Poster #1

Nanoparticles in Tobacco Smoke: Characterization with Single Particle ICP-MS

R. S. Pappas, M. Fresquez
Centers for Disease Control and Prevention

Tobacco smoke aerosol consists of moisture, nicotine, and tar. Viscous aerosol tar contains suspended solids. Insoluble aluminum silicates and silica are the most common solid particulate species observed in mainstream cigarette smoke tar using scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDS) [1]. Inhalation of fine solid particles in the smoke aerosol can cause inflammatory response in the lungs. Solid nanoparticles (< 100 nm), however, are smaller fractions of the particulate that are transported in either fine aerosol or nanoaerosol droplet fractions of mainstream cigarette smoke. Solid nanoparticles readily cross the pulmonary epithelium, enter the interstitial tissue, and may pass into the circulation, potentially contributing to both pulmonary and systemic toxicity [2,3]. The solid nanoparticle fraction of smoke particulate has not been successfully characterized due to difficulties with the water-insoluble tar matrix and analytical interferences.

We suspended insoluble particles from the tar matrix by dissolving the organic aerosol bulk trapped by electrostatic precipitation in N,N-dimethylformamide, and diluted the suspension into ultrapure water for analysis. We applied Single Particle-Triple Quad-Inductively Coupled Plasma-Mass Spectrometry (SP-QQQ-ICP-MS) to the study of the solid nanoparticle fraction of mainstream smoke particulate. High nebulization and particle transport efficiency (greater than 25% based on NIST gold nanoparticle analysis) was obtained with the use of a desolvating introduction system. The desolvating introduction system, together with the use of hydrogen cell gas for elimination of HCN+ and other interferences, and the improved vacuum and argon gas purification systems of the new generation QQQ-ICP-MS instrumentation, permitted improved sensitivity and made possible low background equivalent diameter limits for solid nanoparticle analysis (as low as 5 nm).

Aluminum-, silicon-, and titanium-containing particles in smoke tar were characterized according to size distribution and particle number in mainstream smoke using SP-QQQ-ICP-MS. Size ranges and nanoparticle quantities from mainstream smoke obtained from select commercial cigarettes were determined. Silicon-containing nanoparticle fractions were determined to have median particle diameters in the range of 75 nm, and aluminum-containing fractions below 45 nm. Titanium oxide particles, though fewer in number, were smaller, with median diameters below 20 nm.

This work has provided much-needed data on the characterization of nanocomposition of solids in mainstream smoke aerosol, expanding on previously published concepts of aerosol droplet size to the characterization of solid particles transported in mainstream tobacco smoke.

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Development of Low-Cost Silver Nanoparticle Syntheses for In-Country Manufacturing and Application on Ceramic Water Filters

Z. Shepard, E. Lux, V. Oyanedel-Craver
Department of Civil and Environmental Engineering, University of Rhode Island, Kingston, RI

Silver nanoparticles increase the antimicrobial effectiveness of ceramic water filters (CWFs) to provide clean drinking water to hundreds of thousands of people in developing countries around the world. CWFs have been recognized as effective, socially-sensitive point-of-use water treatment devices. The ceramic pot can be manufactured in country, but the silver nanoparticles are usually synthesized in developed countries, implying a great financial burden and supply chain challenges to CWF factories. Ideally, the CWF manufacturer should have the capability of producing silver nanoparticles in country. This would decrease the cost of the filter and increase the resilience of the production, while maintaining a high degree of effectiveness in terms of bacterial removal. In this project, we work to develop and assess methods for synthesizing antimicrobial silver and copper nanoparticles with materials readily available in developing countries. The nanoparticles are characterized and then applied to ceramic disks in order to test their ability to remove bacteria. We will also compare our in-house manufactured nanoparticles with those commercially available and commonly used by CWF factories.

The physico-chemical characteristics of these nanoparticles will be assessed as well as their antimicrobial performance. Nanoparticles will be analyzed using dynamic light scattering for size and zeta potential. Scanning electron microscopy will allow us to determine the morphology of the synthesized particles, and transmission electron microscopy will determine the extent of aggregation. EPA standard testing methods will be used to test the antimicrobial effectiveness of ceramic disks impregnated with silver and copper nanoparticles. ICP-MS analysis will allow us to determine the extent to which metals are leaching into the effluent of the ceramic filters.

In developing countries, occupational exposure to nanoparticles or hazardous chemicals during the synthesis is an issue due to lack of funding and weak government oversight. The synthesis processes discussed here are evaluated for potential risks to the manufacturer based on National Institute for Occupational Safety and Health (NIOSH) and U.S. Consumer Product Safety Commission (USCPSC) regulations and guidelines. Whenever possible, our syntheses utilize safe, natural products that pose little health concern. Through this research we hope to provide CWF manufacturers with the tools to synthesize silver nanoparticles on site, thus lowering the cost of the filter and bringing greater access to clean water to the developing world.

Chemical Transformations of Silver-Nanomaterial Containing Textiles After Use and Disposal

D. E. Gorka, J. M. Gorham
Materials Measurement Laboratory, National Institute of Standards and Technology, Gaithersburg, MD

Due to their unique properties, nanomaterials have been increasingly incorporated into consumer goods. In particular, silver nanomaterials (AgNMs) have been added to biomedical devices and textiles due to their antimicrobial properties. Increased use of AgNMs in consumer goods will result in increased entrance into the environment. However, the chemical properties of these AgNM-enabled textiles at various stages of their lifecycle need to be studied in greater detail to better predict environmental impact after use and disposal. Here we evaluated the chemical and physical transformations that AgNM-enabled textiles undergo during modeled human use and environmental exposure, using a commercially available AgNM wound dressing (Ag-WD). Ag-WDs were exposed to synthetic sweat or simulated wound fluid (modeled human exposure) or synthetic freshwater or artificial landfill leachate (modeled disposal exposure). The Ag-WD was then analyzed quantitatively and qualitatively using several analytical techniques including scanning electron microscopy with energy dispersive X-ray spectroscopy, X-ray diffraction, X-ray photoelectron spectroscopy, inductively coupled plasma mass spectrometry, UV-visible spectroscopy, and dynamic light scattering. Lastly, to evaluate antimicrobial efficacy, zone of inhibition tests were performed on the Ag-WDs with the bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The results of the work here will help foster a better understanding of the chemical and physical transformation that Ag-WDs undergo during use and disposal, which will help manufacturers and regulators to make more informed decisions on product design and use.

Exposure to Engineered Nanomaterials, Seven Case Studies within the LabEX SERENADE Project: a Safer by Design Approach

C. de Garidel-Thoron[1,2], A. Masion[1,2], M. Auffan[1,2], S. Pekar[1,2], J-Y. Bottero [1,2], J. Rose[1,2], LabEx SERENADE Consortium[1]

1. LabEX SERENADE Consortium, Europole de l'Arbois, Aix en Provence, France; Aix-Marseille University, CNRS, CEA, INSERM, INERIS, INRA, IRD, Grenoble Alpes University, Montpellier University, University Paris-Est-Creteil, Novancia Business School, Allios, Suez-environnement

2. CEREGE CNRS, Aix-Marseille University, CNRS, IRD, INRA, Coll France, Aix en Provence, France

Nanomaterials are expected to be a key to innovation breakthroughs and to lead to many new applications by 2020 and further. To reach the forecasted level of economical development, the public acceptance of nanotechnology is essential not only in terms of human health safety but also concerning the environmental impact. This lead manufacturers to develop sustainable processes of production, taking account safety and acceptance considerations since first steps of the design and during the entire production of new-generation nanomaterials.

The LabEX (Laboratory of Excellence) SERENADE (funded since 2012 by the French "Investissements d'Avenir" project) proposes an integrated scientific and educational approach to develop new concepts and tools for the safer design of next-generation nanomaterials and manufacturing processes. It is supported by a French national multi-disciplinary network of 11 academic partners including most of the French pioneering groups in the field from fundamental to applied research and education and 2 industrial partners (Suez-Environment, ALLIOS).

SERENADE initiated seven integrated case studies aiming at testing the SERENADE "Safer by Design" methodology on various product types (photocatalytic paints, sunscreen, quantum dots, silver nanowire, food packaging) and examining the often neglected end of life stage. We present the first results of the different case studies with a specific focus on the determination of consumer and environmental exposure.

Study of Toxicity of Ceria Nanoparticles on an *In-Vitro* Alveolo-Capillary Barrier

L. P. Beeravalli, J. Starobin, S. Aravamudhan
Joint School of Nanoscience and Nanoengineering, Greensboro, NC

Respiratory diseases due to penetration of ultra-fine particles are steadily increasing. The main portal of entries for such particles is a gas-exchange region of the lung, which has the large absorption area with a thin alveolo-capillary barrier. The goal of this work is to study this barrier and investigate *in-vitro* toxic effects of 15-30 nm nanoceria on A549 epithelial cell line and EA.hy926 endothelial cell line monocultures. We determined cytotoxicity using membrane integrity assays and the cellular uptake using flow cytometry and hyperspectral microscopy. The A549 and EA.hy926 cell lines were cultured on either side of a permeable transwell insert to establish the co-culture and to mimic the complex 3D structure of alveolo-capillary barrier. We found that A549 cells failed to form the tight junctions necessary for forming a functional barrier *in-vitro*, therefore we treated them with dexamethasone. Dexamethasone helped to form the tight junctions, which was confirmed by using Confocal and SEM (Scanning Electron Microscopy). A549 epithelial cells cultured on the apical side of the transwell insert were incubated with nanoceria for 24, 48, and 72 hours to reproduce the physiological environment with supply of nutrients from basal side and the apical pole of the cells exposed to air. The transport of nanoceria across the alveolo-capillary barrier was analyzed taking the endothelial cells cultured on the basal side of the transwell insert using Inductively Coupled Plasma-Optical Emission Spectrometry. In summary, the experiments demonstrated that our co-culture system provides a suitable *in-vitro* model to examine the effects of nanoparticles on the alveolo-capillary barrier and to investigate the mechanism of particulate matter toxicity across this functional barrier.

Detection of Titanium Released from Nano-TiO_2 Concrete Weathered in a Variety of U.S. Climates

K. Challis[1], R. Lankone[2], Y. Bi[3], M. Garland[4], Y. Wang[5], L. M. Gilbertson[5], P. Westerhoff[3], H. Fairbrother[2], A. Kennedy[6], J. Ranville[1]
1. Department of Chemistry and Geochemistry, Colorado School of Mines, Golden, CO
2. Department of Chemistry, Johns Hopkins University, Baltimore, USA
3. School of Sustainable Engineering and the Built Environment, Arizona State University, Tempe, USA
4. Sinnhuber Aquatic Research Laboratory (SARL), Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR
5. Department of Civil and Environmental Engineering, University of Pittsburgh, Pittsburgh, PA
6. Environmental Laboratory, U.S. Army Engineer Research and Development Center, Vicksburg, MS

Titania (TiO_2)-containing cement is a commercial product, TXactive™. It is marketed as self-cleaning due to the photocatalytic properties of TiO_2[1]. Pollutants, such as organic compounds, that contact the surface of the concrete are degraded by TiO_2 photocatalysis in sunlight. TiO_2-containing concrete samples were weathered in an outdoor weathering network as 5 cm diameter disks. A study was designed to determine the magnitude of release of TiO_2 from TXactive™ concrete across a variety of natural weathering environments in the USA. The locations were: Tempe, AZ; Corvallis, OR; Golden, CO; Baltimore, MD; Vicksburg, MS; and Pittsburg, PA. Triplicate samples were suspended in containers that passively collect rainwater for a month. Containers without cement were used as controls. A 40-element analysis, including Ti, of the content of the rainwater was measured using ICP-OES. The particle form of TiO_2 was examined by electron microscopy and single particle ICP-MS (spICP-MS), which detects metal particles as time-resolved pulses. All control (background) and concrete samples contained titanium. For example, the control jars in the first month of exposure, July 2016, had average total Ti release of 5.2, 1.7, and 0.8 µg Ti in Tempe, AZ; Golden, CO; and Baltimore, MD; respectively. The cement samples had slightly more Ti on average with 5.3 µg Ti, 2.6 µg Ti,and 1.1 µg Ti at the same respective locations. A portion of control/background Ti was determined to be particulate by spICPMS. Analysis of the background particles by electron microscopy/EDX showed Ti was present as discrete titania particles. The background results in interference when quantifying engineered titania released from the concrete. Experimental titanium measurements will be summarized.

1. Lehigh Hanson Heidelberg Cement Group. TX ACTIVE. 2018

Quantification of Nanomaterial in Cells After Applying Emulated Consumer Spray Formulations in a Newly Developed Spray System Coupled to an Air-Liquid Interface

F. S. Bierkandt[1], P. Bergelt[2], J. Tentschert[1], H. Jungnickel[1], P. Laux[1], A. Luch[1], L. Hillemann[2]
1. Federal Institute for Risk Assessment (BfR), Department Chemical and Product Safety, Unit Product Research and Nanotechnology, Berlin, Germany
2. Institute of Process Engineering and Environmental Technology, TU Dresden, Dresden, Germany

The growing application of engineered nanomaterials (ENMs) and especially their use as constituents in numerous spray products (e.g., cosmetics, coatings, or cleaning products) increase the possibility of human exposure via inhalation. A detailed investigation of such formulations with regard to their deposition behavior and toxic potential is urgently required. Adverse effects associated with aerosol inhalation are connected to the size and shape of ENM, as shown, e.g., for fibrous materials. Amongst others, these factors influence particle deposition in the lungs. Moreover, the composition of sprayed formulations and the resulting aerosols may impact ENM toxicity due to adsorbed compounds. Such substances may alter ENM properties like cellular penetration or reactivity. Furthermore, certain formulations may carry substances into areas which are normally inaccessible (i.e., Trojan Horse effect). Thus, characterization of spray formulations and their influence on the fate of ENMs in solution as well as after aerosolization is essential and shall be addressed in this work. Moreover, the alteration of the ENMs upon spray application or uptake in cellular systems needs to be investigated in toxicological test systems. In order to address the effects of commercially available products, a spray application of simulated formulations in cell exposure systems is currently developed.

Here, Ag- or CeO_2-ENMs in combination with several additives were used in emulated spray formulations based on pure water and ethanol. These particles were selected due to their frequent use related to their bactericide activity (Ag-ENMs) or their UV-stability (CeO_2-ENMs). They also represent two groups of ENMs with different solubility: while Ag-ENMs are more soluble, CeO_2-ENMs are considered rather insoluble and biodurable. To address nanoscale effects, respirable ENM sizes of < 100 nm and ~ 200 nm in addition to microsized particles were tested. As surface active substances are used as co-formulants in marketed products to improve their spray behavior, the influence of two perfluorinated silanes on ENM toxicity is being tested exemplarily.

A first spray chamber design—a box-like approach—allowed aerosol sampling at different positions relative to the spraying location and target. Nevertheless, the size of this system proved to be disadvantageous for sampling of large aerosol amounts, and for fast and repeated spraying. Therefore, a channel-like approach was implemented, which enables an easy chamber ventilation and an anisokinetic sampling downstream of the spray target.

Exposure of A549-cells was conducted by connecting an Air-Liquid Interface system (ALI) to sampling, while parallel on-line aerosol measurements using condensation particle counter (CPC) or scanning mobility particle sizer (SMPS) instruments enabled characterization of the generated aerosol. Measurements by inductively-coupled plasma mass spectrometry (ICP-MS) after microwave digestion confirmed a successful ENM deposition on the cells as well as on pads intended for future imaging analysis. Although no toxicological tests after aerosolization were done yet, results of *in vitro* studies under submersed conditions stress the importance for future investigation of these ENM- containing formulations.

Estimation of Nanomaterial Weight Fraction in Consumer Products Using Machine Learning Methods

B. L. Thornton, D. E. Carlson, M. R. Wiesner
Department of Environmental Engineering, Duke University, Durham, NC

The increasing prevalence of engineered nanomaterials (ENMs) in consumer products and infinite potential combinations of properties requires more efficient and predictive means for evaluating exposure and hazard. A necessary component of exposure evaluation is the concentration, or weight fraction (WF), of a chemical within a product. Oftentimes manufacturers are not required to publicly report chemical concentrations, making these values difficult to obtain without time-consuming laboratory analysis. Machine learning (ML) methods such as random forest classification have been used to predict WF for bulk-sized organic chemicals (Isaacs et al. 2016), but not for ENMs. These models used physicochemical properties and functional use categories (e.g., hydrophobic agent, fragrance) as descriptor variables, or features, for prediction. The ML model presented here is a proof-of-concept model trained on data from the PEN Consumer Products Inventory (2013) to predict ENM-WF. Classification into three separate weight fraction bins (low at 0.00-0.01; medium at 0.01-0.10; and high at 0.10-1.00) was done through a support vector machine with a non-linear kernel (see Smola and Schölkopf 2004). This outperformed previously applied estimators of WF, averaging 74.5% accuracy over 5-fold cross validation. The current model, however, is data-limited—meaning accuracy is expected to improve by increasing the size of the dataset. A regression model to predict numeric ENM-WF values is also in development. In addition to physicochemical properties and functional use categories developed by the Organisation for Economic Co-operation and Development (OECD), the use of product categories refined by the U.S. Environmental Protection Agency (e.g., hand soap, sports equipment) as model features improved performance. Functional use categories were still the most important predictors of WF by far, in concordance with Isaacs et al. (2016). The significance of functional use categories over chemical properties provides support for future work exploring the supplementation of ENM-WF model coefficients with those from a much richer dataset of organic chemicals via transfer learning (a subset of ML). Models such as this can provide prediction values for first-pass chemical safety evaluations and prioritizing research efforts.

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Thermal Decomposition/Incineration of Nano-Enabled Coatings and Effects of Nanofiller/Matrix Properties and Operational Conditions on Byproduct Release Dynamics: Potential Environmental Health Implications

D. Singh, W. Wohlleben, P. Demokritou
Center for Nanotechnology and Nanotoxicology, T.H. Chan School of Public Health, Harvard University, Boston, MA

Nano-enabled coatings (NECs) are a growing class of nanomaterials used in architectural applications that are likely to be thermally decomposed at their end- of-life via incineration of construction/demolition/renovation waste or accidental fires in buildings, thereby increasing concern over the potential release of engineered nanomaterials (ENMs) used as nanofillers. This is especially important for NECs as they have a larger surface-to-volume ratio compared to more traditional nano-enabled products. In addition, questions remain about effects of nanofiller properties such as chemical composition, size, and loading on NECs' thermal decomposition behavior. Different incineration operational conditions (final temperature, oxygen content, aerosol residence time) also may significantly modify the released byproduct (released aerosol, residual ash, off-gases) properties.

In this study, the recently developed Harvard Integrated Exposure Generation System (INEXS) was used to address the above hypotheses, to identify important factors governing thermal decomposition of commercially relevant NECs and to assess potential environmental health implications.

Findings confirm that nanofiller chemical composition, size, and mass loading, as well as incineration conditions, influence the release of nanofiller into the aerosol. Conversely, the host coating polymer matrix and the incineration conditions together determine the released aerosol number concentration. The morphology, mass-size distribution, and the overall partitioning of elemental and organic carbon of the released aerosol are also primarily governed by the host coating polymer matrix. On the other hand, the physicochemical and morphological properties of the remaining residual ash are strongly influenced by the nanofiller chemical composition, morphology, and size.

Overall, the study highlights important byproduct release dynamics and exposure characteristics at the end-of-life of NECs. Further studies on understanding the synergistic interactions between the released byproducts, their fate and transformations, and their toxicological and environmental health implications are warranted.

Quantifying Nanomaterials Release of Nanocomposites Following Natural Weathering

R. S. Lankone[1], K. Challis[2], L. Pourzahedi[3,4], D. P. Durkin[5], Y. Bi[6], D. Hanigan[7,6], Y. Wang[3], M. Garland[8], R. B. Reed[2,6], T. Zaikova[9], F. Brown[10], K. Hristovski[10], J. E. Hutchison[9], R. Tanguay[8], P. Westerhoff[6], G. Lowry[4], L. M. Gilbertson[3], J. Ranville[2], H. Fairbrother[1]
1. Department of Chemistry, Johns Hopkins University, Baltimore, MD
2. Department of Chemistry and Geochemistry, Colorado School of Mines, Golden, CO
3. Department of Civil and Environmental Engineering, University of Pittsburgh, Pittsburgh, PA
4. Department of Civil and Environmental Engineering, Carnegie Mellon University, Pittsburgh, PA
5. Department of Chemistry, United States Naval Academy, Annapolis, MD
6. School of Sustainable Engineering and the Built Environment, Arizona State University, Tempe, AZ
7. Department of Civil and Environmental Engineering, University of Nevada, Reno, NV
8. Sinnhuber Aquatic Research Laboratory, Environmental & Molecular Toxicology, Oregon State University, Corvallis, OR
9. Department of Chemistry and Biochemistry, University of Oregon, Eugene, OR
10. Engineering and Environmental & Resource Management Programs, Arizona State University, Mesa, AZ

Nanocomposites are manufactured by inclusion of nanoparticles into a material matrix. These nanocomposites express a wide range of useful properties, including improved strength, conductivity, flame-retardancy, and antimicrobial efficacy, which are increasingly exploited in consumer products. A consequence of the steady rise in the development, production, and application of nano-enabled consumer products, however, is the increased likelihood of environmental and human exposure to engineered nanomaterials (ENMs) following release from their surrounding matrix. This is of particular concern for products intended for outdoor use, as weathering will be a persistent degradation and release pathway. To address concerns of ENM exposure, and more specifically ENM release from nanocomposites, we have developed an original outdoor weathering methodology in order to quantify ENM release from a wide range of nanocomposites during natural weathering in unique climate zones across the U.S. The methodology and experiments are coordinated and carried out in collaboration with life cycle assessment (LCA) practitioners to ensure that the data acquired is useful to advancing LCA of nano-enabled products. Importantly, these experiments support the generation of data where it is currently scarce—the use and end of life stages.

The specimens used in this weathering study include lab-prepared carbon nanotube and silver polymer nanocomposites (CNT-PNCs & Ag-PNCs) as well as commercially available pressure treated lumber (containing CuNPs). These samples were secured in custom-designed sample holders, placed on roofs at five different geographic locations (Baltimore, MD; Tempe, AZ;. Golden, CO; Pittsburgh, PA; and Corvallis, OR), and allowed to weather for over a year. ENM release was quantified each month and the total release measured over the 18-month period was analyzed in tandem with site-specific weather data to determine which climate factor(s) —temperature, precipitation, solar fluence, and UV index—are most important in driving ENM release in the natural environment. To complement outdoor weathering studies, lab-based studies were performed in which single particle ICP-MS (sp-ICP-MS) was utilized to characterize the form (nanoparticle vs. ion) of released nanomaterial and how it evolves as a function of aqueous exposure time. Additionally, the leaching potential during the disposal stage of pressure treated lumber was determined by the toxicity characteristic leaching procedure (TCLP). Findings from our study will be presented with respect to how such data will enhance the realism of LCA impact assessment results and provide more accurate insight into the kinetics of ENM release in the environment.

Predicting Nanomaterials Toxicity using 3D Bioprinted Cell Cultures

G. Ao, M.Y. Lee
Department of Chemical & Biomedical Engineering, Cleveland State University, Cleveland, OH

The development of *in vitro* three-dimensional (3D) cell-based assays for high-throughput screening (HTS) of engineered nanomaterials is important to better assess the potential toxicity and enhanced exposure of humans of new nanomaterials. Our research goal is to create *in vivo*-like miniature tissues via microarray 3D bioprinting as highly predictive cellular models to study nanomaterials toxicity. In this work, structurally sorted, pure-chirality, DNA-wrapped single-wall carbon nanotubes (SWCNTs) were selected as model one-dimensional, high-aspect-ratio, tubular nanomaterials. The DNA-SWCNT hybrids are multifunctional materials with exceptional optical, electronic, and physicochemical properties with enhanced biocompatibility. Due to the intrinsic photoluminescence of nanotubes in the tissue-transparent near-infrared region, DNA-SWCNTs can be potentially used as spectrally coded, multi-color fluorescent probes for biological applications, such as biosensing and imaging. We observed that in 10% v/v fetal bovine serum (FBS) containing RPMI cell culture media, the temporal change in fluorescence intensity of three distinct, pure-chirality DNA-SWCNTs is strongly dependent on the combination of the DNA sequence and specific SWCNT species. This indicates a clear difference in the dynamic behavior of cell culture media interacting with a specific DNA-SWCNT hybrid. In addition, we demonstrated the development of various microarray chip platforms for bioprinting, including a novel 384-pillar plate with four sidewalls and four slits (384PillarPlate), to support organotypic 3D cell cultures for performing various miniaturized biochemical and cell-based assays. Our next step is to use 384PillarPlate to culture organotypic liver cells in biomimetic hydrogels and test the toxicity of DNA-SWCNTs for predicting their adverse responses *in vivo*. This will enable us to predict the long-term potential risk and toxicity of surface-engineered SWCNTs with precise functionalities and facilitate the development of novel nanomaterials for biomedical applications.

Microbial Transformation and Plant Uptake of Multi-Walled Carbon Nanotubes

Y. Yang[1], Y. You[1], K. Das[1], V. Nava[1], J. W. Chan[2], F. Barrios-Masias[3], P. Verburg[3], S. R. Poulson[4], X. Wang[5], X. Wang[6], J. C. Chow[6], B. Xing[7]
1. Department of Civil and Environmental Engineering, University of Nevada Reno, Reno, NV
2. Center for Biophotonics, University of California Davis Medical Center, Sacramento, CA
3. Department of Natural Resources and Environmental Science, University of Nevada Reno, Reno, NV
4. Department of Geological Sciences & Engineering, University of Nevada Reno, Reno, NV
5. Laboratory for Earth Surface Processes, College of Urban and Environmental Sciences, Peking University, Beijing, China
6. Division of Atmospheric Sciences, Desert Research Institute, Reno, NV
7. Stockbridge School of Agriculture, University of Massachusetts Amherst, Amherst, MA

Due to their unique physiochemical properties, carbonaceous nanomaterials are widely used in industry and consumer products, but concerns have been raised regarding their release into the environment and subsequent impacts on ecosystems and human health. Many efforts have been devoted to understanding the environmental fate of carbonaceous nanomaterials, but to date, information about microbial transformation and plant uptake of these emerging contaminants is rare. Our study explored interactions between carbon nanotubes and a polycyclic aromatic hydrocarbon-degrading bacterium, *Mycobacterium vanbaalenii* PYR-1. By applying multiple analytical methods, we found that *M. vanbaalenii* PYR-1 was able to degrade both pristine and carboxyl-functionalized multi-walled carbon nanotubes (MWCNTs), with carboxyl-MWCNT showing greater degradation and a mineralization rate of up to 0.25% carbon per day. In addition, we developed a method for detection of MWCNTs in plant tissues, by coupling digestion and Raman analysis. We then applied this method to lettuce (*Lactuca sativa*) plants grown hydroponically with 0, 5, 10, and 20 mg/L solutions of pristine (p-) or carboxyl-functionalized (c-) MWCNTs. Both p-MWCNTs and c-MWCNTs were detected in the root, stem, and leaf tissues of most exposed lettuce plants, indicating the uptake and translocation of both MWCNTs. For quantification of MWCNTs in plant tissues, a novel method of digestion coupled with programmed thermal analysis (PTA) was developed. Background interferences were minimized by the digestion, and detection limit was achieved at 64.9 μg of CNT-C/g of plant tissues. Spectroscopic analysis has also been used for rapid quantification of CNTs in plant tissues. Our results provided novel insight into the microbial transformation and plant uptake of carbonaceous nanomaterials in a soil-plant system.

Influence of Different Acids on the Transport of CdSe Quantum Dots from Polymer Nanocomposites to Food Simulants

P. J. Gray[1], J. E. Hornick[2], A. Sharma[3], R. G. Weiner[1], T. V. Duncan[1]
1. Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Bedford Park, IL
2. Biological Imaging Facility, Northwestern University, Evanston, IL
3. Department of Food Science and Nutrition, Illinois Institute of Technology, Bedford Park, IL

There is great interest in understanding the impacts polymer nanocomposites (PNCs) may have on human health and the environment. For PNCs that may be used as food contact materials, more information on how food chemistry impacts release of nanoparticles embedded in polymer host materials is needed to generate comprehensive exposure models. We developed model PNCs made from low-density poly(ethylene) and CdSe quantum dots (QDs) to explore how long-term storage in different acidic foods could impact potential consumer exposure. Exposure to three acidic food simulants under accelerated time/temperature conditions at pH = 2.5 was evaluated: citric acid, acetic acid, and hydrochloric acid. While low-level release of dissolved QDs into all the test environments was observed, exposure to dilute acetic acid resulted in the release of more than double the amount of QD mass than exposure to dilute mineral acid at the same pH. Conversely, citric acid suppressed QD release compared to other acidic simulants. Permeation experiments and confocal microscopy revealed mechanistic details underlying these mass transfer phenomena. From these experiments, we conclude that pH is not the sole determinant of consumer exposure to mass deriving from nanoparticles embedded in plastics. This result underscores the complexity of exposure dynamics for nanotechnology-enabled materials and may also have implications for plastics incorporating non-nanoscale fillers.

Polymer/Noble Metal Nanocomposites: A Model System to Assess Exposure to Nanotechnology-Enabled Food Packaging Materials

R. G. Weiner
Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Bedford Park, IL

Polymer nanocomposites (PNCs), in which nanofillers are added into a polymer matrix, can be engineered to show improved barrier properties and increased mechanical strength. PNCs also have potential use as intelligent chemical or biological sensors. With respect to the use of PNCs as food packaging materials, there is a need for additional information about the possibility of release of nanofillers into food or the environment. Many previous studies on nanofiller exposure have used PNCs incorporating polydisperse samples of nanoparticles (NPs), and thus yielded results that can be difficult to interpret. Therefore, more systematic studies of NP release into the environment would be useful. Here, several sizes of Ag NPs were synthesized and mixed into low-density polyethylene (LDPE) to create PNC films with well-controlled nanofiller characteristics. The release of Ag NPs or their components into model food contact substances, such as water and dilute acetic acid, was tested and quantified by ICP-MS, STEM, and optical measurements. To determine the role of NP stability and composition, Ag_2S and Au NP/LDPE PNCs were tested as well. This work is an example of an experimentally controlled lifecycle analysis when it comes to PNCs in the environment.

In Vitro Intestinal Toxicity of Copper Nanoparticles in Rat and Human Cell Models

M. F. Hughes[1], T. E. Henson[2], J. Navratilova[3], A. H. Tennant[1], K. D. Bradham[4], K. R. Rogers[4]
1. U.S. Environmental Protection Agency (EPA), Office of Research and Development (ORD), National Health and Environmental Effects Research Laboratory, Research Triangle Park, NC
2. Student Services Contractor, Research Triangle Park, NC
3. National Research Council, Research Triangle Park, NC
4. U.S. EPA, ORD, National Exposure Research Laboratory, Research Triangle Park, NC

Human oral exposure to copper oxide nanoparticles (NPs) may occur following ingestion, hand-to-mouth activity, or mucociliary transport following inhalation. This study assessed the cytotoxicity of CuO and Cu_2O-polyvinylpyrrolidone (PVP) coated NPs and Cu^{2+} ions in rat (IEC-6) and human intestinal cells, two- and three-dimensional models, respectively. The effect of pre-treatment of CuO NPs with simulated gastrointestinal (GI) fluids on IEC-6 cell cytotoxicity was also investigated. Both dose- and time-dependent decreases in viability of rat and human cells with CuO and Cu_2O-PVP NPs and Cu^{2+} ions was observed. In the rat cells, CuO NPs had greater cytotoxicity. The rat cells were also more sensitive to CuO NPs than the human cells. Concentrations of H_2O_2 and glutathione increased and decreased, respectively, in IEC-6 cells after a 4-h exposure to CuO NPs, suggesting formation of reactive oxygen species (ROS). These ROS may have damaged the mitochondrial membrane of the IEC-6 cells causing a depolarization, as a dose-related loss of a fluorescent mitochondrial marker was observed following a 4-h exposure to CuO NPs. Dissolution studies showed that Cu_2O-PVP NPs formed soluble Cu whereas CuO NPs essentially remained intact. For GI fluid-treated CuO NPs, there was a slight increase in cytotoxicity at low doses relative to non-treated NPs. In summary, copper oxide NPs were cytotoxic to rat and human intestinal cells in a dose- and time-dependent manner. The data suggests CuO NPs have inherent cytotoxicity, without dissolving and forming toxic Cu^{2+} ions, whereas Cu_2O-PVP NPs are toxic due to their dissolution to these ions. (This abstract does not represent U.S. EPA policy.)

Tiered Multiscale Modeling of Population Exposures Resulting from Nanotechnology-Based Products

Z. Mi1[2,4], L. Chao[1,2,3], D. Mukherjee[1,2,3], P. Georgopoulos[1,2,3,4]
1. Computational Chemodynamics Laboratory, Environmental and Occupational Health Sciences Institute, Rutgers University, NJ
2. Department of Environmental and Occupational Health, Rutgers School of Public Heath, NJ
3. Department of Chemical and Biochemical Engineering, Rutgers University, NJ
4. Department of Environmental Sciences, Rutgers University, NJ

Human exposures to manufactured nanoparticles (MNPs) can be the result of both (a) near-field emissions from usage of consumer products containing MNPs and (b) dispersion from far-field emission sources. Significant information gaps in exposure-related data exist for most situations relevant to MNPs. A tiered modeling system, Prioritization/Ranking of Toxic Exposures with GIS (Geographic Information System) Extension (PRoTEGE), has been under continuing development: it employs a product Life Cycle Analysis (LCA) approach coupled with simplified human Life Stage Analysis (LSA) to characterize potential exposures to chemicals of current and emerging concern, including nanomaterials. PRoTEGE utilizes available MNP production, usage, and properties databases, along with laboratory measurements of emissions, to characterize MNP exposures from ambient and indoor sources. It uses probabilistic material flow analysis to account for releases during product manufacturing, transport, usage, disposal, etc. Human exposure and dose characterization further employ screening microenvironmental and intake fraction calculations combined with LSA for potentially exposed populations, to assess differences associated with gender, age, sociodemographics, and climate regions. PRoTEGE is designed for coupling with a novel mechanistic multiscale human toxicokinetic and toxicodynamic modeling system to further characterize risks of MNP exposures. Two case studies are presented as implementations of PRoTEGE, one involving nanosilver (nAg) used in consumer products (near-field emission sources) and another for nanocerium (nCe) used as diesel engine additive (both near- and far-field emission sources), to demonstrate simulations of exposure scenarios for age-stratified U.S. populations.

Detection and Quantification of Engineered Nanoparticles in Surface Waters Impacted by Sewage Spills in Columbia, South Carolina

M. Baalousha, F. Loosli, Z. Yi, J. Wang
Center for Environmental Nanoscience and Risk, Department of Environmental Health Sciences, Arnold School of Public Health, University of South Carolina, Columbia, SC

Whereas there are several analytical techniques suitable for analysis of pristine engineered nanoparticles (ENPs), few analytical approaches are adequate for detection and quantification of ENPs in complex environmental samples. Thus, there is currently a significant knowledge gap in the assessment of environmental exposures (concentrations) of ENPs. This is due to the significant un-met challenges in detection and quantification of ENPs in the natural environment. Measuring ENPs in environmental samples can be impeded by 1) high background concentration of natural nanoparticles (NNPs), 2) low environmental concentrations of ENPs, 3) similarity of the physicochemical properties of ENPs and NNPs, 4) transformation processes altering the properties of ENPs, and 5) underdeveloped methodologies for accurately characterizing ENPs and NNPs with sufficient specificity and sensitivity.

This poster will discuss the quantification of TiO_2 ENPs in surface waters impacted by sewage spills in Columbia, South Carolina, using a multimethod approach to differentiate ENPs from NNPs by tracing their physiochemical properties (e.g., elemental composition, elemental ratios, and morphology). Nanoparticle elemental composition and elemental ratios were determined by inductively coupled plasma-mass spectroscopy (ICP-MS) following total digestion; multi-element single-particle inductively coupled plasma-mass spectroscopy; and X-ray energy dispersive spectroscopy (X-EDS) coupled to transmission electron microscopy (TEM). Nanoparticle morphology was determined by TEM. Our findings suggest that sewage spills result in the release of high concentrations of titanium dioxide ENPs (up to 100 $\mu g\ L^{-1}$) and low concentration of silver ENPs ($< 0.1\ \mu g\ L^{-1}$) to surface waters.

Electrochemical Detection and Characterization of Silver Nanoparticles Towards the Understanding of Nano-Silver Surface Chemistry

J. Sikes, I. Niyonshuti, M. Magness, J. Chen, I. Fritsch
Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR

Silver nanoparticles are capable of releasing silver ions into their surroundings, making them toxic to the surrounding microbiomes. Due to their antimicrobial properties, silver nanoparticles have been heavily incorporated into consumer goods. However, the usage of silver particles in consumer goods has far preceded the understanding of the fundamental chemical properties of the particles themselves. Electrochemical methods can be employed to provide insight into the chemical surface of silver nanoparticles by investigating the interactions of individual nanoparticles with an electrode surface. Individual silver nanoparticles can be detected and analyzed through the usage of chronoamperometry (CA), as the particles produce a brief spike in current upon oxidation at the electrode surface. The signals produced can be explored to obtain information about the kinetics of particle oxidation at the electrode surface.

Changes in the particles' chemical properties produced by changes in the particles' size, shape, solution environment, and capping ligand can be studied by tracking changes in the chronoamperometric peak sizes, shapes, and frequencies of occurrence. Understanding the surface properties and chemistry of silver nanoparticles can provide further insight into the biological properties, particle release potential, and particle dissolution mechanisms of silver nanoparticles. Additionally, *in-situ* release potential of silver particles from consumer goods can be developed once the electrochemical properties of silver nanoparticles are more well understood, due to the ability to detect individual nanoparticles through the usage of electrochemistry.

This research investigates the time-dependent oxidation of silver nanoparticles at a platinum microdisk electrode using silver nanoparticles dispersed in aqueous solutions. The behavior of electrochemical silver nanoparticle oxidation in the presence of potassium chloride (KCl) has been of particular interest due to the biological and environmental abundance of potassium and chloride, as well as the catalytic effects that chloride exhibits on the oxidative dissolution of silver nanoparticles. In addition to KCl, potassium nitrate (KNO_3), and lithium perchlorate ($LiClO_4$) have been employed for the electrochemical oxidation of silver nanoparticles. The effect that changing electrode potential, electrolyte composition, electrolyte concentration, particle shape, and capping ligand have with regards to the time-dependent oxidation is being investigated, and preliminary data will be presented. Additionally, the average quantity of silver ions released upon oxidation of each individual particle under the experimental conditions will be presented.

Quantitative Evaluation of Nanomaterial Release from Multi-Walled Carbon Nanotubes Epoxy Composite after Weathering Treatment

Y. Zhao[1], G. Ramakrishnan[2], D. Goodwin[3], L. Sung[3], E. Petersen[4], A. Orlov[2]
1. Department of Chemistry, Stony Brook University, Stony Brook, NY
2. Department of Materials Science and Chemical Engineering, Stony Brook University, Stony Brook, NY
3. Engineering Laboratory, National Institute of Standards and Technology (NIST), Gaithersburg, MD
4. Material Measurement Laboratory, NIST, Gaithersburg, MD

Carbon nanotubes (CNTs) exhibit high thermal and electrical conductivity, flexibility, stiffness, tensile strength, and light weight. For this reason, CNTs have been employed as additives or nanofillers to enhance the properties of polymeric materials. Several studies have shown that the polymer matrix degradation can occur as a result of environmental factors such as ultraviolet (UV) radiation, moisture, mechanical stress, and/or a combination thereof. Such degradation can lead to surface accumulation, protrusion, and potential release of CNTs. However, only few studies have quantified the rate of CNT release. In this study, we investigated the effect of environmental factors on the degradation of MWCNT/epoxy nanocomposites with a MWCNT content of 1.0% by mass. Chemical changes resulting from epoxy matrix degradation were investigated by attenuated total reflectance infrared spectroscopy (ATR-FTIR), and morphological changes of the samples were assessed by scanning electron microscopy (SEM). In order to determine the rate of CNT release, we incorporated 14C-labelled MWCNTs into the nanocomposites and measured their release with liquid scintillation counting (LSC) after weathering treatment. Degradation of epoxy resulted in matrix removal, exposure of CNT network, and release of detectable amounts of CNTs to the environment.

Fluorescent Cellulose Nanomaterials: Preparation, Characterization and Application

I. Patel[1,2], J. Woodcock[2], J.A. Shatkin[3], S. Stranick[2], S. Harper, J. Gilman[2], D. Fox[1]
1. Department of Chemistry, American University, Washington, DC
2. Materials Measurement Laboratory, National Institute of Standards and Technology, Gaithersburg, MD
3. Vireo Advisors, Boston, MA
4. School of Chemical, Biological, and Environmental Engineering, and Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR

Cellulose nanomaterials (CNs) have received significant amounts of attention recently due to their unique combination of features: renewability and large application space. However, the availability of data regarding the impact of CNs to human health and environmental safety is deficient. A reliable characterization methodology, to locate and track these materials *in vivo* and *in vitro*, is based on use of fluorescently labelled CNs. To address this issue, a new fluorescent probe has been developed as an easily attached, high quantum yield, photo-stable, pH-resistant, chemically stable, and non-toxic fluorescent tag. The cellulose nanofibrils (CNFs) were surface modified using fluorescent probe in alkaline condition at room temperature. In this work, time-correlated single photon counting fluorescence lifetime microscopy (FLIM) is used to assay commercially produced CNFs and their purity. Preliminary results show that labeling chemistry preferentially reacts with bound lignin over hydroxy group of CNFs. This indicates that chemical composition of CN and the method applied to produce CN have a significant impact and can easily lead to erroneous results in subsequent studies. For ecotoxicity purpose, the fluorescent CNF were exposed to embryos of zebra fish. No change in toxicity can be noted following conjugation of CNF with the mDTEB fluorophore. As a result, we have developed a labeling strategy for cellulose that remains attached and stable across the varying pH of the digestive tract.