UC Center for Environmental Implications of Nanotechnology

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The mission of the University of California Center for Environmental Implications of Nanotechnology (UC CEIN)[1] is to ensure that nanotechnology is introduced in a responsible and environmentally safe manner, thereby allowing the US and International Communities to leverage the benefits of nanotechnology for global economic and social benefit. This mission is being accomplished by developing decision tools based on models of predictive toxicology premised on nanomaterial properties that determine fate, transport, exposure, and biological injury in cells, organisms, and populations. Since its founding in September 2008, the UC CEIN has successfully integrated the expertise of engineers, chemists, material scientists, ecologists, marine biologists, cell biologists, bacteriologists, toxicologists, computer scientists, biostatisticians, and social scientists to develop a predictive platform that informs about engineered nanomaterial (ENM) hazards and how exposure reduction, lifecycle analysis and safe-by-design strategies can reduce the environmental impact of nanotechnology

The key components of the *predictive and inter-related scientific models* include: (i) the establishment of ENM libraries based on a consideration of production volumes and the material types most likely to come into contact with the environment; (ii) ENM distribution to the environment as determined by modes of release, physicochemical and transport properties, contact and interaction with biological receptors; (iii) representative ecological life forms serving as early sentinels to monitor the spread and bio-accumulation of hazardous ENMs; (iv) mesocosm, population, and dynamic energy budget theory of freshwater, marine, and terrestrial environments; (v) high throughput screening assays using cells, bacteria, embryos, etc., to generate information about hazardous ENM properties (included in compositional and combinatorial ENM libraries) to plan environmental health effect studies on increasing complex organisms in the environment; and (vi) a series of *in silico* decision making tools and computational models to predict ENM interactions at the nano-bio interface and in the environment. These research activities are being combined with educational programs to inform the public, future generations of scientists, public agencies, and industrial stakeholders of the impact of our work.

The research of the UC CEIN is carried out by 44 distinct but interactive research projects across seven interdisciplinary research themes:

- Theme 1: Compositional and Combinatorial ENM Libraries for Property-Activity Analysis
- Theme 2: Molecular, Cellular, and Organism High-Throughput Screening for Hazard Assessment
- Theme 3: Fate, Transport, Exposure, and Life Cycle Assessment
- Theme 4: Terrestrial Ecosystems Impact and Hazard Assessment
- Theme 5: Marine and Freshwater Ecosystems Impact and Toxicology
- Theme 6: Environmental Decision Analysis for ENMs
- Theme 7: Societal Implications, Risk Perception, and Outreach Activities

One of the major accomplishments of the Center to date has been the synthesis, characterization, and implementation of metal oxide libraries across the Center, allowing a series of multidisciplinary and integrated efforts that have generated a major body of knowledge regarding the environmental behavior of these materials. The major impacts of these collaborative efforts have been reviewed in our ACS Nano Focus review (Vol 5, 13-20, 2011). Not only is it possible to develop reproducible physicochemical characterization and assessment of dispersion of metal oxide ENMs in multiple biological and environmental use media, but we were also able to develop a number of dispersing agents to conduct biological experiments with well-dispersed and agglomerated ENMs. Through the implementation of high throughput screening and assessment of appropriate injury pathways in mammalian and fish tissue culture cells as well as bacteria, it is possible to perform hazard ranking of these materials. We can also compare the *in vitro* screening results with biological outcomes in a variety of environmental life forms, including phytoplankton, oyster embryos, algae, and zebrafish embryos. Finally, the high volume data sets that are being generated have allowed us to develop *in silico* software tools that allow feature recognition, hazard ranking, and establishment of quantitative property-activity analysis.

Noteworthy research accomplishments in the past year include:

- Theme 1 (Core B) maintains a library of more than 100 different ENMs that provide the basis for mechanistic and high throughput studies designed to study the mechanisms cellular, organism, and ecosystem toxicity. Characterization of the materials is actively conducted as they are synthesized or acquired.
- Theme 1 developed a new compositional library containing 24 metal oxide ENMs that will project the valence and conduction band energies in terms of biological redox potentials in mammalian and bacterial systems.
- Theme 1 established a silica library to study the impact that surface reconstruction and silanol surface density display on toxicity, including showing that fumed silica is the most toxic of the materials.
- Theme 2 demonstrated that our high content multiparametric assay can be advanced to the level of HTS to screen large batches of ENMs. This assay allows us to assess cytotoxicity, oxidative stress and organellar injury in response to a diverse number of NMs that are capable of stimulating ROS generation, toxic ion release, lysosomal and mitochondrial injury. Our cellular hazard ranking was also compared to screening for zebrafish embryo toxicity. While ZnO, Pt, and quantum dots were equally hazardous in embryos as in the mammalian cells, nano-Ag induced severe toxicity in zebrafish embryos but showed little effect on mammalian cells.
- Working with feature selection tools developed by Theme 6, Theme 2 established the use of self organizing maps (SOM) that allow comparison of similarities between different ENM types as well as response differences between different cells types submitted to HTS. Out of an initial set of 14 fundamental particle physicochemical parameters, four descriptors emerged that could predict cytotoxicity (atomization energy, periodicity, NP primary size, and NP volume).
- Theme 2 used zebrafish embryos and larvae to perform toxicological screening of the effects of ZnO, CuO, NiO, and Co_3O_4. While the zebrafish embryo studies failed to demonstrate a relationship to oxidative stress, ENM solubility and shedding of transition metals were well correlated to interference in embryo hatching. The more soluble materials (ZnO, Cu, NiO) disrupted embryo hatching due to shedding of metal ions that interfere in the hatching enzyme. A major development during the execution of these studies has been the development and implementation of high content brightfield and fluorescence-based imaging platforms for analysis of hatching interference and morphological abnormalities in embryos as well as transgenic larvae.
- Fate and transport studies have shown that natural organic matter (NOM), in the form of humic acid or alginate, can stabilize metal oxides such as CuO, metallic ENMs such as Pd and Pt, as well as carbonaceous ENMs like CNTs. NOM reduces the attachment of metal oxide ENMs to mineral surfaces, even if they eventually aggregate and deposit in sediments. NOM plays a role in facilitating transport of metal oxide ENMs (specifically TiO_2) in groundwater, except under high

ionic strength conditions. The implication of this increased stability and mobility is that these ENMs will be more bioavailable in the presence of NOM.

- Theme 3 explored the effect of different particle characteristics on aggregation, transport and photoactivity. Studies of ZnO showed morphology plays an important role in the ability to predict aggregation, with spherical ZnO behaving closer to theoretical predictions than plate ZnO ENMs which aggregate under all conditions. Sixteen different TiO_2 NPs are currently being studied to evaluate the effect of size, shape (spheres, wires/rods, nanodots) and crystalline structure (anatase, rutile, and combinations). TiO_2 nanowires aggregate more than nanospheres and nanodots under all conditions. For rods, increased size leads to higher aggregation. Hydroxyl radical production is greatest in spheres and wires, while nanodots have limited photoactivity. These results highlight the need to incorporate additional NP characteristics into our fate and transport modeling.
- To understand the effects of ENMs on soil processes, Theme 4's work with unplanted soil microcosms was expended to reveal which taxa are impaired by exposure to ZnO and TiO_2. Using a sensitive photosequencing approach, functionally-narrow taxa were discovered to be particularly sensitive to these ENMs. Specifically we discovered that free-living-N_2-fixing taxa, methane-oxidizing taxa, and other taxa known for their important roles in organic matter decomposition were relatively less abundant as a consequence of metal oxide NP exposures. By studying these effected taxa in mechanistic and HTS bacterial studies, we can now determine if these taxa are sentinel taxa that may be used as bioindicators of ENM environmental impacts.
- Theme 4 has greatly expanded our work with hydroponic plants to include corn, cucumber, and native mesquite. For example, results from the absorption, distribution, and possible biotransformation of Zn in mesquite treated with ZnO showed that this material increased catalase activity (CAT) in roots, stems, and leaves, while ascorbate perioxidase (APX) increased only in stems and leaves. Combined with studies on corn and cucumber, we are generating a significant body of knowledge regarding plant uptake, bioprocessing, and effects of metal oxide ENMs.
- Theme 4 has expanded the studies of ENM effects on bacteria, integrating five assays (ROS, superoxide, membrane potential, dehydrogenase activity, and membrane activity) and optimizing their use as a system. We discovered that metal oxides were inhibiting *E.coli* population growth by ROS-mediated membrane damage. We also observed membrane potential loss and increased electron transport activity - the latter of which is an indication of cellular stress. This system of assays is currently being translated to a HTS platform within the MSSR high-throughput screening facility at UCLA. The Dynamic Energy Budget (DEB) models of bacteria have matured to include ROS as a variable. Application of this model to Cd(II) toxicity to bacteria will be applicable to many bacterial-nanomaterial combinations.
- Theme 5 launched marine organism studies to explore the mechanisms by which CNT cause injury to marine mussel hemocytes have been carried out. *In vitro* exposure of hemocytes to CNTs causes increased production of ROS and in some cases can impair the phagocytes ability to phagocytose foreign particles. The effects occur at different concentrations, dependent on the type of CNT exposed.
- Theme 5 exposed two species of marine phytoplankton to nano-ZnO, CuO, and Ag to examine multiple cytotoxicological endpoints. All the materials tested were found to have a negative impact at some concentration, although the response mechanisms were different for each species and material. For example ZnO had significant impact on the mitochondrial membrane potential of *Dunaliella*, whereas in *Isochrysis* there was no mitochondrial effect but membrane damage was observed. Response differences are related to differences in cell wall structure

and/or physiology and future experiments are being designed to identify specific morphological/physiological mechanisms involved. ZnO exposure was found to significantly reduce rates of phytoplankton growth at concentrations ranging from 230-1000 ug L-1, while Ag and CuO were much less toxic, reducing growth rates only at relatively higher concentrations due to slower dissolution rates.

- Theme 6 developed a classification-based cytotoxicity nano-SAR based on a set of nine metal oxide ENMs. This classification nano-SAR enables the identification of decision boundaries which are crucial for use in hazard ranking of ENMs and are being incorporated into our ongoing environmental impact analysis (EIA). Collaborations with Themes 1 and 2 suggest that band gap energy is an important descriptor to consider in the prediction of toxicity of metal oxides.
- Detailed theoretical calculations of ENM agglomeration by Theme 6 demonstrated decreased average size and a larger tail of smaller aggregates with increasing primary particle size. This suggests that exposure to a larger concentration of smaller aggregates may occur with increased primary size. Expanded computer based simulations are in progress to map out this behavior. Computations are based on a novel Constant-Number Monte-Carlo/DLVP ENM agglomeration model that accounts for particle sedimentation. Our model is the first to quantitatively predict the agglomeration state of ENMs which we validated via comparison of predictions with literature reported data for TiO_2, CeO_2, and C_{60} showing good agreement over a wide range of environmental conditions. These model simulations are being incorporated into our environmental impact analysis methods and multimedia fate and transport modeling.
- Theme 7 conducted a study of environmental risk management and regulation in the global ENM industry to look at the relationship between industry leaders' perceived risk, attitudes about regulation, and their companies' safety practices. There is a high degree of contradiction between beliefs and practices within industry, which highlights the lack of effectiveness in published guidance documents for workplace ENM safety.
- In a survey of ENM experts (material engineers, nano EHS researchers, and regulators), Theme 7 demonstrated that there are significant and consistent differences in the views of ENM risk across these groups. Regulators were found to be most risk averse and nanoscience engineers most risk tolerant. All groups viewed regulatory agencies as "unprepared" to manage the risk of ENMs. The results have drawn significant interest from government, EH&S researchers, and the NNI.

A major goal of the UC CEIN is to train the next generation of nano-scale scientists, engineers, and regulators to anticipate and mitigate potential future environmental hazards associated with nanotechnology. Our educational programs are developed to broaden the knowledge base of the environmental implications of nanotechnology through academic coursework, world-class research, training courses for industrial practitioners, public outreach, and a journalist–scientist communication program.

Over the past year, we have focused our attention on activities that will both enhance the mentoring and training that our students and postdoctoral researchers receive, as well as developing impactful ways of communicating our research findings to the broader scientific, educational, and policy communities, both nationally and internationally.

The UC CEIN has developed a 13 lecture online course in Nanoecotoxicology. The course provides an introduction of the multidisciplinary research in the Center, and includes presentations on ENM: manufacturing; physicochemical properties; fate and transport in the environment; impact on cells, organisms, populations, and stability of ecosystems; synthetic approaches to reduce biological harm;

and the social and governance implications of nanotechnology. The course is supplemented by a series of assigned readings and includes quizzes to aid in knowledge evaluation. After a pilot testing of the course by students and faculty at CINVESTAV in Mexico City, we conducted a 5 day intensive workshop where a dozen researchers from Mexico and the US spent a week learning the fundamentals behind the lectures to allow them to integrate aspects of the course into their own curriculum. The online course is available to all CEIN partner institutions and will be made available more broadly to US institutions. In Winter 2012, UC Santa Barbara is offering the online course for credit to Graduate Students in the Environmental Sciences, and Northern Arizona University is incorporating a number of the lectures into an undergraduate Chemistry course being taught by Professor Marin Robinson.

Career skills assessment and training is a key part of the Center's Postdoctoral Training plan, and our mentoring and skills development activities are offered to our Center postdoctoral researchers, graduate students, and technical staff from all of our partner institutions. In March 2012, we hosted a 2-day retreat for 24 of our trainees with a focus on "The Academic Job Search." Additional workshops this year have included "Communicating your Science to the Public" hosted by Sharon Dunwoody of the University of Wisconsin, "Searching for Postdoctoral Research Positions" hosted by Hilary Godwin and David Avery, and "Writing Science" by Joshua Schimel from UC Santa Barbara which focuses on how to write impactful research papers and research proposals. Additionally, we implemented a cross-campus student seminar series where trainees conduct seminars on their personal research projects to audiences across themes and campuses. In May 2011, we co-hosted the 3rd International Conference on Environmental Implications of Nanotechnology (with CEINT), held at Duke University. Over 3 days, more than 200 participants from academia, government, and industry came together to review the latest advances in environmental ENM research. UC CEIN trainees presented nine research talks and sixteen posters over the course of the meeting.

Our Public Outreach programs are strengthening partnerships with the California Science Center, the Santa Monica Public Library, the California NanoSystems Institute at UCLA, and the Brentwood School (K-12). Our 2011 events, which include Nano Days 2011 at the California Science Center, "Nanotechnology: Small is Big" lecture series at the Santa Monica Library, participation in the CNSI Art/Sci program and high school nanotechnology summer institute, and the "Explore Your Universe" public science day at UCLA, helped us to reach over 1000 K-12 students and several hundred adults through our demonstrations and lectures. The focus of all of these activities is to introduce the concepts of nanotechnology and discuss the potential effects of ENM in our environment.

The UC CEIN continues to play an active role in using our science and discovery to assist State and National decision makers in the governance of nanotechnology safety. We have partnered with the University of California Environmental Health and Safety Division and the California Department of Toxic Substances Control to assist the State in the evaluation of the California statewide call-in on carbon nanotubes , and provided advice on strategies for future call-ins for metal oxides. We have also developed and are testing guidance for the safe handling of ENMs in academic laboratory settings through our partnership with the California Nano EH&S working group. Once tested, this guidance will be made available to academic institutions nationwide.

Select Publications - 2011

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[1]For additional information about the Center, its projects, and a full list of publications, please visit http://www.cein.ucla.edu.

[2]The UC CEIN is headquartered at UC Los Angeles in partnership with UC Santa Barbara, UC Davis, UC Riverside, Columbia University, University of Texas El Paso, University of New Mexico/Sandia National Laboratory, Northwestern University, Nanyang Technological University (Singapore), the Molecular Foundry at Lawrence Berkeley National Laboratory, Lawrence Livermore National Laboratory , the University of Bremen (Germany), University of British Colombia (Canada), Cardiff University (Wales), University College Dublin (Ireland), and the Universitat Rovira i Virgili (Spain).