ICEIN 2011 – EPA Nano Grantees Conference
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ABSTRACT BOOK

SESSION 1: RISK ASSESSMENT

Assessing exposure and effects for environmental risk assessment: The challenge of engineered nanoparticles

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[1] University of York

Keynote Presentation
11:10 PM, Monday

The increasing use of engineered nanoparticles in a range of product types means that it is inevitable that selected ENPs will be released to the natural environment. It is therefore critical that we begin to establish the risks of ENPs to aquatic and terrestrial systems. A range of environmental risk assessment schemes are already in place for 'traditional' chemical substances. However, questions have been raised over the application of these approaches to ENPs. This presentation will provide a broad overview of the typical environmental risk assessment approach for a traditional chemical and illustrate the approach using a case study. The talk will then move on to compare and contrast the behaviour and effects of traditional chemicals and ENPs in the natural environment and explore the implications of any differences for the risk assessment process. Areas where we should be focusing our efforts to better assess the risks of ENPs will highlighted.

Implementation of life cycle assessment methodologies for commercializing nano-enabled products

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Oral Presentation
2:00 PM, Monday

Goal 4 of the National Nanotechnology Initiative Strategic Plan 2011 advocates the responsible development of nanotechnology as central to advancing the NNI's overarching mission of promoting scalable nanomanufacturing and product commercialization. The Strategic Plan calls for research to "incorporate safety evaluation of nanomaterials into the product life cycle, foster responsible development, and where appropriate, sustainability across the nanotechnology innovation pipeline." In sum, the NNI sees life cycle assessment (LCA) as integral to the process of moving nanomanufacturing to sustainable commercial scale production.

At the Nanoscale Science and Engineering Center for High-rate Nanomanufacturing, research is ongoing to develop novel directed self-assembly processes and techniques for nanomanufacturing of nano-enabled products such as chemical- and bio- sensors, batteries and electromagnetic interference (EMI) shielding (1-4). Concurrently LCA case studies for several of these emerging products have been underway.

Life cycle assessment is a four-stage process, consisting of 1) scope, 2) inventory, 3) impact assessment and 4) valuation for decision-making. Although we can collect inventories for CNT applications under development in CHN laboratories (stage 2), there is little to no information on the environmental or health impacts of CNTs (stage 3). Therefore it will be necessary to develop impact assessment characterization indices for various nanomaterials before results from LCA can be generated in stage 3. With respect to LCA stage 4, there are issues in valuation of the results of impact assessment, owing to differences in stakeholder perceptions about environmental and health impacts and priorities. Therefore we are exploring means for comparing valuation of resulting LCA outcomes in light of decision making. Further, data obtained from LCAs do not automatically translate into actions by company or government decision-makers that guide responsible product stewardship. Meaningful pathways for LCA findings are sought to elucidate the implications for how policymakers understand end-of-life recycling infrastructures. This aspect is critical insofar as US federal agencies are beginning to mandate use of LCA as part of their assessment practices.

Using several carbon nanotube enabled products as examples, environmental footprints for each are compared to the existing technologies that they would replace. A CNT switch is assumed to replace present-day flash memory, and CNT-polymer mesh is presumed to be used as electro-magnetic interference (EMI) shielding. End-of-life (EOL) issues for CNT products include identification of potential barriers or advantages to recycling products containing CNTs (5-8).

Responsible commercialization of nano-enabled products (NEPs) hinges on three interwoven dynamics: 1) the successful development of new manufacturing techniques; 2) the creation of an economically viable product that meets societal needs; and 3) a conscious and systematic consideration of short and long-term societal impacts to avoid unintended and unduly harmful consequences (9-11). This work reflects unique perspectives from an interdisciplinary team working concurrently with scientists and engineers during design and development processes to assess product lifecycle impacts leading to responsible commercialization.

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Control strategies for occupational and environmental exposure of nanomaterials

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Oral Presentation
2:00 PM, Monday

We have been addressing sustainable nanotechnology occupational and environmental health and safety *from the beginning*. It is extremely important that all engineered nanoparticle researchers and manufacturers incorporate sustainable practices into their work. We evaluated nanoparticle exposures in a variety of settings, and assessed the effectiveness of current control strategies to set the stage for the next phase, which we believe to be the development of consensus methods for engineered nanoparticle exposure assessment and control.

Our research has investigated various occupational and environmental exposure issues and developed effective control strategies. The precautionary principle and sustainable development guided our research philosophy since its earliest stage. We adopted cleaner production principles to eliminate contamination at the source from many different nanotechnology research and production activities. We followed the hierarchy of engineering controls, i.e. isolation, filtration and ventilation, to develop control strategies.

We will review our results regarding current knowledge about occupational and environmental exposures to engineered nanoparticles and effective techniques for evaluating and controlling such exposures. Case studies from our research will be discussed including the control strategies found to be effective. Research laboratories and industrial manufacturers were in need of economic and straightforward control methods; we found that modifications to currently-available controls were required for the nano-industry to move forward to the next phase. In addition, the current consensus on best practices to reduce exposure and the challenges presented by the next phase will be presented and discussed.

The currently-available nanoparticle control methods can provide excellent performance when used properly and efficiently. Understanding the behavior of airborne nanomaterials, such as their response to the surrounding airflow pattern, is one of the key elements for controlling exposure. The knowledge gained to date can guide the further development of effective consensus standards for engineered nanoparticle health and safety.

Benefits, risks, bias and nanomaterial regulation: results of an expert survey

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Oral Presentation
2:20 PM, Monday

Research Significance: Engineered nanoscale materials (ENMs) present a difficult challenge for risk assessors. Continuing uncertainty about the potential risks of ENMs means that expert opinion will play an important role in assessing and regulating risk. This research aims to shed light on the views of various groups of 'nano experts' to better understand which nanomaterials or applications are viewed as more risky than others, and to understand the implications of these findings for future nanomaterial regulation. Furthermore, this work aims to characterize differences in judgments between experts to evaluate how experience, research institution, disciplinary background, and other demographic variables might influence the perceptions of risks and benefits from emerging nanotechnologies.

Methods: A web-based survey (n=1,800) was conducted with three pools of US and Canadian experts: nano scientists and engineers (NSE), nano EHS scientists and toxicologists (NTOX), and scientists and regulators in government agencies (NREG).

Results: Our research found that nanomaterial releases in occupational settings and in emissions from production facilities were seen as most risky compared to other release scenarios and product applications. Furthermore, significant differences in risk perceptions were found across expert groups and other variables including gender and race, with experts in regulatory agencies judging risks as significantly higher than other groups.

Conclusion: This research provides insight into experts' judgments of the benefits and risks of nanomaterials and identifies a number of areas of concern. Several variables were also found to describe differences in perceptions of risks of nanomaterial applications and release scenarios. These findings can help risk assessors and regulators to prioritize efforts and to identify potential biasing factors that can affect expert judgment of risk.

Intuitive cognition in the perception of air, water and soil as they interact with engineered nanomaterials: A study of US public views

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Oral Presentation
3:00 PM, Monday

This paper examines both how people make sense of the multiple possibilities of engineered nanomaterials (ENMs), and the basis through which these materials are judged as effecting environmental media. Drawing from a recent national web survey conducted in the US by IRG 7 in the UC CEIN, we developed psychometric scales to characterize the qualities that people intuitively assign to air, water, and soil and the value-based logics and available cues they use to evaluate the toxicology of these media. Four factors emerged as characterizing judgments: (1) the perceived tangibility of media; (2) the resilience of media in the face of impact; (3) the sensory detectability of media; and (4) the ephemeral quality of media. In contrast, the 'value and cues' question set loaded into a single 'lay toxicology' factor. These factors, along with race and gender, were then used to predict the acceptability of 14 different ENM applications, each designed in consultation with UC CEIN NSE and toxicologists. A full tutorial about nanotechnology preceded questions specific to these. Material descriptions included their application domain (environmental, medical, and military); the type of material used (e.g., carbon nanotubes), and a qualitative assessment of the environmental risks involved (minimal, uncertain, moderate, significant). Acceptability judgments of these were found to be driven by the stated risk level, and not by application domain or material type. The factor resilience, which also correlates highly with lay toxicology values, predicts well the acceptability of these materials. Nonwhite males, unusually, are more inclined to regard the applications as acceptable, white males a little less so, whereas white and nonwhite females, view risks as similarly unacceptable.

Comprehensive environmental assessment of nanomaterials

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Oral Presentation
4:00 PM, Monday

To better evaluate the possible ecological, human health, and other impacts of nanomaterials, the U.S. Environmental Protection Agency (EPA) has been developing and applying an approach known as Comprehensive Environmental Assessment (CEA) to this emerging technology. CEA is both a framework and a process for holistically and systematically evaluating environmental trade-offs of technology choices (1). It includes a product life cycle perspective but goes beyond typical life cycle assessments by also considering environmental fate and transport processes, exposure-dose, and ecological, human health, and other potential impacts, both indirect and direct, in qualitative as well as quantitative terms. An essential feature of CEA is the use of a formal decision science process to evaluate complex and sometimes conflicting evidence. This process requires a "critical mass" of diverse technical expertise and stakeholder perspectives, and the resulting assessment may thus identify potential adverse effects not brought out in other types of assessments that rely on a narrower group of risk assessors.

EPA has applied the CEA framework in developing case studies of selected nanomaterials to identify and prioritize research directions that would support future assessments of nanomaterials. Thus far, EPA has developed case studies of nanoscale titanium dioxide (nano-TiO2) used for drinking water treatment and for topical sunscreen (2) and nanoscale silver (nano-Ag) used for disinfectant spray (3). These case studies have served as the starting points for the CEA process, which used structured workshops with participants selected to achieve a balanced distribution across sectors (academic, government, industry, non-governmental and other organizations) and technical disciplines (e.g., materials characterization, product life cycle, environmental fate and transport, exposure-dose, ecological effects, human health, risk assessment/management/policy).

A series of case study workshops is planned, two of which have been held thus far: in September 2009 focusing on the nano-TiO2 case studies, and in January 2011 focusing on the nano-Ag case study. In these meetings a formal method called Nominal Group Technique (NGT) was used to structure the process of identifying and prioritizing information gaps (4). Broadly stated, the top priority issues have pertained to physicochemical characterization, detection and measurement of ecological and human health effects, and exposure assessment. More detailed statements of information gaps reflect varying levels of specificity: some refer to particular applications of a specific nanomaterial; others refer to specific nanomaterials without regard to application; and still others refer to nanomaterials more generally.

The results of these workshops are being used by EPA as part of an ongoing effort to refine a long-term research strategy to support the comprehensive environmental assessment of nanomaterials. In addition, the decision support aspects of CEA are being explored for use in near-term risk management decision-making.

Disclaimer

The views expressed in this abstract are those of the authors and do not necessarily represent policies of the U.S. EPA.

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Informing selection of nanomaterial concentrations for ToxCast *in vitro* testing based on occupational exposure potential

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Oral Presentation
4:20 PM, Monday

Currently, little justification is provided for nanomaterial testing concentrations in *in vitro* assays. The *in vitro* concentrations typically used may be higher than those experienced by exposed humans. Selection of concentration levels for hazard evaluation based on real-world exposure scenarios is desirable. Nanomaterial concentrations in air (particle mass or count per air volume) are being measured in manufacturing and R&D lab settings. We reviewed nanomaterial levels reported across facility types for different nanomaterial classes. Using particle number concentration data from these studies, we calculated nanomaterial mass retained in the trachobronchial and alveolar regions of the human lung using the open-source Multiple-Path Particle Dosimetry (MPPD) model. These estimates of inhalation dosimetry were performed for carbon nanotubes (CNTs), titanium dioxide (TiO2) and silver (Ag) nanoparticles. The key model input parameters that affect the alveolar mass retained after 24 hours of nanoparticle exposure were particle size and size geometric standard deviation, aspect ratio, breathing conditions (resting, light or heavy exercise), and aerosol concentration. These key parameters were varied to further calculate alveolar mass retained per alveolar surface area (μg/cm2) for different particle sizes (ranging from 5 to 100 nm), aerosol concentrations (0.1 and 1 mg/m3), and exposure times (24 hours and a full working lifetime of 45 years at 8 hours per day, 5 days per week of aerosol inhalation). The alveolar mass retained per surface area for Ag and TiO2 nanoparticles and CNTs for *a full working lifetime* exposure duration was similar to the high-end concentrations (~ 30-400 μg/mL) typical of *in vitro* testing. Analyses performed and results obtained here are generally applicable to inform the design of nanomaterial *in vitro* hazard screening studies. *This abstract may not necessarily reflect U.S. EPA policy*.

Aerosol emissions from nanotechnology-related consumer products

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Oral Presentation
4:40 PM, Monday

It is well established that inhalation of nanoparticles is associated with adverse health effects [1-3], yet the introduction of new products that have the potential to aerosolize engineered nanoparticles is proceeding swiftly. Normal use of several types of nanotechnology-related consumer products, such as sprays, mists, humidifiers, and hairdryers, may to lead to inhalation exposure of engineered nanoparticles, but the amount and size of the aerosolized particles—important for assessing risk—is unknown. The objective of this work is to characterize the emissions of airborne particles from consumer products that claim to contain silver nanoparticles or ions.

Three spray products were chosen for this study based on their claim of containing elemental silver and potential for generating aerosols during normal use: an anti-odor spray for hunters, a surface disinfecting spray, and a throat spray. Two different bottles of the "hunter spray" were tested individually for aerosol emissions.

Products were sprayed from their own bottles into a 0.52-m^3 polyethylene chamber that was initially filled with filtered, particle-free air at low relative humidity. We then used a scanning mobility particle sizer and an optical particle counter to measure the aerosol concentrations and size distributions, and we collected aerosol samples on filters using a 4-stage cascade impactor. We extracted silver from the filters and quantified it by inductively coupled plasma mass spectrometry. We also collected aerosol samples onto TEM grids using a thermophoretic precipitator built by the authors for this study.

The chamber was modeled as a continuously-stirred tank reactor (CSTR) for which particle wall losses were quantified. Using the CSTR model and size-resolved particle concentrations, we developed emission factors describing the total amount of particles emitted per spray action of each product (Figure 1a).. Using the size-resolved silver concentrations, we also calculated mass emission factors of silver per spray action. (Figure 1b).

The disinfecting spray and the first bottle of hunter spray produced bi-modal particle size distributions, peaking in the nucleation size range (around 20 nm) and then again in the accumulation mode (around 500 nm). The second bottle of hunter spray and the throat spray presented similar aerosol size distributions, with most particles in the accumulation and coarse (>1000 nm) modes. Emissions of silver (Figure 1b) varied greatly among the products. Even though all products had similar total silver concentrations in liquid form (20 – 30 ppm), the amount aerosolized was much greater with the throat spray compared to the other products. Based on size-dependent deposition efficiencies of particles in the respiratory system, these results indicate that airborne silver generated by the products tested will deposit in the nasopharyngeal region (>500 nm) and the alveolar region (100–500 nm) if inhaled.

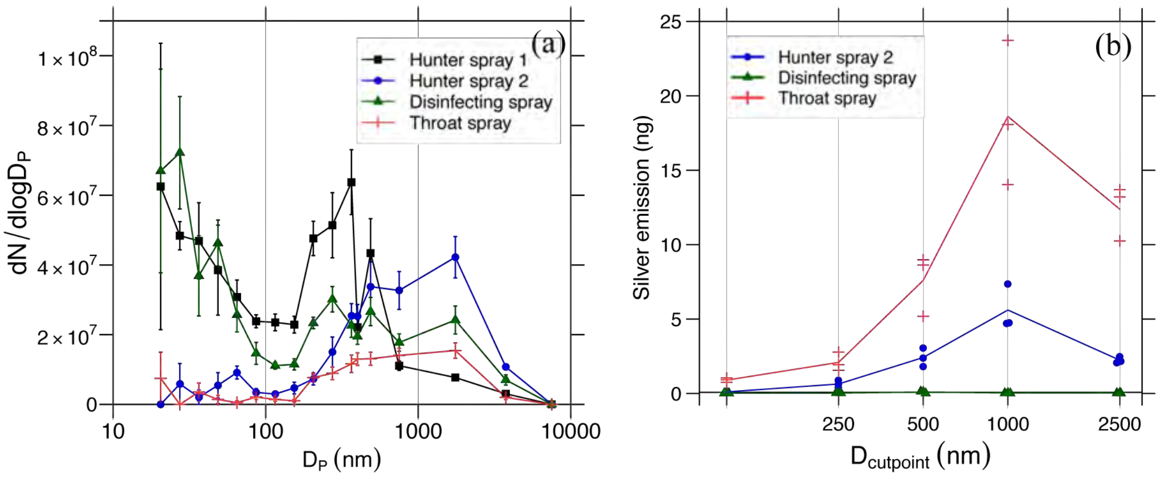

Figure 1: Size-resolved aerosol emission rates (a) and silver mass emission rate (b), per spray action.

Single-particle chemical and morphological properties are being determined through scanning and transmission electron microscopy with energy dispersive spectroscopy. Results can be used to guide the selection of relevant particle doses in nanotoxicity testing, to predict exposure to nanoproduct emissions in indoor air quality models, and to develop regulations to ensure consumer safety.

Acknowledgments

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Ecological risk forecasting of nanomaterials using Bayesian networks: A case study of nano-Ag exposure potential in a North Carolina river basin

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Oral Presentation
5:00 PM, Monday

Assessing the behavior and potential risks of nanomaterials in an environmental setting is a complex task. Particle characteristics and environmental conditions can directly impact particle behavior and the subsequent exposure and hazard in environmental systems. There is also a great deal of scientific uncertainty surrounding these mechanisms. Despite this uncertainty, there is still a need to create an informative model of potential nanomaterial risks (1).

Ecological risk characterization for nanomaterials (or any substance) requires knowledge about the potential exposure and resulting hazard once the material enters the environment. Typically, the riskiness of a particular substance is expressed in terms of a deterministic ratio between the expected concentration in the environment (exposure) and the lowest concentration that causes an adverse effect (the hazard) on the most sensitive species of a particular group of organisms. The exposure and hazard are determined from field measurements, estimations, and toxicological studies.

One of the issues with a deterministic approach to risk is the lack of knowledge about the uncertainty present. One way to deal with this uncertinty is to use a probabilistic approach to risk assessment and risk characterization that we have termed risk forecasting. In this sense, Bayesian networks provide a robust tool containing several features that make them an ideal approach for assessing the environmental risks of nanomaterials. Bayesian networks provide both a conceptual and mathematical framework that can integrate multiple knowledge bases, explicitly account for uncertainty, adapt to changes in knowledge, and mathematically update model outcomes as new data become available (2).

This presentation uses the exposure component of the newly developed FINE (Forecasting the Impacts of Nanomaterials in the Environment) model to illustrate the use of Bayesian networks for ecological risk forecasting of nanomaterials (3; Fig. 1). FINE is a Bayesian network that incorporates nanoparticle characteristics, environmental conditions, exposure, hazard and risk into a single analysis framework. $\text{FINE}_{\text{Nano-Ag}}$ has been built to characterize Nano-Ag risk in an aquatic environment.

The case study focuses on the Neuse River Basin in eastern North Carolina. Concentrations of nano-Ag in wastewater effluent were estimated for major wastewater treatment plants within the basin. These concentrations were then used as input into the FINE model to predict the exposure potential for several scenarios based on existing environmental conditions within the basin. The presentation concludes with additional thoughts on model validation and ongoing research for prioritization and expansion to other nanomaterials.

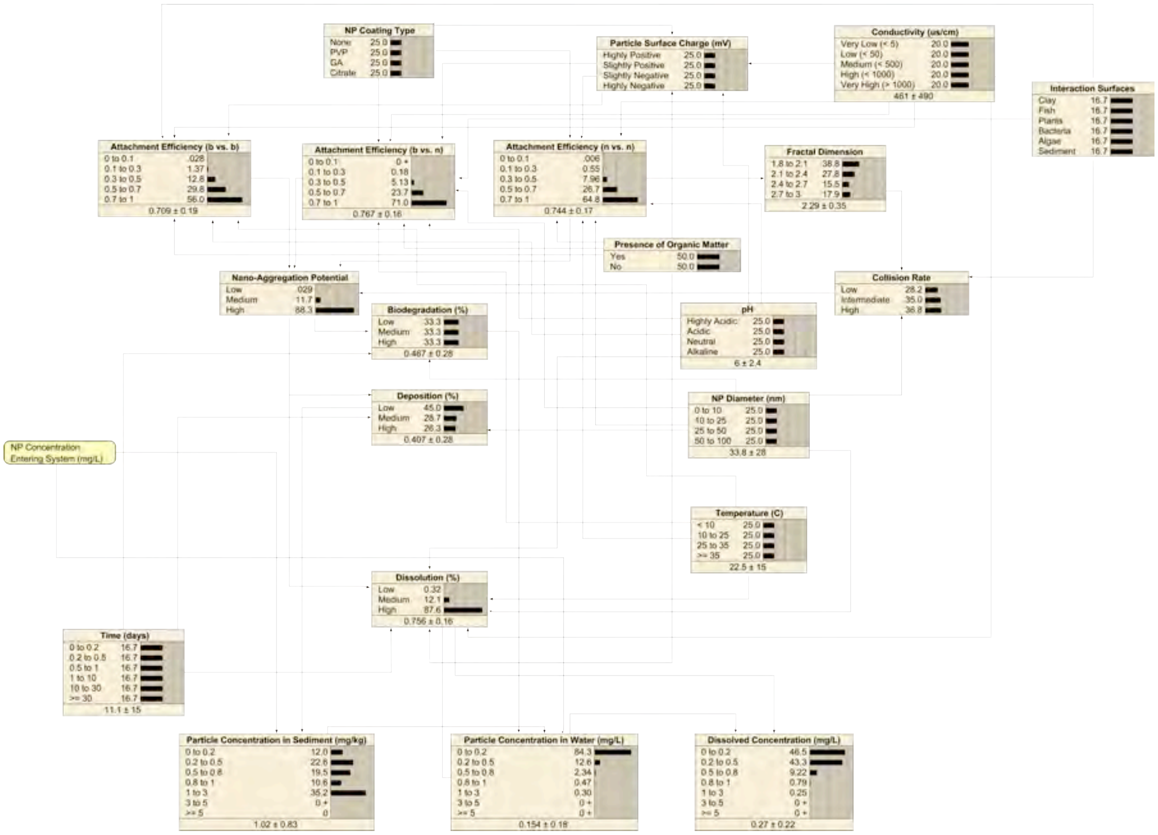

Figure 1: FINE$_{\text{Nano-Ag}}$ Exposure Module. The yellow box indicates incoming nanoparticle concentration from external sources (i.e., WWTP effluent). The bars represent baseline probability distributions for each variable prior to incorporating additional knowledge bases (i.e., data).

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Identification of information rich attributes for nanoparticle toxicological risk assessment

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Oral Presentation
5:20 PM, Monday

Classification and regression tree analysis of published nanoparticle toxicity studies has revealed quantitative rankings of information value of a series of nanoparticle attributes and experimental parameters. This meta-analysis of nanotoxicity experiments provides objective clarification and commentary on proposed minimum characterization standards for nanomaterials by identifying different attributes as being information-rich, or information-poor. These results address some debates regarding proper experiment design and questions over the relative importance of specific measures of geometry, dose, chemistry, and exposure methods. While the limitations of the current data set preclude definitive attribute categorization, this research demonstrates the importance to future nanoparticle toxicity studies of characterizing the small set of nanoparticle attributes associated with high information value and those displaying significant sensitivity of information value to measurement frequency.

A set of data from *in vivo* and cellular toxicity experiments were assembled and analyzed with the use of classification and regression trees (CART) (1). CART is a machine learning algorithm that creates a hierarchal series of divisions of outcome observations based on the input attribute with the greatest information gain. In this analysis, input attributes include physical and chemical nanoparticle properties and experimental parameters affecting exposure. Outcome observations include quantitative measures of toxicity such as broncoaveolar lavage (BAL) cell counts and lactate dehydrogenase (LDH) concentrations as an indicator of cell membrane damage in cellular assays. A total of 13 toxicity outcomes were included in the analysis. Published studies were selected for inclusion based on nanoparticle characterization measures conducted and on having quantitative toxicity outcomes in common with at least one other study.

An example regression tree for BAL total cell count (one toxicity outcome for one particle type and exposure route) is shown below in Figure 1. The regression trees outperform linear regression models without over-fitting (as evaluated through cross-validation) allowing better resolution in differentiating between the value of competing variables. The overall importance or information gain contained within each measured nanoparticle attribute and experimental condition was evaluated by creating a series of regression trees randomly including sub-sets of the total group of input variables, known as a random forest predictor. The ten most important attributes for BAL total cell count following exposure to

CNTs are displayed in Figure 2. These analyses repeated for the full set of toxicity outcomes and different particle types reveal important consistencies among the experimental results even in the face of inconsistent nanoparticle characterization procedures among the studies.

Commonality among the important variables associated with different toxicity indicators provides confirmation in the value of those characterization measures, while those characteristics not displaying importance even when well documented will have limited value for the development of toxicity risk models in the future.

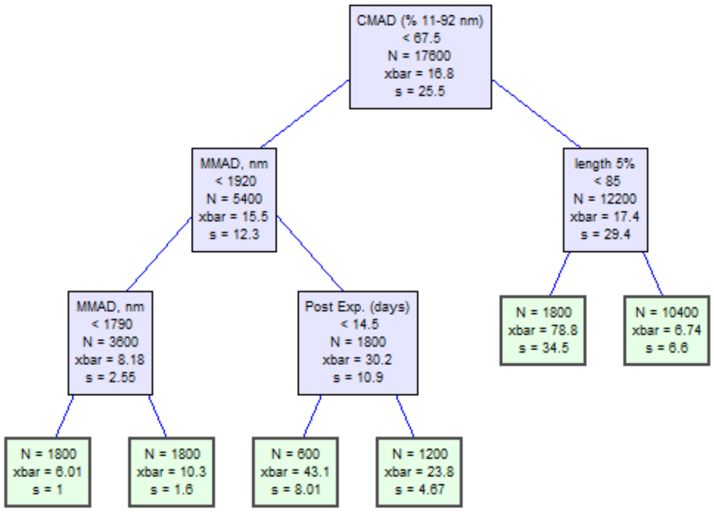

Figure 1: Regression tree for BAL total cell count (number of cells in millions) following pulmonary exposure to carbon nanotubes in rats displaying dependence on small particle fraction (CMAD % 11-92 nm), aggregate diameter (MMAD, nm), carbon nanotube length (length 5th%), and post-exposure recovery period (post. exp., days)

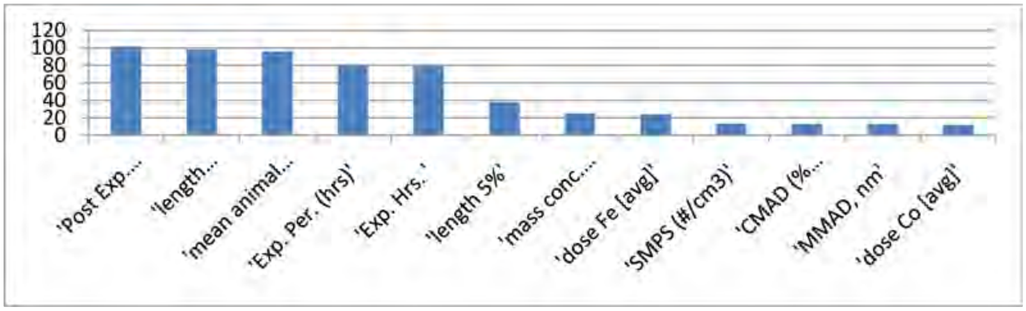

Figure 2: Total information gain (variable importance) associated with BAL total cell count following pulmonary exposure to carbon nanotubes in rats and mice.

Acknowledgements

This material is based upon work supported by the National Science Foundation (NSF) and the Environmental Protection Agency (EPA) under NSF Cooperative Agreement EF-0830093, Center for the Environmental Implications of NanoTechnology (CEINT).

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SESSION 2: ECOTOXICITY

Assessing the bioavailability and toxicity of engineered nanoparticles in aquatic environments using enriched stable isotope tracers and biodynamic modeling

Marie-Noële Croteau[1], Samuel N. Luoma

[1] USGS

Keynote Presentation
11:10 AM, Monday

The environmental risk of engineered nanoparticles (NPs) is a current and highly topical focus of concern. Yet, very little is known about the environmental fate, bioavailability and effects of exposure to metal-bearing NPs. Understanding bioavailability is a pre-requisite for predicting bioaccumulation and toxicity of NPs in the aquatic environment. Bioavailability is defined by uptake rates from food and water, as well as loss and detoxification rates from an organism. Knowledge of those rates for a species allows comparisons of NPs formulations and modeling of potential for bioaccumulation at environmentally realistic concentrations. Quantifying the rates is possible in relatively simple experiments that require a tracer. However, research is hampered by lack of reliable tools to detect, visualize and trace NPs movement in complex environmental and biological systems. Reliable tracing is of a particular concern for NPs comprising of metals such as Zn which is essential to organisms but also a common pollutant (elevated background concentrations). We previously showed that labeling of particles with an enriched stable isotope appears as an effective way to tackle this problem. Metal-bearing NPs can be synthesized with unique stable isotope ratios and employed in biodynamics experiments. Here we present results for Ag NPs with different caps, ZnO NPs and Ni NPs in an aluminium oxide matrix, and show that the biology of the organism, the characteristics of the nanoparticle and the interaction of the nanoparticle in the environment all combine to affect bioavailability and resultant toxicity, sometimes in unanticipated ways.

Short-term fate and toxicity of silver nanoparticles in wetland mesocosms

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Oral Presentation
2:00 PM, Monday

Silver nanoparticles (AgNPs) have been shown to be toxic to bacteria, fungi, plants, vertebrates, and invertebrates in laboratory studies. There is thus concern among environmental scientists about potential AgNP impacts on natural systems, yet to date there have been no field experiments to examine ecosystem scale impacts. Here we report results from the first thirty days of an ongoing fully-replicated field mesocosm experiment in which we dosed large (1.2x1.2x3.7m) wetland mesocosms with two different types of AgNPs (6nm gum arabic coated nanoparticles, GA-AgNPs, and 40nm polyvinylpyrrolidone coated particles, PVP-AgNPs; 3 reps each). A suite of ecological responses were compared between these treatments relative to negative controls (deionized water addition; 4 reps), coating controls (to assess GA or PVP affects; 3 reps) and positive controls (equal mass of Ag as $AgNO_3$; 3 reps). For all treatments, dosing solutions were added to the 350L aquatic portion of each mesocosm to achieve a final concentration of 2.5mg L^{-1} Ag.

Concentrations of Ag dropped three orders of magnitude over the course of the first 7 days for all silver treatments. The dominant fate of silver was in the soil/sediment ecosystem compartment where silver was transformed from Ag^+ (in AgNO3 treatments) or Ag^0 (in AgNP treatments) to Ag_2S or Ag-thiol, suggesting that sulfide and thiol groups are a strong sink for silver.

The aquatic plants Egeria densa and Potamogeton diversifolius—abundant in the mesocosms at the time of dosing—were immediately affected by exposure to both $AgNO_3$ and GA coated AgNPs. Both forms of Ag addition led to plant dieback within 24 hours and concomitant increases in DOC concentrations from a background of 12 mg C L^{-1} to 34 and 31 mg DOC L^{-1} for the $AgNO_3$ and GA-AgNP treatments, respectively. PVP coated AgNPs led to less dramatic plant dieback and more modest DOC increases to 19 mg DOC L^{-1}.

Watercolumn dissolved oxygen concentrations decreased, with depressed oxygen persisting out to 30 days, despite the fact that plants had begun to recover. Dissolved concentrations of carbon dioxide, methane, and N2O increased dramatically following dosing. Methane in particular rose from an average of 58 to an average of 1800ppm in two days. This methane response was short lived, though, decreasing back to the level of the controls by 8 days for $AgNO_3$ and GA-AgNPs, and by day 20 for the PVP-AgNPs.

Thus the ecosystem response over the first thirty days of this experiment showed an initial phase of acute toxicity to aquatic plants and release of DOC, which was then associated with an increase in microbial activity, decrease in O_2, and increase in trace gas production. As silver concentrations in the watercolumn decreased, the magnitude of these responses decreased, so it may be that the long term impacts will may depend on both the long term fate and bioavailability of silver in sediments, and the long term trajectory of ecosystem recovery to the disturbance of silver addition.

Acknowledgements:
This work would not have been possible without the hard work in the lab and in the field of the following individuals: Brooke Hassett, Medora Burke-Scoll, Rich Di Giulio, Cole Matson, Audrey Bone, Mariah Arnold, Mathieu Thérézien, Shihong Lin, Tammy Stern, Corinne Williams, Sarah Anciaux, Eddie Liu, Katie Harrold, and Christina Arnoaut.

Silver nanoparticle toxicity to Atlantic killifish (*Fundulus heteroclitus*) and zebrafish *(Danio rerio*) in complex environmental media: A comparison of laboratory, mesocosm, and microcosm studies

Audrey Bone, Cole Matson, Ben Colman, Richard Di Giulio

Oral Presentation
2:20 PM, Monday

Nicholas School of the Environment and Center for the Environmental Implications of NanoTechnology, Duke University, Durham, NCThe increasing use of silver nanoparticles (AgNPs) in consumer products and industrial applications has raised concern over their inevitable release into the aquatic environment. In this study, the effect of complex environmental media on AgNP toxicity was investigated in mesocosms using silver nitrate ($AgNO_3$), and AgNPs coated with gum arabic (Ag-GA NP) and polyvinylpyrollidone (Ag-PVP NP). Water samples were taken from the mesocosms 24 h after dosing and compared to lab-incubated samples prepared by spiking control mesocosm water with Ag-GA NP, Ag-PVP NP and $AgNO_3$, at the same concentrations observed in the mesocosms at 24 h. Samples were compared using acute toxicity testing on early life stage Atlantic killifish and zebrafish. Differences in toxicity between the lab-incubated water and the mesocosm samples were attributed to the more complex environment created by introducing plants, sediment, and variable environmental conditions (including natural light/UV). In general, lab-incubated samples were more toxic than samples taken from the mesocosms. However, for Ag-PVP NP, mesocosm samples were more toxic than lab-incubated samples, indicating that an environmental factor unique to the mesocosms caused increased toxicity of Ag-PVP NP. In addition, for mesocosm samples, both AgNPs were more toxic than $AgNO_3$, on an equal mass basis. Therefore, the complex media is acting differently on Ag^+ to reduce its toxicity in a manner that does not translate to AgNPs. A follow-up microcosm experiment was used to investigate the role of different sources of dissolved organic material (DOM) on AgNP toxicity. Microcosm experiments using the same particles with four different environmental scenarios indicate that plants were the strongest protective factor and that sediment may have contributed to the increased toxicity of Ag-PVP NP. These results suggest that conventional laboratory testing and equation of AgNPs to Ag^+ on a mass basis may be inadequate for risk assessment.

Phytoplankton as sentinels of nanomaterial toxicity in marine ecosystems

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Oral Presentation
2:40 PM, Monday

Phytoplankton forms the foundation of marine food webs and drives biogeochemical cycles in the oceans through uptake, remineralization, and sequestration of chemical elements. These abundant, small, primary producers have high surface-to-volume ratios, and consequently are capable of rapid nutrient uptake and high growth rates. Such characteristics make phytoplankton likely to be among the first organisms to encounter suspended nanomaterials that are discharged into marine ecosystems, and effects on phytoplankton populations could cascade through marine food webs. Their vulnerability and importance to marine ecosystems thus make phytoplankton ideal sentinels for experiments measuring effects of nanomaterials on marine organisms. Our work on phytoplankton, one of four major research emphasis of UC CEIN marine nanoecotoxicology, includes three integrated components: determination of population-level effects, high-content screening for potential mechanisms of toxicity, and evaluation of trophic interactions (trophic transfer, bioaccumulation, biotransformation) - with copepods and mussels. Here we present results from each of these component areas.

Biotransformation studies of CeO2 and ZnO NPs in mesquite plants

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Oral Presentation
3:00 PM, Monday

The impact of metal nanoparticles (NPs) on biological systems is still not well understood. The aim of this research was to determine the effects of zinc oxide (ZnO) and cerium oxide (CeO_2) NPs in velvet mesquite (*Prosopis juliflora-velutina*). Seedlings were in hydroponics with ZnO NPs (10 nm) and CeO_2 NPs (10 nm) at concentrations varying from 500 to 4000 mg L^{-1}. Cerium and Zinc concentrations in tissues were determined by ICP-OES ; while biotransformation and elemental distribution were determined by XAS and µXRF, respectively. The ICP-OES data showed that plants exposed to 500 mg ZnO NPs L^{-1} had 3837 ± 161, 1061 ± 70, and 423 ± 77 mg Zn kg^{-1} d wt in roots, stems, and leaves, respectively. While plants treated with 2000 mg CeO_2 NPs L^{-1} had 4504 ± 520, 935 ± 90, and 438 ± 121 mg kg^{-1} d wt in roots, stems, and leaves, respectively. None of the NPs reduced plant growth. XANES spectra demonstrated clear evidence of the presence of CeO_2 NPs within tissues but ZnO NPs were not observed. Furthermore Zn was found as Zn(II), resembling the spectra of $Zn(NO_3)_2$. The µXRF analysis confirmed the presence of Zn in the vascular system of roots and leaves of ZnO NP treated plants.

Modeling the ecotoxicity of nanomaterials through dynamic energy budgets

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Oral Presentation
4:00 PM, Monday

Engineered nanomaterials (ENMs) entering the natural environment may have ecological effects by influencing the fluxes of energy and material that regulate the abundance, distribution, and dynamics of organisms. Empirical data alone cannot provide the understanding to guide policy or management action, so theory is essential. Dynamic Energy Budget (DEB) theory offers a conceptual framework for describing the interplay of physico-chemical processes with the physiological ecology of living organisms. This has been long recognized in ecotoxicology; indeed some of the earliest work on DEB theory was motivated by the recognition of the limited ecological insight that follows from standardized toxicity tests. DEB theory has the potential to model mechanistically the effects of ENMs on single organisms and on populations. The sophistication of the theory that is required varies greatly among applications, and will be illustrated using examples that include: (i) estimation of toxicity parameters for metal oxide effects on phytoplankton; (ii) models of the influence of quantum dots on bacterial population dynamics; (iii) detailed modeling of physiological responses of a marine invertebrate exposed to a metal oxide; and (iv) planned experiments on phytoplankton-zooplankton interactions that require a mix of DEB and other modeling approaches.

High-throughput screening of *E. coli* gene deletion libraries uncovers the mechanistic ecotoxicology of nanomaterials

Angela Ivask[1, 2], E. Suarez[1], Z. Ji, T. Patel[1], D. Boren[1], D. Telesca[1], R. Damoiseaux[1], K. Bradley[1], H. Godwin[1]

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Oral Presentation
4:20 PM, Monday

Nanosized materials have been suspected to induce toxicity in biological systems mainly due to their small size as well as their specific physico-chemical properties. This study presents a development and validation of a high throughput (HT) genome-wide screen that could be applied to study the mechanisms of adverse effects of nanosized materials on *Escherichia coli* cells. We used a selection of >4000 *E.coli* single-gene mutants in HT growth inhibition assay to seek for genes that if absent, result in altered sensitivity towards the nanomaterials. Cationic (amino-functionalized) polystyrene (PS-NH_2) with 62 nm original size that induced toxicity due to both its charge and size, was selected as a model nanomaterial. IC_{50} of the PS-NH_2 62 nm nanospheres was 158 mg L^{-1}, IC_5 was 108 and IC_{90} 190 mg L^{-1}. Individual deletion of 17 non-essential genes resulted in more resistant bacterial phenotype towards PS-NH_2 nanospheres while deletion of 208 genes resulted in increased sensitivity. Most of the genes whose absence increased the resistance were associated with amino acid and carbohydrate transport and metabolism. In contrast, the loss of genes in lipopolysaccharides biosynthesis pathway, the absence of a subset of outer membrane transport channels, deficiency in membrane redox-reactions, stress response and motility resulted in the most sensitive phenotypes towards PS-NH_2 nanomaterial. A database on sensitivity profiles of *E. coli* single gene mutants towards a range of nanomaterials and standard chemical compounds is under development. This database could be used to compare the role of gene deletions in cellular sensitivities to other nanomaterials of interest and by that, elucidate their potential mechanisms of toxic action.

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Effect of bacteria and protozoa on engineered nanomaterials, and vice versa

Tamara McNealy, Tara Raftery, Jason Payne, Sharon Berk

Oral Presentation
4:40 PM, Monday

Microbes will play an important role in what happens to nanomaterials in the environment. Bacterial biofilms may be dramatically affected by nanomaterials settling onto substrates, and protozoa as primary biofilm grazers and filter feeders of planktonic bacteria may have a significant impact on the bioaccumulation and bioconcentration of nanomaterials. We recently demonstrated induced biofilm dispersal events due to exposure of biofilms to gold and platinum nanoparticles. These dispersal events were both size- and concentration-dependent. We showed bacterial uptake and accumulation of gold particles within the bacteria as well as integration of particles within the extracellular polysaccharides. New data show that exposure of biofilms to nanoparticles also leads to altered protozoa grazing and changes in bacteria-amoebae interactions. Protozoa themselves may contribute to accumulation of nanomaterials in the biofilm through the release of fecal pellets containing concentrated nanomaterials, as shown in our experiments with the ciliateTetrahymena feeding on Qdots and nanoGold. Interestingly, certain bacteria stimulate uptake of nanoparticles by the ciliate, resulting in increased numbers of food vacuoles containing nanomaterials and bacteria as well as the release of increased number of fecal pellets into the environment. All of these data suggest that nanomaterials may be 1) concentrated by filter feeding protozoa, 2) disrupting primary biofilms, 3) altering protozoa grazing ability. These three events all have the potential to significantly disrupt ecosystem functioning and health. Additionally, the accumulation of nanomaterials at the source of the food chain will increase the chance for transfer to higher trophic levels.

Understanding the complex response of natural microbial populations to nanomaterial additions

Dana Hunt[1]

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Oral Presentation
5:00 PM, Monday

Despite the growing use of nanomaterials in commercial applications, very little is known about the fate of these nanomaterials once they are released into the environment. Although laboratory studies have shown that nanomaterials can be toxic for certain species of bacteria, it is unclear whether nanomaterials have the same effect on highly divergent bacterial species or natural microbial populations. Here, we use freshwater mescosms to examine the impact of ionic silver and silver nanomaterials (gum arabic coated and PVP-coated silver nanoparticles) on naturally occurring populations of aquatic prokaryotes and eukaryotic phytoplankton. Flow cytometry was used to measure microbial abundance as well as size/spectra of phytoplankton in the silver treated and control systems. Microbial populations exhibited a toxic response in the first several days following silver additions to the mesocosms, exhibiting reduced cell numbers. However over the next several weeks, dramatic blooms occurred, likely in response to nutrient inputs from decaying macroflora and decreased aqueous phase silver. Although they are small, microbes play a key role in primary production and biogeochemical cycling of nutrients, this mesocosm system provides information on the response and recovery of a microbial community following nanomaterial additions.

Acknowledgements:
This project would not have been possible without the efforts of the entire CEINT mesocosm team, but I would like to specifically acknowledge Ben Colman and Christina Arnaout for their help with sampling.

Trophic transfer of Au nanoparticles along a simulated terrestrial food chain

Jason Unrine[1], Aaron Shoults-Wilson[3], Olga Tsyusko[1], Paul Berstch[1, 2]

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Oral Presentation
5:20 PM, Monday

Recent studies have suggested that terrestrial environments will serve as the ultimate sink for several commonly used engineered nanoparticles (NPs), primarily through application of sewage sludge applied as biosolids to agricultural soils (1). Little is known concerning the fate and adverse ecological effects of nanomateirals in terrestrial ecosystems. Our previous studies demonstrate that detritivores (earthworms) accumulate manufactured nanoparticles from soil in their tissues (2, 3) and some nanomaterials, such as Ag NPs, can cause a variety of adverse responses for ecologically relevant endpoints (4-6). We also have shown that herbivorous insect larvae can accumulate higher concentrations of Au NPs in their tissues than the plants upon which they feed (7). In this study, we used Au NPs as a stable probe to determine if nanoparticles in soil can be taken up by detritivores (*Eisenia fetida*) and transmitted to carnivores (*Rana catesbeiana*) in a simulated laboratory food chain. Au NPs were used in order to circumvent difficulties involved in differentiating uptake of intact particles from dissolution products since they are redox stable under ambient conditions. We also tested the hypothesis that Au NPs are more bioaccumulative through trophic exposure than direct exposure by exposing bullfrogs to Au NPs either through the simulated food chain (soil-earthworm-bullfrog) or directly via oral gavage.

We added 12 nm nominal diameter tannic acid coated Au NPs to natural sandy loam (Yager sandy loam, Estill County, KY, USA) which was amended with manure from dairy cows (250 g/kg soil). The manure amended soil served as a culture medium and food source for a mass culture of earthworms (*E.fetida*). A similar culture was established in the same medium but without the addition of Au NPs. We obtained 36 juvenile bullfrogs (*R. catesbeinana*) from a commercial provider (Rana Ranch, Twin Falls, ID, USA). The bullfrogs were maintained in 6 L plastic aquaria containing 2 L of synthetic moderately hard water and a floating sponge to allow them to exit the aqueous medium. The bullfrogs were subjected to three treatments: 1) control earthworms and a control oral gavage (DI water), 2) Au NP dosed earthworms and control oral gavage or 3) Control earthworms and Au NP suspension oral gavage. The bullfrogs were given either oral gavage or a single earthworm on alternating days to avoid regurgitation of the dose. The earthworms were removed from soil and allowed to void their gut contents on moistened paper towels for 24 hours prior to feeding. A sample of the earthworms used for feeding was also analyzed for Au content so that the oral gavage dose on the following day could be adjusted to achieve similar cumulative doses during the course of the study. Following two weeks of exposure, the bullfrogs were sacrificed and the stomach, intestine, spleen, liver, kidneys and leg muscle were removed. These organs along with the homogenized remaining carcass were analyzed for Au content using previously described methods (2). We also continued to analyze earthworms periodically for 60 days and then transferred them to clean soil for an additional 60 days to determine physiological elimination rates.

Gold concentrations in soil were approximately 200 mg/kg. Accumulation of Au NPs in earthworms was characterized by a rapid increase in whole body concentrations for the first 10 days, followed by a rapid decrease in body concentrations over the subsequent 10 days. Whole body concentrations ranged from < 1 ug/g fresh mass on day one to 9.5 ug/g FM at the peak of uptake on day 10. Body concentrations stabilized over the next 40 days. Following transfer to clean soil media, body concentrations did not decrease over a 60 day period indicating slow or nonexistent elimination. Gold was detected in all of the organs of the exposed bullfrogs. The highest concentrations were found in spleen, and they exceeded 2 ug/g dry mass in some samples. The remaining organs had concentrations decreasing in the following order (intestine>kidney>stomach>liver>muscle) and ranged from 24.8 ng/g to 2.4 ug/g. Very little Au was detected in the remaining carcass. Higher stomach and intestine concentrations may be related to particles sorbed to epithelial surfaces in the lumen. For most of the tissues where exterior sorption was not a possibility (liver, kidney, muscle), concentrations of Au NPs were higher for the trophic exposure than the direct exposure. In the spleen, concentrations were not significantly different among treatments. The overall body burdens were reconstructed from organ weights and concentrations. The results demonstrate that, while trophically exposed bullfrogs accumulated 0.04% of the administered dose, directly exposed bullfrogs accumulated only 0.02% of the administered dose. This suggests that nanoparticles are transformed in soil and/or along the food chain in a way that enhances their bioavailability. We did not observe evidence for biomagnification as in our previous study with tobacco horn worms (7). This may relate to differences in life history, life stages that were used, the duration of exposure and physiological differences between the two organisms. Further studies are needed to determine how toxicokinetics of nanoparticles vary among species and are related to their life history traits.

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SESSION 3: FATE, TRANSPORT, & TRANSFORMATION

Engineered nanoparticles in environmental media: How to deal with inherent heterogeneity

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Keynote Presentation
8:30 AM, Tuesday

The appearance and behavior of engineered nanoparticles in the environment determine their distribution, their fate and the exposure of organisms.
This behavior is governed by several well-known parameters as surface potential of the particles, particle size and shape and of course the water chemistry. However a detailed understanding how the inter-connected processes of aggregation, transport, settling and transformation are influenced by these parameters is still missing. From the perspective of risk assessment it would be essential to be able to predict behavior, fate and transformation, but meaningful predictions derived from first principles remain difficult, even in fairly simple systems. For certain engineered nanoparticles like citrate stabilized Gold-NPs for example, which come with a fairly simple surface chemistry, the prediction of reactions may be more straight forward than for others, especially metal-oxide particles. Hence as long as process understanding remains underdeveloped empiric approaches will be necessary. We have addressed this issue by developing a testing approach to generate empiric data for the dispersion stability of nanoparticles in a wide variety of conditions. The testing procedure itself and the comparison of conditions and materials will be presented and discussed with respect to the application to real world conditions including analytical challenges in real surface waters and soils, limitations due to reduced complexity of the set-up, and the problem of heterogeneity and property distributions of the nanoparticles.

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Effect of engineered and natural surface modification of nanoparticles on their interactions with bacteria

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Oral Presentation
9:20 PM, Tuesday

Engineered nanoparticles (NP) are increasingly utilized in industrial processes and manufacturing while their potential interactions with the environment are currently emerging and poorly understood. For example, bare zero-valent iron nanoparticles (NZVI) for are toxic for the gram-negative bacterium Escherichia coli at concentrations as low as a few mg/L. The mechanism of NP toxicity has not been verified, but the inhibition of bacterial growth may be dependent on both reactivity as well as contact with cell membranes. That said, the surfaces of NP used in many applications are modified with polymers prior to usage or may become modified by natural organic matter or reactions in the environment. The objective of this body of work is to examine how surface modifications of NP may affect the interaction between NP and bacteria. Our findings show that both natural and engineered surface coatings on NP greatly reduce or eliminate the cytotoxicity of NP. The coating of NZVI induces electrostatic or electrosteric repulsion between the NP and cell that prevents toxicity by limiting direct contact. In the case of TiO2, the coating acts as a scavenger of reactive oxygen species, rather than a mediator of cell contact and the effect is strongly dependent on the type of coating. Similarly, the coating of AgNP with engineered and natural polymers has little impact on toxicity, but modification of the surface through sufidation decreases toxicity. Surprisingly, TiO2 NP can have the opposite effect on certain growth phases of gram positive bacteria; the particles may rescue them from programmed cell death. Our studies imply that surface modifications may be engineered to minimize the detrimental impacts of nanomaterials and that NP may have a broader impact on bacterial cell growth than previously understood.

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Physico-chemical characteristics and control of bioreactivity and fate

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Oral Presentation
9:40 PM, Tuesday

Nanoparticles (NPs) are being introduced into many applications due to their specific physical or chemical properties that differ from their bulk counterparts. Quantitative methods are required to characterize their important physico-chemical properties, since the existing routine methods developed for coarse particles are not often applicable to NPs. Among such properties, surface energy is a critical property that determines their commercial as well as bio-catalytic effects . We see how cells are damaged by nanopartcles of oxides such as titania and zinc oxide but not ceria NP or coarse titania. We have developed a novel technique to quantitatively evaluate the surface energy of these metal oxide NPs for the first time. Our results allow assessment of size effects of anatase, hematite, ZnO, and CeO_2.The surface energy of nanoparticles is related to their wettability. We also applied *in situ* UV-Vis absorption spectroscopy to resolve the controversies about the electronic properties of hematite (a-Fe_2O_3) nanoparticles (NPs) and, on this basis, to rationalize the unusual dependence of aquatic (bio)chemistry of these NPs on NP size. We discovered that the band gap of hematite decreases from 2.95 to 2.18 eV with increasing NP size from 7 nm to 120 nm. Possible impacts of this effect on (photo)catalytic and biochemical properties of ferric (hydr)oxide NPs will be discussed. The ranking of NPs according to the surface energy and electronic properties gives a new insight into their interactions with their environmental neighbors, e.g. proteins, and cells.

Fate, transport and bioprocessing of CeO2 and ZnO nanoparticles by mussels

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Oral Presentation
11:00 PM, Tuesday

Engineered nanomaterials are continuously and increasingly being introduced in to the environment. Although there is some information about their physicochemical properties, their fate and transport in marine environments is unknown. Considering that mussels are commonly used as sentinel organisms for contaminant monitoring, they were chosen to study the fate and transport of metal oxide nanoparticles. CeO_2 and ZnO ranging from 1 mg L^{-1} to 10 mg · L^{-1} along with feed in seawater were added to filtered sea water for acute exposure, and to 2 mg · L^{-1} ZnO for chronic exposure experiments. *T. pseudonana* cells were exposed to 10 mg · L^{-1} CeO_2 and ZnO nanoparticles in order to determine their interactions. Effects of CeO_2 and ZnO on *M. galloprovincialis and T. pseudonana* were observed from total metal quantification measurements, as well as growth and survival trends. Mussel uptake and excretion of CeO_2 and ZnO showed an increased accumulation of metal in tissues. The results show that total cerium and zinc present in mussel tissues and pseudofeces increased with increasing concentrations of CeO_2 and ZnO in the seawater. Mussels exposed to 10 mg · L^{-1} of CeO_2 and ZnO, accumulated 24.7 and 469 μg · g^{-1} of dry mussel tissue, respectively. However, the total metal found in the pseudofeces showed Ce concentrations of 44,107 μg · g^{-1} and Zn concentrations of 65,026 μg · g^{-1}, respectively. The different concentrations of metals found in tissues show that Zn is more easily processed and accumulated by mussels than Ce, and that the majority of the nanoparticles in the media are excluded and packed in pseudofeces. Moreover, chronic exposure to ZnO nanoparticles caused adverse physiological effects on *M. galloprovincialis* including decreased growth and survival. Our data suggests that differences in nanoparticle composition and physicochemical properties affect their bioaccumulation in mussels, as well as their fate and transport in the marine environment.

Addressing challenges of single-walled carbon nanotube detection in environmental samples

P. Ariette Schierz[1,2], Ashley N. Parks[3], P. Lee Ferguson[1,3]

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Oral Presentation
11:20 PM, Tuesday

Single-walled carbon nanotubes (SWNT) are considered a new class of emerging environmental contaminants. Our recent work and that of others has shown that SWNT have high affinity for natural particulates in aquatic systems and are thus expected to concentrate in sediments after discharge to receiving waters. However, investigations of fate and toxicity of SWNT in the environment are limited by the lack of reliable methods to detect these materials in complex mixtures at environmentally-relevant concentrations.

NIRF spectroscopy has advanced as a highly selective and information-rich technique for sensitive detection and structural characterization of SWNT materials. We have shown that this technique can be used to quantitatively detect SWNT in sediment and biotic matrices at environmentally relevant concentrations and qualitatively characterize SWNT samples before and after the toxicity, bioavailability and fate studies. Sample preparation prior to NIRF analysis consists of surfactant assisted high power ultrasonication. Different purification, concentration, and separation methods are discussed to reduce matrix complexity and improve the detection limit of SWNT. In addition to concentration, structural information such as shape, length distribution or agglomeration state of SWNT must also be identified and quantified to describe behavior and transport processes as well as biological interactions. Furthermore, asymmetric field flow field fractionation AF4 was applied as a separation method prior to NIRF spectroscopic analysis to determine SWNT length distribution and to reduce matrix complexity by separation of NOM and SWNT. Utilizing "time-resolved" FFF-NIRF spectroscopy compared to static NIRF spectroscopy enabled the detection of SWNT in extracts of SWNT-spiked sediment [> 1 ug/g sediment] at lower concentrations. These results reveal FFF can be used successfully as a sample clean-up tool prior to NIRF analysis to reduce matrix complexity by separation of NOM and SWNT.

Detection and analysis of engineered nanoparticles in environmental waters by dark-field based hyperspectral imaging microscopy

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Oral Presentation
11:40 PM, Tuesday

Detection and analysis of engineered nanoparticles in aquatic systems is critical for making assessments related to their impact on human health and the environment. The phenomenon of scattering and absorption of incident light by metalbased nanoparticles (e.g., silver capped with polyvinylpyrrolidone, titanium dioxide, and cerium oxide) was exploited to map and distinguish these particles from natural colloids by utilizing a novel optical imaging technique, which is a combination of enhanced darkfield microscopy and hyperspectral imagery (HSI). This work hopes to address the problem of first identifying the nanoparticles in the environmental waters and obtain estimates before resorting to high-end microscopy and spectroscopy techniques, which are often time consuming and the information obtained would be only from a few square nanometers area of the sample. Whereas with HIS the field of view is 400 square microns and nanoparticle detection at a high spectral resolution (bandwidth 1.5 nm) is achieved, without the need for sample preparation. Two environmental water matrices (freshwater and wastewater) and a control (ultrapure water) containing model commercial nanoparticles viz. PVP-Ag, TiO_2, and CeO_2 were prepared for the purpose of this study. Each of the particles types were added to ultrapure water (DI), freshwater (aka mesocosm water), and wastewater at environmentally relevant concentrations (10 ppm) and mixed well. Aliquots of the samples were deposited on a clean microscopy slide and imaged under dark-field using hyperspectral imaging camera at a high spectral resolution in the visible and near infrared wavelengths (400-1000 nm). A typical hyperspectral image of PVP-Ag (10 nm) is shown in figure 1. Array of colors associated with the particles in figure 1 represents the particle (or aggregate) size heterogeneity in the sample. These images were subsequently analyzed using Environment for Visualizing Images (ENVI 4.1) tools such as spectral angle mapper (SAM) and mixture-tuned matched filtering (MTMF) techniques. Particle abundances in waters containing nanoparticle mixtures were estimated with aid of unique spectral signatures (endmembers) of nanoparticles dispersed in ultrapure water or experimental waters. The application of dark-field based hyperspectral image analysis provides a non-destructive means for precise identification of nanoparticles in environmental waters.

Probing Ag nanoparticle surface oxidation in contact with (in)organics: An x-ray scattering and fluorescence yield approach

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Oral Presentation
2:00 PM, Tuesday

The development of nanotechnologies has led to increasing concerns about the potential risk of manufactured nanoparticles in natural systems. Characterizing the environmental transformations of such nanoparticles (corrosion, dissolution, aggregation…) under relevant natural conditions is crucial for predicting their behavior in ecosystems. Among the various manufactured nanoparticles used in nanotechnology, silver nanoparticles (Ag-Nps) are the most widely used (1) because of their antimicrobial and antifungal properties, and they are being released in significant amounts in natural systems. A major issue, in particular, is the extent to which these nanoparticles release toxic Ag+ due to their transformations and dissolution caused by interactions with aqueous solutions.

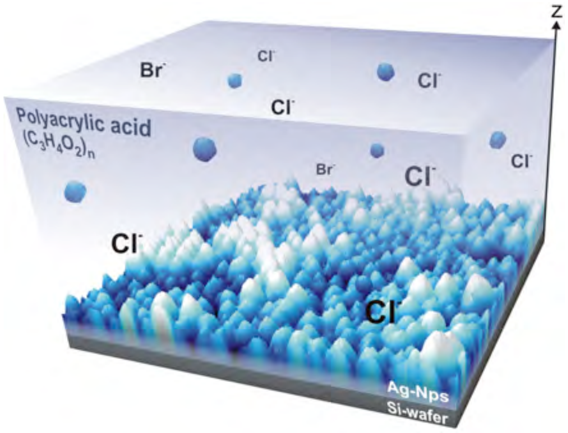

Figure 1. AFM image of PVP-coated Ag nanoparticles deposited in a thin layer on a Si wafer, with a polyacrylic acid film coating the nanoparticle layer. The sample is in contact with water. Chloride ions were present as impurities in the polyacrylic acid.

In this context, we have used a unique approach to study the corrosion processes of polyvynilpyrrolidone (PVP)-coated Ag-Nps (the most common type of manufactured Ag-Nps) in contact with a simplified proxy of natural organic matter (polyacrylic acid – PAA) and an aqueous solution. This system was designed to represent two of the most common components of natural waters) (Figure 1). A fundamental question is whether PVP or other organic coatings act to stabilize the Ag-Np surfaces and prevent their corrosion. Characterization of the reaction products at the interfaces requires powerful tools, with good spatial resolution. The aim of this project is to develop a novel approach to examine corrosion phenomena occurring at the surface of nanoparticles.

Our approach coupled synchrotron-based X-ray scattering (both grazing incidence and x-ray standing wave-generated) and long-period X-ray standing wave fluorescence yield (XSW-FY) spectroscopy to determine phase identity and phase and elemental distribution at the Ag-Nps-PAA/water interface after 24h of reaction under ambient PT conditions. Our grazing-incidence XRD and XSW-based XRD measurements show that although most of the released Ag+ ions are associated with Cl- ions (from the PAA) in the form of crystalline AgCl particles, a significant fraction is present as an amorphous AgCl phase based on the XRD data. Thus, we have shown that a stable AgCl corrosion product is formed despite the presence of potential surface stabilizers (PVP and PAA). This is an important result in the context of Ag-Nps toxicity. In addition, the strong affinity of Cl for released Ag+ ions may limit their toxic effects on organisms.

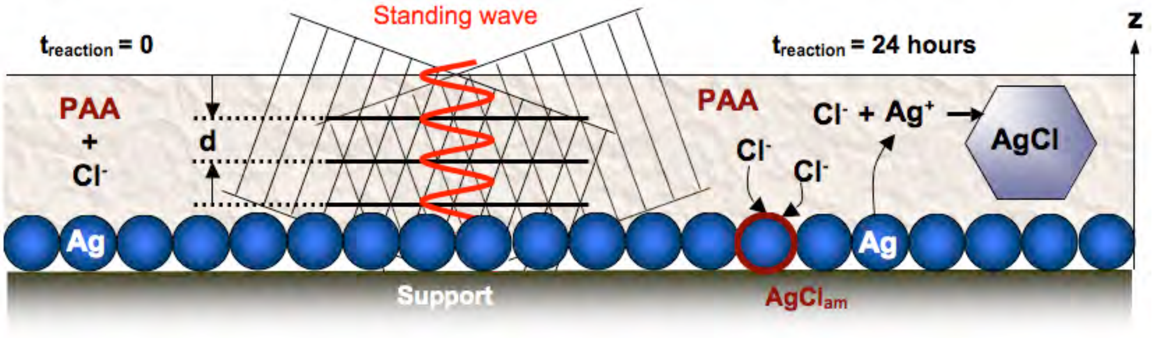

Figure 2. Schematic illustration of the X-ray standing wave phenomenon and the processes occurring at the Ag-NPs/PAA-Cl interface.

The resulting data provide us with a more global picture of the complex processes occurring at the interface between the Ag-Nps and the PAA-containing aqueous solution, including diffusion, dissolution, and precipitation processes with a very good vertical resolution (z axis in Figure 2)

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Sorption of cysteine to silver nanoparticles: Implications for aggregation, dissolution, and silver speciation

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Oral Presentation
2:20 PM, Tuesday

The environmental fate and mobility of coated metallic nanoparticles (NPs) can be strongly influenced by reactions between the coating and natural organic ligands, which may modify particle surfaces and their reactivity during aggregation and dissolution processes. In this work we studied silver (Ag) NPs with two types of coatings: citrate (CIT), which stabilizes the Ag NPs by electrostatic repulsions, and polyvinylpyrollidone (PVP), which causes electrosteric stabilization. We studied the interactions between the nanoparticles and cysteine, a low molecular weight analogue for thiol ligands that are expected to bind dissolved silver in the aquatic environment.

Aggregation experiments were performed with CIT- and PVP-coated silver NPs to demonstrate that thiol-containing ligands such as cysteine (rather than hydroxyl-containing ligands) reduced the colloidal stability of the particles by increasing attachment efficiencies during aggregation. The addition of cysteine to Ag NP suspensions also increased the dissolved Ag concentration. These results indicated that cysteine was simultaneously sorbing to the Ag NPs and promoting their dissolution, resulting in surface modifications that increased aggregation rates.

Measurements of silver speciation were performed using Ag L-III edge X-ray absorption near edge spectroscopy. The data showed that the average silver oxidation state increased with exposure time and that silver-cysteine complexes were formed. The extent to which silver was complexed by cysteine depended on the synthetic coating used to manufactured the nanoparticles. PVP-coated Ag nanoparticles were more susceptible to oxidative dissolution relative to CIT-coated NPs. In suspensions of PVP-coated NPs, 20% and 60% of total silver was found to be bound to cysteine after 2 and 24 hours to cysteine, respectively. For CIT-coated nanoparticles, 7% and 55% of the Ag atoms were bound to cysteine during the same time intervals. These results indicate that PVP coatings on the Ag NPs are more labile towards desorption from the NP surface, particular in natural waters that contain Ag+-binding ligands such as cysteine and dissolved organic matter.

Overall, our study highlights the importance of metal-binding organic ligands for modifying the surface of metal nanomaterials in environmental settings. Sorption of natural organics alters the aggregation and solubility of the nanomaterials, and ultimately, will influence their persistence in the environment. We also demonstrate that the synthetic coatings of manufactured nanomaterials are a key component of nanomaterials that influence their surface reactivity towards weathering processes in the environment.

Size controlled dissolution of silver nanoparticles

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Oral Presentation
2:40 PM, Tuesday

The solubility of Ag NPs can affect their toxicity and persistence in the environment. We measured the solubility of silver nanoparticles (Ag NPs) having particle diameters ranging from 4.3 nm to 80 nm that were synthesized using various methods, and with different coatings including citrate, polyvinylpyrrolidone, and gum Arabic. TEM, DLS, and were used to determine Ag NPs size. Synchrotron X-ray Absorption (XAS) and scattering (XRD) techniques were also used to determine particle size and to determine crystal structure information as a function of NP size. Ag NP solubility was size dependent and correlated with TEM size very well as predicted by the modified Kelvin equation. Solubility was relatively unaffected by synthesis method or coating. Based on the Kelvin equation, the surface tension of Ag NPs was ~ 1 J/m^2 which is similar to bulk silver value. The lattice parameters of the fcc crystal structure of Ag NPs did not change with particle size for Ag NPs as small as 6 nm, indicating the absence of strain. These results suggest that Ag NP solubility is affected by curvature for particles less than 50nm in diameter, and that their solubility can be predicted based on TEM particle diameter for particles as small as 6 nm regardless of synthesis method or coating type.

Controlled evaluation of silver nanoparticle dissolution

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Oral Presentation
3:00 PM, Tuesday

Dissolution is a key process in the fate and toxicity of silver nanoparticles (AgNPs) (1). Size, shape, and water chemistry are all expected to play a role in AgNP dissolution; however, polydispersity and nanoparticle aggregation within AgNP suspensions make it difficult to isolate the effects of each of these factors on dissolution. To eliminate the potential confounding effects of nanoparticle aggregation on silver dissolution, we produced silver nanoparticle arrays on a glass substrate using nanosphere lithography (NSL). The arrays produced using this protocol were then characterized using atomic force microscopy (AFM) both before and after treatment with chemicals known to cause nanoparticle dissolution.

Experimental

NSL provides a method whereby uniform nanoparticle arrays of controlled shape and size may be fabricated (2). Carboxylated latex spheres (450 nm mean diameter) that were purchased from Invitrogen (Eugene, OR) were deposited in a hexagonally close-packed monolayer on a glass substrate by restricted meniscus convective self-assembly (3). Metallic silver was thermally evaporated onto the substrate, and then the latex spheres were removed via an ethanol rinse leaving a regular array of AgNPs on the glass substrate.

Two approaches are used to characterize dissolution from the AgNP arrays by AFM. In the first approach, a time-series of tapping-mode AFM height images is captured as solutions of varying chemistries are pumped over the nanoparticle arrays in a fluid cell. This technique allows for changes in morphology of individual nanoparticles to be tracked as the dissolution process proceeds. The other approach is to characterize the AgNP arrays with tapping-mode AFM in air both before and at predetermined time intervals after the arrays have been exposed to different aqueous environments. This high-throughput method allows for statistical analysis of changes in average particle heights. Both techniques will be coupled with inductively coupled plasma mass spectrometry (ICP-MS) for comparison with a macroscopic approach.

Results and Discussion

AgNP arrays produced by NSL for this study have been shown by AFM height analysis to have highly uniform size distributions (e.g., 55.4 ± 2.7 nm). Preliminary experiments using these arrays demonstrate the capacity for AFM to obtain a detailed description of AgNP dissolution under a range of environmentally relevant conditions. Figure 1illustrates the change in the height of an AgNP array soaked overnight in 0.02% nitric acid as measured by AFM. Additional experiments to test the effects of pH, ionic strength, and ligand concentrations (i.e., cysteine, chloride, and bromide) are ongoing. Once studies with unfunctionalized nanoparticles have been completed, the particles will be surface functionalized with citrate, proteins, and polymers (polyethylene glycol) to probe the effects of surface functionalization on nanoparticle dissolution.

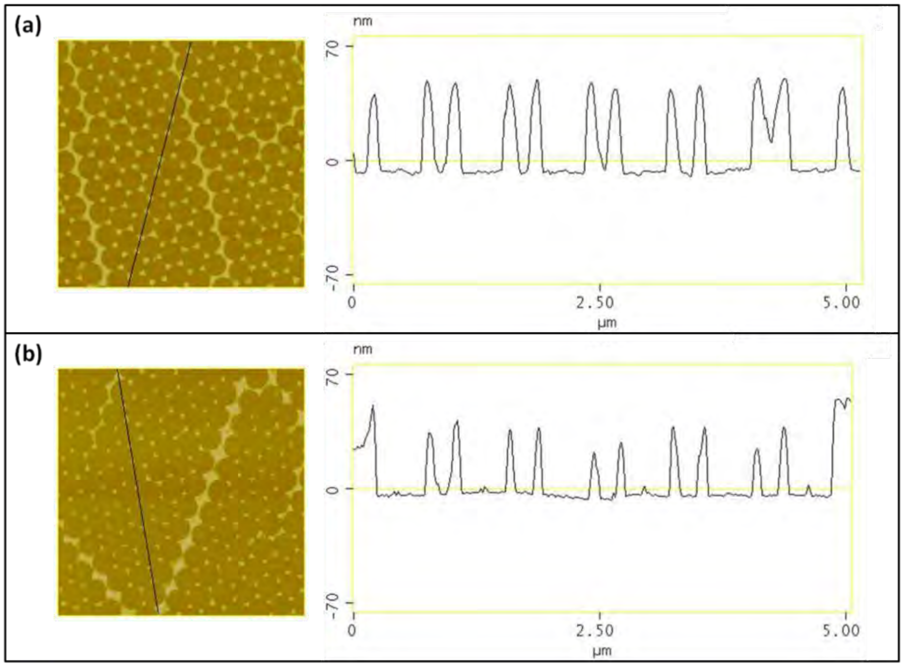

Figure 1: AFM images and cross sections of (a) an unmodified array of silver nanoparticles fabricated by nanosphere lithography and (b) the same array after soaking in 0.02% nitric acid overnight.

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Acknowledgements

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Microbial methylation of mercury sulfide nanoparticles: Influence of particle size and crystallinity on methylation potential

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Oral Presentation
3:20 PM, Tuesday

Monomethylmercury (MeHg) is an extreme neurotoxin that accumulates in food webs and poses a significant risk to human health. The predominant mechanism of MeHg production is microbial methylation of inorganic mercury (Hg) by sulfate-reducing bacteria (SRB) in natural aquatic sediments. MeHg production rates are largely controlled by the availability of Hg species for uptake into SRB, in addition to microbial activity. In sediments, sulfide controls Hg speciation due to its high abundance and strong affinity for Hg. Previous studies have revealed that natural organic matter (NOM) interferes with the precipitation of HgS(s) by preventing aggregation of HgS nanoparticles that are formed during the initial stages of precipitation. As a result of this kinetically-limited reaction, HgS nanoparticles are expected to persist in sediment porewater. We hypothesize that these nanoparticles exhibit higher methylation potential than micro-scale crystalline HgS particles (e.g., metacinnabar) due to the large specific surface areas of nanoparticles. Additionally, recent field studies showed that 'newly' deposited Hg appeared to be more readily transformed to MeHg than the 'older' Hg pool that existed in sediments, which implies a previously unrecognized role of the 'aging' effects in Hg methylation.

Thus, this research investigated how HgS precipitation, a known aging process of Hg, affected MeHg production. We conducted methylation bioassays using pure cultures of two methylating SRB, *Desulfobulbus propionicus* (1pr3) and *Desulfovibrio desulfuricans* (ND132). The bacterial cultures were exposed to different forms of Hg-sulfides, including dissolved $Hg(NO_3)_2$ and Na_2S, NOM-coated HgS nanoparticles (<30 nm), and metacinnabar particles (>1000 nm), which were formulated to represent three different 'aging' states of mercury in sulfidic sediments. Our results showed that methylation rates were greatest with the dissolved Hg-sulfide treatment. In the treatments with HgS nanoparticles, MeHg production was observed at a rate that was significantly faster than the metacinnabar treatment. These results may be due to: 1) the relatively poor crystallnity of HgS nanoparticles compared to bulk minerals that are inherently less soluble; 2) the larger extent of nanoparticle-bacteria surface contact that allows nanoparticulate Hg to be spatially more accessible for the methylating bacteria than micro-scale metacinnabar. Moreover, methylation potential appeared to decrease as HgS nanoparticles 'aged' (16 hrs~1 week), which may be attributed to the enhanced crystallinity of nanoparticles during aging.

The speciation of silver nanoparticles in CEINT mesocosm soils and Kentucky WWTP biosolids using synchrotron X-rays

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Oral Presentation
4:20 PM, Tuesday

It has been predicted through thermodynamics that the dominant form of silver in the environment from the release of engineered silver nanoparticles (AgNPs) will be the low temperature form of Ag2S, named acanthite (1,2). It has also been predicted that the major pathway of release of commercial sources of AgNPs into the environment is from wastewater treatment plant (WWTP) biosolid waste, eventually deposited onto cropland as fertilizer or discarded into a landfill (3). Until now, the speciation of anthropogenic forms of silver aged in environmental conditions at relevant concentrations was not confirmed. The objective of this study was to compare the speciation of engineered AgNPs that had been aged in mesocosm terrestrial soils, mesocosm surficial sediments, and biosolids, from a Kentucky WWTP. Engineered AgNPs were applied to mesocosms and aged for over one year and then the terrestrial soil (above the water level), and surficial sediment soil regimes were sampled for analysis. AgNPs were also aged in a slurry containing biosolids and sampled over the course of two weeks. Synchrotron-based X-ray absorption spectroscopy (XAS) was used to identify and quantify the oxidation products of said ageing. Sulfur-silver species were dominant, but differences between the exposure types resulted. AgNPs aged in mesocosms contained both inorganic and organic sulfur-silver species, whereas the products from biosolid ageing were nearly all Ag_2S. These results have implications for the fate, transport, potential toxicity, and persistence of AgNPs in the environment.

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Racing particles: Start your nanos!

Erica Trump[1]

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Oral Presentation
4:40 PM, Tuesday

Condensation is the dominant physical process controlling the size and composition of sub-micron atmospheric particulate matter. The ability of organic species to condense onto a specific nanoparticle (or population of particles) is dependent on condensation dynamics, thermodynamic mixing (Raoult's Law), and the volatility of the condensing organic species relative to the organics in the background atmosphere. In this way, nanoparticle growth is a more nuanced problem than bulk organic condensation.

We develop a dynamic mass-balance model to treat the role of organic composition (volatility) in driving the net condensation of semi-volatile organics onto particles suspended in the atmosphere. We consider the condensational growth rate of a synthetic nanoparticle and describe sets of atmospheric conditions that lead to vastly different nanoparticle growth rates using two illustrative cases.

In the first case, vapors added to the atmosphere have a vapor pressure higher than the vapor pressure of the background organics. Organic growth is extremely limited because the background organics serve to suppress the partial pressure of the added organics. In the second case, atmospheric organics undergo vapor-phase oxidation. These oxidized vapors have a lower vapor pressure than the background organics and will condense onto nanoparticles. The system maintains equilibrium by forcing additional condensation from the background particles, causing dramatically larger nanoparticle growth.

Transport studies of nano-titanium dioxide in porous medium

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Oral Presentation
5:00 PM, Tuesday

Column studies were performed in order to determine the attachment and transport characteristics of of titania nanoparticles, *nano*TiO2 through iron oxide-coated (IOC) silica beads under different conditions of pH and ionic strength.The *nano*TiO_2 was dispersed by sonication in an aqueous medium, and the average particle size and zeta potential were measured at various pH values using a DLS zetasizer. The sizes of the unsonicated nanoparticles in aqueous media ranged from 300-500 nm, whereas after sonication the range decreases to 150-200 nm. The isoelectric point (IEP) of *nano*TiO_2 was 5.5.

Column studies were conducted to elucidate the sorption behavior of *nano*TiO_2 onto IOC beads as a function of pH (4, 5.5 and 8.3), ionic strength (0.001, 0.01, 0.03 M), adsorbent (bead) characteristics, and adsorbate (nanoTiO2) feed concentrations. The nanoTiO2 feed concentrations were chosen to be environmentally relevant and ranged from 100 to 500ppb. At a solution pH of 4, the affinity of the *nano*TiO_2 for the IOC-silica beads was the highest, whereas affinity for the IOC decreased with higher pH values, as the surface charge of both materials became increasingly negative, as expected from DLVO theory. At pH 4 and 5.5, the attachment efficiencies increased with increasing ionic strength, whereas, as pH 4, the attachment efficiency did not change significantly with increasing ionic strength. In slightly acidic soils it can be expected that *nano*TiO_2 will not travel as far, whereas *nano*TiO_2 is expected to be highly mobile at pH 8.3, the pH of many aquatic environments.

Exact Analytical Expressions for the Potential of Electrical Double Layer Interactions for a Sphere Plate System

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Oral Presentation
5:20 PM, Tuesday

Exact, closed-form analytical expressions are presented for evaluating the potential energy of electrical double layer (EDL) interactions between a sphere and an infinite flat plate for three different types of interactions: constant potential, constant charge, and an intermediate case as given by the linear superposition approximation (LSA). By taking advantage of the simpler sphere−plate geometry, simplifying assumptions used in the original Derjaguin approximation (DA) for sphere−sphere interaction are avoided, yielding expressions that are more accurate and applicable over the full range of κa. These analytical expressions are significant improvements over the existing equations in the literature that are valid only for large κa because the new equations facilitate the modeling of EDL interactions between nanoscale particles and surfaces over a wide range of ionic strength. In addition, with the improved expression, we are able to demonstrate theoretically that for nano-sized particles the particle stability would reach a maximum instead of monotonically increasing when the ionic strength is increased.

Carboxymethyl cellulose-modified zero-valent iron nanoparticle transport through sand packed columns

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Oral Presentation
3:40 PM, Tuesday

In-situ remediation of chlorinated solvent-contaminated aquifers using reactive iron nanoparticles is an emerging technology. Several studies have assessed the mobility of various surface-modified iron nanoparticles in model aquifer systems. The mechanisms contributing to the retention of polymer-stabilized NZVI in granular porous media are however not well understood.

In this study, transport and deposition of carboxymethyl cellulose-modified zero-valent iron nanoparticles (CMC-NZVI) are investigated in laboratory-scale sand packed columns using four different sands of mean diameter of 775, 510, 250 and 150 μm and three different influent concentrations of 0.085, 0.035 and 1.80 gL^{-1}. The CMC-NZVI mass retention along the column length was also measured.

Results will be presented that show that CMC-NZVI effluent concentrations decreased with the mean sand grain diameter in columns packed with sands of mean diameter ranging from 775 to 250 μm. Sectioning of the sand packed columns demonstrated unusually high CMC-NZVI mass retention near the inlet. The experimental data were fitted to colloid transport equations accounting for: (1) particle deposition on the sand collector surfaces only, or (2) straining between sand grains in addition to particle deposition. The trends in the experimental data as well as the fit to the transport equations suggest that in addition to deposition on collector surfaces, straining is a dominant particle retention mechanism for a range of CMC-NZVI diameter to sand grain size ratios.

SESSION 4: TOXICITY

High-content/high-throughput zebrafish assays to define nanoparticle bioactivity

Robert Tanguay[1,2], Lisa Truong[1,2], Tatiana Zaikova[2,3], Jim Hutchison[2,3]

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Keynote Presentation
8:30 AM, Tuesday

The rapid rate of discovery and development in the nanotechnology field will undoubtedly increase both human and environmental exposures to engineered nanoparticles. Whether these exposures pose a significant risk remains uncertain. Despite recent collective progress there remains a gap in our understanding of the nanomaterials physiochemical properties that drive or dictate biological responses. The development and implementation of rapid relevant and efficient testing strategies to assess these emerging materials prior to large-scale exposures could help advance this exciting field. The embryonic zebrafish assay represents a dynamic *in vivo* zebrafish platform to define the biological responses to nanoparticle exposures. Early developmental life stages are often uniquely sensitive to environmental insults, 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 nanomaterials perturb normal biological pathways. Through automation and rapid throughput approaches a systematic and iterative strategy has been deployed to help elucidate the nanomaterials properties that drive biological responses.

Use of *in vivo* zebrafish model for nanoparticle toxicity screening

Tian Xia[1], Saji George[1], Sijie Lin[1], David Schoenfeld[2], Yan Zhao[2], Ivy Ji[3], Jeffrey Zink[3,4], Shuo Lin[2], Andre Nel[2,3,5,6],

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Oral Presentation
9:20 AM, Tuesday

To understand the toxic potential of the increasing number of nanomaterials (NMs), we have developed a multi-parametric rapid throughput screening assay for quick assessment of the toxicity of a large number of NMs by *in vitro* assays using mammalian cells. While providing valuable information, the toxicity profiles of NMs obtained *in vitro* have to be checked *in vivo* in order to show their predictive value. However, *in vivo* animal experiments using rodents are expensive and time consuming, so there is an urgent need to develop an alternative *in vivo* model that can be used to rapidly test the results obtained from *in vitro* cellular assays. We determined an *in vivo* zebrafish model can be used for toxicity screening of nanomaterials. After screening of the toxicity of metal, metal oxide, and quantum dot nanoparticles (Ag, Au, Pt, Al_2O_3, Fe_3O_4, SiO_2, QD, TiO_2, CeO_2, ZnO and Fe-doped ZnO (1-10% Fe-doping)) *in vitro*, we tested their toxicity to zebrafish embryos by determining the toxicity endpoints in embryonic and larval development of fish, such as hatching rate, mortality rate, heart rate, and abnormal morphology. For ZnO, we found that it could inhibit hatching of zebrafish embryos; Fe-doping decreased the dissolution rate of ZnO and reversed ZnO-induced hatching inhibition. ZnO, Ag, Pt, and QD demonstrated substantial toxicity to zebrafish embryos while NMs in the rest of the group are non-toxic. These *in vivo* zebrafish results largely agree with the nanoparticle toxicity profiles we obtained from *in vitro* rapid throughput toxicity screening except that Ag did not show lethal effects *in vitro*. In conclusion, zebrafish is a good *in vivo* model that can be correlated to *in vitro* toxicity screening; evaluation of *in vitro* cellular data and *in vivo* zebrafish data can provide more accurate toxicity ranking of NMs and help to prioritize *in vivo* assays in rodents, which is necessary to build predictive nanotoxicology.

Effects of silver nanoparticles on early life stages of Japanese medaka: Inferences of different coating materials

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Oral Presentation
9:40 AM, Tuesday

Silver nanoparticles (AgNPs), among the current engineered nanomaterials, are widely used due to their antibacterial properties. Almost all AgNPs are coated with organic materials to enhance dispersion in water, but the coatings' effects on the subsequent fate and toxicity of the NPs are not well understood. Acute studies with medaka eleutheroembryos using 11 AgNPs and chronic studies using 4 AgNPs of different sizes and coating materials (e.g. citrate, gum arabic (GA), polyvinylpyrrolidone (PVP)) showed significantly different toxicity between the NPs of different coatings. GA coated AgNPs were consistently the most toxic while PVP coated NPs from different sources had significantly different toxicity. Such differences in toxicity were not fully explained by size, dissolution or rate of NP aggregation in test media. While all AgNPs were 3-10 times less toxic than dissolved silver (mass-based concentration). Toxicity of silver NPs was not fully explained by concentration of dissolved silver in NP suspensions, suggesting the presence of AgNP-specific toxicity. Further evidence for NP-specific toxicity was observed in increased incidences of spinal malformation of exposed medaka compared to dissolved silver treatment. Using hyperspectral imaging with dark field microscopy (Cytoviva) on 5-6 µm paraffin sections of exposed fish, we observed AgNP aggregates in internal tissues of fish with distribution mainly in regions of the gills, gut lumen and in other organs including liver (small amounts). Our results overall suggested that there were coating specific interaction between the NP and medaka. These results may be useful for the future design of NPs and their coatings to minimize risk to aquatic life.

Correlating silver nanoparticle functionalization to generation of reactive oxygen species and silver ion release rates for disinfection applications

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Oral Presentation
11:00 AM, Tuesday

Silver nanoparticles, a common antimicrobial agent, are extensively utilized in medical applications, consumer products and water filtration. Given their widespread use and toxic effect on microorganisms, their environmental risk and impact must be established in order to create effective regulations for their safe and effective use. Prior work has suggested that both silver ions and reactive oxygen species (ROS) play a role in the disinfection mechanism of the particles.(1-4) We have investigated the relationship of particle functionalization on the silver ion release rate, the generation of reactive oxygen species and its disinfection of Escherichia coli in the context of drinking water filtration. Three types of nanoparticles were used in this study, citrate capped, starch capped and silver proteinate functionalized particles. 20 nm diameter citrate capped particles were synthesized and capped using sodium citrate. 30 nm starch-capped particles were synthesized by our collaborators at Rhode Island University and coated with maltose. Commercially available silver proteinate nanoparticles of ~15 nm diameter were purchased from Laboratorios Argenol s.l. Particle sizes and distributions were measured using transmission electron microscopy (TEM) and dynamic light scattering (DLS) methods. All experiments were carried out in temperature controlled vessels in low light to dark conditions. A silver ion specific probe was used to measure silver ion release rates. Preliminary results suggest a release rate of ranging 0.02 - 13 μmol / m^2*hr depending on the particle functionalization and total silver concentration. Silver proteinate particles displayed the highest ion release rate. Fluorescent confocal microscopy on ROS sensitive Dihydrorhodamine 6G dye was used to measure the generation rate of reactive oxygen species. Experimental results suggest that ROS generation ranges from 0.01 - 400 μmol / m^2*hr depending on the particle functionalization and concentration, as seen in Figure 1. ROS generation was found to be directly proportional to available surface area and to vary significantly with different capping agents. Citrate and starch coated particles display a higher ROS generation rate when compared to the proteinate stabilized nanoparticles. The similar trend of ROS generation with increasing concentration of total silver for the various functionalizations suggests a similar mechanism for ROS generation. While the generation of ROS exceeds the production of Ag ions, their lifetime within an aqueous environment is significantly shorter, making their toxicity difficult to directly assess. As a result, ROS is believed to be localized around the surface of the nanoparticle while Ag ions are free to diffuse into the bulk environment. Release rates of both species are correlated to microbial disinfection rates using live/dead cell assay. Initial disinfection results suggest that overall disinfection of the silver nanoparticles cannot be attributed solely to silver ion release. Disinfection is inhibited by the addition of higher salt content, since it results in particle aggregation. Disinfection is also inhibited by the presence of natural organic matter, which does not cause particle aggregation, but likely scavenges ROS and Ag+ species. Future work will focus on quantifying Ag ion release and ROS generation for various functionalized particle coatings on ceramic filters.

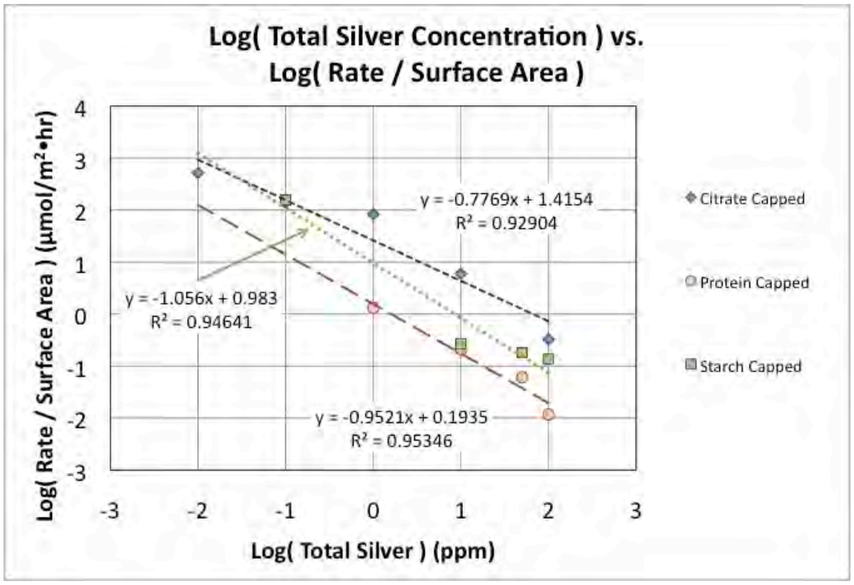

Figure 1. ROS generation for various particle types

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Acknowledgements

Rob MacCuspie, National Institute for Standards and Technology, Ceramics Division, Nanomechanical Properties Group, Gaithersburg, Maryland, USA.

Vinka Craver, University of Rhode Island, Department of Environmental Engineering, Kingston, Rhode Island, USA.

Transcriptomic effects of Au-NP to Caenorhabditis elegans

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Oral Presentation
11:20 AM, Tuesday

Gold nanoparticles (Au-NPs) are being used for medical and imaging applications, but they also can serve as a stable probe for particle specific toxic effects of manufactured nanoparticles. While previous *in vitro* studies demonstrate limited toxicity of Au-NPs, our research using the free living nematode *Caenorhabditis elegans* reveals that 4 nm citrate capped Au-NPs causes mortality at concentrations as low as 1 mg Au L^{-1}. Microfocused x-ray absorption spectroscopy demonstrates that *C. elegans* exposed to Au-NP absorb Au as intact particles. We have examined the toxicogenomic responses of *C. elegans* to 4 nm Au NPs to determine if particle exposure illicits specific gene expression patterns as well as to explore the mechanisms of toxicity.

Age-synchronized wild type N2 *C. elegans* at the L2 larval stage were exposed to four concentrations of Au-NP in 50% K-Medium (32 mM KCl and 51 mM NaCl) for 12 hours to establish a concentration-response relationship. For microarray studies, about 2000 nematodes per replicate with 3 replicates per treatment were exposed to the LC_{10} (5.9 mg L^{-1}) for 12 hours. RNA was extracted from each of the replicates and sent to the Microarray Core facility for labeling and hybridization to Affymetix microarrays containing probes for 22,500 genes. Partek software was used for data normalization, hierarchical clustering and ANOVA to detect differentially expressed genes displaying > 1.5 or < -1.5 fold changes at $p < 0.05$. The list consisting of 1041 differentially expressed genes was imported into Ingenuity Pathway Analysis (IPA) for identification of biologically relevant pathways involving these genes. Microarray results were confirmed by qRT-PCR on samples from independent exposure experiments for 3 selected genes, the apl-1 (amyloid precursor like protein), *dyn-1* (dynamin), and *abu-11* (activated in blocked unfolded protein response). Knockdowns were created using RNAi for *abu-11* and *dyn-1* to test whether some of the selected differentially expressed genes were linked to the observed toxicity.

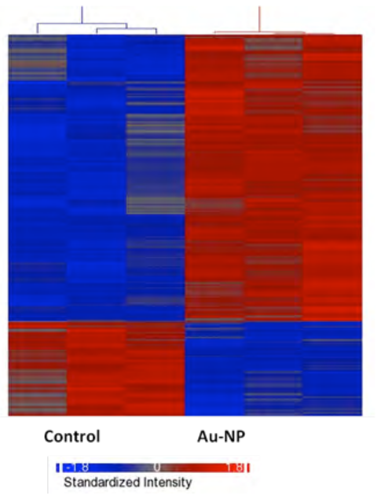

Figure 1. Clustering diagram for Control and Au-NP

There were total of 1041 differentially expressed genes meeting the aforementioned selection criteria, with majority (773 genes) being up-regulated. A treatment independent clustering histogram reveals that the control and exposed replicates are grouped within its cluster, clearly emphasizing the response of *C. elegans* to Au-NPs (Fig. 1). Data analysis using the IPA revealed 5 human-related pathways that were significantly affected by exposure to Au-NPs. Among these pathways are amyloid processing, citric acid cycle, clathryn-mediated endocytosis, apoptosis, and G-protein signaling pathways with p-values ranging from 0.00744 to 0.0289. The up-regulation of *apl-1* and *dyn-1*, which are known to be involved in amyloid processing and endocytosis, was confirmed with qRT-PCR. *Apl-1* is a *C. elegans* gene homologous to human amyloid precursor protein (app-1). Changes in *app-1* expression in humans are associated with the accumulation of β-amyloid and the related cascade of events leading to Alzheimer's disease. In *C. elegans*, *apl-1* is expressed in multiple tissues, being important for molting, morphogenesis, and larval survival [1]. Together with *feh-6*, which was also up-regulated in response to Au-NPs, the *apl-1* forms a complex controlling the rate of pharyngeal pumping. There were 8 up-regulated genes that have been implicated in clathryn-mediated endocytosis. One of the confirmed genes, *dyn- 1*, is involved in the early stages of endocytosis and is responsible for membrane fusion. Another known function of *dyn-1* is in phagocytosis of apoptotic and/or necrotic cells [2]. The activity of *dyn-1* during phagocytosis is controlled by *ced-1*, which was also significantly up-regulated by Au-NPs.

IPA is specific to the human-mouse database and, as a result, 817 significantly up- or down regulated genes were excluded from the pathway analysis. From this population of genes, we identified a group of 24 up-regulated *abu/pqn* genes with fold changes ranging from 1.5 to 8. The *abu/pqn* genes have been shown to be involved in *C. elegans* response to pathogens [3]. These genes are activated in response to endoplasmic reticulum stress (ERS) as a back up to a compromised canonical unfolded protein response (UPR) pathway. ERS is characterized by accumulation of unfolded / misfolded proteins. Up-regulation of the molecular chaperons, *hsp-70* and *hsp-16.1* was also observed and this may be indicative of the UPR. It has been previously hypothesized that Au-NPs may cause denaturation and missfolding of proteins [4]. The response to ERS can also involve apoptosis and/or necrosis of the cells with accumulated misfolded proteins. Several genes involved in regulation of apoptosis and/or necrosis (*ced-1, dyn-1*) were also found to be up-regulated. We are currently conducting RNAi experiments to test whether *abu-11* and *dyn-1* are involved in the defense mechanism against Au-NPs toxicity.

Results from this study highlight the fact that intact Au-NPs are bioavailable, can induce specific biological pathways, and are capable of causing adverse effects on whole organisms.

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Development of a new toxicogenomic index for nanotoxicity assessment

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Oral Presentation
11:40 AM, Tuesday

The growing production and use of engineered nanomaterials (ENMs) has raised considerable concern regarding their implicated environmental and health risks. Therefore, there is an urgent need to understand the toxic effects and mechanisms of these ENMs. Toxicogenomic research (genomic, proteomic, and metabonomic), which measures the global molecular-level activity in response to environmental stressors, provides a significant advance in nanotoxicity evaluation, understanding nanotoxicity mechanisms and pathways. However, one of the greatest initial challenges is how to convert the rich toxicogenomic information into a readily usable and transferable format that can be potentially linked to regulatory endpoints and incorporated into a decision-making framework. Furthermore, currently there is no accepted quantitative toxicogenomic endpoint that incorporates the important time-dependence of the genomic response into a toxicant assessment framework.

In this study, we examined the nanotoxicity induced transcriptional level activities using a GFP-infusion library consisting of 91 selected genes that covers most of the known stress-responsive genes in *E. coli*, such as general stress genes, SOS DNA repair system genes, redox stress genes, detoxification genes and others with specific functions[iii]. Three-dimensional real-time gene expression profiles were obtained for four NMs, including nano-silver (nAg), nano-titanium dioxide anatase ($nTiO_2$_a), nano-titanium dioxide rutile ($nTiO_2$_r), and fullerene root (fullerene). The 3-D altered gene expression profiles are distinctive for the four NMs, suggesting compound-specific cellular responses likely resulted from their different mode of actions (MOAs)

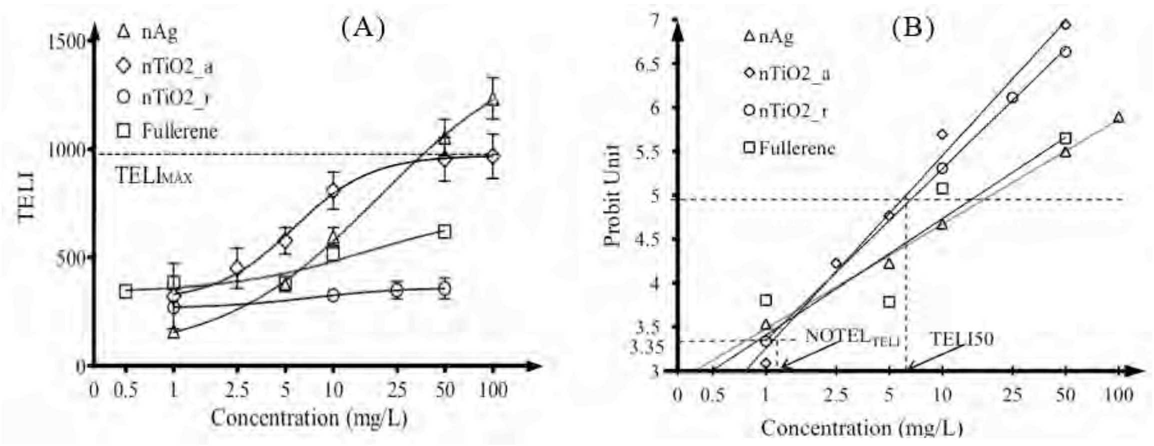

Figure 1. Dose response curves based on TELI versus dose concentration for nAg, nTiO$_2$_a, nTiO$_2$_r, and fullerene, fitted by Four-parameter Logistic Equation. Data points with error bar represent the TELI value determined at each dose concentration. (A) TELI versus dose concentrations for the NMs studied. TELI$_{MAX}$, the maximal value of TELI for each assay, is determined through model fitting. (B) Transformed dose-response curve based on probability unit. TELI values are normalized to the TELI$_{MAX}$. TELI50, NOTEL$_{TELI}$, and SlopeTELI are determined via a linear fitting of *probit unit* versus dose concentration.

To link the toxicogenomic results with regulatory benchmarks and conventional toxicity assessment endpoints, a Transcriptional Effect Level Index (TELI) that incorporates both the extent and magnitude of altered genes expression induced by NMs as well as the factor of exposure time are developed. Those TELI values exhibit concentration-dependent pattern (Figure 1) and allows for the establishment of dose-response curve. The parameters derived from TELI-dose response relationship, as TELI$_{MAX}$, TELI50, NOTEL$_{TELI}$ and TELI$_{slope}$, are compared with those current used endpoints, as EC50, BOD (Biological Oxidative Damage) and NOTEL (Non Transcriptional Effect Level) (Table 1). Employment of multiple endpoints as proposed above can describe the dose response behavior and characteristics more completely and holistically. For example, the NOTEL$_{TELI}$ and TELI$_{50}$ values for TiO$_2$_r and TiO$_2$_a are comparable, however, the dose-response curves in Figure 2 clearly showed distinctive toxic response behaviors, which can be reflected by the TELI$_{MAX}$ and TELI$_{slope}$. This is consistent with traditional toxicology that both threshold doses and the slope of dose-response curve are indicative and characteristic for the toxic response of a toxicant. Partcularly, the TELI$_{MAX}$ values among the NMs seem to be correlated relatively well with the EC50 (r=-0.82). This correlation implies that "phenotype anchoring" is possible with our proposed TELI$_{MAX}$, therefore allowing for the linking of phenotype changes with cellular response.

Table 1 Correlation of TELI-derived toxicity endpoints with currently used toxicity endpoints

Corrlation Coefficient	$TELI_{MAX}$	Slope	TELI50	$NOTEL_{TELI}$
NOTEL	-0.51	-0.04	0.20	*
EC50	-0.82	0.39	-0.61	0.78
BOD	0.98	-0.63	0.80	-0.95

*NOTEL and $NOTEL_{TELI}$ both represented the toxin level with no response, the absolute value of these two endpoints are consistently as the *p*-value varies from 0.92 to 1 for all the ENMs in our test.

TELI values can also be determined for genes in each stress response category (e.g. oxidative stress, DNA repair) indicate MOA-related comparative transcriptional level toxicity among compounds and it can reveal detailed information of toxic response pathways such as different DNA damage and repair mechanisms among the NMs. For example, although both nAg and $nTiO_2$_a seem to cause DNA stress, they lead to different DNA damage type and repair pathways.

Our results demonstrated that the prokaryotic toxicogenomic approach using whole-cell arrays could be potentially applied as a feasible method for qualitative and qualitative and quantitative toxicity assessment of NMs. TELI convert the rich toxicogenomic information into readily usable and transferable format that can be potentially link to regulation endpoints and incorporated into decision-making framework may serve as such an effective index.

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Evaluation of compatibility of ToxCast high-throughput/high-content screening assays with engineered nanomaterials

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Oral Presentation
2:00 PM, Tuesday

High-throughput and high-content screens are attractive approaches for prioritizing nanomaterial hazards and informing targeted testing due to the impracticality of using traditional toxicological testing on the large numbers and varieties of nanomaterials. The ToxCast program at EPA has used various high-throughput assays and developed computational tools to help assess potential toxicity and identify toxicity pathways of hundreds of traditional chemicals. The ToxCast phase I data are publicly accessible (1) and used by researchers to investigate links between chemicals and diseases (2). We investigated the compatibility of ToxCast cell-based high-throughput screening assays on engineered nanomaterials, with the ultimate goals of identifying toxicity/biological pathways affected by nanomaterials and finding correlations among nanomaterial physic-chemical characteristics, testing conditions, and nanomaterial toxicities/bioactivities.

We initially evaluated 15 nanomaterials and reference materials (4 nano-Ag with different coatings, 6 CNTs, nano-Au, AgNO3, micro-Ag, micro-quartz, and dispersant for a nano-Ag) on 6 assays. These 6 assays measured cell growth kinetics, transcription factor activity, cellular toxicity phenotypes, secreted and cell surface protein profiles, and cellular signaling pathways in various cultured cells, as well as developmental toxicity in zebrafish embryos (EPA). Our preliminary results showed that, at the same mass concentration, AgNO3 was most toxic, followed by nano-Ag, while CNT, nano-Au, micro-Ag and micro-quartz were not toxic in most cell-based assays. Similarly, AgNO3 was more toxic to zebrafish embryos than nano-Ag, and no obvious dose-dependent developmental toxicity was observed for any of the materials under tested conditions. Nano-Ag, micro-Ag and AgNO3 data are consistent with other studies, while the lack of detected CNT toxicity may be due to the type of CNT used as well as limited compatibility of current assays for CNT. In terms of pathways affected, AgNO3 and nano-Ag elicited changes associated with oxidative stress/antioxidant, metal exposure, heat shock, stress, DNA damage, and apoptosis, indicating that the use of high-throughput screenings can illustrate mechanistic information. We continue testing other classes of nanomaterials, such as nano-TiO2, nano-ZnO, and nano-SeO2, and evaluating the compatibility of high-throughput/high-content screening assays with these nanomaterials. *This abstract may not necessarily reflect U.S. EPA policy.*

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(2) http://cerhr.niehs.nih.gov/evals/diabetesobesity/

Towards an HTS toxicity assessment program with environmentally-relevant bacteria

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Oral Presentation
2:20 PM, Tuesday

The rate at which engineered nanoparticles (ENPs) are being synthesized and incorporated into mainstream consumer products is increasing, and their inevitable release into the environment has raised concern about the potential risks of ENPs to organisms and ecosystems. The gap between ENPs being produced and our understanding of their toxicity continues to widen, as traditional low-throughput methods for toxicity assessment are insufficient to study the vast number of emerging ENPs. As such, there is a need to transfer current low-throughput methods to highly automated, high-throughput (HT) toxicity assessment programs. HT toxicity studies require organisms or cells that are amenable to low volume, rapid toxicity assessment. Bacteria exhibit rapid growth and response to stress, and are therefore ideal for HT formats. Further, bacteria are ubiquitous in nature and play a fundamental role in biogeochemical processes, so understanding bacterial response to ENPs is of environmental importance.

The initial interaction between ENPs and bacteria in nature will occur at the cell membrane. Previously, we observed association of various metal-oxide ENPs with gram-negative and gram-positive bacterial surfaces, which corresponded to decreased cell yield and specific growth rates. We hypothesized that direct oxidation of cell membranes, by transfer of electrons from bacteria to NPs, may alter membrane function or integrity. In particular, electron transport chain function and establishment of a membrane potential could be inhibited as electrons necessary for both are transferred to NPs near or at the cell surface. NP-derived reactive oxygen species (ROS) could also cause oxidation of membrane components or physically compromise membrane structure. Based on our hypotheses, we selected five existing assays as candidates for transfer to HT-formats, for quantification of electron transport chain function, membrane potential, membrane permeability, total ROS and superoxide. A systematic approach was developed to optimize assays in microplate format, test for direct or indirect interference of ENPs with assay reagents, and unify parameters across multiple assays for transfer to an automated HT platform. Ultimately, this work is towards establishing a largely automated, HT toxicity assessment program for environmental bacteria exposed to ENPs. The systematic approach we have developed for LT-to-HT assay transfer can be applied to a wide range of fluorescence and colorimetric assays, nanoparticle chemistries, and cell types.

A living cell biosensor for continuous monitoring of cytotoxic cell responses to engineered nanomaterials (ENMs)

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Oral Presentation
2:40 PM, Tuesday

Hundreds of engineered nanomaterials (ENMs) are in use and new ENMs are being synthesized and modified at rapid rates (1, 2). Determining the toxicity of newly developed ENMs before they become widely utilized represents a major challenge for the responsible development of nanotechnologies (2, 3). Cellular testing remains the predominant evaluation platform for ENMs; however, these platforms have serious limitations (4, 5). Many cellular or biochemical toxicity assays have limitations for ENM testing because: they have limited throughput capacity; ENMs may interfere with the reagents and instrumentation used; and, finally, methods are not designed to look continuously or at multiple cumulative sublethal dose exposure scenarios. To address these challenges, we have developed a biosensor that can detect and measure in real time the responses of living cells to ENM exposures. The device is called a living cell quartz crystal microbalance (QCM) biosensor since it uses cells coupled to an oscillating quartz crystal which together produce a stable oscillation frequency. If cells are treated with different materials, at different doses, and either cell stress or death occurs, this can be rapidly detected and measured as signature shifts/patterns in altered oscillation frequency.

Using this biosensor system, we analyzed the response of live macrophages to the addition of either Zymosan A or polystyrene beads, both benign substances, or single walled carbon nanotubes (SWCNT) (3-150 ug/ml) and monitored the response of the cells over 18 hrs in the sensor as well as in parallel tissue culture growth chambers. After 18 hrs, macrophages in growth chambers were fixed and stained to detect cell death or apoptosis and the medias were measured for release of lactate dehydrogenase (LDH). In the biosensor, we observed a stable unchanging crystal oscillation frequency when we used cells alone, Zymosan A or beads alone, or SWCNTs without cells at the highest dose in the sensor. However, with living cells in the sensor, when either benign substances or SWCNTs were added, we saw a statistical drop in crystal oscillation frequency in the first 6 hrs which was dose dependent. With benign substances or low dose ENMs (3-30 ug/ml), this trend reversed and the oscillation frequency returned to pre-treatment levels, indicative of recovery of the cells; this interpretation was confirmed in parallel tissue culture assays. Depending on dose of SWCNTs, the lag time to see this QCM reversal was dose dependent. Lastly, at higher doses of ENMs (50-150 ug/ml), the frequency never recovered and apoptosis and/or necrosis were documented to occur in parallel cell culture experiments.

These experiments introduce a new device called the living cell QCM biosensor that can detect and provide unique information of peak, sub-lethal and toxic exposures of living cells to ENMs in real time and could be developed for deployment in a ENM manufacturing or use setting.

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Data mining, pathways and network analyses of nanoparticle toxicity based on high throughput screening data

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[2] University of California, Los Angeles

Oral Presentation
3:00 PM, Tuesday

Nano-sized materials are increasingly utilized in many industrial products and processes, with over 1000 commercial products utilizing engineered nanomaterials (eNMs), primarily due to their unique nano-scale properties. Despite the many beneficial uses of nanomaterials, there is a growing public concern regarding the potential release of eNMs to the environment and the potential adverse impacts of exposures to eNMs. In this regard, high throughput toxicity screening is critical for characterization of the potential hazard of eNMs, which is in turn indispensable information for subsequent risk assessment and development of environmental and health regulatory policies. In the present work, an approach to knowledge extraction from high throughput screening (HTS) of nanoparticle toxicity is presented, based on RAW and BEAS-2B mammalian cell lines, focusing on identification of toxicity outcomes (i.e., "hit identification"), development of predictive nano-quantitative-structure-activity relations (nano-SARs), identification of pathway linkages and correlation of cell signaling pathways and cytotoxicity. Based on newly developed feature selection approach, a classification based nano-SAR was developed for cytotoxicity of metal and metal oxide nanoparticles. The approach was demonstrated for a library of metal and metal oxide nanoparticles to develop to identify clusters corresponding to sub-lethal pro-inflammatory responses associated with ROS generation, lethal genotoxic responses, and cytotoxicity. Also, complex network theory methods were applied to identify relationships between cell responses (pathway activation and cytotoxicity measures) as well as physicochemical properties of nanoparticles. For example, the analysis based on the above cell lines revealed a hierarchical activity-activity pattern where sub-lethal effects such as ROS generation and the intracellular Ca^{2+} flux are strongly related to lethal effects (e.g. cell membrane damage and cell death) and that eNM primary size, aggregation and its dissolution tendency were critical parameters affecting the observed toxicity behavior. Cytotoxicity data from HTS measurement in particular can aid in identifying decision boundaries with respect to hazard ranking of nanoparticles.

Classification nano-SAR development for cytotoxicity of nanoparticles

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Oral Presentation
3:20 PM, Tuesday

Nano-sized materials are increasingly utilized in many industrial products and processes, with over 1000 commercial products utilizing engineered nanomaterials (eNMs), primarily due to their unique nano-scale properties. Despite the many beneficial uses of nanomaterials, there is a growing public concern regarding the potential release of eNMs to the environment and the potential adverse impacts of exposures to eNMs. In this regard, high throughput toxicity screening is critical for characterization of the potential hazard of eNMs, which is in turn indispensable information for subsequent risk assessment and development of environmental and health regulatory policies. In the present work, an approach to knowledge extraction from high throughput screening (HTS) of nanoparticle toxicity is presented, based on RAW and BEAS-2B mammalian cell lines, focusing on identification of toxicity outcomes (i.e., “hit identification”), development of predictive nano-quantitative-structure-activity relations (nano-SARs), identification of pathway linkages and correlation of cell signaling pathways and cytotoxicity. Based on newly developed feature selection approach, a classification based nano-SAR was developed for cytotoxicity of metal and metal oxide nanoparticles. The approach was demonstrated for a library of metal and metal oxide nanoparticles to develop to identify clusters corresponding to sub-lethal pro-inflammatory responses associated with ROS generation, lethal genotoxic responses, and cytotoxicity. Also, complex network theory methods were applied to identify relationships between cell responses (pathway activation and cytotoxicity measures) as well as physicochemical properties of nanoparticles. For example, the analysis based on the above cell lines revealed a hierarchical activity-activity pattern where sub-lethal effects such as ROS generation and the intracellular Ca^{2+} flux are strongly related to lethal effects (e.g. cell membrane damage and cell death) and that eNM primary size, aggregation and its dissolution tendency were critical parameters affecting the observed toxicity behavior. Cytotoxicity data from HTS measurement in particular can aid in identifying decision boundaries with respect to hazard ranking of nanoparticles.

SESSION 5: NANOCHEMISTRY

Synthesis and characterizations of monodisperse well-defined titania and silica nanoparticles

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Oral Presentation
4:20 PM, Tuesday

Amorphous silicon dioxide (silica) nanoparticles - either fumed silica, formed at high temperature by flame pyrolysis, or precipitated silica, formed by nucleation and growth within aqueous solution, are produced commercially in tonnage quantities. Crystalline silica is the most ubiquitous mineral on earth. Thus, understanding silica toxicity is important for both technological and environmental reasons. Studies of nanoparticle toxicity require mono-sized particles with engineered purity, shape, surface chemistry, and crystallinity. To this end, we have synthesized monodisperse amorphous and crystalline metal-free silica nanoparticles through careful control of synthesis and processing conditions, and we have characterized these materials, along with commercially-prepared fumed silica, using a variety of analysis methods. Amorphous silica colloids were synthesized by a modified Stöber method. Using these uniform Stöber silica colloids as templates, we have prepared well-dispersed mondisperse single-crystal alpha-quartz nanospheres through hydrothermal devitrification – thereby preserving the size and shape of the parent Stöber silica colloids. Also, uniform single-crystal cristobalite nanoparticles were synthesized by carbon deposition on aerosolized Stöber silica particles, followed by devitrification of the coated silica at high temperature and carbon removal by oxidation. Particle morphology, phase, size, structure, surface chemistry, and reactivity of silica particles were characterized by a range of structural and chemical analysis methods, including transmission and scanning electron microscopies, x-ray diffraction, thermal gravimetric analysis, nitrogen sorption, fluorescence spectroscopy, dynamic light scattering, and vibrational spectroscopy (mid- and near-infrared absorbance, and Raman scattering). Silica nanoparticle surfaces can be terminated with siloxanes (Si-O-Si), silanols, or energetic tri-coordinated silicon atoms and non-bridging atoms. Equilibrated in water all amorphous silicas have a hydroxyl coverage of about 5 OH/nm^2. Despite this, preliminary studies indicate higher toxicities of fumed silica for comparably sized particles.

As fumed silica is heated, infrared and Raman spectroscopies demonstrate a gradual surface dehydroxylation accompanied by a redistribution of surface silanol environments and the formation of strained three-membered silica rings at the particle surface. These surface structural changes correlate with toxicity measured by reactive oxygen species, intracellular calcium flux, mitochondrial membrane depolarization and membrane disruption. This talk will outline how processing strongly influences amorphous silica structure and accordingly toxicity – we conclude that not all silica is created equal.

Development of standardized dispersion and characterization methods for the environmental risk assessment of nanomaterials

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Oral Presentation
4:40 PM, Tuesday

The evaluation of the environmental and health risks of engineered nanomaterials (ENMs) requires the dispersion of ENMs in relevant matrices (e.g., cell culture media). For ENMs in dry powder form, the source material must first be de-agglomerated in the test medium. The widespread use of ultrasonic treatment for the dispersion of ENM powders is inconsistently applied across laboratories, and the lack of standardization has further contributed to observed variability. The present work addresses the need for standardized and reproducible dispersion methods for powdered ENMs in relevant biological media. We discuss the effects of sonication and medium specific parameters on stability of ENM dispersions. Results presented here will focus on nanoscale TiO2 in aqueous media, phosphate buffered saline, and cell culture medium (Dulbecco's Modified Eagle Medium). Under optimized conditions, reproducible, nanoscale dispersions in biological media are obtained, using bovine serum albumin as a biocompatible surfactant. The resulting dispersions remain stable for 48 hours under relevant incubation conditions. Multiple measurement techniques have been utilized to validate the dispersion procedures.

Imogolites: New inorganic nanotubes for nanotechnology hazard evaluation

C. Levard[1], P. Maillet, Liu Wei[2,3], M. Auffan[2,3], A. Masion[2,3], J. Rose[2,3], A. Thill[4,3], E. Doelsch[2,3,5], P. Chaurand[2,3], O. Spalla[4,3], O. Prous[6,3], J.L. Hazemann[6,3], D. Borschneck[2,3], N. Menguy[7], **Jean-Yves Bottero[2,3],**

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Oral Presentation
5:00 PM, Tuesday

Since the discovery of carbon nanotubes (NTs), there has been great interest in the synthesis and characterization of similar shaped structures like inorganic nanotubes, nanorods, or nanowires. However, limitations such as purity, complexity of the protocol, low-yield production, and size polydispersity still remain major impediments for industrial-scale applications. Imogolites ($Al_2SiO_3(OH)_4$) are natural aluminosilicate single wall nanotubes. To date, only Ge-Al imogolites analogues have been successfully synthesized 100 times more concentrated than Si-Al imogolites.

The growth mechanisms of imogolite-like aluminogermanate nanotubes have been examined at various stages of their formation. The accurate determination of the nucleation stage was examined using a combination of local (XAS at the Ge-Kedge and 27AINMR) and semilocal scale technique (in situ SAXS). For the first time, a model is proposed for the precursors of the nanotubular structure and consist in rooftile-shaped particles, up to 5 nm in size, with ca 26% of Ge vacancies and varying curvatures. These precursors assemble to form short nanotubes/nanorings observed during the aging process. The final products are most probably obtained by an edge-edge assembly of these short nanotube segments.

Two structure are revealed by SAXS: at 0.25M of Al the Al-Ge imolite NT are double-walled NTs with a low dispersion in diameter consisting of two concentric tubes of equal length and identical wall structure. At higher concentration (0.5 M), both SAXS and cryo-TEM data confirm the formation of single-walled NTs.

First tests to reveal cyto (test WST1) and genotoxicity (micro nuclei) on various vertebrates cells (human fibroblasts and CHO-K1) are interesting. They show a genotoxicity decreasing from proto to long length DWNT but appearing from 8 10^{-5} G/L.

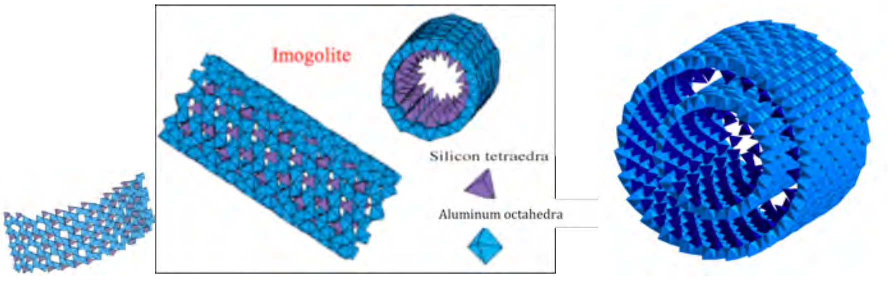

Figure 1. Structure of Al-GE proto and single and Double Wall Imogolite

Fine tuning size and shape of nanomaterials by hydrothermal synthesis

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Oral Presentation
5:20 PM, Tuesday

Since its first use in 1840s to simulate the natural geological process in the formation of rocks and minerals, the hydrothermal process has become one of the most commonly used methods for mineral and bulk crystal synthesis. With the help of advanced analytical techniques such as Fourier Transform Infrared Spectroscopy, Raman Spectroscopy, and High Resolution Scanning Electron Microscopy, starting from 1990, hydrothermal technology has occupied a unique place in the processing of advanced materials with controlled size, shape, crystallinity, and other physicochemical properties. Monodispersed nanoparticles of metals, metal oxides, metal suphides, carbon materials, and other nanocomposites prepared by the hydrothermal method have been reported extensively in the literature. In this study, the hydrothermal method was introduced to generate UC CEIN combinatorial nanomaterial libraries. By controlling synthesis composition and conditions, fine tuning of CeO_2 nanoparticle size, shape, and phase was achieved. It is expected that different defect concentrations and exposed crystal planes in the prepared CeO_2 nanocubes, nanorods, and nanowires will potentially lead to different toxicity responses. Additionally, this method has been extended to create other metal oxide (e.g., CuO, V_2O_5, MnO_2) combinatorial libraries.

Evaluation of the colloidal persistence of gold nanoparticles by UV-Vis spectroscopy and dynamic light scattering

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Oral Presentation
5:40 PM, Tuesday

Introduction

Numerous studies have shown the tendency of nanoscale particles to form larger aggregates when introduced to biological media or natural aquatic systems (1), and the dependence of aggregation processes on a number of factors including particle concentration, media ionic composition, pH, organic matter composition, and the presence of stabilizing surface coatings such as proteins and polymers is well established (2,3). The kinetics of aggregation processes have been commonly examined using dynamic light scattering (DLS) and UV-Vis spectroscopy (UV-Vis), yet few studies have attempted to establish quantitative relationships between these uniquely complementary characterization techniques. The current study combines DLS and UV-Vis spectroscopy to examine the stability and the localized surface plasmon resonance (SPR) activity of citrate-stabilized gold nanoparticles (cit-AuNP) as a function of primary particle diameter and media ionic composition. Our results have implications on the development of metrics to characterize the persistence of nanoscale materials in biological and environmental systems.

Experimental

Suspensions of citrate-stabilized AuNP (cit-AuNP) of 7.8, 15, 30, and 46 nm mean primary particle diameter (d_{TEM}, n>100) were prepared according to synthesis procedures reported in the literature (4-6). Time-resolved UV-Vis and DLS were used to evaluate the colloidal stability of nanoparticle suspensions in US EPA moderately hard synthetic (MHS) freshwater (7) as well as in solutions of the individual mono- and di-valent salts from which EPA MHS is prepared—sodium bicarbonate ($NaHCO_3$), potassium chloride (KCl), magnesium sulfate ($MgSO_4$), and calcium sulfate ($CaSO_4$). One additional salt, $CaCl_2$, was included for comparison to $CaSO_4$. For DLS measurements, hydrodynamic diameter ($Z_{AVG, nm}$) was monitored as a function of time and electrolyte concentration using a Malvern NanoZS. To evaluate colloidal stability by UV-Vis spectroscopy, the absorbance intensity at the SPR band (Abs_{SPR}) for each cit-AuNP suspension was monitored over time using a Cary 5000 UV-Vis spectrophotometer.

Results

Upon addition to divalent salt solutions, aggregate formation is evident for all four sizes of cit-AuNP. Aggregation rates determined from regression analysis of $Z_{AVG,\,nm}$ measured for all cit-AuNP suspensions in high concentrations of $MgSO_4$ (5 mM) indicate a positive linear relationship between primary particle diameter and $Z_{AVG,\,nm}$. Surprisingly, this relationship is reversed at low $MgSO_4$ concentrations (<1 mM), with aggregation rates being inversely related to primary particle diameter. Time-resolved UV-Vis spectroscopy indicates that at salt concentrations exceeding 100 mM, all divalent cation solutions investigated ($MgSO_4$, $CaSO_4$, $CaCl_2$) induce measurable changes in the UV-Vis spectra of all sizes of cit-AuNP. These changes include a decrease in absorbance intensity at the primary SPR band coupled with the formation of a red-shifted secondary SPR band located between 600 and 800 nm. Comparing results obtained by DLS and UV-Vis, kinetic changes in the Abs_{SPR} are evident at salt concentrations that are too low (i.e., ≤0.5 mM) to induce measurable changes in hydrodynamic diameter after 30 mins (Figure 1a-b), suggesting that Abs_{SPR} may be a more sensitive indicator of changes occurring in the localized dielectric environment, which may precede growth of aggregates measurable by DLS. Nevertheless, at higher electrolyte concentrations a significant negative relationship exists between $Z_{ave,\,nm}$ and Abs_{SPR} (Figure 1c) indicating that decreases in optical absorbance at the primary SPR band correlate well with increases in aggregate size as measured by DLS.

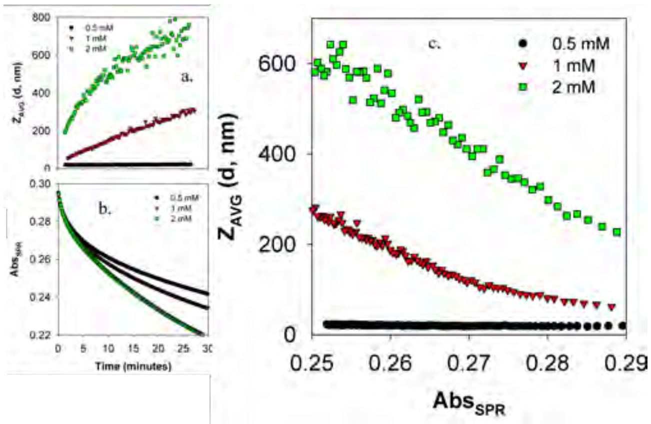

Figure 1. Relationship between hydrodynamic diameter ($Z_{AVG,d}$) and absorbance at the surface Plasmon resonance band (Abs_{SPR}) for 15 nm citrate-AuNP at varying salt concentrations. Panels a) and b) show the $Z_{AVG,d}$ as measured by DLS and the Abs_{SPR} as measured by UV-Vis spectroscopy, respectively, for each of the three $CaCl_2$ concentrations—0.5, 1, and 2 mM—over 30 minutes. Panel c) plots the inverse relationship between the untransformed values of $Z_{AVG,d}$ and Abs_{SPR} from panels a) and b).

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SESSION 6: EPA FATE, TRANSPORT AND TRANSFORMATION

Impact of sunlight and humic acid on the deposition kinetics of aqueous fullerene nanoparticles and multiwalled carbon nanotubes

Xiaolei Qu, Pedro J. Alvarez, **Qilin Li**[1]

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Oral Presentation
8:30 AM, Wednesday

We have shown in our previous study that sunlight and humic acid are key environmental components that interact with aqueous fullerene nanoparticles (nC60) and subsequently affect their transport behavior. We found that nC60 nanoparticles were subject to photochemical transformation under solar irradiation, which leads to oxidation of nC60 surface. Such surface oxidation greatly increases nC60 colloidal stability in NaCl solutions, but had an opposite effect in the presence of calcium ions.

In the current study, the roles of sunlight and humic acid in the deposition of nC60 and multiwalled carbon nanotubes (MwCNTs) were investigated using a combination of packed-bed column experiments and the quartz crystal microbalance with dissipation monitoring (QCM-D) technique under similar hydrodynamic conditions. The effects of both dissolved and surface immobilized humic acid were investigated.

The deposition attachment efficiencies of nC60 on bare silica surface measured in QCM-D system were in good agreement with those from packed column experiments, suggesting that QCM-D is a reasonable alternative to column experiments in studying nC60 transport in saturated porous media. Irradiation in the UVA range greatly decreased nC60 deposition in NaCl solutions due to the oxidation of nC60 surface. The critical deposition concentration (CDC) of nC60 was found to exhibit a systematical dependence on its surface oxygen concentration. Dissolved humic acid had a two-fold effect on nC60 deposition. It stabilized nC60 nanoparticles against aggregation, resulting in enhanced mass transfer towards the silica surface at high ionic strength. On the other hand, it hindered the deposition through the steric hindrance effect. The overall effect of dissolved humic acid depended on the dissolved humic acid concentration and the ionic strength. The interactions between nC60 and surface immobilized humic acid were found to be mainly controlled by electrostatic and steric hindrance forces, both depending on the total ionic strength. Overall, our results suggest that dissolved humic acid plays a more important role in nC60 deposition compared to surface immobilized humic acid.

UVA irradiation on carboxylated multi-walled carbon nanotubes (CMWCNTs) resulted in notable decrease in surface oxygen content of the CMWCNTs, likely through decarboxylation. Such change in surface properties greatly enhanced aggregation of CMWCNTs in NaCl solutions, consistent with the decreased negative surface charge. Accordingly, the irradiated CMWCNTs had higher deposition rates than the pristine CMWCNT in NaCl solutions. In CaCl2 solutions, however, no noticeable changes in either particle surface charge or stability were observed after irradiation. This is attributed to the two contradictory effects of surface carboxyl groups: rending the surface negative charges and complexing with Ca2+. These results suggest CMWCNTs may gradually lose their stability after exposure to sunlight in surface water.

Influence of surface chemistry on the sorption properties and colloidal stability of carbon nanotubes in the presence of NOM

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Oral Presentation
8:50 AM, Wednesday

Introduction: When an engineered nanoparticle (ENP) enters an aquatic environment, its surface will be modified by the adsorption of natural organic matter (NOM). Although the adsorption of NOM onto ENPs will certainly influence the particle's colloidal stability in aquatic environments, an important and currently unresolved question is the extent to which (if any) the nascent properties of the ENP influence the adsorption properties and stabilizing effects of NOM. Amongst the various physicochemical properties (e.g. size and shape) which define an ENP, surface properties are likely to play a particularly important role in determining NOM-ENP interactions. To explore the interplay that exists between nascent ENP surface chemistry and ENP interactions with NOM, we have studied the sorption properties and stabilizing effects of NOM with carbon nanotubes (CNTs) that exhibit systematic variations in the concentration of surface oxygen (see **Figure 1**). These hydrophilic oxygen-containing functional groups are often present on the graphenic surface of CNTs as a result of either deliberate functionalization strategies or incidental exposure to oxidizing conditions. In previous studies, we have shown that the degree of surface oxidation exerts a pronounced effect on the colloidal stability of CNTs in simple solutions that do not contain NOM. (1,2)

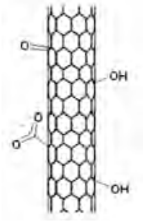

Figure 1: Oxides on CNTs

Sorption Studies: We have shown that CNT surface oxides decrease the CNT's sorption capacity towards naphthalene while increasing the sorption capacity towards metal cations. (3,4) Because NOM exhibits both hydrophobic and charged segments, it is therefore reasonable to hypothesis that CNT surface chemistry will also influence NOM adsorption. Using water from the Great Dismal Swamp as a reasonably well characterized source of NOM we measured sorption isotherms on several multi-walled carbon nanotubes (MWNTs) that exhibit different levels of surface oxygen. Results from these studies showed a systematic decrease in NOM sorption with increasing concentrations of MWNT surface oxides (**Figure 2**). This indicates the

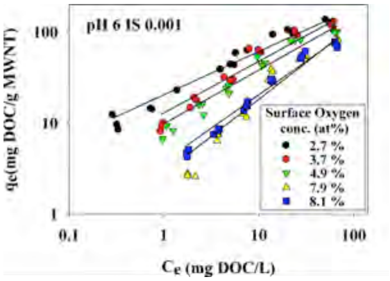

Figure 2. NOM sorption isotherms onto MWNTs that exhibit different surface oxygen concentrations. Lines represent fits to the Freundlich isotherm.

presence of electrostatic repulsion between negatively charged carboxylic acid groups on the MWNT and the NOM macromolecules whose magnitude will increase as the surface oxygen concentration on the MWNT increases.

Colloidal Stability: Time resolved dynamic light scattering (TR-DLS) data has shown that the colloidal stability of MWNTs was, as expected, significantly enhanced in the presence of NOM (**Figure 3**) and increasing pH. In general, the colloidal stability of all MWNTs were similar in the presence of NOM. However, at pH values > 6, bench-top sedimentation and TR-DLS studies indicated that the decrease in NOM sorption on more heavily oxidized MWNTs (**Figure 3**) caused these particles to become somewhat less stable than MWNTs with lower concentrations of surface oxides, which are able to adsorb more NOM. This suggests that an ENP's nascent surface chemistry can influence the particle's ultimate colloidal stability in the real environment, and furthermore that ENPs which are relatively more stable in a laboratory setting may actually be relatively less stable once they enter the natural environment.

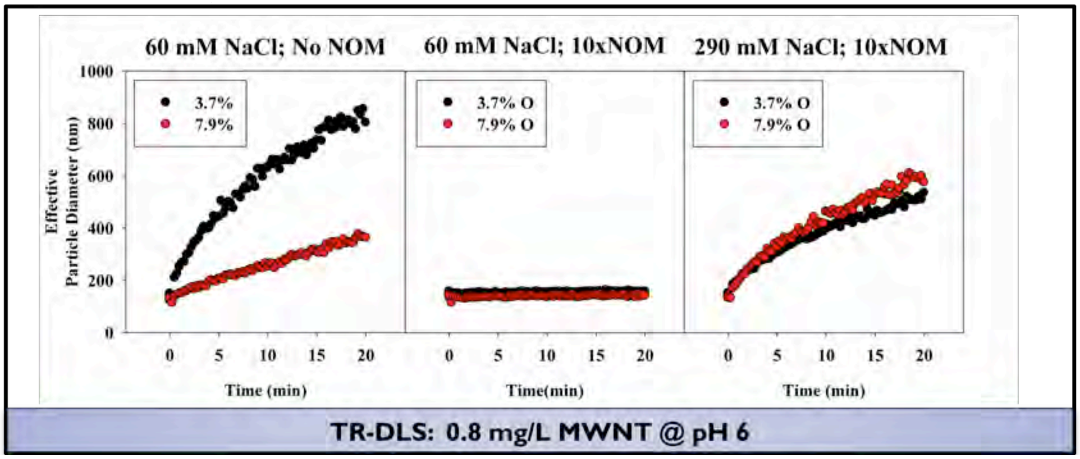

Figure 3: Time-resolved dynamic light scattering (TR-DLS) comparing the colloidal stability of two MWNTs with different levels of surface oxygen (3.7% and 7.9%) in different environments; (left) pure water and 60mM NaCl, (middle) NOM-laden water and 60 mM NaCl, and (right) NOM-laden water and 290 mM NaCl.

Acknowledgements: The authors acknowledge support from the Environmental Protection Agency (grant # RD-83385701-0), National Science Foundation (grant # BES0731147), and Institute for Nanobiotechnology (INBT) at Johns Hopkins University.

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Transport of oxidized multi-walled carbon nanotubes through porous media

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Oral Presentation
9:10 AM, Wednesday

During the process of production and application, carbon nanotubes (CNTs) are frequently dispersed in water or other polar solvents, which requires surface modification, one method for which is surface oxidation through acid treatment(1). To better understand the effect of such oxidation on CNT transport in groundwater and sand filters, we have undertaken a study of the effect of surface oxidation on the transport of MWCNTs through porous media under different conditions of aquatic chemistry.

In this study, MWCNTs purchased from Nanolabs Technology (Nanolabs, Inc.) were oxidized with nitric acid at one of two concentrations ($53\%_{w/w}$ or $15\%_{w/w}$). The fractional surface content with regard to different oxygen containing functional groups was then measured using X-ray Photoelectron Spectroscopy (XPS). Column experiments were conducted on the transport of these materials through glass beads of diameter 0.335 – 0.425 mm, including studies of pH and ionic strength (IS) effect (at constant CNT concentration and approach velocity) and studies of the effect of CNT concentration and approach velocity at constant pH and IS. For most experiments, approximately 0.6 μg of colloidal O-MWCNTs were injected into the column as a short pulse using 0.024 cm/s as the baseline injection velocity. In order to obtain consistent and accurate results, the collectors were used only once after purchase and were cleaned thoroughly by careful sonication of pre-wet materials prior to every experiment. Pulse breakthough curves were then collected using UV-vis detection. Results were analyzed using a simple one dimensional convection-dispersion transport (**eq. 1**). The hydrodynamic dispersion coefficient *D* and the filtration deposition coefficient k_d were obtained by fitting the experimental results using a non-linear least sqare analysis (Eq 3), and the attachment efficiency α was then calculated by comparing the fitted k_d to "$k_{d,fast}$" estimates that were obtained under favorable deposition (high IS) conditions)—see equation [4]

$$\frac{\partial \mathrm{C}}{\partial \mathrm{t}} = \mathrm{D}\left(\frac{\partial^2 \mathrm{C}}{\partial \mathrm{x}^2}\right) - \mathrm{v}\left(\frac{\partial \mathrm{C}}{\partial \mathrm{x}}\right) - k_d \mathrm{C} \quad \textbf{[Eq. 1]}$$

$$k_d = -\frac{1}{\mathrm{t_p}} \ln\left(\frac{\mathrm{C_f}}{\mathrm{C_o}}\right) \quad \textbf{[Eq. 2]}$$

$$C(x,t) = n_0 \frac{x}{2\sqrt{\pi t^3 D}} \exp(-k_d t) \exp\left[-\frac{(x-vt)^2}{4Dt}\right] \quad \textbf{[Eq. 3]}$$

$$\alpha = \frac{k_d}{k_{d,\mathrm{fast}}} \quad \textbf{[Eq. 4]}$$

Transport studies in packed columns confirmed that the attachment efficiency of MWNTs is strongly affected by ionic strength, and pH in a manner generally consistent with expectations (**Figures 1 and 2**). Results were found to be independent of the injection amount of MWCNT dispersions (**Figure 3**), which indicated that the aggregation of MWCNTs was not significant in this experiment. Surprisingly, the oxidation degree of O-MWCNTs was found to have comparatively little effect on the attachment efficiency (compare **Figures 1 and 2**). The relationship of the approach velocity v and the deposition rate coefficient k_d can be expressed by the equation: $k_d = 1.22v^{0.89}$ **[Eq. 5]** and **Figure 4.** These results suggest that collisions of MWCNTs and glass beads was likely caused by direct interception rather than by diffusion or sedimentation (2).

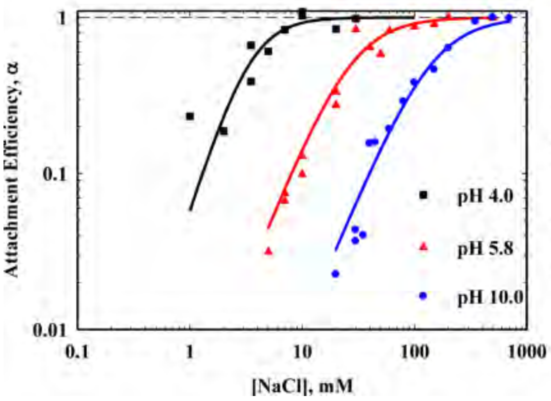

Figure 1. Effect of pH and IS on a for **O-MWCNTs treated with 53% HNO_3** at pH 4.0± 0.2, 5.8 ± 0.2, and 10.0± 0.2.

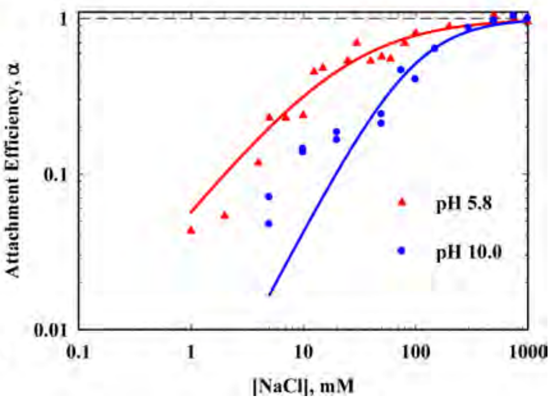

Figure 2. Effect of pH and IS on α for **O-MWCNTs treated with 15% HNO_3** at pH 5.8 ± 0.2 and 10.0± 0.2.

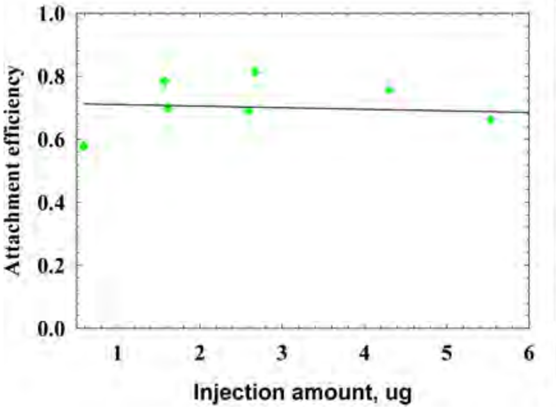

Figure 3. Attachment efficiency of O-MWCNTs treated with 53% HNO_3 at different injection amount at 40 mM NaCl. pH 5.8 ± 0.2

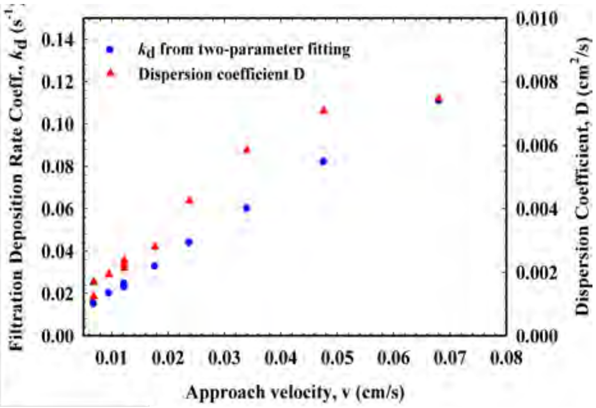

Figure 4. Filtration Deposition Rate coefficient k_d and dispersion coefficient D at different approach velocities

Acknowledgements

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Ion release and aggregation kinetics of citrate-coated silver nanoparticles in aqueous environment

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Oral Presentation
9:30 AM, Wednesday

Silver ion release and aggregation are two important environmental behaviors of silver nanoparticles (AgNPs), which can influence their bioavailability and cytotoxicity. Characterization of these two kinetics processes is critical for better understanding the environmental fate, transport, and biological impacts of AgNPs. The ion release kinetics of AgNPs with three primary diameters (20, 40, and 80 nm) was studied by dispersing them in quarter-strength Hoagland medium at two initial concentrations (300 and 600 μg/L). Silver ion release rates were found to depend on primary particle size and concentration, while other environmental factors (e.g., dissolved oxygen and proton) were kept constant. A kinetics model was developed to describe the silver ion release based on the hard sphere theory using the Arrhenius equation. The model fitted the experimental data very well with correlation coefficients of 0.97-0.99, and the dependence of ion release kinetics on the primary particle size and concentration is well interpreted with the model. Moreover, effects of environmental factors (e.g., dissolved oxygen, pH, temperature, and salinity) can potentially be interpreted too. This model provides fundamental insight into the ion release kinetics of AgNPs in aqueous environments, which could allow us to understand and predict the nanotoxicity of AgNPs better.

Aggregation state of AgNPs was dependent on various factors such as pH, ionic strength, electrolyte type, which is widely rationalized by classic Derjaguin−Landau−Verwey−Overbeek (DLVO) theory. Although these factors may play a role in AgNP aggregation, the results presented herein suggest that dissolved oxygen (DO) is another governing factor for particles like AgNPs that react with DO in aqueous environments. This study employed time-resolved dynamic light scattering (TR-DLS) technique to investigate the aggregation kinetics of citrate-coated AgNPs. Our results clearly demonstrate that when DO was present in suspensions, AgNPs released Ag^+, and the aggregation rate became much faster (e.g., 3-8 times) than that without DO. The hydrodynamic sizes of AgNPs had a linear growth within the initial 4-6 h and after the linear growth, the hydrodynamic sizes became random for AgNPs in the presence of DO, whereas without the presence of DO the hydrodynamic sizes grew smoothly and steadily. Furthermore, the effects of primary particles sizes (20, 40, and 80 nm) and initial concentrations of AgNPs on aggregation kinetics were also investigated. These two factors are crucial in determining the aggregation state of NPs and are either ignored or not explicitly mentioned in most of the reported toxicity studies.

Behavior of quantum dots in the terrestrial environment: Soil column leaching and plant uptake studies

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Oral Presentation
10:30 AM, Wednesday

Interest on the environmental impacts of engineered nanomaterials has rapidly increased over the past 5 years. With the extensive work being done on developing materials in the "nano-scale", these materials will eventually be released into the environment. Fate and transport studies on QDs (or nanomaterials in general) involving solid matrices are still very limited. The present work provides results from our recent studies to investigate the potential of QDs to leach into the groundwater or be taken up by plants when these nanomaterials are discharged into the terrestrial environment.

Differences in the partitioning and mobility of CdSe and CdSe/ZnS QDs were examined using small-scale soil columns;10 mM CaCl2 was used to model artificial rain, while 10 mM Na_2EDTA was used to model chelating acids. Potential for QD plant uptake were investigated using *Arabidopsis thaliana* as model organism. Our results suggest that while QDs may exhibit limited soil mobility (in 10 mM $CaCl_2$), the presence of chelating acids in soil can enhance the leaching potential of intact QDs (in 10 mM Na_2EDTA). Mobilization of Cd-containing species was observed when EDTA solution was applied; 98% of the total Cd^{2+} loaded leached out from the Cd^{2+}- spiked soil, while only 30% and 75% leached out from the CdSe and CdSe/ZnS QDspiked soil, respectively. Longer incubation (15 days) of QDs in soil also indicated some QD degradation. Nevertheless, intact QDs were detected in the Na_2EDTA leachates with or without aging. An example of a soil column Cd^{2+} distribution profile for the different QDs is provided in **Figure 1**; Cd concentrations were determined by ICP-MS. Transmission electron microscopy image and electron diffraction pattern of collected leachates which verify the presence of intact QDs are shown in **Figure 2**.

Indeed, retention of intact QD in soil could be a concern for terrestrial plants. Results from the plant uptake experiments revealed that *Arabidopsis* exposed to nanoparticle suspensions for 1-7 days did not show any internalization of intact QDs. QDs remained adsorbed onto the root surfaces with or without humic acid addition. ICP-MS analysis of the roots and leaves suggest that QDs were indeed only found in the roots and not translocated in the leaves. Despite no evidence for uptake, plant glutathione levels (GSH/GSSG) decreased in the presence of QDs (with HA); this could have been caused by the nanoparticle or the probable leaching of toxic Cd^{2+} or SeO_3^{2-}. Fluorescence microscopy images illustrating adsorption of QDs onto the plant root surfaces are in **Figure 3**.

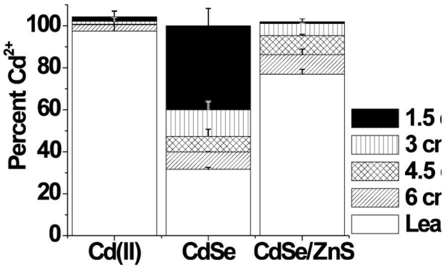

Figure 1. Soil column profile for the leaching of Cd(II), CdSe QD and CdSe/ZnS QD spiked soils using 10 mM EDTA solution. Shaded squares pertain to different soil sections from top to bottom.

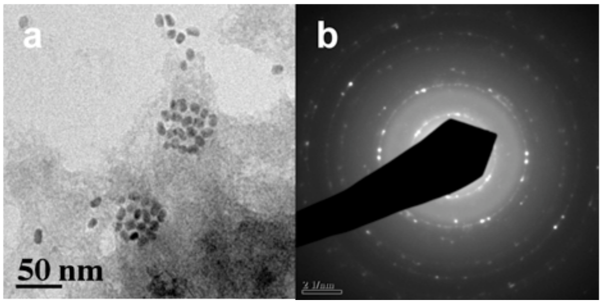

Figure 2. (a) TEM image of a leachate collected from a soil column spiked with CdSe/ZnS QDs. (b) SAED of the same leachate sample showing concentric rings indicating crystallinity of the particles.

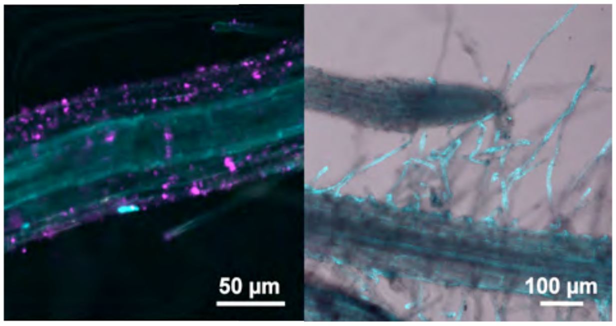

Figure 3. Superposition of fluorescence and light microscopy images of roots from plants (a) exposed to QD suspensions in Hoagland's nutrient solution compared to (b) control plants.

** The authors would like to acknowledge Dr. Yueling Qin for the TEM analysis and Alan Siegel for the fluorescence microscopy measurements.*

Speciation and transformations of platinum in environmental materials

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Oral Presentation
10:50 AM, Wednesday

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

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

Road dust and road-side aerosol samples from multiple sites in cities across the country (including Atlanta, Denver, Los Angeles, Milwaukee) exhibit elevated levels of total platinum (200–800 ng/g). Significant (8-23% of total) soluble pools of platinum, with measureable anionic character, were measured in these vehicle emission receptor samples. Our sXAS studies (ANL-APS, 20-BM) of aerosol emissions (PM) from diesel engines burning a Pt/Ce-based FBC reveal a large fraction of oxidized platinum. Spectral fitting suggests that a platinum(IV)oxide-hydrate is the dominant oxidized platinum species in the engine PM. Similarly, a substantial component of the platinum pool in used TWCs was found to be oxidized. The majority of the primary emissions of platinum from diesel engines burning a Pt-FBC was present in fine and ultra-fine particle-size fractions. We have advanced the HPLC-IC-SFICPMS analytical methodology for separation and detection of hexa- and tetra-chloroplatinate to achieve quantification limits of lower than 5 ng/L – an order-of-magnitude better than reported in the literature – and we are working to further improve these limits.

Through our multidisciplinary approach we expect to substantially advance our understanding of the sources, speciation, transformation, and potential human exposures to nano-platinum materials in the environment. We expect to provide some of the first measurements of the recognized toxic species of platinum in environmental media. Vital information on the concentrations **and** chemical species of platinum in mobile source emissions and important environmental receptors will be provided. Fundamental data on rates of species transformation will be acquired. The chemical speciation and exposure data will enable enhanced assessments of the toxicological relevance of environmental nano-platinum species.

Deposition and transport of Ag nanoparticles on silica as a function of environmental conditions

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Oral Presentation
11:10 AM, Wednesday

In this project, we are using two methods to characterize the attachment of Ag nanoparticles on silica: thin layer chromatography (TLC) and quartz crystal microbalance with dissipation (QCM-D), both under similar pH and ionic strength conditions. The nanoparticle sizes in the various aqueous matrices are determined by dynamic light scattering. We will discuss the connections between the mobility and deposition behaviors of nanoparticles based on these two characterization techniques by applying the classical and extended theory of DLVO colloidal stability in the absence and presence of natural organic matter respectively.

An integrated approach toward understanding the impact of aggregation and dissolution of metal and metal oxide nanoparticles

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Oral Presentation
11:30 AM, Wednesday

Nanoparticles, the primary building blocks of many nanomaterials, may become suspended in air or get into water systems, e.g. drinking water systems, ground water systems, estuaries and lakes etc. Therefore, manufactured nanoparticles can become a component of the air we breathe or the water we drink. One important issue in understanding the environmental fate, transport, toxicity and occupational health hazards of nanoparticles is in characterizing the nature and state of nanoparticles in air, water or in vivo. For the nanoparticles of interest in these studies, metals and metal oxides, it can be asked: (i) will metal oxide and metal nanoparticles be present in air or water as isolated particles or in the form of aggregates?; (ii) will metal oxide and metal nanoparticles dissolve in aqueous solution or in vivo?; and (iii) under what conditions will metal oxide and metal nanoparticles aggregate or dissolve? As the size regime will be very different depending on the state of the nanoparticles, as dissolved ions, isolated nanoparticles or nanoparticle aggregates, these questions are important to address as it impacts the size regime that needs to be considered or modeled in for example environmental transport or lung deposition models. Furthermore, the effect on biological systems including nanoparticle-biological interactions and toxicity will depend on the state of nanoparticles. In the studies discussed here, macroscopic and molecular-based probes that includes quantitative solution phase adsorption measurements, molecular based probes, light scattering and zeta-potential measurements to investigate the behavior of nanoparticles in aqueous suspensions. We have focused on several different metal and metal oxide nanoparticles in including Fe, Ag, Zn, Cu, Ce and Ti. Some of our newest results, including detailed size-dependent studies, which focus on aggregation and dissolution, including detailed size-dependent studies, in the presence and absence of organic acids will be discussed. This research is beneficial as it significantly contributes to the growing database as to the potential environmental and health implications of nanoscience and nanotechnology and how nanomaterials will behave in the environment and impact human health.

SESSION 7: EPA TOXICITY

Phototoxicity of TiO2 nanoparticles in developing zebrafish

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Oral Presentation
1:00 PM, Wednesday

Exposure of humans and other organisms to nanomaterials is an inevitable consequence of nanomaterial usefulness. It is important, but difficult, to predict the biological consequences of these exposures. We hypothesized that the unique chemical properties that make nanoparticles useful might also be the key in predicting their biological impact. To investigate this, we chose titanium dioxide nanoparticles (TiO_2NPs) and developing zebrafish embryos as model systems. TiO_2NPs absorb photons to generate electron-hole pairs that react with water and oxygen to form cytotoxic reactive oxygen species (ROS). To test this hypothesis we conducted dose-response experiments in which zebrafish embryos were exposed to a solution containing graded doses of commercially available TiO_2 nanoparticles. The fish were divided into 2 groups. In one group, the fish were illuminated with a bright light source using a 14h/10h light/dark cycle. This illumination was designed to simulate the slightly blue-shifted spectrum of sunlight in clear shallow water. The other group was kept in dim tungsten filament lighting using the same light/dark cycle. After 5 days of exposure, we observed toxicity that was clearly photo-dependent. The non-illuminated group showed almost no lethality at any concentration tested. The nominal individual particle size was 21 nm; however, the particles rapidly aggregated in solution to produce aggregates of approximately 1 micron in size. Nonetheless, these particles were internalized by the developing zebrafish; however, the egg chorion was a barrier to uptake. Studies of uptake using inductively coupled plasma optical emission spectrometry (ICP-OES) and transmission electron microscopy (TEM) showed uptake throughout the tissues of the developing zebrafish. Chemical probes and a transgenic zebrafish line in which a GFP reporter is expressed from Antioxidant Response Elements (AREs). The *Tg(are:eGFP)* line showed elevated reporter expression when the fish were exposed to both the TiO_2 nanoparticles and illumination, but not in response to either stimulus alone. Addition of an ROS scavenger rescued fish embryos from toxicity. Our study highlights the relationship between the property of the material that makes it useful and the biological effect that is produced. This concept should serve as a guide for future nanotoxicological studies aiming to identify potential hazardous effects on organisms.

Characterization of the potential toxicity of metal nanoparticles in marine ecosystems

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Oral Presentation
1:20 PM, Wednesday

There are many uncertainties regarding the potential toxicity of nanoparticles, but as their production and commercial applications increase, there is an increased likelihood of significant exposures to marine organisms. There are also many uncertainties about their cellular interactions and targets. Our model system has been oysters, *Crassostrea virginica* – a filter-feeding bivalve, and some of the cellular responses that have been studied are lysosomal integrity, oxidative damage, and antioxidant responses. Studies with adults, embryos, and isolated tissue studies have been used to address important issues associated with nanoparticle toxicity, e.g. does shape affect toxicity, are toxic responses to metal nanoparticles due to the metal ions released as the particles degrade or do metal nanoparticles cause nanoparticle –specific toxicity, are there tissue specific differences? The relative sensitivities of adults and embryos have been compared, and the value of isolated tissues for assessing toxicity potential has been considered. Therefore oysters (adults, embryos, hepatopancreas and gill tissues) were exposed to various silver nanoparticle preparations and comparable concentrations of silver nitrate. The effects on different cellular biomarker responses (lysosomal destabilization, glutathione, lipid peroxidation, antioxidant enzymes) were evaluated in gill and hepatopancreas tissues. The results indicate shape-specific differences in toxicity, and tissue-specific differences between the responses to nanoparticles and dissolved silver (silver nitrate). For example, after short term exposures (48 hours) to silver nitrate, lipid peroxidation levels of gill tissues were significantly higher than controls, but no significant increases were observed in hepatopancreas tissues; in contrast, exposing oysters to silver nanoparticles caused a significant increase in lipid peroxidation in hepatopancreas tissues but not in gill tissues. Prism-shaped silver nanoparticles were found to be slightly more toxic than spheres or plates in studies with embryos and adults. These results suggest that metal nanoparticle toxicity may not be readily predicted by studies based on metal salts. While nanoparticles represent a new class of contaminant, there are numerous issues regarding how they interact with biological systems that need careful investigation.

Effects of functionalization on the bioavailability of carbon nanotubes to Daphnia magna

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Oral Presentation
1:40 PM, Wednesday

Engineered carbon nanostructures, such as carbon nanotubes (CNTs), are inherently hydrophobic and are not stable in aqueous systems. However CNTs can be surface modified with functional groups that will affect aqueous stability. Furthermore, the aqueous stability and bioavailability of these nanotubes may be influenced by many water quality parameters, such as natural organic matter (NOM), and inputs from wastewater treatment plant (WWTP) effluents. NOM has been shown to bind to the surface of CNTS and result in a functionalized surface that will increase aqueous stability. Proteins are a main constituent of WWTP effluents and have been shown to bind to the surface of CNTs much like NOM. This enhanced stability is likely to lead to an increased residence time in the water column and increased exposure times for pelagic organisms. While our previous work demonstrated that multiwalled carbon nanotubes (MWNT) stayed within the gut tract of *D. magna*, some researchers have suggested that the small diameter of single walled nanotubes (SWNTS) may allow them to cross cell membranes (1-3). In the present study NOM from Suwannee River (SR-NOM) increased the stability of OH, SiO_2, polyethylene glycol (PEG), and polyaminobenzene sulfonic acid (PABS) functionalized SWNT suspensions. Fetal bovine serum (FBS) was used as a protein source to determine the effects of protein functionalization on the toxicity and bioavailability of OH-SWNTs. Standard 96 hr static renewal *D. magna* toxicity tests were conducted to evaluate the acute toxicity of these functionalized SWNTs. Particles were characterized using zeta potential and transmission electron microscopy (TEM). Confocal microscopy, micro Raman spectroscopy, and TEM coupled with electron emission loss spectroscopy (EELS) were also used to inspect the gut tracts of *D. magna* to determine the fate of ingested functionalized SWNTs. Functionalized SWNT exposure to *D. magna* caused little acute toxicity compared to published data on MWNT (4). Confocal microscopy and micro Raman techniques provided evidence that functionalized SWNTs greatly impact the gut tract once ingested, but were unable to work at the scale needed to determine material absorption. TEM coupled with EELS provided the best evidence for material absorption across the gut tract.

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Carbon dot uptake, transfer, and toxicity in Daphnia magna and zebrafish (Danio rerio)

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Oral Presentation
2:30 PM, Wednesday

Carbon dots are photoluminescent forms of carbon nanomaterials (CNM) developed as alternatives to the potentially more toxic heavy metal-based quantum dots. However, nanomaterials have been shown to exhibit physical and chemical properties appreciably different from those of the parent compounds. For instance, surface modified CNM, such as fullerene C70, have been shown to have toxic effects to aquatic organisms, including the onset of oxidative stress. However, it is difficult to assess the ability for these CNM to translocate across the gut membrane to elicit effects directly to tissues. Like quantum dots, carbon dots are fluorescent as a factor of their size and not from chemical alteration. Carbon dot fluorescence was detected in *Daphnia magna* and zebrafish by confocal fluorescence microscopy. Carbon dots were observed to adsorb primarily to the carapace of exposed *D. magna*. However, carbon dots were positively detected within internal *D. magna* tissue. Carbon dots were unable to be detected within eggs of gravid *D. magna* exposed for up to 48h. Carbon dots did not sorb to zebrafish eggs, but evidence suggest uptake by larvae exposed post hatch. This study is a component of an investigation of nanoparticle size dependence on the uptake and effects carbon dots in aquatic organisms as compared to CdSe/ZnS quantum dots.

Nanosilver inhibited anaerobic digestion under bioreactor landfill operations

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Oral Presentation
2:50 PM, Wednesday

Silver nanoparticles (AgNPs, nanosilver) released from industrial activities and consumer products may be disposed directly or indirectly in sanitary landfills. To examine the effect of these nano particles on anaerobic digestion of landfill waste, we loaded municipal solid waste (MSW) in identical landfill bioreactors (9 L volume each) and exposed to AgNPs (average particle size = 21 nm) at the concentrations of 0, 1, and 10 mg silver/kg solid. The landfill anaerobic digestion was carried out for more than 250 days, during which time the cumulative biogas production was recorded automatically and the chemical property changes of leachates were analyzed. There were no significant differences in the cumulative gas volume or gas production rate between the bioreactor exposed to nanosilver at the concentration of 1 mg AgNPs/kg and the control bioreactor. However, landfill solids exposed to AgNPs at 10 mg/kg resulted in reduced biogas production and the accumulation of volatile fatty acids (including acetic acid) while the leachate pH was maintained consistently between 5 and 6. Total Ag in the leachates of the bioreactor treated with 10 mg AgNPs/kg decreased rapidly from an initial concentration of 14.8 mg/L to below 2 mg/L after 112 days. Quantitative PCR results after day 100 indicated that the total copy numbers of 16S RNA gene of methanogen was in the range of $5.79 \pm 2.83 \times 10^5$ (copies/mL) in the bioreactor treated with 10 mg AgNPs/kg. For comparison, these numbers were $1.97 \pm 0.21 \times 10^7$ and $0.90 \pm 0.03 \times 10^7$ (copies/mL) in the bioreactor treated with 1 mg AgNPs/kg and the control bioreactor, respectively. The results suggest that AgNPs at the concentration of 1 mg/kg have minimal impact on landfill biogas production, but a concentration at 10 mg/kg or higher may result in reduced biogas production and inhibition of methanogenic assemblages.

The pharmacokinetics of ceria (a model engineered nanomaterial) with emphasis on the brain: Comparison to other engineered nanomaterials

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Oral Presentation
3:10 PM, Wednesday

Background: Nanoscale ceria has extensive commercial uses which can contribute to its environmental release, including its use as a diesel fuel additive. We are studying it as a model metal oxide engineered nanomaterial (ENM).

Research needs: Nanoscale ceria was nominated by the NIEHS for evaluations of chemical disposition and toxicokinetics and is on the priority list of the OECD for measurement, toxicology and risk assessment studies.

Objective: To characterize the distribution, biopersistence, and resultant effects of ceria ENMs, with emphasis on the brain, compared to reports of other ENMs.

Methods: Studies were conducted with in-house produced and characterized ~ 5, 15, 30 and 65 nm citrate-coated ceria ENMs, iv infused as a single dose into rats. They were terminated 1 h or 1, 7, 30 or 90 days later and blood, cerebrospinal fluid, and tissues were collected for cerium (Ce) determination by inductively coupled plasma-mass spectrometry (ICP-MS). To determine ceria distribution in blood, samples were obtained over time and Ce determined in whole blood, serum and clot (as an indication of association with blood cells). Urinary and fecal Ce outputs were determined for 2 weeks. Effects of ceria ENM administration included blood-brain barrier (BBB) integrity; oxidative stress in 3 brain regions and selected peripheral organs, and when significant, antioxidant enzymes; and ceria distribution and associated cell changes viewed by light and electron microscopy.

Results: More than 98% of the ceria ENMs that were larger than 5 nm were cleared from blood within 10 min of their infusion. The 5 nm ceria persisted much longer, presumably because it was too large to be filtered by the kidney, but not large enough to be readily recognized by macrophages. Daily urinary and fecal Ce excretion was less than 1% of a 30 nm ceria dose. As seen with most carbon-based, metal, and metal oxide ENMs, they were primarily cleared from blood into reticuloendothelial system (RES) tissues. There was no significant clearance of the 30 nm ceria ENM for up to 90 days, consistent with the persistence of other insoluble ENMs. The rapid clearance of ceria ENMs from blood, very limited distribution into the brain, and their deposition as persistent/insoluble agglomerations in the RES are properties seen with many other ENMs. The rapid clearance from blood, persistence in RES tissues, and an increase in the blood concentration ~ 2 to 4 h after iv infusion of some ceria ENMs are not well described by traditional pharmacokinetic parameter calculations or PBPK models. Changes in the distribution of ceria ENM in blood over the 4 h following its iv infusion suggest changes in its surface properties, consistent with reports showing rapid ENM opsonization by blood proteins. As no other work has been reported of ENM distribution in blood, these results may predict the behavior of other ENMs. The ceria ENMs generally, but non-significantly, increased BBB permeability. Persistence of ceria ENM agglomerations in the liver and spleen were associated with abnormalities, including granulomas and some oxidative stress.

Conclusions: Insoluble ENMs are rapidly removed from blood and stored in RES tissues, from which they are very slowly or not eliminated over time. Ceria ENMs behave similarly to other metal and metal oxide as well as carbon-based ENMs. Their persistence supports the concern that this may result in adverse effects.

Some results of this research have been published: Hardas, S.S., Butterfield, D.A., Sultana, R.L., Tseng, M.T., Dan, M., Florence, R., Unrine, J.M., Graham, U.M., Wu, P., Grulke, E.A., Yokel, R.A., Brain distribution and toxicological evaluation of a systemically delivered engineered nanoscale ceria, Toxicological Sciences 116(2), 562-576, 2010; doi: 10.1093/toxsci/kfq137. Others have been submitted for publication.

Benefits to the EPA: These results of ENM translocation, biopersistence and hazard identification in the mammal provide data for ENM risk characterization.

Supported by US EPA STAR Grant RD-833772.

Phase inverted poly (amic acid) membranes for sensing and isolating engineered nanoparticles

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Oral Presentation
3:30 PM, Wednesday

There is urgent demand for rapid screening methods to isolate, detect, and monitor engineered nanomaterials in the environment. Conventional methods for characterizing nanomaterials such as transmission electron microscopy, scanning electron microscopy and atomic force microscopy tend to be bulky and inadequate for field & rapid screening of free nanomaterials. Our Group has discovered that a new class of flexible, stand-alone polyamic acid (PAA) membranes could be successfully used as both sensors and nanofilters. A new nanofilters device based on PAA membranes is hereby introduced. The nanofilters were derived from phase-inverted, copolymers of PAA and other polymers, with the surface and pore sizes systematically controlled by varying the conditions of the synthesis. This presentation will focus on the use of PAA membranes for simultaneous removal and electrochemical detection of silver nanoparticles, quantum dots and titanium dioxide nanocrystal from environmental samples.

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