erionite



Longest dimension (μm)

Integrated chemiluminescence intensity upon macrophage-particle (NR8383) interaction

	Median size (micrometer)	10 micrograms	50 micrograms	250 micrograms
Mordenite	3.7	na	503±183	649±303
Fractionated mordenite	1	946.5±139	1846±1134	8382±1855
Erionite	10	na	695±288	1166±589
Fractionated erionite	3	na	965±361	1110±333
Fine erionite	0.8	na	1904	na

na: not analyzed

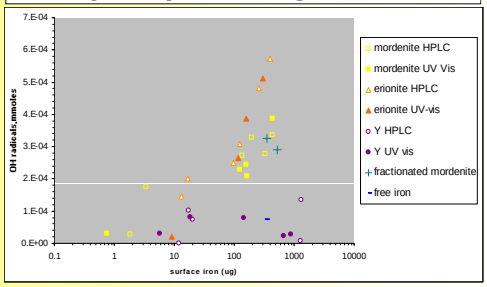
- ROS relatively particle independent
- Smaller particles produce greater oxidative burst
- Increase loading greater oxidative burst

What about the chemical role?

Fe^{2+} (surface) + H_2O_2 → Fe^{3+} + OH^* + OH^-

- Surface iron loading
- Hydroxyl radical production

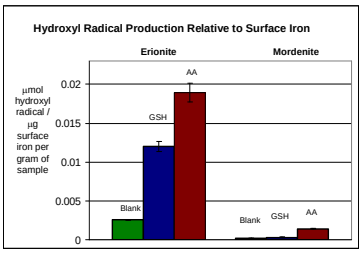
Hydroxyl radical generation



1. OH radicals generation increases with surface iron amounts
2. Small hydroxyl radical production compared to amounts of surface iron (not all iron species are in the right redox state and environment)
3. Erionite-bound iron> Mordenite-bound iron>zeolite Y-bound iron

Fe(III) + reductant → Fe(II)

Ascorbic Acid, Glutathione

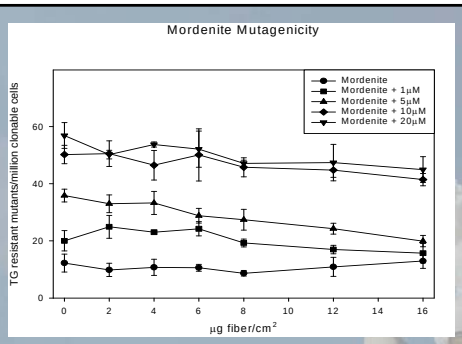


Comparison of hydroxyl radical production in the presence of reductants.

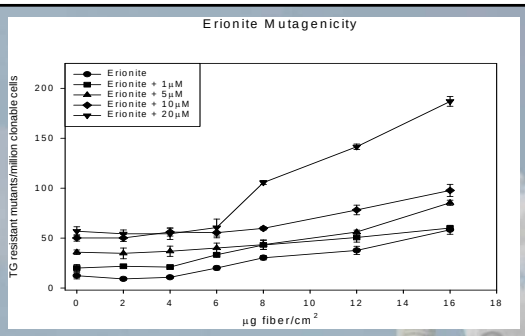
Mutagenesis Experiments

- Cell Line: AS52 cells
- Cells examined for TG resistance clones

(spontaneous mutation frequency =10.5 ± 2.7 TGr/10^{10} clonable cells)

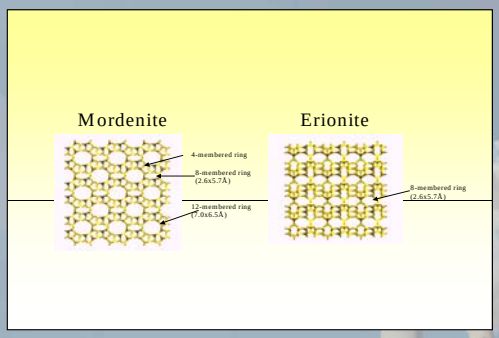


- Effects of mordenite insignificant as compared to controls



- Above 8 µg fiber/cm^2, erionite + Fe^{2+} increased mutation rate
- 16 µg fiber/cm^2 + 20 µM, 3.3x increase

Surface Structures of Erionite and Mordenite



- **Coordination environment can modify the iron redox potential**
- **Chemical reactivity differences result in different biological reactivity**

Manufactured C nanoparticles

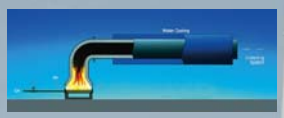
- Carbon black production: 10^6 metric tons, 1999 (J. Occup. Health 2001; 43: 118-128)

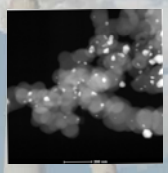
"Furnace Black"

~14nm

"Lamp Black"

~100nm

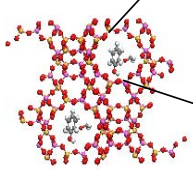


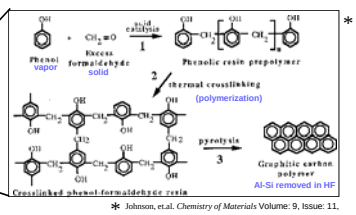


http://www.degussa.com/

Templated synthesis of carbon-based model particulates

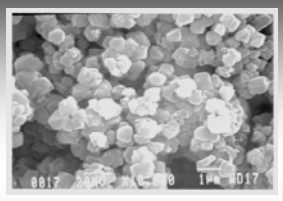
1 µm aluminosilicate zeolite particles



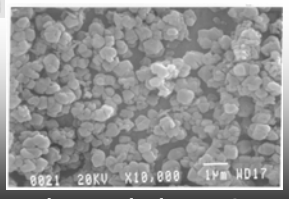


* Johnson, et.al. *Chemistry of Materials* Volume: 9, Issue: 11, November 18, 1997, pp. 2448-2458

carbon particulates



zeolite Y ~ 1 µm

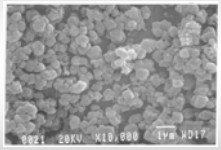


carbon particulates ~ 1 µm

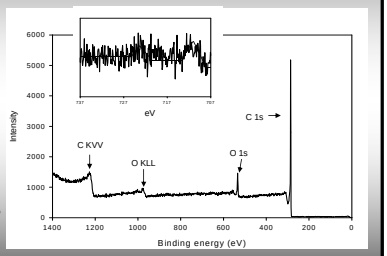
Carbon-iron particulates (C-Fe)

Template has ion exchange capability.
Fe incorporated into template.

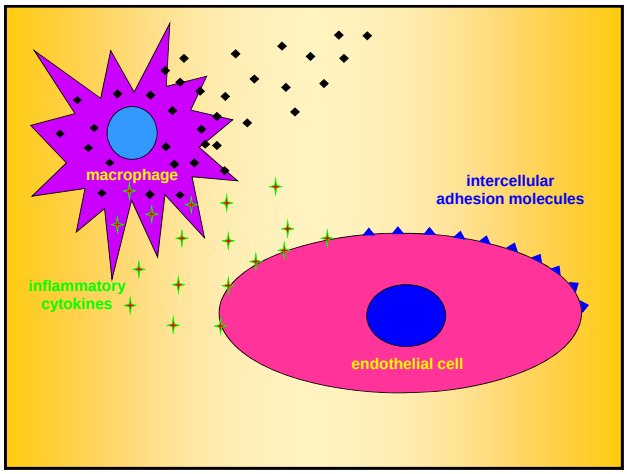
SEM: C-Fe

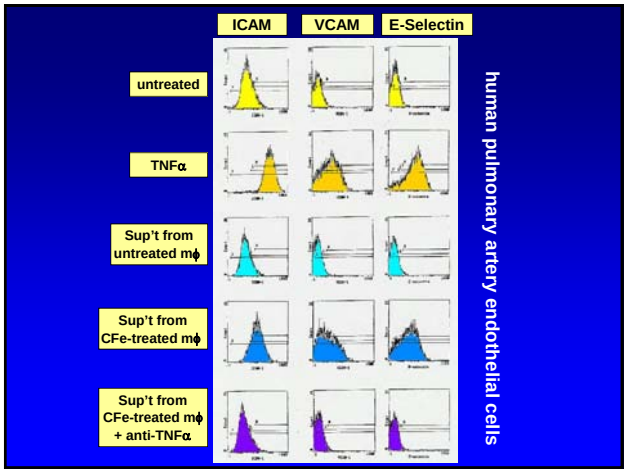


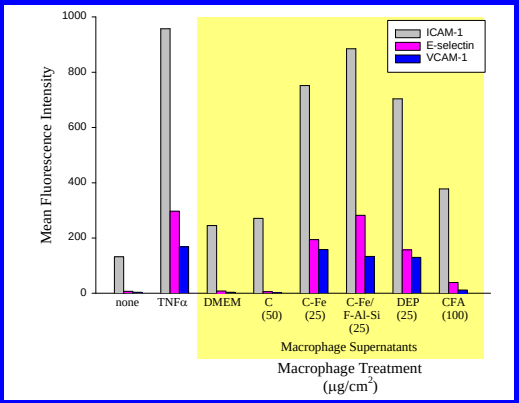
iron loadings low after template etching

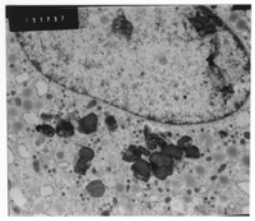


XPS – surface elemental analysis
inset: iron region



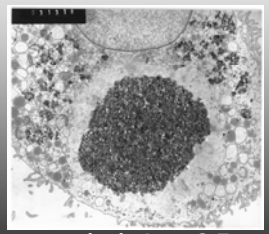






synthetic 1 μm C
24 hrs post-exposure

TEM
human monocyte-derived macrophages



synthetic 1 μm C-Fe
24 hrs post-exposure

Hydroxyl Radical Production

$Fe(III) + H_2O_2 \rightarrow Fe(II) + HO_2^{*} + H^{+}$

$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^{*} + OH^{-}$

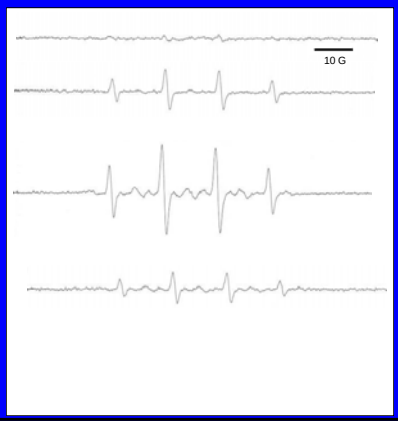
DMPO + OH^{*} → DMPO-OH

EPR active!!! (1:2:2:1 quartet)

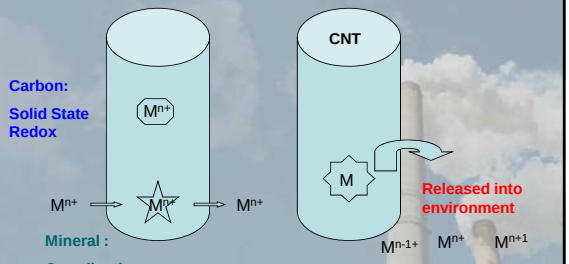
DMPO = 5, 5-dimethylpyroline-N-oxide

1) Valvanidis et. al. Atmospheric Environment 34(2000) 2379-2386

DMPO Spin Adducts



Materials that release metal ions ?

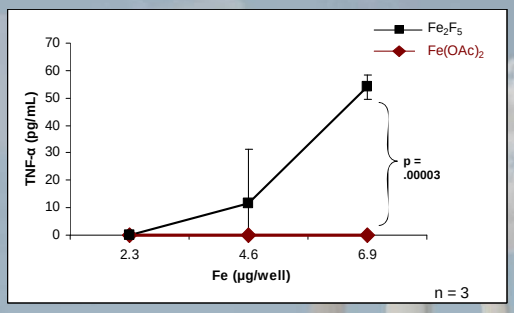


What is the role of n in M^{n+}?

Role of n^+?

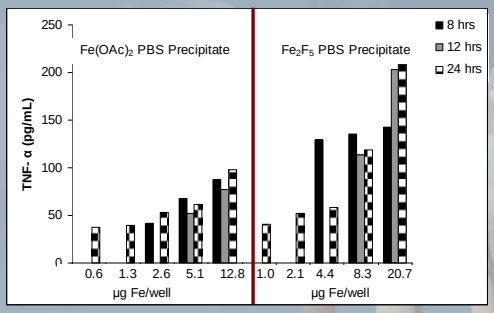
- Investigated two compounds
  Fe (II) Acetate (OAc) and Fe (II). $Fe(III)F_5$
- Both compounds form a precipitate in presence of phosphate buffer
- For Fe (II) OAc : 50% of Fe(II) precipitated
- For Fe (II). $Fe(III)F_5$: 100% of Fe(III) precipitated
- So, solution species of both comparable: Fe(II)

TNF-α production after 12 hour exposure of Murine Alveolar macrophages to the two Fe sources in phosphate buffer



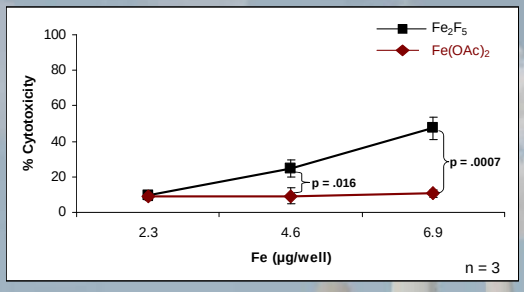
Fe (III) sample more inflammatory

TNF-α production for Murine Alveolar macrophages treated with the two different iron phosphate precipitates



Differences arising from the precipitates

Cytotoxicity after 12 hour exposure



Significant difference between the 2 iron sources

- Fe(III) precipitate more cytotoxic than Fe(II)
- Fe(III) precipitate more inflammatory than Fe(II)

Hypothesis : Redox state of the element released is important

Acknowledgements

- NSF-EMSI
- NIH

Collaborators: W. James Waldman
Marshall Williams
John Long

Students: Estelle Fach
Robert Kristovich
Amber Nagy
Brian Peebles

A Rapid In Vivo System for Determining the Toxicity of Nanomaterials

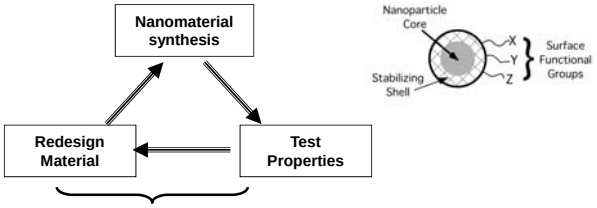
Robert Tanguay
Department of Environmental and Molecular Toxicology
Environmental Health Sciences Center
Oregon Nanoscience and Microtechnologies Institute (ONAMI)
- Safer Nanomaterials and Nanomanufacturing Initiative

The Opportunities

Proactively guide the development of safer nanomaterials to reduce hazard

- **Identify the physicochemical properties that drive biological responses–take a broader view**
- **Think nanoscience - not toxicology**
- **Develop predictive models from experimental data.**
- **Feed the Nanomaterial Biological Interactions (NBI) knowledgebase**

Designing Safer Nanoparticles



Structure/Property Relationships:
Physicochemical properties and biological responses

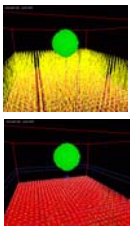
Nanoparticles have *widely* tunable properties - the key is to enhance performance and safety at the same time.

Platforms to Define Nanobiological Interactions and Responses

- ***In vitro***
  - **Continuous cell culture system**
  - **Primary cell culture system**
  - **Stem cells**
- ***In vivo – High content studies***
  - **Whole animal studies**
    - **Rodents**
    - ***Fish***
    - **Flies**
    - **Worms**

….The field is at the discovery phase.

Why do we chose not cultured cells?



Response

Proliferation
Cell death
Metabolism
Gene expression
Phenotypic change

As a discovery platform …Too many "blind spots"

Cell cultures -What blind spots?

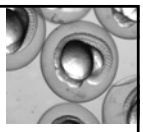
- **Different cell-cell interactions cannot be evaluated**
- **Indirect effects cannot be evaluated**
- **Cells in culture can only respond using their unique repertoire of expressed gene products – limited potential targets**
- **Tremendous potential for missed data – missed opportunities**

In vivo systems may offer significant advantages if amenable to efficient assessments

Why evaluate responses during early embryonic development?

- Vertebrate embryonic development is the most complex biological system.
- Processes of development are remarkably conserved
- Comparative genomics data supports overall conservation of potential "targets"
- Generally more responsive to insult
  - Most dynamic life stage…and the full signaling repertoire is expressed and active, therefore fewer blind spots.. Highest potential to detect interactions
- If a chemical or nanomaterial is developmentally toxic it must influence the activity of a molecular pathway or process.. i.e. hit or influence a "Toxicity Pathway"

Why Zebrafish?



- Share many developmental, anatomical, and physiological characteristics with mammals
- Genome is "completely" sequenced
- Molecular signaling is conserved
- Technical advantages of cell culture - power of in vivo
- Amenable to rapid whole animal mechanistic evaluations
- Hundreds of laboratories are exploiting this model - shared resources

Consider startpoints - not endpoints

- Signaling pathways and molecular events are conserved
- ..But fish are not rodents or humans
- Consequences of disrupted signaling often species specific
- ……..the mechanism by which a "target" is hit is likely conserved, but the <u>consequence</u> of the "hit" may be distinct

Assay Considerations

- The goal is to investigate interactions and responses.
- Embryonic development serves as a "biological sensor and amplifier"
- These are "forced" interactions!
- Remove chorion "potential barrier"

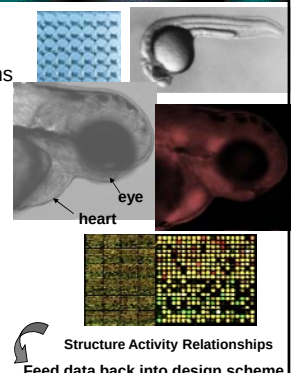
- HAZARD Identification, not risk assessment!

Assessing Biological - Nanomaterials Interactions and responses

<u>Tier 1:</u> Toxicity Screening
- Toxicity testing whole organisms
  - *In vivo* - zebrafish

<u>Tier 2:</u> Cellular Targets and Distribution
- Defined *in vivo*
  - Fluorescent nanomaterials
  - Targeted assays

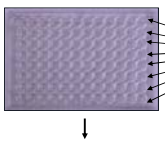
<u>Tier 3:</u> Molecular Expression
- Genomic Responses
  - Whole animal gene expression profiles



Structure Activity Relationships

Feed data back into design scheme

Tier 1 Testing



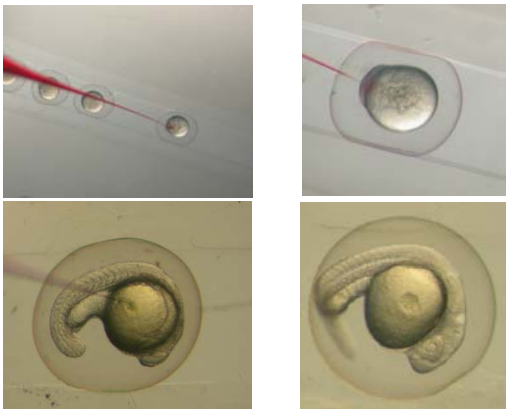
Multi-well plates

Test Materials

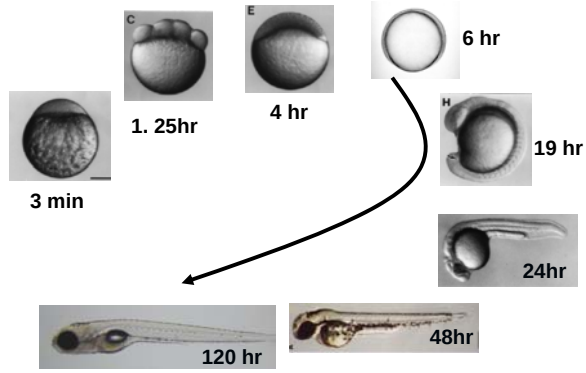
- Purified well characterized
- Forms actually in use
- Aged- i.e. environmentally

Screening for responses 1-5 days

Alternate Exposure Route - Microinjection



Development Stages of Assessments



High Content Tier 1 Endpoints
(Assessed between 24 and 120 hpf)

Morphological

Malformations
- i.e. pericardial edema, yolk sac edema, body axis
- fin malformations, eye diameter

Circulation

Heart beat (rate)

Developmental progression

Embryo viability

Behavioral

spontaneous movement (18-24 hpf) onset and frequency

touch response (27 hpf)

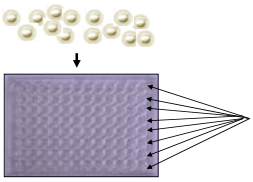
motility

Nanoparticles Assessed - to Date

Over 200 fully evaluated through tier 1.

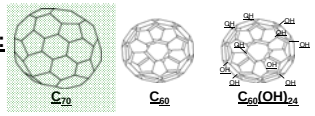
C_{60}, $C_{60}(OH)_{24}$, C_{70}, SWCNT, DWCNT, dendrimers, metal oxides, Q-dots, gold nanoparticles, viral derived……

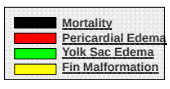
- Gold nanoparticles
- Fullerenes

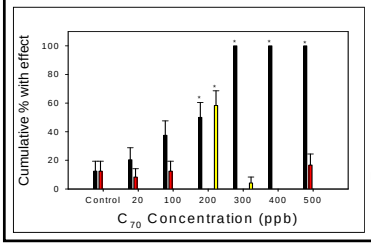


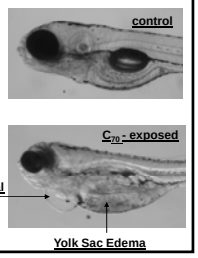
Toxic Potential
Size and Surface Functionalization

Carbon Fullerenes:



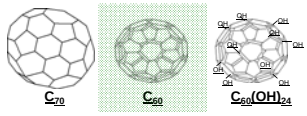


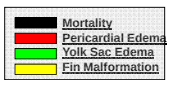


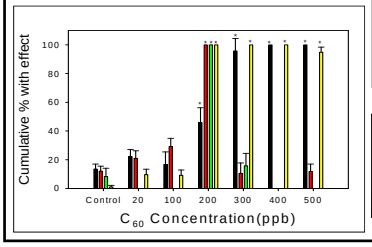


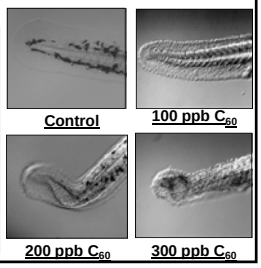
Toxic Potential
Size and Surface Functionalization

Carbon Fullerenes:



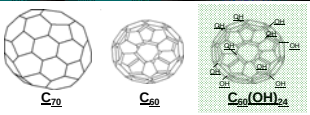




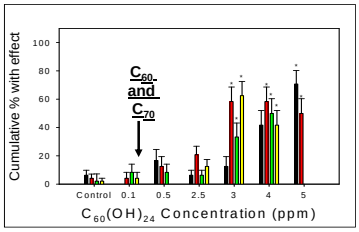


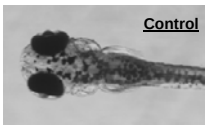
Toxic Potential
Size and Surface Functionalization

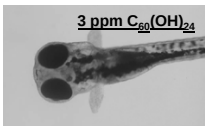
Carbon Fullerenes:



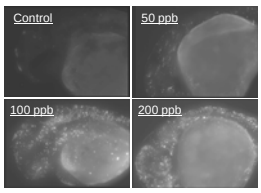
Mortality
Pericardial Edema
Yolk Sac Edema
Fin Malformation

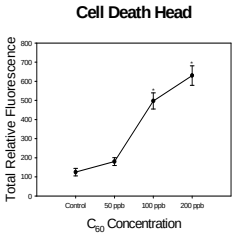




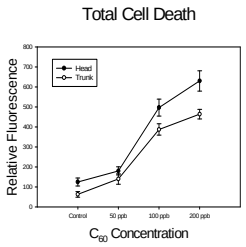


C60 Exposures Increases Cellular Death
Acridine Orange – *In vivo assessment*

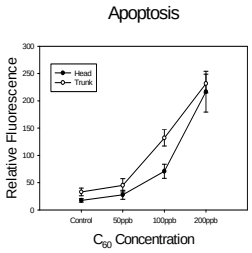




C60-Induced Cell Death

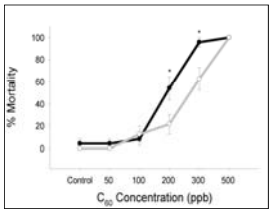


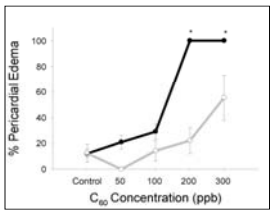
Acridine Orange



TUNEL

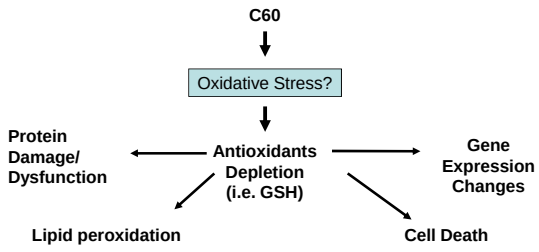
Light Exposure Increases C60 Toxicity



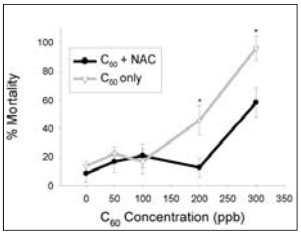


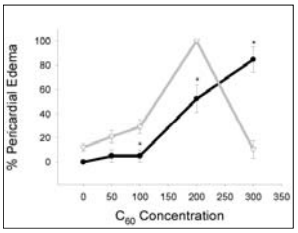
C60
C60 dark

Oxidative Stress Response (Tier 2)

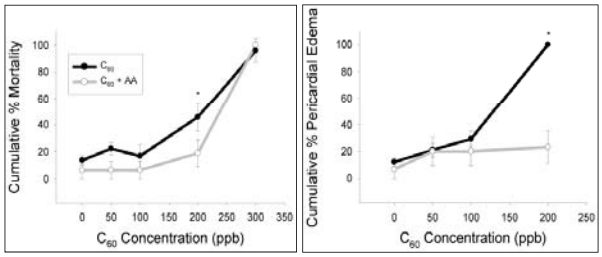


GSH Precursor -NAC Offers Partial Protection



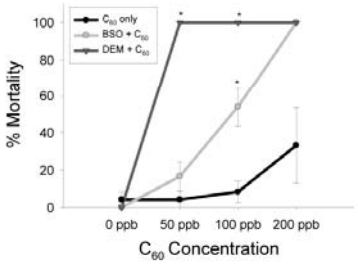


The Antioxidant Ascorbic Acid Offers Partial Protection

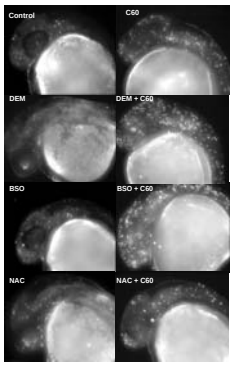


Chemical Depletion of Glutathione

Embryos Are More Sensitive to C_{60}



Oxidative Environment Impacts In vivo Cellular Death Response



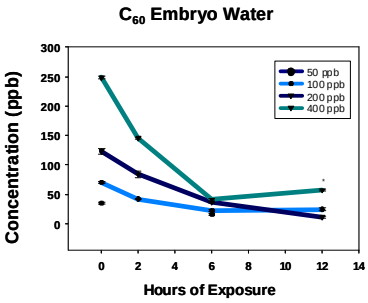
Determining Nanomaterial Dose

- **Defining Dose is challenging – regardless of the platform**
  - **Numerous obstacles**
    - **Agglomeration parameters in aqueous media unknown**
    - **Uptake and distribution unknown**
    - **Few labeled materials**
    - **Must define dose for comparative studies**

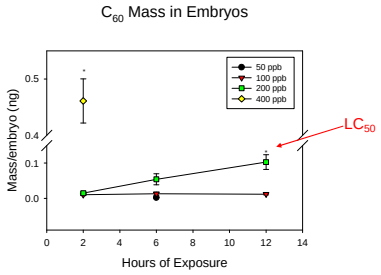
C_{60} Dose Determination

- **Goal: to develop a method for detecting and quantifying C_{60} associated with biological and aqueous samples.**
- **Analytical quantification of C_{60} using LC-MS (Collaboration with Dr. Carl Isaacson and Dr. Jennifer Field – OSU EMT)**
- **Pooled 100 embryos per replicate**
- **Use of ^{13}C-labeled C_{60} surrogate to calculate losses during extraction method.**

Water Concentration Declines Over Time



C_{60} Dose Determination

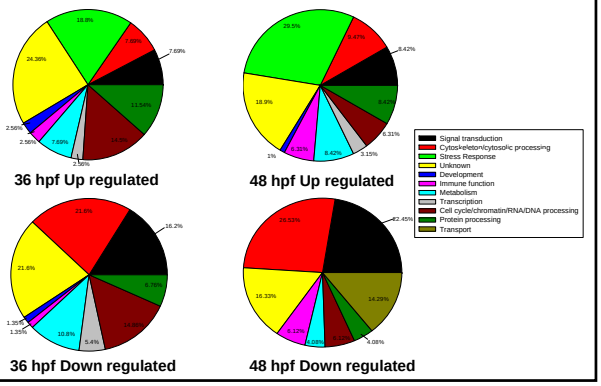


The C_{60} LD_{50} in embryonic zebrafish is 0.1 ng/mg.

Global Gene Expression (Tier 3)

- **Zebrafish oligo arrays used to evaluate gene expression changes following C60 exposure (>14,000 genes)**
- **200 ppb C_{60} and 1% DMSO controls**
- **Expression evaluated at 12 & 24 hrs post exposure**

Results by Functional Category



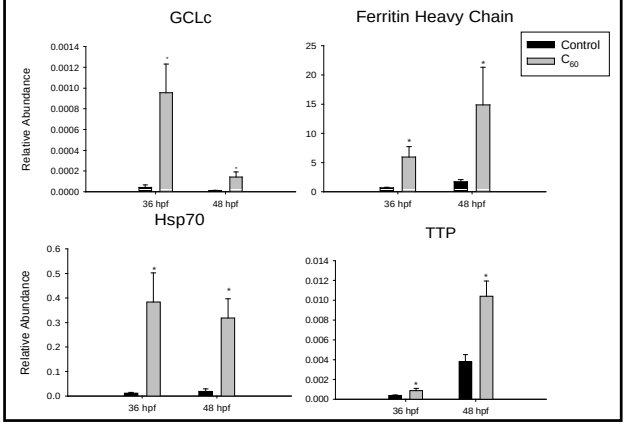
36 hpf Up regulated

48 hpf Up regulated

36 hpf Down regulated

48 hpf Down regulated

Embryonic Stress Response - Q-RT-PCR



Conclusions

- **Cannot predict biological responses without data.**
- **Many advantage by evaluating interactions/responses in vivo - multiple levels of organization**
- **Zebrafish: a discovery platform to define nanomaterial/biological Interactions from diverse sources**
- **Opportunities to define structure response relationships**
- **Extremely well-suited for whole animal mechanistic studies.**

SNNI SAFER NANOMATERIALS AND NANOMANUFACTURING INITIATIVE

ONAMI

Acknowledgements

- **Dr. Stacey Harper**
- **Crystal Usenko**
- **Lisa Truong**
- **Kate Saili**
- **Dr. Jennifer Field**
- **Dr. Carl Isaacson**
- **Oregon State Radiation Center**

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•NIEHS T32 Toxicology Training Grant

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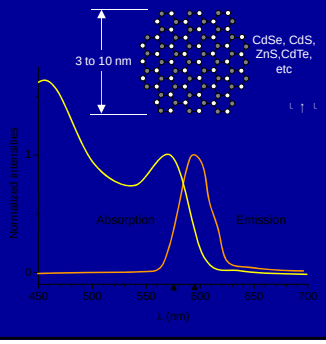
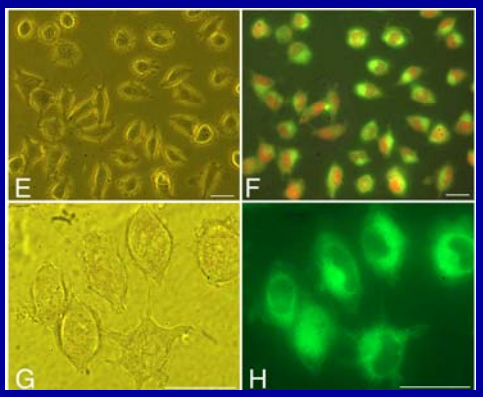
Quantum Dot Toxicity in Zebrafish

Greg Mayer - Texas Tech University
Jay Nadeau - McGill University
Anja Nohe - University of Delaware

Why QDs?

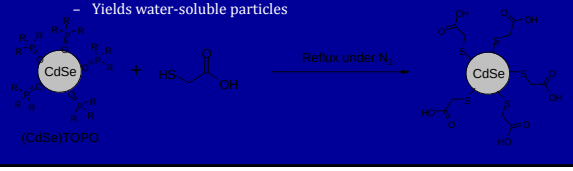


- Emission wavelength is related to the size of the crystal
- Slow to photobleach and radiation resistant
- Emission can be quenched/modulated by attaching electron donors or acceptors to the surface
- Can be suspended in aqueous and non-aqueous environments
- Many colors obtained with a single UV excitation source
- Surface can be conjugated to chemically and biologically important molecules



QD Synthesis/Solubilization

- CdSe/ZnS core-shell
- Synthesis via a two-step, single flask method.
  - Injection of Selenium precursor into hot coordinating solvent containing the cadmium precursor, CdO.
  - Leads to nucleation and growth of particles
  - Injection of Zn and S solutions arrests growth, forms cap around particles.
- Water solubilization is done by TOPO cap exchange with thiol mercaptosuccinic acid (MSA) or mercaptoacetic acid (MAA)
  - Reflux in methanol for 6 hours
  - Yields water-soluble particles

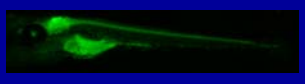


Objectives of Investigation

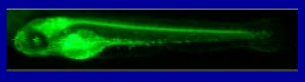
- Compare molecular responses elicited by organism from exposure to heavy metals and semiconductor nanoparticles
- Determine how semiconductor nanoparticles facilitate resulting cytotoxicity

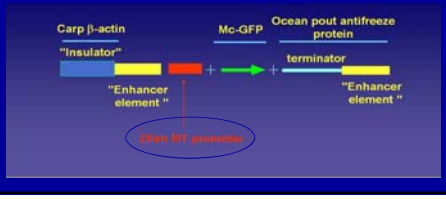
ZM9 Strain

Control 6dpf



50μM Zn 6dpf

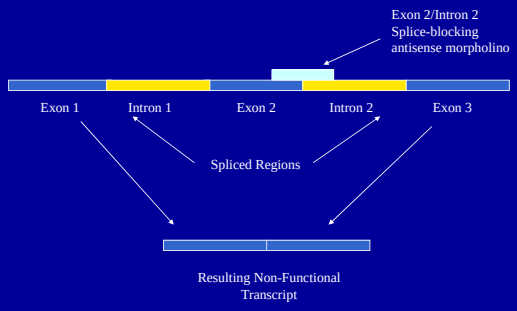




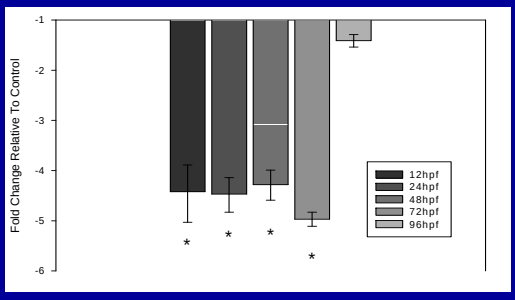
MTF-1 Knockdown Model

- Determine extent of MTF-1 knockdown in wild type
- Observe subsequent MT reduction in wild type
- Knockdown MTF-1 in transgenic model and monitor heavy metal response

Morpholino Design

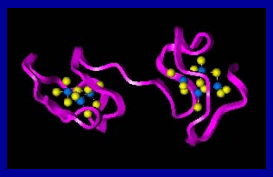


Morpholino Efficiency

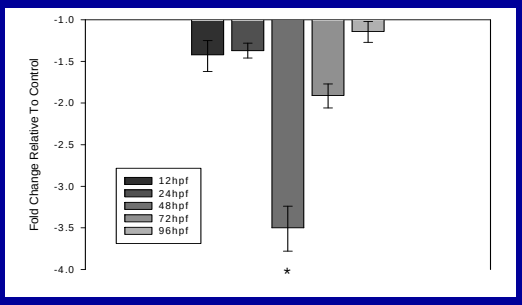


MTF-1 Target Genes

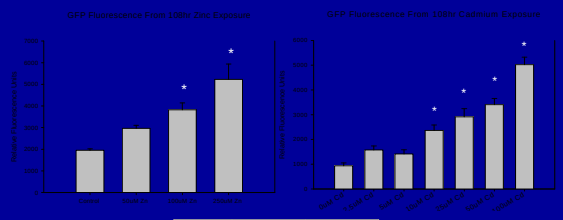
- Metallothionein (MT)
  - Heavy metal and free radical scavenger
  - Well-conserved
  - Increases with elevated group I-IIB heavy metal load



Target Genes - MT

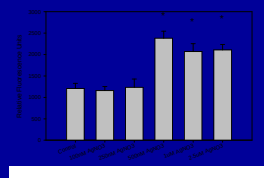


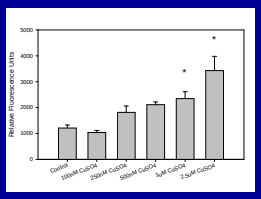
Zinc and Cadmium Tox Curves

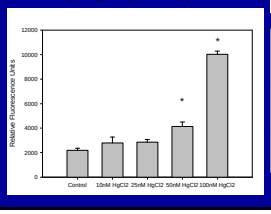


	Zinc Sulfate	Cadmium Chloride
Average	725.742 µM	193.419 µM
S.E.	+/- 44.24	+/- 7.244

Other Group IB and IIB Metals

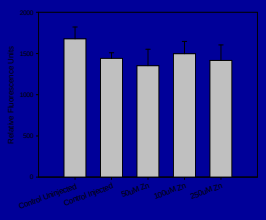


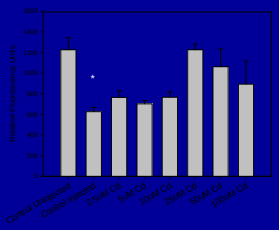




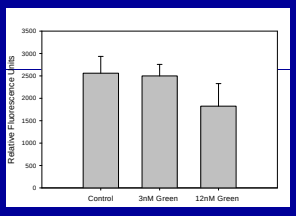
	Copper Sulfate	Mercuric Chloride	Silver Nitrate
Average	13.058 µM	254.429 nM	1.240 µM
S.E.	+/- 2.179	+/- 5.961	+/- 0.103

Effect of Morpholino On Transgenic Model

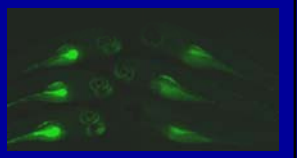




Effect of Quantum Dot Exposure

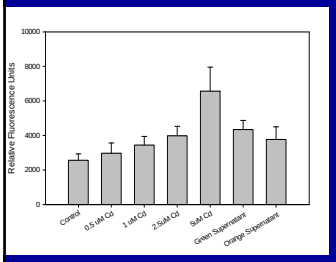


Control QD Exposed



24 Hour Exposure (5-6 dpf)

Quantum Dot Solution Supernatant

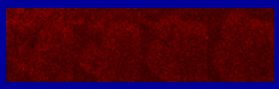


Quantum Dot Supernatant	Cadmium (µM)	Zinc (µM)
Orange	0.5	6.0
Green	0.4	8.2

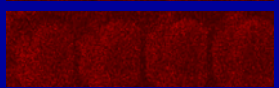
ICP-MS Analysis

24 Hour Exposure (5-6 dpf)

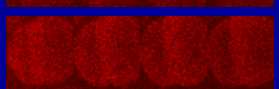
Quantum Dot Accumulation In Zebrafish Embryo



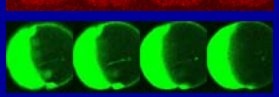
40 Minutes



1 hr 40 Minutes



2 hrs 40 Minutes

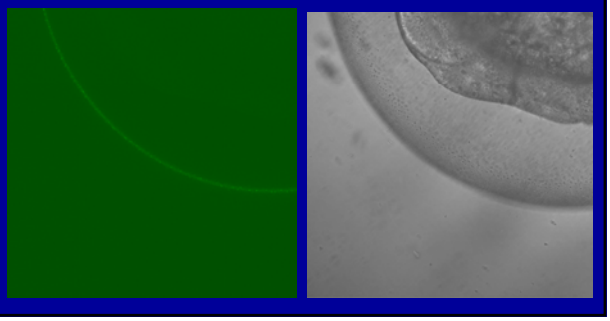


embryo uptake

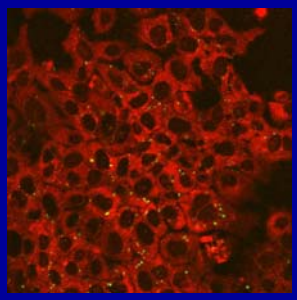
~45 minutes post fertilization

embryo uptake

~2 hrs. post fertilization



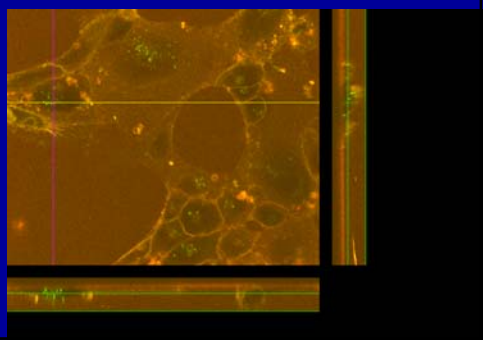
Quantum Dot Interaction With Zebrafish Liver Cells



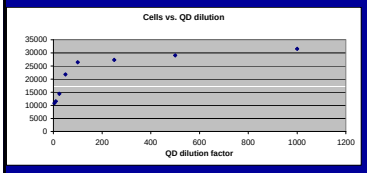
10nM Green QD for 24 hr

Membrane Stain w/ BODIPY ceramide

Cellular compartmentalization



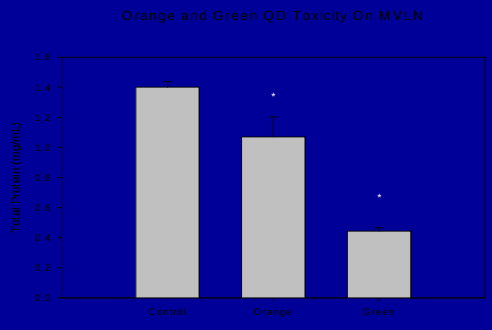
ZFL cellular toxicity



Starting concentration of ~5mM

Dilutions were 2x, 5x, 10x, 50x, 100x, etc.

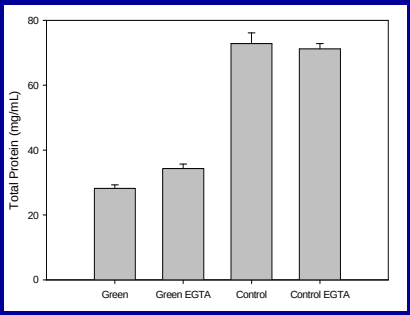
Cellular Toxicity



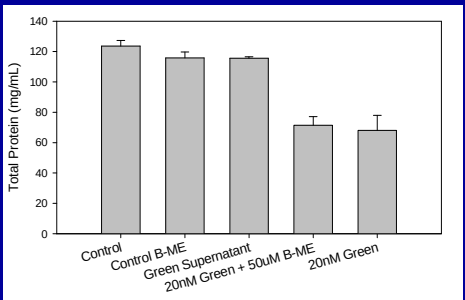
Quantum Dot Toxicity

- Primary reasoning
  - Heavy metal liberation
  - Free radical generation → oxidative stress
  - Membrane damage/disruption

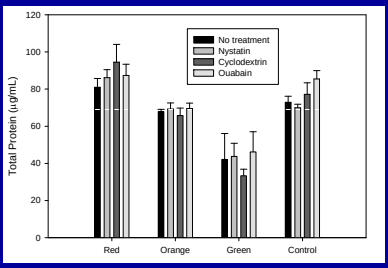
Heavy Metal Chelation



Free Radical Elimination



Endocytic/Clathrin Inhibitors



Similar results with amantidine and Cytochalasin D

No significant difference observed with inhibition of calveolin-mediated or clathrin-mediated uptake

No alteration of toxicity with suppressed active uptake mechanisms

Conclusions

- Semiconductor nanoparticles accumulate in zebrafish embryos
  - Potentially damage hepatic system
- Bind to cellular membrane
- Do not enter cell through clathrin-dependent endocytosis
- Diameter correlates with overall toxicity
- Toxicity not induced by heavy metal release or free radical generation
- Degrade and liberate free heavy metal ions ?

Acknowledgements

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Dr. Anja Nohe
Jeremy Boner

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U.S. Environmental Protection Agency
Interagency Environmental Nanotechnology Grantees Workshop

Sheraton Tampa Riverwalk Hotel
Tampa, FL

November 19 – 21, 2008

EXECUTIVE SUMMARY

NOVEMBER 19, 2008

INTRODUCTION AND OVERVIEW

The 2008 Interagency Environmental Nanotechnology Grantees Workshop was held November 19-21, 2008, in Tampa, Florida, and was hosted by the U.S. Environmental Protection Agency (EPA), Office of Research and Development (ORD), National Center for Environmental Research (NCER). The workshop brought together research grantees funded by the EPA Science To Achieve Results (STAR) Program, the National Science Foundation (NSF), the National Institute of Environmental Health Sciences (NIEHS), and the National Institute for Occupational Safety and Health (NIOSH). Grantees discussed the latest science regarding the potential effects of engineered nanomaterials (ENMs) on human health and the environment. Additional talks were given by federal agency program officials. The goal of the workshop was to stimulate communication and collaboration among scientists and engineers investigating the potential implications of ENMs. Approximately 100 participants attended the workshop.

Welcome
Nora Savage, EPA, NCER

Dr. Nora Savage welcomed participants to the meeting and provided background about her job and colleagues at NCER, within EPA's ORD. She reviewed the agenda for the meeting, noting some changes. She explained the logistics of the meeting and introduced the contractor staff, including individuals from The Scientific Consulting Group, Inc. (SCG). She encouraged participants to complete the meeting evaluation form and return it to SCG staff; EPA would like input about future co-location of this meeting with the Society of Environmental Toxicology and Chemistry (SETAC) Annual Meeting or other professional society meetings. She introduced Mr. Christopher Zarba, the Deputy Director of NCER.

Sponsored Research at U.S. EPA NCER
Christopher Zarba, EPA, NCER

This year, as in the past 5 years, nanotechnology is the number one research priority. The area of nanotechnology receives most of the funding, which illustrates how important this issue is to EPA. The customers assist in writing the Requests for Applications (RFAs), and the proposals received in response to the RFA are reviewed and ranked by an external peer review panel. Only those proposals that receive excellent or very good scores move on to the next level. Customers select and prioritize proposals, with approximately 10 to 20 percent of proposals funded. Scientists that receive EPA STAR funding are the best and brightest, working on world-class environmental issues.

There are approximately 1,800 employees in ORD. ORD's budget in the 2009 President's Budget is $54.1 million, which has not changed much in the last 12 years. There are 13 laboratories and research

facilities around the country. ORD's mission is to give their customers the scientific information they need to write regulations and to set policies. The requests for research are about 10-fold more than the available resources. National Program Directors (NPDs) are independent scientists who report to the Assistant Administrator. They look at both extramural and intramural research being conducted in their program areas. Since the creation of the NPDs, there has been an increasing emphasis on the use of STAR grants, particularly for new and emerging programs. The Agency is developing a Nanomaterial Research Strategy (NRS). This document covers broad themes and general approaches for extramural and in-house nanotechnology research. ORD has identified four key research themes and seven key scientific questions where ORD can provide leadership for the federal government research programs and support the science needs of the Agency. The NRS should be available within 2–3 months. There is a possibility that an NPD will be assigned for nanotechnology.

Established in 1995, the STAR Program is the extramural funding arm of EPA's ORD. There is significant Agency and cross-agency involvement in the solicitation writing and review of proposals and all solicitations are competitive. The STAR Program awards about $66–100 million annually and currently is managing about 800 active research grants and fellowships. About 25 RFAs are issued each year. Each year the STAR Program receives 3,000 grant applications and makes about 200 new STAR awards. EPA tries to collaborate with other agencies; nanotechnology is a good example as EPA has collaborations with the NSF, NIEHS, NIOSH, and the Department of Energy (DOE).

EPA is interested in nanoscale materials for a number of reasons, including the following: (1) the unique chemical properties of nanoscale materials makes traditional risk management techniques and regulations unsuitable in many situations; (2) these materials have potential environmental applications, such as cleaning up past environmental problems, improving present processes, and preventing future environmental problems; (3) the Agency has regulatory responsibilities because these products are in the marketplace and may pose risks to human health, the environment, or both; and (4) opportunities exist to maximize the environmental benefits and minimize impacts from the beginning, as new technologies are developed. Specific areas of interest for the STAR Program in nanotechnology include research on implications (e.g., potential toxicity; potential exposure; fate, transport, and transformation; and bioavailability and bioaccumulation) and applications (e.g., pollution remediation and treatment, pollutant or microbe monitoring and detection, and the development of environmentally benign processes for pollution prevention).

The nanotechnology program was initiated in 2002 with $5 million. The STAR Program began by funding exploratory research, primarily on applications of nanotechnology, in 2001; the program shifted to exploratory research on the implications of nanotechnology in 2003. EPA's Small Business Innovation Research (SBIR) Program also has solicited research on nanotechnology. The goal of the SBIR Program is to bring new, innovative environmental technologies to market. In the STAR Program, grants can be converted into cooperative agreements. This funding mechanism allows researchers within ORD to work more collaboratively with STAR grantees. EPA and NSF have made awards to establish two Centers for the Environmental Implications of Nanotechnology (CEIN). The centers, led by the University of California, Los Angeles (UCLA) and Duke University, will study how nanomaterials interact with the environment and with living systems, and will translate this knowledge into risk assessment and mitigation strategies useful in the development of nanotechnology.

Discussion

A participant asked Mr. Zarba to describe EPA's customers. Mr. Zarba responded that their customers are the EPA program offices (e.g., Office of Air, Office of Water) which write regulations, set Agency policy, write criteria, etc., and need the research conducted to support their work.

National Science Foundation (NSF)
Mihail (Mike) Roco

Since 2000, nano science and engineering has expanded to many disciplines and approximately $14 billion is spent worldwide on nanotechnology research and development. Nanotechnology is working at the atomic, molecular, and supramolecular levels, in the length scale of approximately 1–100 nm range, to understand and create materials, devices, and systems with fundamentally new properties and functions because of their small structure. The definition encourages the following new contributions that were not possible before: (1) understanding and exploitation of novel phenomena, properties, and functions at nanoscale, which are nonscalable outside of the nanomaterial domain; (2) the ability to measure/control/manipulate matter at the nanoscale to change those properties and functions; and (3) integration along length scales and fields of application. A timeline was developed for the four generations of nanotechnology products and processes by considering the beginning of industrial prototyping and nanotechnology commercialization. The first generation products (2000–2004) were passive nanostructures, such as nanostructured coatings, nanoparticles (NPs), nanostructured metals, polymers, and ceramics. The second generation products (2005–2009) include active nanostructures such as 3-D transistors, amplifiers, targeted drugs, actuators, and adaptive structures. Third generation products (2010–2015) will be nanosystems such as guided assembly, 3-D networking, new hierarchical architectures, and robotics. The fourth generation (after 2015) will include molecular nanosystems such as molecular devices "by design," atomic design, and systems with emerging behavior.

NSF supports 26 large research and education centers on nanotechnology and two user facilities. Currently, there are 4,000 active research awards, and approximately 10,000 students and teachers are trained each year. The current year's nano budget at NSF is approximately $400 million. NSF spends about 7 percent ($28 million) of its nanotechnology budget on environmental health and safety concerns through single investigator projects, small groups, and centers. Collaborations and partnering are important to NSF. NSF has had a number of program collaborations with EPA as well interactions with the National Institutes of Health (NIH), DOE, NIOSH, and other agencies. In 2007, NSF collaborated with EPA and DOE on a solicitation that focused on exposure from manufactured nanomaterials. The collaborations and partnerships for the nano centers, networks, and user facilities were described.

Both immediate and continuing societal implications issues as well as long-term concerns must be addressed earlier in research programs. An anticipatory and corrective approach that is both transforming and responsible in addressing societal implications for each major nanotechnology research and development program from the beginning is needed. Risk governance of nanotechnology is becoming increasingly important at the national and international levels.

National Institute for Occupational Safety and Health
William (Allen) Robison, NIOSH

Dr. W. Allen Robison explained that NIOSH is small institute within the Centers for Disease Control and Prevention (CDC) with an overall annual extramural budget of $82 million. The purposes of NIOSH's Nanotechnology Program are to: (1) increase knowledge of nanotechnology and manufactured nanomaterials, (2) examine the occupational safety and health aspects of nanotechnology, and (3) examine application and implications of nanotechnology. The program complements the intramural program. Since 2001, NIOSH has used R01, R03, and R43/44 funding mechanisms to fund nanotechnology projects. NIOSH utilizes program announcements and has participated in joint RFAs with EPA, NSF, and the National Institute of Environmental Health Sciences (NIEHS). Dr. Robison highlighted the annual extramural funding amounts since 2001; the largest annual amount was $1.46 million in 2005. Funding for the current year is $800,000 and approximately $5 million has been granted in external funding since 2001. In 2008, R01, R03, and R44 funding mechanisms were used to fund 13

projects that deal with a variety of topics including sensors for portable monitors, lung oxidative stress and inflammation, and toxicity of inhaled NPs. The extramural process is a competitive, peer-reviewed process; proposals must be relevant to occupational safety and health. There is an emphasis on research to practice (i.e., show how research can be used to improve the workplace). More information regarding nanotechnology research can be found in the 2007 NIOSH report, Progress Toward Safe Nanotechnology in the Workplace, on the NIOSH Web Site at http://www.cdc.gov/niosh/topics/nanotech, and in the NIOSH online Nanoparticle Information Library at http://www2a.cdc.gov/niosh-nil/index.asp.

National Institute of Environmental Health Sciences Activities on Nanotechnology: Applications and Implications
Srikanth Nadadur, NIEHS

Each of the NIH's 26 institutes and centers has a nanotechnology research program. NIH created an intramural nano task force comprised of representatives from each of the 26 institutes and centers to work with extramural experts to identify priority research areas for nanomedicine and health. Some of the critical research areas include: nano delivery systems; bioimaging and informatics; organ-tissue nanoengineering; medical devices; biocompatibility and toxicity; and environmental health and safety. NIEHS is solely responsible for developing research programs to evaluate the environmental health implications and safety of nanomaterials. The creation of the National Nanotechnology Initiative in 2001 helped spur an increase in funding for nanotechnology research. Last year, NIH spent approximately $200 million on nanotechnology research and approximately $30 million of that total was spent on nano environmental health safety research.

For the study of health implications, NIEHS' work includes both basic and exposure research. Exposure research is focused on determining routes of exposure and systemic distribution, correlating physical and chemical characteristics of ENMs with biological response, identifying biomarkers of exposure and biological response, and developing models to evaluate and predict biological response. Basic research includes projects studying the interaction of ENMs with biomolecules; studying transmembrane transport, cellular uptake, subcellular localization and retention; identifying cell- and organ-specific toxicity response pathways; and studying the effects of structural and surface modifications.

There are three research programs within NIEHS: extramural, intramural, and the National Toxicology Program (NTP). Extramural research is funded by NIEHS through the Division of Extramural Research and Training in three areas: (1) nanotechnology-health implications; (2) nanotechnology-based applications; and (3) remediation devices. Health implications research ranges from efforts to understand basic interactions between nanomaterials and biological systems to organ-specific toxicity. Research in enabling technologies addresses the applications of nanotechnology, including the development of: (1) deployable environmental sensors for a broad range of environmental exposures; (2) biological sensors to link exposure with disease etiology; and (3) intervention devices, such as drug delivery devices and other therapeutic nanoscale materials. Remediation devices include nanotechnology-based devices for the superfund research program aimed at eliminating exposure. Researchers in the Division of Intramural Research (DIR), such as those in the NTP, investigate the applications of nanotechnology and characterize nanomaterials. Materials characterized by the NTP are available to researchers for collaborative efforts. DIR investigator-initiated research addresses the application of nanotechnology in the areas of environment, health, and safety. The NTP's areas of emphasis include: (1) exposure and dose metrics; (2) internal dose-pharmacokinetics in biological systems; (3) early biological effects and altered structure or function; and (4) adverse effects related to exposure to nanomaterials. The scientific focus of the NTP Nanotechnology Safety Initiative is to identify key physical-chemical features that govern nanomaterial safety. Materials currently under evaluation by NTP include quantum dots (QDs), titanium dioxide (TiO_2), carbon fullerenes, nanoscale silver, multi-walled carbon nanotubes (MWCNTs), nanoscale gold, and dendrimers.

Discussion

A participant asked which study sections at NIH focus on the issues discussed. Dr. Nadadur said that there is a standing study section named NANO that reviews research in the areas of nanotechnology, and there is also a new special emphasis panel, Systemic Injury to Environmental Exposures (SIEE) that has the required expertise to review grant proposals on nano environmental health safety.

Department of Energy Nanoscale Science Research Centers (NSRCs): User Facilities for the Scientific Community
Neal D. Shinn, Sandia National Laboratories

Dr. Neal Shinn is affiliated with one of the DOE NSRCs and presented information on each center and how each may benefit researchers. The five NSRCs, located across the United States and opened between 2006 and 2008, are research facilities for the synthesis, processing, analysis, and characterization of nanoscale materials. They provide specialized equipment, unique tools, and dedicated support and scientific staff. The NSRCs are operated as user facilities and are available to all researchers, with access determined through peer review of proposals. There is no user fee for nonproprietary work leading to publication; federal law, however, requires that costs be recovered for proprietary work. All NSRCs are co-located at DOE National Laboratories with existing major user facilities, including synchrotron radiation light sources, neutron scattering facilities, and other specialized facilities. Although most NSRCs offer similar expertise, some have unique capabilities and expertise. The expectation for the NSRCs is that they help foster impactful science and create a community of successful users. This is reflected in metrics such as publications, citations, size of the user population, and so on.

The Center for Nanophase Materials Sciences is located at the Oak Ridge National Laboratory and has a variety of research capabilities. The Laboratory has world-class capabilities in polymer synthesis, computation and visualization, and computational nanotoxicology, which determines the environmental impacts of nanomaterials. The Molecular Foundry is located at Lawrence Berkeley National Laboratory and includes six facilities, with a principal scientist for each facility and a team of scientists working within each facility. The Center for Nanoscale Materials is located at the Argonne National Laboratory and is working on six integrated scientific themes, including "nanobio" interfaces, nanophotonics, theory and modeling, X-ray microscopy, nanofabrication and devices, and electronic and magnetic materials and devices. The Center for Integrated Nanotechnologies is a partnership between Sandia National Laboratories and Los Alamos National Laboratory. It is focused on the integration of nanostructured materials to exploit their special properties and the need to move nanosystems into real-world applications. In its two facilities, the Center for Integrated Nanotechnologies has the capabilities for synthesis, characterization, and integration and has four science thrusts: (1) nanophotonics and optical nanomaterials; (2) nanoscale electronics and mechanics; (3) soft, biological, and composite nanomaterials; and (4) theory and simulation of nanoscale phenomena. The Center for Functional Nanomaterials is located at the Brookhaven National Laboratory and has five scientific themes: (1) nanocatalysis; (2) electronic nanomaterials; (3) soft and biological nanomaterials; (4) electron microscopy; and (5) theory and computation. Its focus is on energy applications (e.g., functional nanomaterials for exploiting renewable energy sources, energy storage, and utilization).

The role of the NSRCs is to make specialized capabilities and expertise available to outside researchers, and the DOE looks to the centers for technical input with respect to the developing area of engineered nanomaterials safety. Dr. Shinn explained that operational policies currently are being crafted, and he invited participants to be involved and have an impact on how DOE sets policy. The five NSRCs have received approximately 800 to 1,000 user proposals and have had more than 1,000 researchers working at the centers. There are semi-annual calls for proposals, with other mechanisms for brief access for time-sensitive projects. Historically, there is a 55 to 93 percent likelihood of a proposal being accepted. If a

proposal is rejected, in most cases feedback is provided and the researcher is encouraged to resubmit in the next cycle. Proposals are first evaluated for feasibility and then peer-reviewed for scientific quality and expected impact. Each proposal must include a statement of work that is reviewed by an external panel that assesses what is clear and achievable. Projects can be 1 day to 1 year, but it must be clear what the researcher would like to accomplish. The researcher's institution must sign an agreement that allows the researcher to work at the NSRCs and publish. The centers are in place to help make researchers' work successful.

Discussion

A participant stated that students that work at Argonne National Laboratory are required to complete extensive safety training. What type of safety training is in place through this program? Dr. Shinn responded that all users must complete safety training, but the specific training would depend on the project.

A participant noted that early on, the environmental science community had a difficult time receiving high rankings in proposals because review panels did not understand the science and asked whether the DOE has considered how it is populating its review panels. Dr. Shinn responded that all of the centers list their reviewers on their Web sites; if researchers find that they are lacking in expertise, they are encouraged to provide this feedback to the individual center or the DOE. He added that peer-review judgments are inherently qualitative, and reviewers could have trouble with proposals if they are not well written.

METALS, METAL OXIDES: REMEDIATION AND EXPOSURE

National Exposure Research Laboratory (NERL) Nanomaterials Research Program
Michelle Conlon, EPA, NERL

Ms. Michele Conlon discussed the Agency's intramural nanomaterials research in which funds are used to address Agency-driven problems. The goals of this research are to assess the impact to environment and human health and research beneficial environmental applications. The key issues under these research goals are the uniqueness of nanomaterials as contaminants, risk assessment approaches, mitigation strategies, and the use of environmental nanomaterial technology. Nanomaterials are of interest because they exhibit different characteristics than their larger size counterparts. With ENMs in particular, the issue is that they have been changed from their natural state.

Nanotechnology research is driven by the Nanotechnology Environmental and Health Implications (NEHI) Working Group, an interagency strategy for collaboration; the Organization for Economic Cooperation and Development (OECD), an international cooperative program; the EPA Office of Pollution Prevention and Toxics (OPPT) Nanoscale Material Stewardship Program, which involves inter-Agency working groups; the EPA STAR grants program; and ORD's NRS. The purpose of the NRS is to guide nanomaterials research within ORD; the final draft is under review and is expected to be finalized within the next few months. NERL is working on sources, fate and transport, and exposure. It is collaborating with EPA's National Health and Environmental Effects Research Laboratory (NHEERL) on human health and ecological effects, with EPA's National Center for Environmental Assessment (NCEA) on risk assessment and case studies, and with EPA's National Risk Management Research Laboratory (NRMRL) on preventing and mitigating risks. EPA selected five nanomaterial classes on which to focus its efforts: titanium dioxide (TiO_2), zero-valent iron (ZVI), nanosilver, nanocarbon, and cerium oxide (CeO_2).

Regarding sources, fate and transport, and exposure, NERL developed five research goals, and initial research has been focused on identifying, characterizing, and quantifying nanomaterials in soil, water, and biota media. The eventual goals are to model transport and exposure and characterize multimedia and cross-media fate and transport. Within the next 2 to 3 years, NERL would like to: (1) separate and characterize certain nanomaterials in soil and water matrices; (2) evaluate the detection of nanomaterials by at least six physical and chemical methods; (3) understand the influence of certain environmental factors on nanomaterials; (4) identify and prioritize the research needed for NCEA's comprehensive environmental assessments; and (5) describe the properties of certain nanomaterials in the environment. The long-term research goals are to: (1) model deposition of airborne nanomaterials; (2) model nanomaterial behavior in surface water; and (3) design a nanomaterial exposure modeling approach. All of this work is aimed at addressing the following major questions: Do nanomaterials move through the environment? Is there exposure potential for humans and/or ecosystems? Do nanomaterials pose unique exposure problems?

Reactive Composites for Targeted Remediation of Trichloroethylene (TCE)
Vijay John, Tulane University

This research project is attempting to devise new methods to remediate TCE. TCE is a dense nonaqueous phase liquid (DNAPL). DNAPLs are a major problem, and TCE materials escape into groundwater and create flumes that are difficult to clean up because they sink so far into the ground. ZVI is an effective reductant for the remediation of TCE that is environmental friendly, highly efficient, and inexpensive. The challenge is that ZVI particles have poor mobility because of their magnetic properties, so new techniques are being created to disperse them. Because effective *in situ* remediation of TCE requires the successful delivery of reactive nanoscale iron particles (RNIPs) through soil, the goal of the research is to engineer reactive particles that have good mobility through soils and directly target TCE. Particles must be synthesized that are reactive to TCE, will partition to TCE or to the TCE/water interface, and are of the correct size range for optimal mobility through sediments. The idea is to incorporate nanoscale iron into porous submicron silica particles that are functionalized with alkyl groups; the accompanying hypothesis is that organic functional groups adsorb dissolved TCE facilitating contact with ZVI and also extend in the organic phase to help particle stability. Using silica allows for the correct size range for optimal mobility through sediments; almost all iron/ethyl-silica particles are in the size range for optimal mobility and have optimal collector efficiency. Experiments show that: (1) the iron/ethyl-silica suspension transports through the soil readily, whereas most of the RNIPs are retained at the top of the column; (2) approximately two-thirds of iron/ethyl-silica particles are eluted through the sediment, whereas RNIP does not elute; and (3) bare RNIP accumulates at the capillary inlet, whereas iron/ethyl-silica particles move through the capillary. The researchers then examined a simpler technology and using carbons prepared from sugars, incorporated the ZVI on the carbon surface for reaction. Following preparation, electron microscopy showed prepared carbon as monodispersed uniform spherical particles. Pyrolysis and activated carbons exhibited nearly 100 percent TCE adsorption. ZVI particles are dispersed on the carbon surface, and the weight ratio between carbon and iron is controllable. The elution profiles and capillary results of pyrolysis carbons indicate good elution of the materials. Furthermore, the researchers found that: (1) iron/ethyl-silica particles may preferentially accumulate and localize at the TCE-water interface, making dechlorination more efficient; (2) adsorption of TCE on the particles leads to a dramatic reduction in solution TCE concentration; and (3) composite particles can be used in *in situ* remediation and the development of reactive barriers. Currently, alternate technologies for adsorptive-reactive supported nanoscale ZVI particles are in development.

Discussion

A participant noted that optimum size appears to be important and asked what size range is most optimal and whether it would change based on the material used. Dr. John responded that silica particles are very

different from carbon materials so the comparison is difficult. The participant then asked whether the optimum size would be a function of porous media, and Dr. John replied that it would.
A participant commented that there is a group in Oklahoma performing work on groundwater and this might be a source of collaboration.

A participant asked how sticking is controlled with sugar-based carbons and how they are mobile. Dr. John responded that they do not appear to aggregate much.

Synthesis and Application of Polysaccharide-Stabilized Fe-Pd Nanoparticles for* In Situ *Dechlorination in Soil and Groundwater
Donye Zhao and Chris Roberts, Auburn University

Contaminated plumes often are difficult to reach. The idea to deliver NPs to contaminants *in situ* first was proposed in 1997, but there were no mobile NPs at that time. The primary accomplishments during Year 3 of this project were that: (1) batch and column tests for degradation of TCE sorbed and/or trapped in soils using carboxymethyl cellulose (CMC)-stabilized ZVI NPs were conducted; (2) transport behaviors of CMC-stabilized ZVI NPs in porous media were tested and modeled; and (3) *in situ* dechlorination in soils using CMC-stabilized ZVI NPs was pilot tested. The researchers modified the traditional process by starting nanoparticle synthesis by adding polysaccharide starch or carboxymethyl cellulose (CMC) before the nanoparticles were formed (via the reduction of Fe^{2+} via the addition of electron donors). Following Pd coating, the result was the formation of stabilized and soil-dispersible iron-palladium bimetallic NPs. Researchers showed that CMC can facilitate the synthesis of nearly monodispersed palladium NPs that can catalyze TCE degradation. Dr. Zhao described the experimental set up and results of several experiments that demonstrated that: (1) CMC can facilitate size-controlled synthesis of ZVI NPs, (2) transport of CMC-stabilized iron NPs are controllable and can be modeled by the convection-dispersion equation and filtration theory, and (3) CMC-stabilized ZVI can degrade TCE in soil but must overcome mass transfer and sorption limitation and dissolved organic matter inhibition.

Discussion

A participant asked what Dr. Zhao thought the reactive lifetime of particles is and whether, when injections are performed, excess CMC is injected. To the first question, Dr. Zhao responded that the lifetime depends on the composition, concentration, particle size, and conditions. If kept refrigerated, the NP dispersion's reactivity can last for months, but all particles will be oxidized eventually. To the second question, he responded that there always is some excess CMC, and the maximal CMC:iron ratio is determined. The researchers try to use no more than is required for stabilization, which is approximately 0.2 percent per 0.2 g of iron.

Characteristics, Stability, and Aquatic Toxicity of Cadmium Selenide/Zinc Sulfide (CdSe/ZnS) Quantum Dots (QDs)
James Ranville, Colorado School of Mines

CdSe/ZnS QDs are bright, photostable fluorophores that are used in biological imaging, optics, and other applications. This project is examining them because cadmium, selenium, and zinc metal-containing QDs are known to be toxic and they could escape into the environment in a variety of ways. The objective of this research project is to characterize the environmental fate of QDs in the aquatic environment. Characterization is key to this effort, and the research approach utilized ultraviolet and visible (UV-Vis) absorption spectroscopy, fluorescence, transmission electron microscopy, inductively coupled plasma (ICP)-atomic emission spectrometry (AES), and field-flow fractionation (FFF) to characterize the core, shell, and polymer. Researchers also examined short- and long-term stability. *Daphnia magna* is being used to determine acute toxicity and uptake. Four types of QDs were used in the experiments; the optical

properties of each depend on core size. Researchers found that there is a large excess of cadmium associated with QDs, given the assumed stoichiometry of 1:1 Cd to Se. The FFF results strongly suggested that Cd is associated with the polymer coating. The researchers investigated the implications of the characterization results for stability and toxicity and observed that: (1) mercapto-undecanoic acid (MUA) toxicity appears to be a mass-based phenomenon; (2) there are dissolved metals present at 48 hours post-test; (3) there is enough dissolved cadmium to cause observed death; and (4) the rate of metal release is important. In terms of poly(ethylene oxide) (PEO) toxicity, researchers observed that: (1) this toxicity appears to be a particle number phenomenon; (2) smaller QDs are more toxic on a mass basis; (3) although no detectable dissolved metals were found in solution at 48 hours, toxicity was observed; (4) cadmium is not completely bioavailable as dissolved cadmium is more toxic than both PEO QDs on an equivalent cadmium basis; and (5) dissolved zinc is potentially the toxic agent for the red PEO QDs. In terms of acute toxicity, the researchers concluded that: (1) stability has a strong influence on QD toxicity; (2) dissolved cadmium can explain the observed toxicity for MUA QDs; and (3) the lack of dissolved metals found with PEO QDs suggests an alternate pathway of toxicity. The laboratory will continue its characterization, stability, and toxicity experiments.

Discussion

A participant asked what the approach was for measuring dissolved cadmium. Dr. Ranville responded that the researchers used filtration as a measure to dissolve cadmium.

Dr. Savage noted that EPA is attempting to establish a partnership with the United Kingdom. The RFA will specify a joint U.S.-U.K. team and will be funded at $2 million each year for 4 years. If the partnership does not work out, the usual amount of $600,000 will be offered.

METALS, METAL OXIDES: FATE AND TRANSPORT

Effect of Surface Coating on the Fate of NZVI and Fe-Oxide NPs
Greg Lowry, Carnegie Mellon University

There are releases from nanomaterial-related products into air, soil, and water. To develop NPs that can be placed underground, it is necessary to coat the particle. Most nanomaterials are coated, and these coatings are important because they affect the manner in which they behave in the environment. In previous studies, researchers have shown that a polyaspartate (PAP) coating decreases reactive oxygen species (ROS) and cytotoxicity in glial cells and neurons. Fresh particles have an effect at low concentrations but oxidation and coating of particles can affect particle toxicity. The goal is to understand how the coating affects the fate of these particles. The key questions are: What is the oxidation rate of nanoscale ZVI in the environment? What is the fate of the coatings? Do aging and coatings affect bactericidal properties? Is there synergy between nanoscale ZVI, coatings, and bacteria that enhances remediation? The researchers investigated the rate and extent of desorption of adsorbed polyelectrolyte from nanoscale ZVI during a 4-month period. Dr. Lowry briefly described the methods used to achieve this. Researchers found that lower molecular weight coatings have higher rates of desorption; greater than 30 percent of the polyelectrolyte stays on the surface. Bare particles do not move; PAP, CMC, and poly(styrene sulfonate) (PSS) were immediately mobile and remained mobile after 8 months. The researchers also examined how polymer and natural organic matter coatings, oxidation state, and environmental conditions affected the bactericidal effects and toxicity of nanoscale ZVI using *Escherichia coli*. The findings showed that aerobic cultures were less affected than anaerobic cultures, indicating that Fe^0 content is less important than the presence of oxygen. Fe^0 oxidizes quickly in an aerobic environment, and it appears that under aerobic conditions a different iron oxide shell is formed on

the outside of the particle. Results also indicated that PSS, PAP, and natural organic matter coatings eliminated bactericidal effects, and coatings decreased contact between bacteria and nanoscale ZVI.
In summary, high molecular weight coatings do not readily desorb from nanoscale ZVI, and coatings and aerobic conditions appear to decrease bactericidal effects. Under realistic groundwater conditions, these NPs appear fairly immobile.

Discussion

A participant asked whether surface coatings are changing reduction-oxidation chemical properties, and if this would be a problem in the real world. Dr. Lowry explained that a coating is being placed on the particle that slows down but does not completely stop its reactivity. Even coated particles will oxidize over time; the factor that is blocking electron transfer is the different iron oxide coatings.

A participant asked whether the coated particles can last 8 months in water. Dr. Lowry replied that iron zero content plays a large role; if it is depleted, the particles are less likely to agglomerate. The desired outcome is for the coating to come off so that the particles do not move, but this is not happening. Therefore, the particles could continue to be mobile under the correct hydrogeochemical conditions.

A participant asked whether the degradation rate of the coating was checked. Dr. Lowry responded that an undergraduate student currently is comparing the biodegradation rates of free coating polymers. The participant asked whether a synergistic effect of anaerobic degradation was observed. Dr. Lowry responded that the laboratory is working on this.

A participant asked whether the ROS were analyzed in the presence of oxygen, which could explain the observed antimicrobial effects. Dr. Lowry responded that the laboratory has not measured this specifically, but the results are counter to this as anaerobic conditions have greater antimicrobial conditions.

Bioavailability and Toxicity of Nanosized Metal Particles Along a Simulated Terrestrial Food Chain
Jason Unrine, University of Kentucky

Dr. Jason Unrine explained that their laboratory is examining ecotoxicological effects of NPs in the terrestrial system with a focus on detritivores. Detritivore food chains dominate in soil ecosystems, and materials taken up by detritivores can move up the food chain. The overall objectives of the project are to: (1) determine the interactions between particle size and particle composition in determining absorption, distribution, metabolism, excretion, and toxicity in earthworms and amphibians; (2) investigate the plausibility of nanomaterial trophic transfer along a simulated laboratory food chain; and (3) determine whether simulated environmental and biological modifications influence bioavailability and toxicity. The hypotheses are that: (1) nanomaterials have relatively low bioavailability in soils; (2) uptake from soils, toxicity, and distribution of nanomaterials within organisms is size- and material-dependent; and (3) biological responses are related to the release of metal ions. The laboratory is focusing on mechanistic and ecologically relevant endpoints and used copper, silver, and gold as test materials. Results showed that gold particles are delivered throughout the body of earthworms. Results of earthworm subchronic toxicity and reproduction experiments indicated that in most cases, copper, silver, and gold do not cause high mortality in earthworms, but silver nitrate ($AgNO_3$) at a soil concentration of less than 20 mg/kg has a mortality rate of 100 percent in earthworms. The earthworms bioaccumulated all three types of metal NPs in a size-dependent manner, and a decrease in reproductive success was seen; large particles showed a trend of decreased reproductive success with increased exposure. Researchers also examined changes in gene expression related to metal homeostasis, oxidative stress, and molecular chaperones. Results indicated that metallothionein gene expression, a measure of metal homeostasis, was significantly altered following exposure to copper and silver NPs. In the future, the laboratory plans to: (1) determine the

uptake and elimination rates in earthworms, (2) determine the toxicity of smaller particles at higher concentrations, (3) further develop methods for *in situ* characterization of particles/metals in soils and tissues, and (4) investigate amphibians as another trophic level.

Discussion

A participant asked how $AgNO_3$ caused the mortality. Dr. Unrine responded that the mechanism had not yet been determined, and there were no obvious molecular markers. His theory is that it somehow interferes with earthworm ion regulation.

The Bioavailability, Toxicity, and Trophic Transfer of Manufactured ZnO_2 Nanoparticles: A View From the Bottom
Paul Bertsch, University of Georgia

The overall objectives of this research project are to examine: (1) the bioavailability and toxicity of manufactured NPs (i.e., nanoparticle zinc oxide [ZnO-np]), as a function of particle size to model soil bacteria (*Burkholderia vietnamiensis*) and (*Cupriavidus necator)*, and the model detritivore *Caenorhabditis elegans* as referenced against aqueous zinc (i.e., Zn^{2+}); (2) the ability of manufactured ZnO-np to be transferred from one trophic level to the next as assessed in the simple food chain consisting of pre-exposed *B. vietnamiensis* and *C. elegans*; and (3) the synergistic or antagonistic effects of manufactured ZnO-np on the toxicity of copper to *B. vietnamiensis* and *C. elegans*. The researchers hypothesize that: (1) the bioavailability and toxicity of manufactured ZnO-np increases with decreasing particle size; (2) the toxicity of ZnO-np to *B. vietnamiensis* and *C. elegans* is lower than an equivalent concentration of dissolved Zn^{2+}; (3) the bioavailability and toxicity of ZnO-np introduced via trophic transfer differs from that introduced via direct exposure; and (4) ZnO-np alters the bioavailability and toxicity of dissolved metals. The first year of research focused on characterization of commercial ZnO-nps and found evidence for at least three acetate populations. This is important because acetate inhibits surface reactivity; removing acetate significantly increases surface reactivity. Additionally, there is much greater surface reactivity of larger (80 nm) versus smaller (2 nm) nanoparticles. In terms of characterization, the researchers found that: (1) size determination and surface chemistry are critical issues; (2) transmission electron microscopy may not be the best method for size determination for small metal oxide nanomaterials; (3) acetate controls smaller ZnO-np reactivity and passivates surface sites, but this is not the case for larger particles; and (4) removal of acetate leads to flocculation/aggregation of small ZnO-np primary particles but promotes surface reactivity. Results from bacterial exposure experiments showed that: (1) there is no significant difference in the growth rate of *C. necator* and *B. vietnamiensis* following exposure to ZnO-np and aqueous zinc; (2) *C. necator* displays higher acetate utilization rates with aqueous zinc compared to ZnO-np, indicating a possible difference in bioavailability; and (3) there are a greater number of compromised cell membranes associated with ZnO-np than with the free ion. Experiments with nematodes indicated that: (1) mortality is not significantly different between aqueous zinc and ZnO-np; and (2) at higher zinc concentrations ($> 100\ mg.L^{-1}$), ZnO-np decreases copper toxicity compared to aqueous zinc. Finally, there was no evidence for significant trophic transfer in the bacterial-nematode model (although this may be more related to experimental challenges), and ZnO-np is bioavailable from soils as demonstrated in earthworm exposures.

Discussion

Dr. Randy Wentsel (EPA) commented that, in terms of linkage between EPA intramural and extramural research, Dr. Bertsch should consider working with EPA researchers regarding ecoeffects and ecological risk assessment of these materials. Dr. Bertsch responded that he has had discussions with EPA researchers at the Athens, Georgia, and Cincinnati, Ohio, facilities. His group also is fortunate to be part of the Duke-Carnegie Mellon Center for Environmental Implications of Nanotechnology.

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

As nanomaterials enter the environment, a major question is whether NPs are toxic to bacteria and eukaryotic cells. This research focuses on how NPs interact with cellular organisms, including quantifying cellular-scale processes that affect nanoparticle entry, stability, and toxicity. Researchers are examining two materials, CdSe QDs and TiO_2 NPs. Researchers chose to work with bacteria because they are abundant, biodiverse, and act as catalysts. Previous cell labeling experiments led researchers to ask the following questions: Is light necessary? Are bare QDs internalized? Is external binding a prerequisite? What are the quantitative fates of QDs? How are they toxic? Experimental results displayed a typical dose-response relationship for *Pseudomonas aeruginosa* growth in response to exposure to both Cd(II) and CdSe QDs. Additionally, bare QDs dissolve relatively quickly but not completely, and QDs add to Cd(II) toxicity above a certain threshold. Above this threshold, researchers noted membrane damage, increased intracellular ROS, and metal uptake in cells. Multiple evidence points to the probability that QDs cause membrane damage, enter cells, and are highly reactive within the cells. Researchers concluded that QDs appear to be more toxic than Cd(II) above a threshold, and sorption to the membrane is not a prerequisite. *Pseudomonas* appears to alter the fate of QDs: intracellularly QDs appear mostly broken down, whereas extracellularly QDs are relatively stabilized. Researchers also attempted to grow *P. putida* in the presence of TiO_2 NPs and determine whether the growth rate is affected by the particles. Initially, in rich media, the particles are highly agglomerated, but after 12 hours they are highly dispersed. The researchers hypothesized that this could be caused by: (1) the cells metabolizing the factor in the media causing agglomeration, (2) bacterial biosurfactant production, or (3) specific adhesion. Further experiments showed that the dispersion is caused by specific adhesion; the cells have a higher affinity to the NPs than they have for each other. In the future, the researchers plan to examine the mechanisms behind their observations, employ high-throughput methods, and scale up their research to include soil ecosystem processes and biota.

Discussion

A participant asked whether QD fluorescence could be used to measure the concentration of intact QDs within the cells. Dr. Holden responded that from a purist standpoint, she did not believe so. Labeling indicates that as the QDs are being processed in the cells their fluorescence is changing.

METALS, METAL OXIDES: TOXICITY

ORD NHEERL Manufactured-Engineered Nanomaterial Health Effects Research Program
Kevin Dreher, EPA, NHEERL

ORD's strategic plan for nanotechnology flows from the 2007 EPA Nanotechnology White Paper, the National Nanotechnology Initiative (NNI), Woodrow Wilson International Center for Scholars documents regarding the environmental health and safety implications of nanotechnology, the National Academy of Sciences publication *Toxicity Testing in the 21st Century: A Vision and a Strategy*, and OECD's nanotechnology document. EPA's health laboratories plan to develop an implementation plan for the ORD strategy, which includes four basic themes. NHEERL nanotechnology research falls under the theme of risk assessment and risk management, but all of the themes inform each other. NHEERL must develop long-term goals to address the research question of determining the health effects of manufactured-engineered nanomaterials and their applications and how these effects can be quantified and ultimately predicted. High priority research areas include: (1) toxicology, hazard identification, mechanisms of injury, and modes of action of nanomaterials and nanotechnology; (2) dosimetry, biokinetics, and response modifiers of nanomaterials; and (3) the adequacy of existing test methods and development of predictive approaches to assess toxicity of nanomaterials and nanotechnology. The long-

term goal is to ultimately quantify and predict adverse health outcomes, and researchers initially are examining manufactured nanomaterials in pursuit of this goal.

NHEERL has formed the "Nano" Health Effects Team, which includes 15 investigators representing each NHEERL health division and a variety of expertise, to develop the implementation plan. The team also is examining the systemic effects of inhaled or ingested nanoparticles. NHEERL's nanomaterials health effects research employs an integrated multidisciplinary approach in its assessment of a common set of well-characterized manufactured-engineered nanomaterials. Various types and sizes of TiO_2, CeO_2, and carbon nanotubes have undergone independent physical and chemical characterization. This independent characterization of commercially available nanomaterials showed significant differences from the vendor's product information and underscores the need to conduct independent physical and chemical characterizations of commercially available nanomaterials prior to conducting effects research. In terms of alternative testing methods, NHEERL is involved in several projects that examine biochemical interactions and surface properties via non-cellular and cellular-based assays that mimic pulmonary, cardiovascular, liver, gastrointestinal, neuro, and ocular toxicities. In summary, to address some of the challenges associated with assessing the health effects of manufactured-engineered nanomaterials, ORD has developed a multidisciplinary strategy to screen and prioritize nanomaterials for *in vivo* toxicity testing in a manner that ultimately will identify and develop validated alternative toxicity testing methods for nanomaterials that predict *in vivo* toxicity.

Discussion

A participant asked why human health was considered a priority versus ecological concerns in regard to nanosilver, because nanosilver is not as toxic to humans compared to aquatic organisms. Dr. Dreher responded that Dr. Steve Diamond could answer this question better during his presentation. In terms of human health, there will be a significant OECD effort regarding nanosilver, and NHEERL will fill in the gaps. Nanosilver can be toxic to humans. NERL also is performing ecological work on nanosilver.

Microbial Impacts of Engineered NPs
Shaily Mahendra, Rice University

This research examines the effects of engineered nanoparticles on bacteria. Bacteria are important in ecotoxicological studies because they are at the foundation of all known ecosystems, and as simpler organisms, they can be indicative of the potential toxic effects on more complex organisms. Although C_{60} is insoluble in water, it can form a suspension, termed nC_{60}, when introduced to water via a solvent; nC_{60} is an important form of C_{60} in the aqueous environment and is a potent, broad-spectrum antibacterial agent that affects a variety of organisms. In comparing the bacterial toxicity of nC_{60} to other nanomaterials, nC_{60} is among the most toxic. The researchers examined the effects of nC_{60} particle size and found that particles were 100 times more toxic when particle size was reduced by one-half. Researchers also observed that salt promotes aggregation (increase in particle size) of nC_{60} particles, indicating that the particles would be more toxic in freshwater than in seawater. Natural organic matter, however, reduces nC_{60} bioavailability and toxicity. Researchers also reviewed possible toxicity mechanisms to determine how nC_{60} causes toxicity and tested three hypotheses involving changes in membrane permeability, increased oxidative stress, or disruption of membrane oxidation/electron transport phosphorylation. Results showed that nC_{60} did not appear to induce ROS-mediated damage in bacteria, but nC_{60} did significantly collapse membrane potential, suggesting that nC_{60} results in oxidative damage and can directly oxidize proteins. Researchers concluded that there is oxidative damage that is not mediated by ROS but is most likely a result of oxidative stress on direct contact of nC_{60} with the cells. In terms of potential applications, photocatalytic NPs could enhance UV disinfection of drinking water. Fullerol, a hydroxylated form of C_{60}, enhanced virus removal by UV irradiation, shortening the contact time by a factor of three. Because nC_{60} is bactericidal, release or improper disposal could have important

environmental implications. Fortunately, this can be mitigated by natural organic matter and salinity. Alternatively, nC_{60}'s antimicrobial activity can be exploited to protect public health by preventing microbial growth in water distribution and storage systems or enhancing UV disinfection practices.

Discussion

Dr. Lowry asked, if it is not ROS that implies a direct electron transfer, whether that means that nC_{60} must be attached to the particle. Dr. Mahendra responded that this was the case, and the data support the fact that there should be direct contact between the cell wall and the nanoparticle. Dr. Steve Diamond added that a good deal of work conducted in the laboratory has found that the activation process must occur in tissues.

Engineered Nanomaterial Ecological Effects Research Within ORD's NHEERL
Steve Diamond, EPA, NHEERL

The EPA's NHEERL is divided into health and ecology components and is one of the laboratories within EPA's Office of Research and Development (ORD). Three of the four ecology divisions within NHEERL (Atlantic [AED], Mid-Continent [MED], and Western [WED]) are involved in work with nanomaterials. Research planning within ORD and NHEERL is based on documents prepared by NNI and NEHI, the 2007 EPA Nanotechnology White Paper, and the draft version of ORD's Nanotechnology Research Strategy. Each of the three ecology divisions working on nanotechnology has completed a formal research plan. MED will focus on freshwater systems, including freshwater sediments; AED will focus on marine systems, including marine sediments; and WED will focus on terrestrial systems, including soils. Ecological effects nanomaterials research aims to: (1) evaluate current methods for assessing hazard; (2) assess hazard for nanomaterials; (3) identify nanomaterial characteristics that predict toxicity; (4) identify mechanisms of action, accumulation, distribution, metabolism, and elimination; and (5) incorporate knowledge of production volume and potential pathways of exposure within a product life cycle framework. NHEERL scientists work in close collaboration with other ORD laboratories in these efforts. Early efforts of scientists within NHEERL's ecology divisions included coordinating the review of toxicity testing guidelines for both the Organization for Economic Cooperation and Development (OECD) and EPA's Office of Pesticide Programs and Toxic Substances (OPPTS). Reviewers included all of the nanotechnology principal investigators from the AED, MED, and WED as well as researchers from the U.S. Army Corps of Engineers (USACE) and the U.S. Geological Survey (USGS). The OPPTS review found that the toxicological principles and endpoint aspects of current testing guidelines were adequate; however, media preparation, physical/chemical properties of materials, quantification of exposure, and exposure metrology aspects of the current testing guidelines were inadequate. The inadequacies identified were generally related to the particulate and fibrous nature of nanomaterials and the colloidal nature of exposure media.

Preliminary research at MED has focused on approaches to producing consistent nanomaterial exposure media for aquatic toxicity testing. The effect of ionic strength on the particle size of titanium dioxide has been quantified, as well as settling rates and resulting stable bulk concentrations. The effect of UV exposure on the toxicity of C_{60} and titanium dioxides is being studied in collaboration with USACE scientists. MED researchers also have initiated work on nanosilver, which is increasingly being used in consumer products. Preliminary assays have been completed, and researchers have successfully imaged nanosilver in organisms using two-photon, scanning, and confocal microscopy. Single- and multiwall carbon nanotubes have been obtained from Nikkiso Company, Ltd. (Japan) to be used in OECD Sponsorship Program assays. Scientists from WED have coauthored a manuscript regarding the effects of single-walled carbon nanotubes (SWCNTs) on root elongation of crop species in the journal *Environmental Toxicology and Chemistry*. In the near term, NHEERL will continue its involvement in OECD planning, review, and testing; its collaborations with South Carolina University, Oregon State

University, USACE, and USGS; and its provision of assistance and technical support to EPA regulatory offices.

Discussion

A participant asked whether collaborations were formal or informal. Dr. Diamond responded that most collaborations currently are informal. There is one formal collaboration, which is an ongoing Cooperative Agreement with the University of Minnesota.

Characterization of the Potential Toxicity of Metal NPs in Marine Ecosystems Using Oysters
Amy Ringwood, University of North Carolina at Charlotte

While more nanomaterials are being released into the environment, there are numerous potential environmental risks of engineered NPs that are not well characterized or understood. This research focuses on oysters (*Crassostrea virginica),* a widely-distributed estuarine bivalve species that lives in a wide range of salinities. Filter-feeding bivalves are good models for characterizing the potential risk of nanoparticles, because they are highly effective at removing particles, have high filtration rates, and sample water column and surface/resuspended sediments. Additionally, there is extensive background information regarding their toxic responses to metals and organic contaminants. The potential toxicity of nanoparticle exposure to adult oysters is being investigated based on lysosomal destabilization, lipid peroxidation, antioxidant responses, and cellular and tissue accumulation. The potential effects on oyster embryos also are being investigated to compare the relative sensitivity of developmental stages and adults. Nanoparticle exposure experiments were conducted with nanosilver seeds, which are approximately 15 nm in diameter. Short-term (2-day) exposures were conducted in which adult oysters were exposed to a range of Ag nanoparticle concentrations; and similarly, 48-hour embryo development assays were conducted. The range of exposure concentrations selected for these studies was relatively low. The results of the adult oyster exposures indicated increased rates of lysosomal destabilization associated with Ag nanoparticle exposure. Furthermore, the levels of destabilization observed are associated with reproductive failure. Results of lipid peroxidation studies indicated that gills did not show oxidative damage, but hepatopancreas tissues did, and the response was more threshold-dependent than dose-dependent. There was no evidence of depleted or altered glutathione status in either tissue. For embryos, adverse effects were not seen until the highest dose was given, indicating a similar threshold response. Dr. Ringwood summarized that, in terms of lysosomal destabilization in adult oysters, there are significant adverse effects, and dose-dependent responses are based on exposure and tissue concentrations. In regard to adult oxidative damage, there were significant increases in lipid peroxidation with hepatopancreas tissues at the same concentrations at which adverse effects on lysosomal destabilization were observed. There was, however, no significant oxidative damage to the gill tissues. Next steps include characterization in seawater, investigations with other nanosilver preparations (e.g., rods, etc.), examination of antioxidant responses, and investigation of metallothioneins.

Discussion

A participant asked whether there was a nanosize effect. Dr. Ringwood responded that some work has been done with the ion itself, which appeared to be less toxic than the NPs. She reminded the audience that this is a work in progress.

Acute and Developmental Toxicity of Metal Oxide NPs in Fish and Frogs
Chris Theodorakis, Southern Illinois University

The objectives of this research project are to determine the environmental hazard of metal oxide NPs (Fe_2O_3, ZnO, CuO, and TiO_2) in terms of acute and chronic toxicity of these particles to fathead minnows

(FMs) and African clawed frogs. The researchers hypothesized that nanoparticle exposure would affect the survival, growth, development, egg hatchability, and metamorphosis of FM and African clawed frogs. In experiments conducted to date, mortality was seen in the frogs at 1.0 and 2.02 mg/L (nominal concentrations). As expected, chronic exposure resulted in a higher mortality than acute exposure did. Frog growth was accelerated by low doses of ZnO and slowed by higher doses of ZnO. CuO and Fe_2O_3 NPs are highly toxic to FMs, while TiO_2 and ZnO were not shown to be toxic in standard 96-hour tests. Future work will include: measuring metal concentrations, characterizing nanoparticle size distribution, determining the contribution of dissolved versus particulate metals to toxicity, comparing the toxicity of metal NPs to dissolved ionic metals, comparing the Lethal Concentration 50 (LC50) of the metal oxide NPs to the LC50 of the freely dissolved metal oxide, studying the toxicity of metallic copper to African clawed frogs, and conducting chronic toxicity tests for metallic Cu, CuO, and TiO_2 in FMs.

OTHER NANOMATERIALS: SENSORS AND TREATMENT

A Novel Approach to Prevent Biocide Leaching

Patricia Heiden, Michigan Technological University

With preserved wood, introduction of biocide is necessary, and leach is a potential problem. The hypothesis is that biocide-containing NPs could penetrate the wood interior, enhance service life via a stable and controlled release, and reduce or prevent leach. The objectives of this research are to "fix" biocides into core-shell NPs and control biocide release by matrix hydrophobicity. Dr. Heiden highlighted the initial nanoparticle synthesis, nanoparticle properties, and wood properties targets, comparing them to current results. In terms of nanoparticle size, the initial target was less than 100 nm in diameter; this has been achieved. Currently, the researchers are working on core-shell composition. A significant decrease in leaching has been achieved; obtaining zero leach, however, is not possible at this time. The nanoparticle size is suitable for delivery into wood if the NPs are not aggregated; sonicating before treating wood improves efficiency. Delivery efficiency of 68 percent was achieved. Observed NPs appear to be aggregates of much smaller core-shell NPs, which provides larger ill-defined core-shell NPs; functionally, the NPs appear to work as intended to provide good control over the active ingredient release rate. In terms of controlled release into water, as methyl methacrylate (MMA) is increased, there is a decrease in the rate of release. Additionally, a background loss of mass with NPs is not seen. The control showed significant release initially, whereas nanoparticle-treated wood showed a much smaller initial release; ultimately, nanoparticle-treated wood had 55 percent less leach than the control. The effect of using a polar co-monomer was similar. The biological efficacy is quite good, but researchers would like to replace gelatin with chitosan. Researchers also decided to examine copper-containing NPs, but discontinued their work because of the manufacturer's formulation with unknown components. The new approach utilizes a 1:4 copper:tebuconazole complex (CTC), which has many advantages in that: (1) inorganic/organic biocides are usually used in combination, (2) the complex may leach less than either biocide alone, (3) the complex can be obtained in high yield via simple methods, (4) it can be delivered into wood by various routes, and (5) the complex dissociates in water. CTC nanoparticle size appears to be similar to that of the tebuconazole NPs, but the data need to be replicated. The delivery efficiency of CTC into wood also appears to be similar to gel:MMA NPs with tebuconazole. The researchers plan to optimize the formulation and measure leach, as well as carry out some studies using chitosan instead of gelatin. There are plans to predominantly evaluate and optimize leach in the remaining studies. Researchers also will evaluate the biological efficacy or lowest leaching samples.

November 20, 2008

CARBON-BASED SENSORS AND EXPOSURE

Single Conducting Polymer Nanowire Immunosensors
Ashok Mulchandani, University of California, Riverside

Conducting polymers exhibit electrical, electronic, magnetic, and optical properties of metals or semiconductors while retaining attractive mechanical properties and processing advantages. They can be applied as conductometric, potentiometric, amperometric, and voltammetric transducers and as active layers of field-effect transistors (FETs), and they can be synthesized electrochemically. Benign conditions enable the direct deposition of conducting-polymer materials with embedded bioreceptors in one step. Conductivity can be modulated over 15 orders of magnitude. The objective of this research project is to develop new methods for cost-effective fabrication of single nanowire conducting polymer affinity-based sensor arrays for label-free, highly sensitive, selective, precise, and accurate detection of bioagents such as toxins, viruses, and bacteria at point-of-use. The approach to the research includes: (1) *in situ* fabrication of conducting polymer nanowires in e-beam lithography patterned nanochannels between a pair of electrodes; (2) magnetic alignment of template synthesized multi-segmented nanowire on prefabricated electrodes; and (3) AC dielectrophoretic positioning and maskless assembly of template synthesized nanowire on prefabricated electrodes. *In situ* fabrication has the advantage of biological functionalization during fabrication and sequential site-specific deposition into individual channels. It is, however, expensive due to the need for e-beam lithography. The magnetic alignment and assembly identified the following limitations: (1) magnetic (Ni) segment integration is required; (2) the multi-segmented nanowire architecture results in mechanical weakness, especially at the interfaces; (3) the low aspect ratio can potentially result in lower dynamic range; and (4) the sodium hydroxide required for template dissolution over-oxidized the polypyrrole segment, resulting in lower conductivity and possibly in lower sensing performance. The maskless assembly is the most cost-effective method. Future work includes: (1) demonstrating an immunosensor for viruses; (2) demonstrating a nucleic acid nanosensor; (3) integrating micro-fluidics for improved handling and real-time sensing; and (4) demonstrating a multi-analyte sensor array.

CARBON-BASED FATE/TRANSPORT

Carbon Nanotubes (CNTs): Environmental Dispersion States, Transport, Fate, and Bioavailability
Elijah Petersen, University of Michigan

The overarching goal is to evaluate factors that control the environmental dispersion states, transport, fate, and bioavailability of CNTs, thereby providing a foundation for human and ecological risk assessment. Specifically, single-walled and multi-walled ^{14}C-labeled CNTs will be synthesized, purified, and characterized using techniques previously established in the researchers' laboratory. These radio-labeled materials will then be used to systematically investigate: (1) the dispersion states of these nanomaterials under typical environmental conditions; (2) their transport behaviors within and through a series of different types of soil and sediment media; and (3) their bioavailability to selected critical aquatic and terrestrial food-chain organisms. The researchers have developed and refined a means for producing single-walled and multi-walled ^{14}C-labeled CNTs by using radioactively labeled methane as a feedstock for the synthesis of CNTs via chemical vapor deposition methods. CNT bioavailability to *Daphnia magna*, an aquatic worm, and an earthworm was tested in lab-scale systems to examine the potential of these nanomaterials to enter food chains in different environments and the factors controlling ecological bioavailability. The uptake and depuration behaviors for these bioavailability studies were presented. Results of the research include: (1) changing the hydrophobicity of multi-walled CNTs changes their

octanol-water distribution behavior but does not impact accumulation by earthworms or aquatic worms; (2) adding CNTs to soils affects the uptake of soil-borne pyrene by earthworms in a concentration-dependent manner (low concentrations of nanotubes show no impact but higher concentrations decrease pyrene accumulation and act similarly to black carbons); (3) polyethyleneimine was covalently bonded to multi-walled CNTs to form nanotubes with positive, negative, or neutral surface changes, and the cellular toxicity of these nanotubes was tested; and (4) a novel method to quantify fullerenes in ecological receptors was developed and the test results showed significant accumulation and limited depuration by *Daphnia magna*.

Aggregation and Deposition Behavior of CNTs in Aquatic Environments
Menachem Elimelech, Yale University

The use of engineered carbon-based nanomaterials has grown exponentially in recent years, but their environmental and health impacts are not known. This research project is studying the aggregation and deposition behavior of carbon-based nanomaterials as this will determine the fate and transport of these nanomaterials through the environment. Experiments have shown SWCNTs to be much more toxic than MWCNTs. The electrokinetic properties of MWCNTs were characterized to understand their aggregation behavior and humic acid was found to stabilize MWCNTs. The deposition behavior of SWCNTs was studied; long SWCNTs were found to be strained. Findings to date include: electrostatic interactions control the aggregation behavior of CNTs; humic substances stabilize CNTs by electrosteric repulsion; and CNT transport in porous media is relatively limited because of straining.

Discussion

A participant asked if a new method of measuring the surface charge of SWCNTs was needed. Dr. Elimelech responded that his group measures size, which indicates the transfer properties of the SWCNTs.

Cross-Media Environmental Transport, Transformation, and Fate of Carbonaceous Nanomaterials
Peter Vikesland, Virginia Polytechnic Institute and State University

Little is known about the unintended health or environmental effects of manufactured nanomaterials, but some evidence suggests that they may be toxic. For example, nC_{60} produced using the tetrahydrofuran (THF) method is suggested to cause oxidative stress in fish brain tissue and is potentially toxic to human cell lines. The goal of this research project is to examine carbonaceous nanomaterial fate and transport in the environment. The researchers focused on the question: How do atmospheric transformations of NPs affect their fate in water and soil? The project focused on the characterization of the aqueous aggregates of C_{60} fullerene. Due to its shape and electronic structure, C_{60} is highly reactive towards nucleophiles, exhibits a sizable electron affinity, and can be photosensitized. C_{60} is extremely insoluble in water, but it can form stabled water suspensions through the use of transitional solvents or long-term stirring in water; this environmentally relevant form of fullerenes is called nC_{60}. Natural water and physiological fluid components are expected to alter the mechanism(s) responsible for nC_{60} formation and stability. These components include: electrolytes, organic macromolecules (proteins, lipids, carbohydrates, humic and fulvic acids), and low molecular weight organics (nucleic acids, amino acids, carboxylic acids). The nC_{60} aggregate size decreases in the presence of natural organic matter isolates. Carboxylic acid groups are prevalent in many organic groups. Citrate is a well known stabilizer of many nanomaterials. Sodium citrate increases the negative surface charge of these particles at low concentrations, but decreases the negative surface charge at higher concentrations. The research conclusions are: (1) citrate stabilized nC_{60} (cit-nC_{60}) is a new form of nC_{60} with unique properties; (2) carbonyl-π interactions stabilize these molecular crystals—these interactions are relatively weak and can be broken by alterations to solution conditions, filtration, etc.; (3) molecular C_{60} is an important intermediate in carboxylic acid/nC_{60}

suspensions; and (4) aerosolization of nC_{60} results in a decrease in aggregate size. The implications of the weakly stabilized molecular crystals on the fate and transport of C_{60} are unknown.

Transport and Retention of Fullerene NPs in Quartz Sands and Natural Soils
Kurt Pennell, Georgia Institute of Technology

The objectives of this research project are to: (1) investigate the transport and retention of nC_{60} aggregates in water-saturated soils as a function of soil properties and systems parameters; (2) assess the effects of nC_{60} aggregates on soil water retention, water flow, and transport in unsaturated soils; and (3) develop and evaluate a numerical simulator(s) to describe nC_{60} aggregate transport, retention, and detachment in subsurface systems. The researchers found that nC_{60} aggregate transport decreases, and retention increases, as grain size or flow rate is decreased. A mathematical model that includes non-equilibrium attachment and maximum retention capacity accurately predicts nC_{60} transport and retention behavior in Ottawa sands. The researchers also found that ionic strength strongly influences nC_{60} aggregate transport and retention; the researchers attributed this primarily to electrostatic interactions. Future work will include: (1) measurement and simulation of nC_{60} transport and retention in unsaturated porous media; (2) investigation of nC_{60} transport and retention in heterogeneous 2-D aquifer cells; and (3) investigation of technologies to image the retained nC_{60} aggregates on quartz sand surfaces (e.g., force-balance microscopy). In a separate project, the researchers will evaluate the neurotoxicity of manufactured nanomaterials in cell culture and mouse models (oxidative stress, dopamine system).

Photochemical Fate of Manufactured Carbon Nanomaterials in the Aquatic Environment
Chad Jafvert, Purdue University

For many organic chemicals, photodegradation is a significant environmental fate process, and information regarding the rates and products of these reactions is therefore important in overall risk assessment analysis. The overall objective of this research is to investigate photochemical transformation of buckminsterfullerene (C_{60}) and SWCNTs under conditions of environmental relevance. Due to the strong light absorbance of these materials within the solar spectrum, photochemical transformation in the environment may lead to potentially more water soluble and easily bioaccumulative products. The three subobjectives of this project are to: (1) measure photochemical transformation rates and products of C_{60} solid films hydrated with aqueous solutions under solar irradiation; (2) measure solar photochemical transformation of C_{60} in aqueous humic acid solutions and as clusters in aqueous solution; and (3) extend these measurements to include the photochemical transformation of SWCNTs under similar conditions. The photochemical transformation of aqueous C_{60} clusters (nC_{60}) in sunlight (West Lafayette, IN, 86° 55' W, 40° 26' N) and lamp light (λ = 300-400 nm) has been investigated. Upon exposure to light, the brown to yellow color of nC_{60} was gradually lost and the cluster size decreased as the irradiation time increased. TOC analysis indicated that nC_{60} products/intermediates were soluble in the aqueous phase and C_{60} may have partially mineralized. The rate of C_{60} loss in sunlight was faster for smaller clusters compared to larger clusters (i.e., $k_{obs} = 3.66 \times 10^{-2}$ h^{-1} and 1.42×10^{-2} h^{-1} for C_{60} loss from 150 nm and 500 nm nC_{60} clusters, corresponding to half-lives of 18.9 h and 40.8 h, respectively, at the same initial C_{60} concentration). Dark control samples showed no loss, confirming phototransformation as the underlying degradation process. The presence of 10 mg/L fulvic acid, changes in pH, and the preparation method of nC_{60} clusters had negligible effects on the reaction rate. Deoxygenation resulted in a decreased loss rate, indicating that O_2 played a role in the phototransformation mechanism. These findings suggest that the release of nC_{60} into surface waters will result in photochemical production of currently unknown intermediate compounds. Future work will include: (1) singlet oxygen measurement; (2) functional group-specific X-ray photoelectron spectroscopy (XPS); (3) NMR analysis; (4) head space CO_2 analysis; and (5) the extension of this work to CNTs.

Discussion

A participant asked if the tests were done without any suspended solids or anything to which the fullerenes could absorb. Dr. Jafvert replied that only water was used. In some cases, the researchers did not buffer the solutions and the pH dropped, indicating that they had gotten some carboxyl groups. In some cases, the researchers used phosphate species to buffer the pH. The ionic strength was controlled, and no solid materials were seen other than the C_{60} particles.

A participant asked whether a C_{60} particle absorbed to a mineral surface, some bacteria, or some other biological material would change the rate of the dissolution. Dr. Jafvert responded that it possibly could. The researchers would like to do C_{60} coatings on walls and other materials to see if there are enhanced or decreased rates of reaction.

Fate and Transformation of Carbon Nanomaterials in Water Treatment Processes
Jae-Hong Kim, Georgia Institute of Technology

The objective of this research is to examine the response of water-stable fullerene aggregates to processes that are used in water treatment, using C_{60} and its stable aggregate, nano-C_{60}, as model compounds. The researchers investigated the stability of carbon nanomaterials in natural waters and removal by conventional water treatment processes. The results showed that: (1) natural organic matter (NOM) enhances stabilization of carbon nanomaterials (C_{60}, SWCNT, MWCNT) in natural waters; (2) adsorptive interaction between NOM and nanotubes depends on water quality parameters (e.g., pH and ionic strength) and NOM characteristics; and (3) fullerenes are expected to be well removed by water treatment processes. In the study of the chemical transformation of water stable C_{60} aggregates, the results showed that: (1) ozonation transforms nC_{60} into water soluble fullerene oxide species; (2) ozonated C_{60} appears more toxic than nC_{60}; (3) irradiation of UV (254 nm) transforms nC_{60} into water soluble fullerene oxide species; (4) C_{60} photolysis product appears less toxic than nC_{60}; (5) C_{60} in the aqueous phase reacts with the hydroxyl radical and hydrated electrons with a relatively high rate constant resulting in an unstable product. The results from the study of the photochemical activity of C_{60} in the aqueous phase during UV radiation showed that: (1) the status of the C_{60} dispersion in the aqueous phase affects its ability to transfer absorbed photoenergy to oxygen; (2) C_{60} present in water as a stable aggregate does not produce $^1O_2$ and $O_2^{\bullet}$ under UV illumination, in contrast to pristine C_{60}; (3) when C_{60} is present as an aggregate, the lifetime of key intermediate species for energy transfer is drastically reduced, fundamentally blocking the ROS production mechanism; and (4) peroxide forms during preparation of nC_{60}, which is partially responsible for the reported toxicity.

Discussion

A participant asked whether the NPs entered the cell during the *E. coli* destruction of protein in the cell. Dr. Kim responded that it is not possible to see it in the cell matrix.

CARBON-BASED TOXICITY

The Role of Particle Agglomeration in Nanoparticle Toxicity
Terry Gordon, New York University School of Medicine

The objective of this study is to determine the biological consequences of nanoparticle agglomeration. The hypothesis of this research project is that the toxicity of fresh (predominantly singlet) carbon NPs differs from that of aged (predominantly agglomerated) carbon NPs. The researchers further predicted that this difference also would apply to metal NPs. The objectives were to: (1) measure the agglomeration rate of carbon NPs; (2) identify whether agglomeration is affected by altering exposure conditions, such

as humidity and particle charge; and (3) compare the toxicity of singlet versus agglomerated particles in mice exposed via inhalation. The researchers used a dynamic exposure system to establish the agglomeration of freshly generated carbon NPs at various distances (i.e., aging times) downstream from particle generation. They then exposed mice to NPs generated in an arc furnace at different stages of particle agglomeration and examined lungs for injury and inflammation. The researchers found a dose–response relationship between exposure to carbon and metal NPs and lung inflammation such that the effects of fresh particles were greater than those of aged particles for carbon particles, but not for copper particles. Humidity and particle charge had no effect on the toxicity of carbon NPs. The researchers found that copper and zinc NPs were more toxic than carbon NPs, and copper NPs were more toxic than zinc NPs. In contrast to carbon NPs, copper particles showed only a small difference between fresh and aged NPs. Differences in response among mouse strains suggest that genetic and age-related factors can influence the response to NPs.

Discussion

A participant commented that everyone is looking for a susceptible strain. He asked whether there was any consistent pattern with one strain being more susceptible for even a single endpoint or all endpoints. Dr. Gordon responded that there was no consistent pattern for zinc. All of the strains responded at the concentration that was used. In reviewing the literature, Dr. Gordon found that in comparing ozone, nitrous oxide, and NPs, there was no consistency among strains.

Assessing the Environmental Impact of Nanomaterials on Biota and Ecosystem Functions
Jean-Claude Bonzongo, University of Florida

The hypothesis of this research project is that nanomaterials could lead to environmental dysfunction because of their potential toxicity and the toxicity of their derivatives. Their small size makes them prone to biouptake and bioaccumulation, while their large surface area could allow nanomaterials to act as carriers or deliverers of pollutants that are adsorbed onto them. The objectives of this project are to:
(1) assess the toxicity of nanomaterials using short-term microbiotests and investigate the impacts of nanomaterials on microbe-driven ecological functions; (2) determine the mobility of metal-based and carbon-based nanomaterials in porous media, as well as the toxicity of nanomaterials in soil leachates; and (3) identify possible mechanisms of toxicity for different types of nanomaterials. The combination of experimental and modeling data collected so far shows that when contact is facilitated between hydrophobic carbon-based nanomaterials (e.g., C_{60} and SWCNTs) and organisms by use of organic solvents or surfactants: (1) an easy penetration of the cell membrane occurs; (2) the retention time within the membrane varies with the nanoparticle size and shape; and (3) while C_{60} tends to induce toxicity primarily by lipid peroxidation, carbon nanotube accumulation within cell membranes results in increased pressure within the membrane with negative impacts on cell membrane functions. Additional studies on the toxicity of carbon and metal-based nanomaterials suspended in natural river waters point to the importance of solution chemistry as it affects both the degree of nanoparticle dispersion/suspension and the biological response of model aquatic organisms exposed to such suspensions.

Discussion

A participant asked if toxicity experiments in this study were conducted comparatively by using both river water-stirred nC_{60} (i.e., without use of THF) and suspensions produced by the THF method. Dr. Bonzongo responded that this was the case, adding that DI-water based suspensions were used as controls and THF- C_{60} suspensions were more toxic. The participant asked if something from the THF derivative could be causing the toxicity. Dr. Bonzongo responded that he did not have experimental evidence to support the idea that a potential THF derivative was responsible for the observed trend in toxicity.

ENMs in the Environment: Aggregated C_{60} and Associated Impurities
John Fortner, Rice University

All stakeholders will benefit from an understanding of how fundamental characteristics of engineered NPs control their biological effects. This research project will provide the first structure-function relationships for nanoparticle toxicology. The guiding hypothesis of the research project is that nanoparticle structure (e.g., size and shape) and surface chemistry directly control cytotoxicity. Within that construct, a secondary hypothesis is that, of the four major material parameters in engineered NPs (size, shape, composition, and surface), surface is the most important in governing cellular effects. The specific objectives are to: (1) expand the characterization of nanoparticle structure in biological media that can change aggregation status and surface chemistry (e.g., protein coat surfaces); and (2) characterize the effects of NPs on cell function. The researchers found that fullerenes behave contrary to initial estimations (i.e., there is water stable aggregate formation), and aggregates have been shown to interact with biological systems. Before such work can be done with certainty, however, the purity of engineered particles must be characterized and normalized: nC_{60} formation via THF intermediate can have impurities that are particle associated and unassociated; THF and THF derivatives have been identified, including a THF peroxide; and γ-butyrolactone was less than 2 percent of the total of THF derivatives. The researchers also found that the systems can be cleaned effectively; the stirred cell method provided enhanced control and removal of greater than 99 percent of aqueous impurities. It also was found that standard protocols for synthesis and purification are essential to compare "apples to apples."

Discussion

A participant asked whether C_{60} could enhance the decomposition of THF. Dr. Fortner responded that it could not. Based on negative controls without C_{60}, THF decomposed to a THF peroxide in the presence of light and oxygen regardless of C_{60}.

A participant commented that a number of studies have shown that these conversions do occur and that toxic byproducts are produced. Knowing that the byproducts tend to be toxic and with all of the efforts involved in removing THF, THF should not be used as a method. Dr. Fortner agreed. He also noted, however, that organic impurities are nearly ubiquitous in engineered nanomaterials as they are often used in the intermediate stages of synthesis. Therefore, this issue must be addressed for all particles with potential impurities. Standard protocols for stating impurity levels and identification must be incorporated into particle characterization as these issues are critical for toxicological analyses and comparison.

A participant commented that the solvate formation of THF in the clusters is similar to the solvate formation of other molecules within precipitants of C_{60}. Solvation is a function of temperature; as temperature is increased, there is an increase in C_{60} solubility from the pure crystalline material, not the clusters. As the temperature is further increased, the clusters are desolvated. If the clusters are formed at higher temperatures, it may be possible to get a lot of the THF to not reside in the clusters. Dr. Fortner agreed that this may be possible.

Long-Term Effects of Inhaled Nickel (Ni) NPs on Progression of Atherosclerosis
Gi Soo Kang, New York University

The hypothesis of this project is that inhaled Ni NPs can generate oxidative stress and inflammatory responses not only in the lung, but also in the cardiovascular system, which in the long term can enhance the development and progression of atherosclerosis in a sensitive animal model. An inhalation study was conducted with 5-month-old male $Apoe^{-/-}$ mice. The dose was 80 μg Ni/m^3 for 5 hours/day, 5 days/week, for either 1 week or 5 months. The research results showed that: (1) inhaled Ni NPs, at occupationally realistic levels, can induce oxidative stress not only in the lung but also in the cardiovascular system;

(2) inhaled Ni NPs can induce pulmonary and also systemic inflammatory responses; and (3) long-term exposure to Ni NPs could exacerbate plaque formation in hyperlipidemic mice. An additional study conducted to investigate which physicochemical properties of tested Ni NPs were responsible for the observed toxicity revealed that toxicity may not be explained solely by particle effects or dissolved Ni effects. This is the first long-term inhalation study to investigate cardiovascular effects of NPs, and the results will provide a useful database to establish size-specific regulations in occupational and environmental settings.

Discussion

A participant asked if there was any direct evidence of nickel translocation to the blood stream. Ms. Kang replied that the researchers were not able to find any direct evidence, but pointed out that the exposure concentration in this study was fairly low and the analytical method used might not have been sensitive enough to detect the very low levels of nickel possibly translocated to the blood.

Aquatic Toxicity of Carbon-Based Nanomaterials at Sediment-Water Interfaces
Baolin Deng, University of Missouri–Columbia

The objectives of this research project are to: (1) adapt a proper method for water and sediment toxicity testing of 1-D nanomaterials (CNTs, silicon carbide [SiC]); (2) assess the toxicity of representative 1-D nanomaterials in water or in sediment to representative sediment-dwelling organisms; and (3) identify factors controlling the toxicity toward the sediment-dwelling organisms. The approach includes three phases: (1) 14 -day toxicity screening of CNT in water with four selected organisms; (2) 14-day sediment tests with the CNTs identified as toxic to species in Phase 1 testing (e.g., 1% CNT spiked into sediments); and (3) sediment tests with dilutions of sediment containing CNTs (No Observed Effect Level [NOEL]) and variations with types of sediments. The researchers found that: (1) sonicated or non-sonicated as-produced single-walled and multi-walled CNTs are toxic to amphipods, midge, oligochates and mussels in water; (2) the observed toxicity is partially contributed to toxic metals dissolved from the nanomaterials such as Ni, but also is caused by purified nanomaterials (effect on growth); (3) sediment can reduce, but not totally eliminate, the toxicity of as-produced MWCNTs to amphipods; and (4) sonication significantly increases the toxicity of SiC nanowires to amphipods. Future studies will include: identifying physical and chemical characteristics of the CNTs; phase 2 sediment toxicity testing; phase 3 sediment dilution testing; and mechanisms for the observed toxic effects.

Toxicity of NPs in an Environmentally Relevant Fish Model
Judi Blatt-Nichols, New York University School of Medicine

The objective of this study is to determine the biological consequences of nanoparticle contamination of the aquatic environment. The investigators hypothesize that there will be a particle-type dependent difference in the developmental toxicity of manufactured NPs in aquatic species, and in testing this hypothesis, they will: (1) measure the differential toxicity of several types of NPs in an estuarine species of fish, Atlantic tomcod; and (2) identify whether the embryo and larval stages of development of tomcod are particularly susceptible to carbon nanoparticle or nanotube toxicity. The research results included: (1) fullerenes cause 100 percent mortality at 500 μg/L and hatching was delayed in all exposed doses; (2) functionalized SWCNTs did not result in significantly more mortality to embryos than carbon black particles, although time to hatch was significantly delayed; (3) for metal NPs, Cu was greater than Fe, Zn was greater than Ag and Ni for mortality; (4) toxicity associated with erbium- and yttrium-containing particles for the mix, soot, and sludge was dose-dependent and statistically significant. Future work will: (1) determine if nanoparticle bioavailaility and toxicity is influenced by aquatic media; (2) characterize the particles used in 5 ppt sea water and the natural waters in terms of mean diameter and zeta potential; (3) expose a second species, *Fundulus heteroclitus*, to a subset of particles to determine if the effects

found in tomcod are replicated in other species; and (4) use high-thoughput microarrays to determine dose- and time-dependent changes in gene expression in tomcod and *F. heteroclitus.*

Discussion

A participant asked if the researchers had considered using the carbon materials with erbium and yttrium atoms as tracers to look at the toxicokinetics of the carbon materials. Ms. Blatt-Nichols responded that they would like to do that in the future.

In response to a question from a participant, Ms.. Blatt-Nichols stated that the soot was the most toxic for erbium; the sludge was not as toxic as the soot and the finished products.

A participant asked where the soot and sludge materials were obtained. Ms. Blatt-Nichols responded that Luna Works was the company that supplied the materials.

Ecotoxicology of Fullerenes (C_{60}) in Fish
Theodore Henry, University of Tennessee

The research objectives are to investigate the characteristics of aqueous C_{60} aggregates and the impact of dissolved organic material on the behavior of these aggregates, and to evaluate bioavailability and toxicity of C_{60} (both aqueous C_{60} aggregates and dietary C_{60}) in fish by assessing changes in gene expression, histopathology, and bioaccumulation of C_{60} in tissues. The hypotheses are: (1) bioavailability of aqueous C_{60} aggregates is impacted by nanoparticle characteristics and presence of dissolved organic material; (2) exposure of fish to C_{60} can be detected by changes in expression of biomarker genes; and (3) toxic effects of C_{60} in fish can be detected only after long-term chronic exposure. Zebrafish (*Danio rerio*) and channel catfish (*Ictalurus punctatus*) are the species that will be investigated in this research. Larval zebrafish were exposed to the following treatments: (1) C_{60} aggregates generated by stirring and sonication (72 h) of C_{60} in water (12.5 mg C_{60}/500 mL water); (2) C_{60} aggregates generated by established methods with THF vehicle; (3) THF vehicle (i.e., method 2 without C_{60} added); and (4) "fish water" control. The Affymetrix zebrafish array was used to assess changes in gene expression (14,900 gene transcripts), and results indicate that changes in expression were related to decomposition products of THF rather than to toxicity from C_{60}. Subsequently, the researchers investigated the interaction of other contaminants with C_{60} aggregates and have determined that aggregate characteristics (e.g., size and charge) can change in the presence of a co-contaminant and that C_{60} can alter contaminant bioavailability in zebrafish. The presence of 17α-ehtinylestradiol (EE2) altered the characteristics of C_{60} aggregates. The Zeta potential decreased, and there was more of a tendency to aggregate. Particles were smaller; however, larger particles may have sedimented out of the aqueous phase. C_{60} reduced bioavailability of EE2 (reduced expression of Vitellogenin genes). Aging appeared to increase the association of C_{60} with EE2 and reduced the bioavailability of EE2.

Discussion

A participant asked whether the C_{60} aggregates were penetrating the chorion or whether de-chorionated embryos were used. Dr. Henry responded that larvae were used; the larvae had hatched so the presence of the chorion was not an issue for exposure.

Development of Methods and Models for Nanoparticle Toxicity Screening
Tian Xia, University of California, Los Angeles

This project aims to learn more about the health effects of nanoparticles. To date, approximately 10 particles, including fullerenes prepared by different methods, polystyrene nanoparticles with different

surface charges, and metal oxides with different dissolution rates have been studied. Results to date indicate that the physical characteristics of the particles and oxidative stress play key roles in particle toxicity. Physicochemical characteristics (e.g., shape, size, surface reactivity, dissolution rate) have been thoroughly characterized, and oxidative stress markers from cellular defense response, pro-inflammation, and cell death, have been tested in mammalian cell systems. Tests on fullerenes prepared using THF showed that, at the THF concentration used, the THF itself is not toxic to the cells. The degradation products—formic acid and γ-butyrolactone—were found to be very toxic and to induce cell death, but it was not clear whether fullerenes sped up the degradation process. For the polystyrene nanoparticles, cationic NH_2-PS nanoparticles were found to be toxic, while plain and anionic nanoparticles were found to be nontoxic. The mechanism of toxicity induced by cationic nanoparticles involves particle uptake inside cells via specific endocytic pathways, proton sponge effects inside lysosomes, lysosomal leakage, and mitochondrial-mediated apoptosis. For the metal oxides, ZnO was found to be toxic; the toxicity is mainly induced by the high Zn concentration that results from ZnO dissolution. For toxicity testing, it is important to thoroughly characterize the physicochemical properties of nanoparticles and the suspending solutions. The lessons learned about the mechanisms of cytotoxicity from this study can be used to design nanoparticles to mitigate toxicity. The following are some examples of the lessons learned to date: for fullerenes, be careful of the residual solvents; for carbon nanotubes, decrease the impurities and rigidity and/or functionalize the surface to increase solubility; for cationic particles, decrease the charge density or replace cationic head groups with amphiphilic head groups; and for ZnO, NiO, Ag, and Cu, cap with surfactants, polymers, or complexing ligands to decrease dissolution.

Discussion

A participant asked how cationic particles, which have a negative zeta potential in biological solutions, could cause toxicity. Dr. Xia explained that the positive charge can reappear inside lysosomes because particles are exposed to low pH environments and the protein coatings can come off.

Effects of Nanomaterials on Blood Coagulation

Peter Perrotta, West Virginia University

The goal of this project is to determine the effects of commercially available nanomaterials on the human blood coagulation system. Common human diseases, such as myocardial infarction, are caused by abnormalities of blood coagulation that predisposes a person to thrombosis (clots) and these diseases are clearly influenced by environmental factors. Because of their large surface area and reactivity, nanomaterials that enter the workplace or home have the potential to adversely affect blood coagulation, which could result in clotting abnormalities. The researchers are studying the effects of nanosized materials on the blood coagulation system using a variety of techniques. An important part of these studies involved documenting adequate dispersion of NPs within biological media. Interestingly, nanoparticle size can be verified in plasma-containing solutions by dynamic light scattering when the NPs are of uniform size and shape. Using these well-dispersed nanoparticle-plasma suspensions for clotting studies, it appears that NPs have the effect of shortening clotting times *in vitro*. They also are capable of altering the ability to generate thrombin, the most physiologically relevant clotting enzyme. Based on the importance of thrombin in human coagulation, the investigators have explored several sensor strategies for detecting clotting proteins like thrombin. The investigators recently have begun to study plasma obtained from rats exposed to ultrafine and nanometer-sized particles through inhalation. Differences in endogenous thrombin potential and fibrinogen levels can be identified between exposed and control animals. In addition, global proteomic profiling techniques (differential gel electrophoresis) and more targeted multiplexed (Luminex) panels have demonstrated significant alterations in rat proteins involved in the coagulation and inflammatory systems.

Discussion

A participant asked whether the ability of citrate to complex calcium plays a role and whether citrate would protect nanomaterials, which are intended to be introduced systemically, and make them safer. Dr. Perrotta responded that citrate is very important; it is used to keep blood from clotting. It potentially could be one way to make nanomaterials safer, but the short half-life of citrate may limit its usefulness.

A participant asked whether any evidence of systemic inflammation, such as c-reactive protein (CRP), was found. Dr. Perrotta responded that CRP was definitely increased, as were other markers of an acute inflammatory response.

Physical Characteristics of NPs Affect Interactions with Aquatic Organisms
David Barber, University of Florida

The goals of this research project are to: (1) expand the database of acute toxicity of metallic nanomaterials in aquatic organisms; (2) evaluate the role of particle composition and dissolution in gill toxicity; and (3) determine the role of particle surface charge in uptake and retention of nanomaterials in aquatic organisms. To address the first goal, researchers assessed the toxicity of NPs and their soluble counterparts to aquatic organisms. To address the second goal, researchers exposed zebrafish to TiO_2, silver, or copper particles and evaluated gill metal uptake, histology, and transcriptional changes at 24 and 48 hours. To address the third goal, researchers examined the uptake and retention of PEG, NH_2, and COOH QDs in *Daphnia*. The researchers found that nanometals can be acutely toxic to aquatic organisms, but they are typically less toxic than their soluble counterparts. NPs aggregate rapidly once they are introduced into water. Large numbers of nanosized particles, however, are likely to remain in the water column for long periods of time; this may allow for prolonged exposure after a release of nanomaterials into the environment. Intact NPs are taken up by gill cells and *Daphnia*. Physical properties of NPs have significant impacts on their interaction with biological systems. Charge is an important determinant of nanoparticle uptake and the effect of charge varies among models. Mechanisms of particle uptake for particles with similar properties can differ. Oxidative injury appears to play a role in nanosilver-induced toxicity.

Discussion

A participant commented that there was a question as to the *Daphnia* and whether or not what was being seen by fluorescence after gut clearing was simple adhesion to the carapace. He suggested taking a molt exuviate and exposing it after it has molted to find if it is strictly adhesion to the carapace. The concept of redistribution is very important and what is seen in the gut before and after gut clearing is a critical question. Dr. Barber responded that this was a good idea. The fact that increased fluorescence with the PEG is seen suggests that it is not simply adhesion. (Postmeeting Note: Electron microscopy with EDS was performed and it was confirmed that QDs are being internalized by *Daphnia*.)

A participant asked if strand breaks were seen from silver nitrate. Dr. Barber responded that they have not addressed that yet.

The Cellular and Gene Expression Effects of Manufactured NPs on Primary Cell Cultures of Rainbow Trout Macrophages
Rebecca Klaper, University of Wisconsin–Milwaukee

The overall objective of this research 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 study 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. The research hypothesis is: nanomaterials of dissimilar chemical composition will stimulate different patterns of trout macrophage gene expression, and nanomaterials of similar chemical characteristics (e.g., charge, shape, and functional group) may be grouped with respect to their bioactivity, expressed as a particular gene response pattern. Specifically, the chemical properties of nanomaterials will impact the genomic response of the immune system: nanomaterials of dissimilar chemical composition will stimulate different patterns of macrophage gene expression and the response will be dose-dependent. A range of water-soluble C_{60} and CNTs with different chemical compositions and surface chemistries will be synthesized and tested for their effects on trout macrophages. A trout primary macrophage cell culture system will be used to determine the: (1) dose versus cell viability for each synthesized nanomaterial type; (2) level of expression (by quantitative PCR) of marker genes associated with inflammatory, antiviral, and anti-inflammatory responses with respect to nanomaterial dose at levels that have no deleterious effect on cell viability; and (3) global patterns of gene expression for those materials that cause significant changes in marker genes using custom trout immune microarrays. The results show that: (1) trout macrophages are a sensitive tool to investigate the effects of NPs on gene expression; (2) side-chains attached to NPs may have just as much of a stimulatory effect on the immune system as the NPs; (3) surfactants used to solubilize NPs may have significant effects on gene expression—deoxycholate is a stimulator of inflammatory gene expression in trout macrophages; and (4) C_{60} fullerenes and nanotubes stimulate inflammatory gene expression in trout macrophages.

Discussion

Dr. Klaper responded to comments from others in previous talks and stated that although THF and other surfactants were not used in these experiments, these compounds should not be banned from use in experiments.

A participant commented that there have been a number of studies on whole fish gills showing inflammation. He asked if there was a way to link that whole gill level response to Dr. Klaper's work. Dr. Klaper responded that it would be interesting to see how much of the inflammatory response was due to pure oxidative stress or other immune factors. The researchers would like to study whole organisms as part of their next project.

A participant commented that Dr. Klaper's point about THF was a good one. This is not an academic exercise; researchers are trying to predict what is going on in the real world. This is similar to what went on with pesticides. Do you test the toxicity of the pure compound or what is used in the formulation that is used industrially? Industry is using things to disperse NPs and these releases are mixtures.

A participant asked Dr. Klaper to comment on her microarray study. Dr. Klaper stated that her team used three fish and there was a strong response for the inflammatory genes. There may be some small variation among fish, but the tissue culture system leads to little variation among fish. In addition, the inflammatory response was overwhelming and varied little among individual plates. The researchers would like to review earlier time points and even lower concentrations of each particle; she thinks that they will see a more sensitive measurement of how the treatments may affect the response.

METALS, METAL OXIDES: TOXICITY

Pulmonary and Immune Effects of Inhaled Carbonaceous Materials
Jacob McDonald, Lovelace Respiratory Research Institute

The research objective is to directly compare the biological disposition, persistence, and toxicity of two commercial nanoscale carbonaceous nanomaterials of potential wide utilization to a control material of known toxicity. Concentration matched (by mass) inhalation exposures of CNTs and fullerenes were compared to inhaled crystalline silica. Inhalation of MWCNTs and SWCNTs at particle concentrations up to 1 mg/m^3 did not result in significant lung inflammation or tissue damage, but caused systemic immune function alterations. The effect appears to be regulated from a TGF-beta lung signal that manifests through the COX-2 pathway. C_{60} fullerenes of median size 20 nm were produced by sublimation-condensation. F344 rats were exposed by nose-only inhalation for 6 hours at $1mg/m^3$, and pulmonary/extra pulmonary disposition was monitored for 7 days. Fullerenes were measured in tissues by LC/MS/MS. C_{60} fullerene inhalation showed poor lung clearance and minimal systemic translocation.

Discussion

A participant asked whether the C_{60} translocation could be related to dietary uptake. Dr. McDonald responded that it would not be related; most everything that is inhaled goes into the gut. Dr. McDonald will be conducting oral studies to answer this question.

NOVEMBER 21, 2008

OTHER NANOMATERIALS: LIFE CYCLE ANALYSIS AND REMEDIATION

Nanostructured Membranes for Filtration, Disinfection, and Remediation of Aqueous and Gaseous Systems
Kevin Kit, University of Tennessee

The objectives of this research project are to: (1) develop electrospun nanofiber chitosan membranes to treat aqueous and gaseous environments by actions of filtration, disinfection, and metal binding; (2) understand the electrospinning process for chitosan in order to control membrane structure; (3) investigate the effect of membrane structure on filtration, disinfection, and metal binding; and (4) optimize performance/efficiency of the chitosan membrane. Electrospinning of pure chitosan has proved to be difficult due to limited solubility and a high degree of intermolecular hydrogen bonding. The researchers were able to form nanometer-sized fibers without bead defects by electrospinning chitosan blends with synthetic polymers poly(ethylene oxide) and poly(acrylamide) with up to 95 percent chitosan in blend fibers. To date, researchers have developed a model to predict Cr(VI) binding properties of chitosan fibers; performed a detailed surface analysis of the fiber surface, and found two highly effective chitosan blends, one with good binding capacity and the other showing a 2-3 log reduction in *E. coli* K-12 with much smaller fiber mass.

Discussion

A participant asked if the researchers ran XPS on the film. Dr. Kit responded that they did and the results were the same for the film structure and the fiber structure.

Comparative Life Cycle Analysis of Nano and Bulk Materials in Photovoltaic Energy Generation
Vasilis Fthenakis, Columbia University

The objectives of this research project are to: (1) assess the life cycle mass and energy inventories of two main candidate nanomaterials for thin-film photovoltaic (PV) applications; (2) use process data to compare the materials and solar cell structures; and (3) investigate the applicability of the results to other nanomaterial-based thin-film technologies. Much progress has been made on the first two objectives. To date, researchers have been able to project the mass and energy flows in future nanotechnology-enabled PV, guided by changes in material utilization, purity, deposition rates, film thickness, and electric conversion efficiency. Solution grown nanostructured CdTe solar cells require more extrinsic materials than micro-CdTe solar cells, but less volume and lower purity semiconductor precursors. Plasma-enhanced CVD of nc-Si requires materials for reactor cleaning that are greenhouse gases (GHG). Adding nc-Si layers to a-Si solar cells increases energy and GHG emissions that can be counterbalanced by cell efficiency increases. Future work will include a detailed investigation of solvent use and recycling efficiency, a detailed investigation of energy use in solution-grown materials and in inkjet printing, investigation of CIGS PV production by inkjet printing, and investigation of nanoparticle inks replacing screen-printed silver-glass-frit pastes for Si cell contact metallization.

Discussion

A participant asked if the researchers had considered using water as a solvent in the cadmium synthesis. Dr. Fthenakis responded that the researchers had not, but would be interested in learning more about this potential approach.

Life Cycle of Nanostructured Materials
Thomas Theis, University of Illinois

The life cycle of a nanostructured material includes its manufacture from raw materials to its release into the environment; each of these stages offers opportunities for exposure and efficiency. To date, most research efforts have focused on the end of the life cycle. Bottom-up techniques (creating nanomaterials and then assembling them) were initially thought to be less harmful to the environment, but this has turned out not to be the case. In fact, sources of nanomanufacturing impacts include: strict purity requirements and less tolerance for contamination during processing; low process yields or inefficiencies; repeated processing, postprocessing, or reprocessing steps for a single product or batch; use of toxic/basic/acidic chemicals and organic solvents; the need for moderate to high vacuum and other specialized environments such as high heat or cryogenic processing; use of or generation of GHGs; high water consumption; and chemical exposure potential in the workplace through technological/natural disasters. The more complicated the structure of the nanostructured material, the more energy needed to manufacture it. At the other end of the life cycle, this project has focused on CdSe NPs in aquatic environments. Preliminary results show CdSe NPs to be extremely insoluble, but the expectation is that they will dissolve after entering the environment which will have implications. Ultimately, the impact of nanostructured materials on human and ecosystem function will depend on many factors.

Discussion

A participant asked why economic impact was not included in the life cycle assessment. Dr. Theis responded that the economic aspect would be included later. The participant stressed the importance of including economic impact in the assessment. Dr. Theis stated that while there is a considerable amount of energy used in the manufacture of nanomaterials, this must be balanced with the potential energy savings resulting from the use of these nanomaterials.

Evaluating the Impacts of Nanomanufacturing via Thermodynamic and Life Cycle Analysis
Bhavik Bakshi, The Ohio State University

The overall goal of this research project is to help guide the development of nanotechnology to ensure that it is environmentally benign and sustainable. Understanding the impact of nanomaterials is essential, but not sufficient; a systems view must be adopted. Life cycle analysis (LCA) of emerging technologies poses unique challenges. In particular, life cycle inventory data for nanomanufacturing are not available and the impacts of ENMs on humans and ecosystems are only partially known. The first objective of this research project is to conduct a life cycle evaluation of nanoproducts and processes. To date, the researchers have established life cycle inventory modules for a number of nanomaterials. The second objective is to explore a predictive model for LCA and impact assessment. Specifically, the researchers will examine the relationship between life cycle inputs and impact and the relationship between the properties of NPs and their impacts. The researchers have found that, from cradle to grave, polymer nanocomposites (PNCs) are 1.6-10 times more energy intensive than steel. On a life cycle basis, the product use phase is likely to govern if net energy savings can be realized, and the use of PNCs in automotive body panels may result in net life cycle fossil energy savings. In addition, the life cycle assessment of nano TiO_2 shows significantly less energy use and impact as compared to carbon nanofibers. A recently completed life cycle energy analysis of nano TiO_2 has identified opportunities for improvement. Future work will include: (1) research on other nanoproducts based on carbon nanofibers or nano TiO_2; (2) exploration of the statistical relationship between inputs and impact; and (3) risk analysis.

Discussion

A participant noted that the research did not include an impact assessment beyond energy requirements and asked how a broader impact assessment could be built into these models. Dr. Bakshi responded that there is a dearth of information on the environmental impacts of these NPs and that taking this type of approach would require collaboration.

OTHER NANOMATERIALS: EXPOSURE

Impact of Physicochemical Properties on Skin Absorption of Manufactured Nanomaterials
Xin-Rui Xia, North Carolina State University

Skin is made up of layers, with the top layer serving as the main barrier for small molecules and particulates. The objective of this project is to establish a structure-permeability relationship for skin absorption of manufactured nanomaterials for safety evaluation and risk assessment. Four dominant physicochemical properties (particle size, surface charge, hydrophobicity, and solvent effects) in skin absorption will be studied. Fullerene and its derivatives will be used as model nanomaterials. Results to date show that fullerenes exist as molecular C_{60} or nC_{60} in different solvents and this affects their skin absorption mechanism. In experiments, C_{60}, nC_{60}, and $ANnC_{60}$ were all readily absorbed into the uppermost layer of skin *in vitro* and *in vivo*. Tape-stripping methods can be used to study solvent effects on skin absorption of nanomaterials and to provide partition coefficients and skin permeability for predictive model development.

Discussion

A participant asked if the researchers had studied nC_{60} in dimethyl sulfoxide (DMSO). If so, how does it behave? Dr. Xia responded that nC_{60} is stable in DMSO.

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

The long-term objectives of this project are to determine the physicochemical properties of ENMs that influence their distribution into the cells comprising the blood-brain barrier (BBB) and the brain and to characterize their beneficial and/or hazardous effects on the brain. The researchers are using ceria (CeO_2) as a model insoluble stable metal oxide tracer. Studies conducted to date in rats have shown that ceria is rapidly cleared from the blood by peripheral reticuloendothelial tissues, much less ceria entered the BBB cells or the brain than peripheral tissues, ceria ENM agglomerates *in vivo*, and the ceria induced mild oxidative stress and stress response in the brain. These results provide a foundation to study the impact of the physicochemical properties of ENMs on peripheral organ distribution, brain entry, and neurotoxic or neuroprotective potential.

Discussion

A participant asked if the results suggested that ENMs would aggregate and coagulate quickly in blood. Dr. Yokel responded that the ENMs could potentially aggregate after they reach the blood. He clarified that, in the experiments discussed, the two solutions infused into the rat (the ceria ENM dispersion in water and 1.8% saline) were not combined until they reached the blood.

OTHER NANOMATERIALS: FATE/TRANSPORT

Aggregation, Retention, and Transport Behavior of Magnetite NPs in Porous Media
Yan Jin, University of Delaware

The overall objective of this research project is to develop an understanding of the fate of NPs released into the subsurface environments. Specific project objectives include: (1) determining the agglomeration behavior of selected NPs under different solution chemistry (pH, ionic strength, and presence of dissolved organic matter); (2) measuring the mobility of NPs in model porous media; and (3) elucidating retention mechanisms of NPs at various interfaces at the pore-scale. Work to date has focused on the first two objectives. Experiments have shown that humic acid can modify the surface charge of NPs by forming a coating on the particle surfaces. This shifts the point of zero charge and changes the pH at which aggregation occurs, increases the critical coagulation concentration (making it more stable), reduces deposition, and increases mobility. The next steps will be to determine if this also will be the case with smaller and other types of nanoparticles.

Internalization and Fate of Individual Manufactured Nanomaterials Within Living Cells
Gayla Orr, Pacific Northwest National Laboratory

Accumulating observations suggest that inhaled nanoscale particles (NSPs) are more harmful to human health than larger particles, and these effects have been linked to the surface properties of the nanomaterials. Current observations also suggest that NSPs might directly enter the circulatory system through the epithelial wall. The hypothesis of this research project is that the initial interaction of NSPs with the living cell *in vivo* occurs at the level of individual or small NSP aggregates (< 100 nm), and that the physical and chemical surface properties of the individual NSPs dictate their mechanisms of interaction with the cell, and ultimately govern their level of toxicity. Experiments conducted to date have shown that both 100 nm and 500 nm particles can take advantage of the actin turnover machinery within microvilli to move into alveolar type II epithelial cells, an expected target cell for inhaled submicrometer and nanoscale materials. This pathway, however, is strictly dependent on the positive surface charge of the particles and on the integrity of the actin filaments, unraveling charge-dependent coupling of the particles with the intracellular environment across the cell membrane. To identify the molecules that

capture the particles at the cell surface, the researchers searched for a negatively charged, transmembrane molecule that could mediate the coupling of the particles with the actin filaments and found that syndecan-1, a transmembrane heparan sulfate proteoglycan, mediates the initial interactions of the particles at the cell surface, their coupling with the intracellular environment, and their internalization pathway. These findings reveal a new mechanism by which positive surface charge supports particle recruitment by polarized epithelial cells bearing microvilli, and identify a critical role for syndecan-1 in the cellular interactions and subsequent potential toxicity of these particles.

Discussion

A participant asked how the charge is distributed. Dr. Orr responded that the distribution of the surface charge over the particle surface was not known; the researchers measured zeta potential to approximate the charge.

Methodology Development for Manufactured Nanomaterial Bioaccumulation Test
Yongsheng Chen, Arizona State University

The objectives of this research project are to: (1) develop suitable manufactured nanomaterial bioaccumulation testing procedures to ensure data accuracy and precision, test replication, and the comparative value of test results; (2) evaluate how the forms of these manufactured nanomaterials affect the potential bioavailability and bioconcentration factor (BCF) in phytoplankton; (3) determine the potential biomagnification of manufactured nanomaterials in zooplankton; and (4) determine the potential biomagnification of manufactured nanomaterials in fish. The researchers tested different nanomaterials on algae, daphnia, and adult and embryonic zebrafish to determine which were most toxic to these organisms. For carbon-based NPs, SWCNTs were most toxic, followed by C_{60} and then by MWCNTs. For metal oxides, nZnO was most toxic, followed by $nTiO_2$ and then by nAI_2O_3. nZnO was found to cause oxidative stress in aquatic organisms and sediment could potentially be a mitigating agent to reduce the toxicity caused by ZnO NPs. Future work includes determining the bioaccumulation behavior of NPs under different exposure conditions, determining the distribution (or fate) of NPs in different parts of the exposure system, and conducting long-term experiments on biomagnification and toxicity.

Discussion

A participant asked what species of green algae was studied. Dr. Chen promised to send the participant the paper describing their work. Another participant asked if there were any physical or chemical property changes in the $nTiO_2$ during exposure. Dr. Chen said that physical and chemical property changes did occur, but he did not include this in his presentation because of time limitations.

Experimental and Numerical Simulation of the Fate of Airborne NPs From a Leak in a Manufacturing Process To Assess Worker Exposure
David Pui, University of Minnesota

This project aims to determine the fate of NPs as they are emitted through a leak from a nanoparticle production process into a workplace environment. This NP fate is determined by measuring and modeling changes in particle and aerosol properties, such as number and surface area concentrations, morphology, and chemical composition. To do this, the researchers simulated a leak and studied the particle changes that occurred. A filtration study showed that results from the two types of monitors used to detect NPs correlated very well. With an aerosol mainly composed of NPs, the surface area filter efficiency was found to represent a more health-relevant filter evaluation and a better characterization of the filter. A particle dispersion study showed that the nanoparticle concentration became more uniformly distributed further out from the release location. Future plans include experimentally and numerically investigating

the fate of NPs upon release into a wind tunnel using a burner setup, studying the effects of background particles on nanoparticle fate, and numerically modeling the fate of NPs for a more complete understanding of the coagulation and dispersion processes with high spatial resolution.

Discussion

A participant asked how many manufacturing facilities had been monitored with these instruments. Dr. Pui said that one of the large chemical companies has plans to begin using these instruments for monitoring soon.

Fun with Carbon and TiO_2 NPs
Andrij Holian, University of Montana

Studies to date have shown that carbon nanoparticle toxicity may be dependent on size, size distribution, aggregation, shape, surface chemistry, surface area, and surface charge. All of these properties could be affected by suspension media, but predicting the optimal media for any one particle is not possible because chemistry will be a factor. Experiments performed for this project have shown that carbon nanoparticle toxicity is difficult to predict from conventional *in vitro* assays. Additionally, the dispersion medium affects the outcome for CNTs. The researchers compared TiO_2 nanospheres and nanowires and found the shape of the nanoparticle to be an important determinant of toxicity, with long nanowires being the most toxic and nanospheres being the least toxic. The scavenger receptor macrophage receptor with collagenous structure was found to be an important receptor for NPs, but is not involved in long nanowire toxicity. Redox is probably not involved in long nanowire toxicity. No unique changes in intracellular ROS were found.

Discussion

A participant asked if the researchers observed frustrated phagocytosis. Dr. Holian stated that they did not see this; nanowire contact with cells was enough to induce toxicity.

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

The overall goal of this project is to quantify interactions between manufactured NPs and wastewater biosolids. This will be accomplished through the estimation of sources and loadings of nanomaterials into wastewater treatment plants (WWTP) and through the development of mechanistic models for nanoparticle removal in WWTPs. The researchers 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 nanoparticle concentrations in sewage should have a negligible impact on the WWTP's biological activity or performance. Experiments to date have shown that functional nanomaterials are not removed as well as metal oxides. In sequencing batch reactors, Nano-Ag and TiO_2 had no effect on heterotrophic activity. Results to date suggest that TiO_2 may serve as a sentinel nanomaterial in the environment, indicating where other nanomaterials will eventually occur.

Discussion

A participant pointed out that TiO_2 may not be a sentinel for another nanoparticle if the two NPs have different point uses. Dr. Westerhoff agreed and stated that all TiO_2 cannot be accounted for based solely on what goes through the body; other sources must be considered as well.

OTHER NANOMATERIALS: TOXICITY

Genomics-Based Determination of Nanoparticle Toxicity: Structure-Function Analysis
Alan Bakalinsky, Oregon State University

This project aims to identify genes that mediate toxicity as a first step toward elucidating mechanisms of action and to correlate toxicity with physical/chemical structure. Experiments showed that nC_{60} did not inhibit the growth of *E. coli* or yeast in minimal media and had no real impact on the survival of yeast in water over a 24-hour period although survival decreased slightly when fewer cells were exposed. Survival of *E. coli* was significantly reduced over 24 hours in 0.9 percent saline, particularly at low cell concentration. No obvious correlations were seen between size or zeta potential and cell survival. Studies of gold NPs showed that none of the three Au NPs tested reduced yeast cell yields in minimal medium. Positively charged Au-TMAT reduced yeast survival more than negatively charged or neutral Au derivatives. Specific amounts of these particles appeared to kill a fixed number of cells. To identify genes and mechanisms implicated in Au-TMAT-mediated killing, a yeast gene deletion library was screened for mutants resistant to Au-TMAT relative to the wild-type parent strain. Six resistant clones were isolated from the initial screen of 2,500 mutants. Loss of GYL1, YMR155W, DDR48, and YGR207C was found to result in Au-TMAT resistance, suggesting that these genes play roles in mediating Au-TMAT toxicity. Future work will focus on identifying additional mutant strains.

Discussion

A participant asked if the researchers had studied chromosome or DNA damage. Dr. Bakalinsky responded that they had not, but would like to do so in the future.

Role of Surface Chemistry in the Toxicology of Manufactured NPs
Prabir Dutta and W. James Waldman, The Ohio State University

This project is working to identify correlations between biological activity and physicochemical characteristics of minerals and particulates, including the biological response (oxidative burst), mutagenicity, and the chemical reactivity (Fenton reaction) of zeolite minerals and oxidative stress and inflammatory responses of carbon particulates. Zeolite minerals (aluminosilicates) and carbon particles were chosen for study to evaluate how the surface structure of particles influences their toxicity. The researchers found that the coordination environment can modify the iron redox potential and the chemical reactivity differences result in different biological reactivity. Further experiments using carbon NPs of the same size showed that it is the surface chemistry of the iron that causes the reaction. Results to date have shown that Fe(III) precipitate is more cytotoxic and more inflammatory than Fe(II). The researchers hypothesize that the redox state of the element released is important.

A Rapid In Vivo *System for Determining the Toxicity of Nanomaterials*
Robert Tanguay, Oregon State University

The hypothesis of this study is that the inherent properties of some ENMs make them potentially toxic. To test this hypothesis, the researchers developed an *in vivo* zebrafish toxicity assay to define the *in vivo* response to nanomaterials, and will eventually define structural properties of nanomaterials that lead to adverse biological consequences. A wide range of nanomaterials will be tested to assess toxicity. Those that cause significant adverse effects move on to the next stage of testing in which potential cellular targets and modes of action are defined *in vivo*; nanomaterials are then grouped according to structural indices and effects. Global gene expression profiles will be used to define the genomic responses to these materials. A Nanomaterial Biological Interactions database will be populated with the data collected on the properties of the nanomaterials. To date, more than 200 nanomaterials have been evaluated for

toxicity in zebrafish. Those determined to be toxic have moved on the next stage of testing. The researchers will continue to test nanomaterials for toxicity and ultimately, develop a database populated with the data collected.

Discussion

A participant asked if the researchers were planning to study epigenetic responses. Dr. Tanguay replied that they are planning to do these studies.

Cellular Uptake and Toxicity of Dendritic Nanomaterials: An Integrated Physicochemical and Toxicogenomics Study
Mamadou Diallo, California Institute of Technology

The overall objective of this research project is to improve understanding of the cellular uptake and toxicity of dendritic nanomaterials in aqueous solutions at physiological pH 7.4. The specific objectives are to: (1) characterize the interactions of dendrimers with cell membranes through measurements of physical-chemical surrogates (octanol-water partition coefficients and liposome-water partition coefficients); (2) characterize the interactions of dendrimers with plasma proteins through measurements of dendrimer binding to human serum albumin (HSA) protein; (3) use molecular dynamics simulations, nuclear magnetic resonance spectroscopy, and neutron scattering to characterize the mechanisms of interactions of dendrimers with lipid bilayers and HSA protein; (4) characterize the cytotoxicity of dendrimers through *in vitro* measurements of cell viability and toxicogenomic studies; and (5) conduct correlation analysis. Work to date shows that PAMAM dendrimers with protonated terminal NH_2 groups at pH 7.4 have a higher tendency to bind to liposomes ($LogK_{lipw}$). These dendrimers also show a high level of toxicity due to their tendency to cause membrane leakage. Other molecular mechanisms beyond membrane leakage may be responsible for the higher toxicity of cationic dendrimers. PAMAM dendrimers with neutral and negatively terminal groups have been found to have low to negligible toxicity. Future work includes: quantitative internalization, live imaging 1 ms frame to track the internalization of dendrimers, and performing correlation analysis and developing structure-activity relationships.

Effects of Ingested NPs on Gene Regulation in the Colon
John Veranth, University of Utah

This research project focused on a model of bowel inflammation and used RKO and CaCo human colon-derived cell lines with and without activation by TNFα. The central hypothesis being tested is that ingested manufactured NPs are taken up by inflamed colon cells, translocate to the nucleus, and alter gene transcription, thereby further increasing inflammation and leading ultimately to the development of pathological conditions including cancer. In separate experiments, samples were prepared from multiple types of metal oxide nanoPM and whole genome microarray experiments were conducted. TiO_2 and ZnO displayed transcriptional effects, with ZnO having the most pronounced effect. The data suggest that multiple pathways are activated by the ZnO, including: stress response pathways, Zn metabolism and transport genes, and genes that suggest alterations in redox pathways. NanoZnO displayed the most toxicity and demonstrated the most pronounced transcriptional response. This transcriptional response suggested that part of the exposure to nanoZnO was exposure to elemental Zn, and therefore, perhaps the toxicity was merely Zn toxicity. Therefore, the investigators sought to determine if the nanoZnO toxicity was due to the dissolution of ZnO to elemental Zn and the mechanism of the cell death upon exposure to the nanoZnO. In addition, two size ranges of ZnO PM were utilized to evaluate the effects of size/surface area. The researchers wanted to determine if: (1) cell and PM contact was required for ZnO toxicity; and (2) ZnO dissolution to free Zn was dependent on the cells. A set of three experimental conditions were used: (1) a dialysis device with a 10 kD cutoff was used to separate the ZnO from cellular contact to

ensure no ZnO PM could interact directly with cells; (2) transwells with 0.4 micron pores that would allow greater interactions with cellular products but still separate the cells and the PM were used; and (3) ZnO PM was placed in direct contact with the cells. The Zn concentrations were measured in the media by ICP spectrometry and cell viability by PI exclusion. The ZnO toxicity was only observed when the particles were in contact with the cells, but the Zn levels in the media were equally high in the transwell and direct contact experiments, suggesting that contact and potentially uptake is required for cellular toxicity. It was also found that ZnO induces apoptosis by inducing superoxide production in the mitochondria and disruption of the mitochondrial potential. In addition, all of the toxic effects are dependent on particle size, as the larger ZnO PM always demonstrated reduced toxicity compared to the smaller ZnO NPs.

Discussion

A participant asked what the molecular mechanism of zinc toxicity is. Dr. Veranth said that little is known about mechanisms of zinc toxicity; this should be explored further.

Nanoparticle Toxicity in Zebrafish
Gregory Mayer, Texas Tech University

The objective of this research project is to investigate the toxicity of semiconductor nanocrystals using zebrafish (*Danio rerio*) as an *in vivo* model, and zebrafish liver cells as an *in vitro* system. The approach will monitor, in real-time, the effects of particle composition, size, and charge on uptake and accumulation of nanostructures in multiple cellular compartments. Additionally, the investigators will address the hypothesis that toxicity of metal-cored nanoparticles stems from dissoluting metal ions by using a transgenic zebrafish model that expresses green fluorescent protein (GFP) in the presence of I-B and II-B metal ions. These data will be correlated with embryo development after particle exposure, and the effects will be extrapolated to human health. Finally, the researchers will develop a model to predict particle toxicity that will help to evaluate the potential health risks of the release of differing semiconductor NPs into the environment. Cell cultures have shown cell viability results similar to those found by other researchers. Toxicity appears to be related to the size of the particle, with smaller particles being more toxic. Work conducted to date suggests that nanocrystals may not be gaining entrance to the cell through classic calveolin- or clathrin-mediated pathways. *In vivo*, the toxicity of quantum confined semiconductors does not seem to be attributable to ion dissolution from the particles.

Discussion

A participant asked whether the researchers saw different effects in the different regions of the fish. Dr. Mayer explained that it appears that the ions are moving into the gut, but because this happens before the embryos feed, this may not be attributable to normal gut uptake. In this stage of development, it would be difficult to discern distinct tissue patterns with this method.

Lung Deposition of Highly Agglomerated NPs
Jacob Scheckman and Peter McMurry, University of Minnesota

The objectives of this research project are to: (1) develop a stable, repeatable source of nanoparticle agglomerates with closely controlled properties; and (2) characterize the effects of agglomerate properties on deposition in physical models of the human lung. Transport and physical/chemical properties of nanoparticle agglomerates depend on primary particle size, fractal dimension, and the number of primary particles in the agglomerate. Agglomerate properties were determined by tandem measurements of mobility (differential mobility analyzer [DMA]), mass (aerosol particle mass analyzer [APM]), and morphology (electron microscopy [SEM/TEM]). Nanoparticle agglomerates of silica were generated by

oxidizing hexamethyldisiloxane in a methane/oxygen diffusion flame. Particles leaving the flame were classified by electrical mobility size using a DMA, and their mass measured with an APM. The measured relationship between mass and mobility was used to determine the fractal dimension. The effects of oxygen flow and mass production rates on single particle mass, fractal dimension, and dynamic shape factor were characterized. Electron microscopy was used to determine primary particle size and to provide qualitative information on particle morphology. The generated particles were chain agglomerates with clearly defined primary particles. Increasing the oxygen flow rate was shown to decrease the primary particle size and the fractal dimension and to increase the dynamic shape factor. Increasing the production rate was shown to increase the primary particle size and mass of the product particles without affecting the fractal dimension and to decrease the dynamic shape factor. These results represent the completion of objective 1. Of particular interest are the effects of agglomerate structure on lung deposition. To investigate this, deposition of silica agglomerates through a straight capillary tube model simulating lung generation 22 was compared to that of spheres. Deposition did not depend on particle morphology in the capillary tubes, but deposition of spheres and agglomerates differed significantly in the entrance/exit region of the model. Future work will: investigate increased deposition in the entrance/exit region, characterize the effects of fractal dimension, and measure deposition through more physically realistic lung models.

Dr. Savage thanked all of the participants for their contributions and adjourned the meeting.