Nanomaterials and the Environment & Instrumentation, Metrology, and Analytical Methods

Report of the National Nanotechnology Initiative Workshop
October 6–7, 2009

nanoEHS

About the Nanoscale Science, Engineering, and Technology Subcommittee

The Nanoscale Science, Engineering, and Technology (NSET) Subcommittee is the interagency body responsible for coordinating, planning, implementing, and reviewing the National Nanotechnology Initiative (NNI). The NSET is a subcommittee of the Committee on Technology of the National Science and Technology Council (NSTC), which is one of the principal means by which the President coordinates science and technology policies across the Federal Government. The National Nanotechnology Coordination Office (NNCO) provides technical and administrative support to the NSET Subcommittee and its working groups in the preparation of multiagency planning, budget, and assessment documents, including this report. More information is available at http://www.nano.gov.

About the National Nanotechnology Initiative

The National Nanotechnology Initiative is the Federal nanotechnology R&D program established in 2000 to coordinate Federal nanotechnology research, development, and deployment. The NNI consists of the individual and cooperative nanotechnology-related activities of 25 Federal agencies that have a range of research and regulatory roles and responsibilities. The goals of the NNI fourfold: (1) to advance a world-class nanotechnology research and development program; (2) to foster the transfer of new technologies into products for commercial and public benefit; (3) to develop and sustain educational resources, a skilled workforce, and the supporting infrastructure and tools to advance nanotechnology; and (4) to support responsible development of nanotechnology.

About the Nanotechnology Environmental and Health Implications Working Group

The NSET Subcommittee and its Nanotechnology Environmental and Health Implications (NEHI) Working Group provide leadership in establishing the NNI environmental, health, and safety (EHS) research agenda and in communicating data and information related to the environmental and health aspects of nanotechnology between NNI agencies and with the public. NNI activities support the development the new tools and methods required for the research that will enable risk analysis and assist in regulatory decision making.

About the Report

This document is the Executive Summary of the report of a workshop held October 6–7, 2009. This was the second in a series of four workshops sponsored by the NSET Subcommittee to inform the NNI's long-range planning efforts for environmental, health, and safety research. Any ideas, findings, conclusions, and recommendations presented in this report are those of the workshop participants. This document was designed, assembled, and edited by NNCO staff.

About the Cover

Cover design is by Kathy Tresnak of Koncept, Inc. Book design by staff members of the National Nanotechnology Coordination Office (NNCO). The cover background is a false-color scanning tunneling microscopy image revealing the atomic-scale electronic perturbations caused by a lattice defect in bilayer graphene (courtesy of Joseph Stroscio, National Institute of Standards and Technology, http://cnst.nist.gov).

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Nanomaterials and the Environment, & Instrumentation, Metrology, and Analytical Methods

Report of the National Nanotechnology Initiative Workshop

October 6–7, 2009, Arlington, VA

Part II of IV in the 2009–2010 NNI Environmental, Health, and Safety Workshop Series

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Sponsored by
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Committee on Technology
Subcommittee on Nanoscale Science, Engineering, and Technology

Acknowledgments

The many individuals listed below dedicated considerable time and expertise to make the NNI Nanomaterials and the Environment & Instrumentation, Metrology, and Analytical Methods Workshop a reality and to write and produce this report.

Workshop Planning Team:

- Phil Sayre, Co-Chair (EPA)
- Dianne Poster, Co-Chair (NIST)
- David Andrews (Environmental Working Group)
- John Cowie (American Forest & Paper Association)
- John Gannon (DuPont)
- Rebecca Klaper (School of Freshwater Sciences, University of Wisconsin, Milwaukee)
- Alan Roberson (American Water Works Association)
- Paul Westerhoff (Arizona State University)

This team was responsible for all essential groundwork for the event, and it wrote and reviewed the report.

Workshop Presenters: Pedro Alvarez, David Andrews, David Arthur, Don Baer, William Ball, Gary Casuccio, Shaun Clancy, Lisa DeLouise, Stephen Diamond, Hendrik Emons, Horacio Espinosa, Howard Fairbrother, Vince Hackley, Richard Handy, Daniel Herr, R. David Holbrook, William Johnson, Jae-Hong Kim, Rebecca Klaper, Greg Lowry, Rajan Menon, Gregory Meyers, Ann Miracle, John Monica, Michael Postek, Alan Roberson, Leonard Robinson, Alexander Star, Keith Swain, Robert Tanguay, Paul Tratnyek, Ron Turco, Mark Weisner, and Steve Wilson shared their expert perspectives with workshop participants on the state of the science in nanotechnology-related environmental and instrumentation and metrology research (for affiliations, see Appendix B).

AAAS Fellows: Allegra da Silva, William Miller, Jami Montgomery, Meghan Radtke, Gina Schatteman, and David Tobias took substantive notes at the workshop breakout sessions.

Support Staff: Staff members of the National Nanotechnology Coordination Office (NNCO) executed the planning and organization of the workshop and production of the report. In particular, Heather Evans and Liesl Heeter supported the workshop committee and handled workshop logistics along with Halyna Paikoush. Liesl Heeter is series editor for the NNI Environmental, Health, and Safety reports. Marlowe Epstein, Patricia Foland, Geoff Holdridge, Pat Johnson, Paul Lagasse, Lapedra Tolson, and Ken Vest assisted at the workshop. Kristin Roy formatted the report; Pat Johnson copyedited it; and Kathy Tresnak of Koncept, Inc., designed the cover. Norris Alderson consulted on the development of the report.

Sponsor: The members of the National Science and Technology Council's Subcommittee on Nanoscale Science, Engineering, and Technology (NSET) sponsored the workshop and reviewed the draft report before its publication. The members of the NSET Subcommittee's Nanotechnology Environmental and Health Implications (NEHI) Working Group were particularly involved in planning and realizing the workshop and in vetting the report.

Thanks are due to all the participants in the October 6–7, 2009, workshop, held in Arlington, VA. The substance of the workshop depended upon the thoughtful engagement of the speakers, moderators, and participants, whose presentations and discussions provide the foundation for this report.

Preface

Nanotechnology holds the promise of exciting new solutions to critical scientific, industrial, and commercial challenges through the engineering of application-specific nanomaterials. Some of these applications are entering the global marketplace, and as with any emerging technology, they are raising questions about potential benefits as well as potential risks from nanotechnology to the environment and to human health. In order to foster a better scientific understanding for answering these questions, the National Nanotechnology Initiative has made environmental, health, and safety research an essential component of its research and of U.S. efforts to be the world leader in nanotechnology.

The benefits from the field of nanotechnology and public acceptance of nanotechnology-enabled products will depend on a reliable scientific capability to asssess and manage potential hazards to the environment and to human health. Developing this capability requires a national effort that brings together scientists from many disciplines within the Federal Government and withouth, through the Government's public-private partnerships with academia, industry, and public health and environmental advocates. To that end, the Nanotechnology Environmental and Health Implications (NEHI) Working Group of the National Science and Technology Council's Nanoscale Science, Engineering, and Technology (NSET) Subcommittee created an adaptive management process in its 2008 Strategy for Nanotechnology-Related Environmental, Health, and Safety Research, which called for holding public workshops on the state of the science.

This document reports on discussions at the Nanomaterials and the Environment & Instrumentation, Metrology, and Analytical Methods Workshop held October 6-7, 2009. This was the second in a series of four public workshops organized by a multi-sector planning team that drew participation from government agencies, academia, citizens, industry, nongovernmental organizations, and other stakeholders for a robust discussion on the state of the science for engineered nanomaterials and the environment and accompanying requirements in instrumentation, metrology, and analytical methods. The proceedings from these workshops will inform the NSET Subcommittee and the NEHI Working Group in adaptively managing the process to refine the NNI EHS Research Strategy, which in turn informs the nanotechnology research agendas of the NNI's Federal agency members.

On behalf of the NSET Subcommittee, we thank the workshop co-chairs and the members of the planning team for organizing this workshop and leading the preparation of this report. Our sincere thanks also go to all the speakers, moderators, and participants for their many excellent contributions to the workshop and to this report.

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About the 2009–2010 NNI Series of EHS Workshops and Reports

From February 2009 to March 2010, the Nanoscale Science, Engineering, and Technology (NSET) Subcommittee of the Committee on Technology of the National Science and Technology Council sponsored a four-part series of workshops to solicit stakeholders' input on the National Nanotechnology Initiative (NNI) strategy to address potential environment, health, and safety (EHS) implications of nanotechnology research, development, and deployment:

- Human and Environmental Exposure Assessment
  February 24–25, 2009, Bethesda, MD
  Website: http://www.nano.gov/events/meetings-workshops/exposure
- Nanomaterials and the Environment, & Instrumentation, Metrology, and Analytical Methods
  October 6–7, 2009, Arlington, VA
  Website: http://www.nano.gov/events/meetings-workshops/environment
- Nanomaterials and Human Health, & Instrumentation, Metrology, and Analytical Methods
  November 17–18, 2009, Arlington, VA
  Website: http://www.nano.gov/events/meetings-workshops/humanhealth
- Risk Management Methods, & Ethical, Legal, and Societal Implications of Nanotechnology
  (Capstone Meeting), March 30–31, 2010, Arlington, VA
  Website: http://www.nano.gov/events/meetings-workshops/capstone

The interagency NSET Subcommittee's Working Group on Nanotechnology Environmental and Health Implications (NEHI) led the organization and management of the workshop series, with active participation from stakeholders in academia, industry, nongovernmental organizations, and the general public. Three NNI EHS documents released by the NEHI Working Group for public review provide a backdrop to the 2009–2010 EHS workshops; all are available at http://www.nano.gov/.

1. *Environmental, Health, and Safety Research Needs for Engineered Nanoscale Materials* (2006) evaluated the state of the science and grouped EHS research into five categories: (1) Instrumentation, Metrology, and Analytical Methods; (2) Nanomaterials and Human Health; (3) Nanomaterials and the Environment; (4) Human and Environmental Exposure Assessment of Nanomaterials; and (5) Risk Management Methods. It also described principal research needs within each category.

2. *Prioritization of Environmental, Health, and Safety Research Needs for Engineered Nanoscale Materials: An Interim Document for Public Comment* (2007) was intended to elicit comments from the public, the scientific community, and other stakeholders on how the NSET Subcommittee proposed to approach prioritization of environmental, health, and safety research needs.

3. *Strategy for Nanotechnology-Related Environmental, Health, and Safety Research* (2008) incorporated input from the 2007 prioritization document. The 2008 strategy describes an adaptive management approach for interagency efforts to address EHS implications of nanotechnology, including identifying priority research needs, assessing existing research, analyzing strengths and weaknesses, and periodically updating and revising the strategy. It provides information to agencies that conduct and fund research on nanotechnology. It informs those agencies on critical research needs, and it facilitates collaborative research activities to address those critical research needs.

As part of its adaptive management of the NNI interagency nanotechnology-related EHS research strategy (NNI EHS Research Strategy, the NSET Subcommittee's objectives are to review the state of the science, identify critical gaps, and inform the updating of the strategy, taking into account research advances made in the United States and abroad and the evolving needs of regulatory decision makers. The goals of the NNI EHS Research Strategy are to support nanotechnology risk assessment and risk management, to advance EHS research, and to develop adequate and timely EHS guidelines and regulations so that nanotechnology R&D is sustainable and of long-term benefit to the nation and the world. All four EHS workshops and their proceedings inform the 2011 update of the U.S. Federal Government's NNI EHS Research Strategy.

Table of Contents

Executive Summary

Engineered nanomaterials are already being used in products in the global marketplace, and new nanomaterials continue to be proposed for commercialization. Knowledge of environmental exposure and potential hazards of nanomaterials in the environment allows for the evaluation of risk and for the establishment of appropriate measures to avoid and mitigate risk and to maximize benefit to the environment from nanomaterials. Addressing gaps in our knowledge of the environmental effects caused by nanomaterials will help ensure the safety and health of the environment and to support greater public acceptance of nanotechnology-enabled products. For regulatory agencies to be able to make science-based risk assessment and risk management decisions regarding nanomaterials, it will be necessary to effectively evaluate the fate of engineered nanomaterials in the environment and the potential consequences for environmental receptors of exposure to nanomaterials. This research will require the development of instrumentation, metrology, and analytical methods with the appropriate sensitivity and specificity.

The Nanoscale Science, Engineering, and Technology (NSET) Subcommittee and its Nanotechnology Environmental and Health Implications (NEHI) Working Group organized the October 6–7, 2009, workshop on Nanomaterials and the Environment, & Instrumentation, Metrology, and Analytical Methods to address two of the five priority categories of EHS research laid out in the 2008 *NNI Strategy for Nanotechnology-Related Environmental, Health, and Safety (EHS) Research* (NNI EHS Research Strategy). This workshop was the second in a four-part series aimed at informing and updating the 2008 NNI EHS Research Strategy through dialogue among government agencies, citizens, academia, industry, nongovernmental organizations, and other stakeholder groups. The workshop objectives were to review the state of the science regarding the potential environmental, health, and safety implications of engineered nanomaterials; to identify critical gaps and barriers to advancing the science; and to identify emerging trends and research needs in both the environmental and instrumentation categories. More than 150 stakeholders and Federal staff participated, and 35 viewers joined from other locations via the webcast plenary sessions.

2008 NNI EHS Strategy Research Needs Addressed in the October 2009 Workshop

The October 2009 workshop addressed two of the categories EHS research needs defined in the 2008 NNI EHS Strategy, as listed below (see also Appendix C). Participants analyzed the appropriateness, completeness, and priority order of these needs.

Nanomaterials and the Environment ("ENV") Research Needs (2008)

1. Understand the effects of engineered nanomaterials in individuals of a species and the applicability of testing schemes to measure effects
2. Understand environmental exposures through identification of principal sources of exposure and exposure routes
3. Determine factors affecting the environmental transport of nanomaterials
4. Understand the transformation of nanomaterials under different environmental conditions
5. Evaluate abiotic and ecosystem-wide effects

Instrumentation, Metrology, and Analytical Methods ("IMA") Research Needs (2008)

1. Develop methods to detect nanomaterials in biological matrices, the environment, and the workplace
2. Understand how chemical and physical modifications affect the properties of nanomaterials
3. Develop methods for standardizing assessment of particle size, size distribution, shape, structure, and surface area
4. Develop certified reference materials for chemical and physical characterization of nanomaterials
5. Develop methods to characterize a nanomaterial's spatio-chemical composition, purity, and heterogeneity

Summary of Overarching Themes for Research Needs: Gaps, Barriers, and Recommendations

Most of the research needs identified by participants were present in the 2008 NNI EHS Research Strategy: these included developing target areas such as a strategy for describing materials; standard characterization methods; methods to describe dosages or concentrations; reference materials that can be widely distributed; methods to measure engineered nanomaterials and their environmental transformation products in the environment and in organisms; models for realistic fate and exposure levels over time; and more effects research, including effects for low exposures and chronic assays, and more holistic perspectives on larger-scale impacts.

In considering the ENV and IMA research needs in light of the current state of the science, the participants' discussions and recommendations at times went beyond the stated research needs of the 2008 NNI EHS Research Strategy: these included identifying regulatory needs and consumer protections, measuring engineered nanomaterials in products, documenting unintended releases from products, conducting life cycle analyses of materials released into the environment, reporting engineered nanomaterials in manufacturer's products, considering the waste streams from engineered nanomaterial products, and proactively addressing worker safety. In addition, there was interest in compiling an inventory of engineered nanomaterials being developed and in incorporating better methods for sharing research with the public, including when engineered nanomaterials are found to be nontoxic.

Nanotechnology Terminology Used in this Report

Nanotechnology is the understanding and control of matter at dimensions between approximately 1 and 100 nanometers, where unique phenomena enable novel applications. Encompassing nanoscale science, engineering, and technology, nanotechnology involves imaging, measuring, modeling, and manipulating matter at this length scale.

–*NNI Strategic Plan*, December 2007 (http://www.nano.gov/NNI_Strategic_Plan_2007.pdf)

Usage Note

Throughout this report, the expression *engineered nanomaterials* is used to describe non-naturally occurring nanomaterials, which best reflects remarks made at the time of the workshop and is the expression still in use among the EHS community. Since the workshop, the International Standardization Organization (ISO) has adopted core terms, including specific definitions for *engineered* and *manufactured* nanomaterials. (ISO/TS 80004-1:2010, available at http://cdb.iso.org/).

The Overarching Themes for ENV and IMA Research Needs

The following eight overarching themes for EHS research needs in the ENV and IMA areas emerged clearly from the workshop discussions. As described below, these themes summarize the participants' opinions regarding (a) the major gaps and barriers to advancing knowledge of risks of environmental exposures and potential hazards of nanomaterials (including both ENV and IMA research needs), and (b) the participants' recommendations on addressing those gaps and barriers.

1. **Develop new methods for detecting and tracking engineered nanomaterials in the environment.** Methods for detecting and tracking nanomaterials are important for the assessment of potential fate and exposures, thus leading to a better understanding of potential risks. Such understanding is limited by our inability to detect many types of engineered nanomaterials in a background environmental matrix. Also, methods

are needed immediately to monitor the release of nanomaterials from production facilities and commercial products, because engineered nanomaterials and nanotechnology-enabled products are already in production and available to consumers. Some detection methods from the colloid research community can be adopted, where applicable to separation and concentration of engineered nanomaterials (e.g., flow field fractionation / inductively coupled plasma mass spectroscopy or FFF/ICP-MS), but these methods need refinement, validation, and application to environmental and biological matrices in which nanoparticles are present. Development of analytical tools is needed for the automated characterization of engineered nanoparticles by electron-beam analysis methods. Development of automated microscopic methods for the rapid analysis and screening of a large number of nanomaterials is vital for real-time monitoring in the manufacturing environment. Accurate correlations of electron microscopy with other size-measurement techniques, such as dynamic light scattering or field flow fractionation, are critical for scientists in the field. Additional particle-labeling methods are also needed, particularly for carbon-based particles, so that they can be readily detected in environmental and biological media. Rapid screening methods should be developed that provide particle size distribution information for smaller nanomaterials present in complex environmental matrices such as soil and sediments.
Separation techniques, such as size-exclusion chromatography, capillary electrophoresis, field flow fractionation, or microfluidic-based approaches, may be applicable for determining classifying populations by size and allowing size-specific analysis of physico-chemical properties.

2. **Understand transformation mechanisms and key transformation products.** Transformation products that would impact environmental effects and fate considerations vary for differing classes of nanomaterials. Possible modifications that could determine key transformation products include biodegradation/chemical transformation, physical attenuation such as aggregation, and surface modifications. Development of analytical tools (e.g., techniques and methodology) is needed for identification and characterization of transformed nanomaterials in environmental matrices such as air, water, soil, sediments, sludge, etc. The same nanomaterial with different surface chemistry and/or coatings may require different detection and characterization methods in different environmental matrices.

3. **Develop reference materials for benchmarking and calibration.** Currently there are few available reference materials for priority engineered nanomaterials[1] that are directly relevant to environmental, health, and safety research, and to environmental toxicology and fate studies in particular. Standards that are currently available often have limited applicability or lack sufficient validation (e.g., documentary standards without corresponding interlaboratory evaluations).
The lack of engineered nanomaterial reference standards or well-characterized common test materials is now widely recognized as a substantial bottleneck to progress in accurate toxicity assessments. The development of reference materials is complicated by limited designation of priority engineered nanomaterials and by the time and effort required to develop, optimize, and validate methods for physico-chemical property characterization, and to certify reference materials. Other issues, such as material stability, also complicate progress in this area.
In the short term, reference materials cannot possibly be developed in a manner that would permit every possible research need, material type, matrix, or property measurement to be directly addressed. Instead, the role of reference materials and, perhaps more generally, of standards should be to provide benchmarks, primary measurement validations, and calibrations. Such materials should enable interlaboratory comparisons and increase the overall confidence levels associated with EHS research findings. Standardized methods need to be developed for labeling (e.g., isotopically) selected engineered nanomaterials (e.g.,

1 Priority engineered nanomaterials refer to those that have the greatest potential impact on human health and/or the environment based on such factors as production volume, widespread use in products and known or potential hazards.

engineered carbon-based nanomaterials that may have unique physico-chemical attributes), such that they can be distinguished from background particles in complex environmental and biological matrices.

4. **Ensure that existing regulatory test protocols for environmental effects of materials are adequate for evaluating the environmental effects of engineered nanomaterials**. A deeper understanding is needed in regard to modes of action, dosing, and other issues that will inform the improvement and expansion of regulatory protocols for evaluating engineered nanomaterials. Methods are needed to standardize the current toxicity assays used by regulatory agencies so that the characteristics and behavior of engineered nanomaterials in various media (such as water, soils and sediments) are better understood and controlled. This will involve better characterization of nanoparticles in test media, more consistent methods of dosing that will result in stable concentrations in test media, clarifications to terminology, and possible adjustments to dose metrics. Dose metrics need to be better identified for engineered nanomaterials, since the traditional mass metrics for chemicals may not reflect the most relevant metric relative to the material, receptor, and route of administration for nanomaterials. Despite these limitations, the apical endpoints targeted and the species used in the approximately 74 Organisation of Economic Co-operation and Development / Environmental Protection Agency Office of Prevention, Pesticides, and Toxics Substances (OECD/EPA OPPTS) protocols, are likely appropriate for engineered nanomaterials evaluation. Toxicity assays need to focus on endpoints and impacts which are seen at low doses that may be more realistic relative to those encountered by organisms in the environment. Toxicity assays such as those for aquatic receptors also need to focus on chronic effects. In addition, to provide more data, toxicity assays should emphasize potential mechanisms of action rather than focusing principally on lethality information. In addition to aquatic testing, testing terrestrial and sediment species is also important for many engineered nanomaterials. Such testing should lead to the identification of the physico-chemical characteristic(s) that dominate toxicity so that structure-activity approaches can be developed. For example, there is still a poor understanding of the exact size ranges at which different classes of nanoparticles have divergent toxicological and fate-related properties relative to their bulk counterparts. Biomarkers may provide a useful way to separate and model effects of engineered nanomaterials and may provide a mechanism to track them in the environment, especially where current metrology methods have not developed a way to monitor a particle in environmental matrices or in organisms of interest. High-throughput screening approaches may also be used to strengthen links between effects seen and more generalized conclusions regarding engineered nanomaterial mode of action and structure-activity relationships.

5. **Determine the effects of engineered nanomaterials at a higher level of complexity**. Testing approaches should be included that focus on population-level-effects endpoints in individual species, and on systems that are more representative of ecosystems such as microcosms and mesocosms. In addition, research on these types of testing systems should occur sooner than what is proposed in the 2008 NNI timeline for research. It may be useful to examine dietary exposure and effects as indicators of population-level effects, since the bioenergetics of receptors can be linked to individual and population-level effects. Microcosm and mesocosm tests may provide significant information not only on larger effects but also on the distribution of various particle types in environmental compartments, thus leading to more realistic understanding of exposures and receptors of concern. These tests should include low-dose exposure levels to mimic what are thought to be realistic potential environmental exposure levels.

6. **Understand exposure as it relates to determining environmental risk**. There is a need to understand what factors determine whether a dose of an engineered nanomaterial in the environment is significant in terms of adverse effects. Understanding the expected concentration of an engineered nanomaterial

that could be released from a manufacturing site and its resultant concentrations in waste streams (such as effluents entering receiving streams from a manufacturing site or from landfills) is fundamental to understanding environmental exposures. Little data are available for releases from sites that manufacture either engineered nanomaterials alone, or that incorporate engineered nanomaterials into final commercial products. Data from water and air monitoring and in-product detection of nanoparticles are also limited. These deficiencies need to be addressed. Concentrations of engineered nanomaterials in waters, sediments, air, and soil should also be understood in the context of background levels of nanoparticles from other natural or industrial sources. Better understandings of environmental concentrations of nanomaterials should lead to more realistic dosing of receptor organisms in effects tests in terms of relevant concentrations, material characteristics, and duration.

7. **Develop models that can predict engineered nanomaterial fate, distribution, exposure routes, transformations, and interactions with organisms and ecosystems.** Data on environmental concentrations of nanomaterials such as used to adjust current exposure pathway models for other pollutants to nanomaterials in order to predict likely concentrations of nanomaterials and their transformation products in environmental matrices prior to their release. Better understandings of exposures and effects studies should lead to improved predictions of higher-level impacts that could result.
8. **Post-release life cycle analyses**. Following the evaluation of releases from manufacturing sites and other major waste streams, life cycle analyses should be applied to identify potential exposure routes during the disposal of engineered nanomaterials. The complexities of life cycle analyses are intertwined with the environmental evaluation of and changes in nanoparticle surfaces and surface activity. Life cycle analyses are needed to identify possible additional primary exposure routes that may occur through the disposal or reclamation of engineered nanomaterials or products that contain them. Life cycle analysis will need to include a greater understanding of particle releases from engineered surfaces.

Other Recommendations

In addition to addressing the ENV and IMA research areas and their respective research needs, the breakout groups also made recommendations related to NNI program coordination that is needed to maximize the efficiency of NNI EHS research efforts on the environment. They felt that NNI agencies should:

- Sponsor meetings and workshops that include broader participation from U.S. researchers, regulators, representatives from industry and nongovernmental organizations, and from other countries, to better understand the state of the science and the most pressing research priorities, and to effectively leverage future research.
- Establish consortia that focus on interactions between instrument manufacturers and regulators to inform the development of instruments that better meet the needs of researchers, regulators, and industry.
- Facilitate cooperation between laboratories to conduct interlaboratory comparisons on environmental effects studies in order to increase confidence in property measurements of prototype reference materials, validate the properties of new nanomaterials, and develop interlaboratory networks for the measurement and testing of common materials.
- Establish a network to monitor environmental loads of engineered particles and to identify background levels of nanomaterials in the environment by specific geographic locations. Collaborations are also needed to identify the most relevant risk characterization information, labeling approaches, and approaches to instrumentation development for the rapid analysis of nanomaterials in environmental media.
- Coordinate the work of multidisciplinary teams of experts on research on safety and efficacy that spans the nanomaterial product life cycle.
- Consider developing an inventory of production and use information that is linked to nanomaterial properties of interest (including

persistence, toxicity, transformations, and phase distribution) and nanomaterial concentrations in the environment.

- Establish a priority ranking of engineered nanomaterials to be studied. This process should include the research and regulatory communities as well as other interested stakeholders. This ranking should be based on the greatest potential impact on the environment and human health, the ability to track nanomaterials in the environment, the degree of use of the products, and known or expected hazards.

1. Introduction

About the Workshop

This report summarizes discussions that took place during the National Nanotechnology Initiative (NNI) workshop on Nanomaterials and the Environment, and Instrumentation, Metrology, and Analytical Methods that was held on October 6–7, 2009, in Arlington, VA. This was the second in a series of four workshops aimed at furthering development and adaptation of the U.S. Federal Government research strategy to address potential environmental, health, and safety (EHS) implications of nanotechnology.

The workshop focused on research needed to understand the interaction of engineered nanomaterials with environmental components (both biotic and abiotic) and to determine the instrumentation, metrology, and analytical methods needed to achieve this understanding. The objectives of the workshop were to review the state of the science, identify critical gaps and barriers to advancing the science, identify emerging trends, and further inform the current interagency research strategy as appropriate. The workshop provided a critical venue for the dialogue necessary to advance the nanotechnology environmental, health, and safety research front, to support progress in the development of nanotechnology safety and health guidelines, and to decrease uncertainty about the viability of nanotechnology-enabled products and future liabilities. More than 150 scientists and other stakeholders from national and international government, industry, labor, and other segments participated in person. An additional 35 viewers joined from other locations through the webcast plenary session.

Planning for the workshop began in June 2009 by the interagency Nanotechnology Environmental and Health Implications (NEHI) Working Group operating under the auspices of the interagency NSET Subcommittee of Committee on Technology of the National Science and Technology Council. One of the NEHI Working Group's main tasks was to develop the 2008 NNI EHS Research Strategy in close consultation with the public. In the process of developing this strategy, the group released three key documents for public review (1)*Environmental, Health, and Safety Research Needs for Engineered Nanoscale Materials* (2006) (2) *Prioritization of Environmental, Health, and Safety Research Needs For Engineered Nanoscale Materials—An Interim Document for Public Comment* (2007), and (3) *Strategy for Nanotechnology-Related Environmental, Health, and Safety Research* (2008 NNI EHS Research Strategy).[1] This workshop was the second in a four-part series aimed at informing and updating the 2008 NNI EHS Research Strategy through dialogue among government agencies, citizens, academia, industry, nongovernmental organizations, and other stakeholder groups.

The Environmental Protection Agency (EPA), in recognition of its role as the coordinating agency for nanomaterials and the environment, and the National Institute for Standards and Technology (NIST), as coordinating agency for Instrumentation, Metrology, and Analytical Methods (2008 NNI EHS Research Strategy, p. 49), played leading roles in organizing the Workshop on Nanomaterials and the Environment, and Instrumentation, Metrology, and Analytical Methods. The workshop was organized

1 These documents are described in the front-matter page "About the 2009–2010 NNI Series of EHS Workshops and Reports" and are available on the NNI website at http://www.nano.gov.

by a multisector planning team with partners from academic, industry, and other stakeholder groups. The planning team structured the workshop to facilitate open and effective communication about the state of the science in these two areas of the 2008 NNI EHS Research Strategy by bringing together a broad cross-section of stakeholders to discuss ecological effects, environmental fate, and instrumentation and metrology research needs. The workshop also addressed whether the research needs proposed in the 2008 NNI EHS Research Strategy were still appropriate in terms of both the identified needs and their relative priority and timing.

The Workshop Agenda

The workshop agenda (see Appendix A) was developed by the multisector planning team noted above, composed of representatives from industry, academia, nongovernmental and public health advocacy groups, and the Federal Government who had expertise in each of the five nanomaterials and the environment ("ENV") and five instrumentation, metrology, and analysis ("IMA") research needs to be addressed by the workshop. The planning team selected 31 additional experts, who represented the same stakeholder groups reflected in the planning team membership, to give talks and help lead ten workshop panel discussions related to the ENV and IMA research needs.

Plenary Sessions

In order to set the stage for the ten panel discussions, the first plenary session of the workshop began with welcoming statements and a charge to participants, followed by several speakers, listed below, who presented different stakeholder perspectives. The workshop included public comment periods on both days and closed with a plenary session that compared the findings of the workshop panels to the research priorities and timing in the 2008 NNI EHS Research Strategy. All plenary presentations and the public comment periods were webcast to facilitate broader public participation.

Opening Session

The following plenary session presentations opened the workshop:

- *Industry Perspective*—David Arthur, CEO, Southwest Nanotechnologies
- *Insurance Perspective*—John Monica, Porter, Wright, Morris & Arthur, LLP
- *States' Perspective*—Leonard Robinson, California EPA
- *Public Stakeholders' Perspective*—J. Alan Roberson, AWWA
- *EPA Titanium Dioxide Case Study: A Perspective*—David Andrews, Environmental Working Group

These presentations were followed by a presentation of a case scenario in which a successful and environmentally responsible nanotechnology company (hypothetically) begins to observe environmental problems in the area of its manufacturing site. Several science-based responses to the case scenario followed to stimulate discussions on the types of research concerns that are raised by nanomaterials-related issues and to give state-of-the-science perspectives related to the case scenario. Participants sitting together at round tables were offered the chance, given their own expertise and experiences, to identify a set of top research needs related to the case scenario.

- *Case Scenario*—Paul Westerhoff, Arizona State University
- *Responses to the Case Scenario*
  - Current assessment of the state-of-the-science, gap analysis, and future priorities
  - *Environmental Effects Presentation*—Richard Handy, U. of Plymouth
  - *Fate & Transport Presentation*—Mark Wiesner, Duke University
  - *Instrumentation, Metrology, and Analytical Methods*—Hendrik Emons, European Commission/Joint Research Centre/Institute for Reference Materials and Measurements

Science-in-the-Round Discussion

The case scenario was followed by a session entitled, "*Science in the round: What is your perspective on the #1 need in nanotechnology research*?" during which Rebecca Klaper of the Great Lakes WATER Institute posed the following questions to workshop participants for group discussion:

- With respect to environmental effects of nanomaterials and instrumentation, metrology, and analytical methods, what is your perspective on the #1 need in nanotechnology research, and is this reflected in the NNI EHS research strategy within the appropriate timeframe?
- Are there items or perspectives that are not represented?
- What needs generated by the speakers are not covered in the strategy?

The following general discussion included a review by participants of perspectives derived from the plenary presentations and case scenario.

Participants were asked to describe their view on the current, top need in research and determine whether the NNI EHS Research Strategy reflected this need and laid out the appropriate time frame and also whether there were research needs that were not represented in the 2008 strategy (see Appendix C) . They were asked to consider these questions in light of the presentations made by speakers prior to this session where the current state-of-the-science and policy issues were discussed. The goal of the science-in-the-round session was to have an interdisciplinary discussion in which all participants could contribute feedback on the research needs proposed in the NNI EHS Research Strategy document.

Research needs identified appeared to cover most of the sections of the 2008 NNI EHS Research Strategy and included target areas such as development of a strategy for describing materials; development of standard characterization methods and methods to describe dosages or concentrations; establishment of reference materials that can be widely distributed; methods to measure nanomaterials and their environmental transformation products in the environment and organisms; determining or modeling realistic fate and exposure levels over time; and more effects research, including assays to test low-level and chronic exposures, as well as more holistic perspectives on larger-scale impacts.

Items that participants thought were not covered in the 2008 NNI EHS Research Strategy, but that need to be considered, included topics related to identifying regulatory needs and consumer protections. Included in these discussions were items related to the entire life cycle of a product, such as measuring nanomaterials in products, documenting unintended releases from products, manufacturers reporting nanomaterials in products, the waste stream from nanomaterial products, and worker safety. In addition, there was a desire for an inventory to be made of nanomaterials being produced and a method for sharing research with the public, including when nanomaterials are found to be not toxic. Participants stated the need for a centralized funding initiative for this type of research.

Panel Discussions

The majority of the workshop was taken up by participants examining the ten 2008 ENV and IMA research needs through a series of ten panels. Each panel was led by a planning team member and included invited experts in the panel topic areas. The panel topics were designed to be repetitive to allow each of the ten research topics to be covered at least twice from the different perspectives that resulted from involvement of different panel experts and different combinations of research topics. The panels were asked to keep the case scenario in mind to provide context for the ensuing discussions.

About the Report

The report that follows provides the results of this workshop. The panels' reports for each of the five ENV research needs are summarized in Chapter 2, and those for each of the five IMA research needs are summarized in Chapter 3. Following Chapter 3 in the appendices are the workshop agenda (Appendix A), list of participants (Appendix B) and 2008 NNI Research Strategy timelines and research needs for ENV and IMA (Appendix C).

Appendices D and E provide, for reference, the complete notes of all ten of the panel discussions. Additional workshop outputs and resources for the community are available at http://www.nano.gov.

2. Summary of Findings and Recommendations: Research Needs for Nanomaterials and the Environment (ENV)

This chapter reports the workshop participants' major findings and recommendations pertaining to the five Environmental Research Needs described in the 2008 NNI EHS Research Strategy (see Appendix C). Under each research need is a short description of its research priorities as designated in the strategy. This is followed by an itemized summary of the discussion points for that research need.

ENV 1. Understand the Effects of Engineered Nanomaterials in Individuals of a Species and the Applicability of Testing Schemes to Measure Effects

Background

The 2008 NNI EHS Research Strategy named the following research priorities for understanding effects of engineered nanomaterials in individuals of a species and applicability of testing schemes to measure effects:

- Test protocols
- Dose–response characterization
- Mode of action, leading to predictive tool development
- Tiered testing schemes

Discussion Summary

Participants largely agreed with structuring of research under Environmental Research Need # 1 around the four research priorities from the 2008 Research Strategy as noted above, but had some modifications to the timing and level of detail. Below are the participants' key findings, organized by research priority from the 2008 Research Strategy.

Test Protocols

- Validation of existing effects' testing protocols for ENMs is a near-term priority. Some of the key limitations to existing regulatory testing protocols involve terminology, ENM preparations for testing, and ENM dosing, and monitoring and stability in test systems.
- Methods for detecting and tracking nanomaterials are important for the assessment of exposures in test system matrices such as soil and water, and for determination of tissue burdens in environmental receptors. There is a lack of ability to efficiently detect many types of engineered nanomaterials in such biological and environmental matrices. Additional research is needed in the mid term on transformation of ENMs and impacts of transformation products on environmental matrices and environmental receptors.

- Mid-term research should also include more low-dose exposure testing over longer periods of time since short-term exposures at high doses may not accurately represent environmental exposure regimes.

Dose-Response Characterization

- Mass-based endpoints may not be suitable for measuring dose-response relationships for all ENMs, and alternate dose metrics should be considered (perhaps tied to individual classes of ENMs).

Mode of Action (leading to predictive tool development) and Tiered Testing Schemes

- Research focused on modes of action should be initiated in the near term, and continued in order to identify any toxicity mechanisms that current regulatory protocols may overlook.
- Research on biomarkers of early responses and/or sensitive model organisms should be pursued in the medium term with results anticipated that are relevant to better understandings of modes of action as noted immediately above.
- Research that develops data to support of biological and algorithm-based models that predict potential toxicity of nanomaterials should be a priority
- With regard to tiered testing schemes, microcosm and mesocosm work should begin sooner (in the mid term) to better understand the complex interactions of ENM fate and effects parameters. Please see related discussion under ENV 5.
- This research should include transformation products and biomarkers of sensitive endpoints.

Other Recommendations

- A government-sponsored clearinghouse for toxicity studies would be an asset in organizing toxicity research.
- Toxicity results from all testing conducted to date with ENMs should be made publicly available.
- Research should focus on selected engineered nanomaterials (ENMs), such as those that are found to occur in the environment, rather than all ENMs that may be developed.

ENV 2. Understand Environmental Exposures through Identification of Principle Sources of Exposure and Exposure Routes

Background

The 2008 NNI EHS Research Strategy named the following research priorities for understanding environmental exposures by identifying principle sources of exposure and exposure routes at two different stages:

- Manufacturing and product incorporation
- Life cycle exposures subsequent to product manufacturing

Discussion Summary

Participants largely agreed with the structuring of research under ENV #2 around the two research priorities from the 2008 Research Strategy as noted above, but had additional detail that should be added to the research proposed. Below are the participants' key findings, organized by research priority from the 2008 Research Strategy.

Principal Sources of Exposure and Exposure Routes Due to Manufacturing of the ENM, and Incorporation of the ENM in a Commercial Product

- Without sensitive, specific analytical methods, labeling of nanomaterials and tracking of nanomaterials and transformation products in the environment are not possible. Such work is of high priority for ENV #2.
- Three different approaches could be considered to better understand the sources of ENMs throughout their life cycle: development of an inventory of the production and use of ENMs within defined media/geographical locations; research to determine typical ENM waste production patterns resulting from production, use, and disposal; and temporal and multimedia sampling of release and exposure sites.
- Further research should be conducted on the transformation of ENMs. Exposures to ENMs are influenced by transformations of those materials prior to, and after, release of ENMs to the environment. ENMs may be able to be classified in accordance with specific transformation pathways.

- ENMs are likely to be present in the solids of waste water treatment plants as a result of production, use, and/or disposal. Near-term research should focus on the initial fate of ENMs as they enter waste streams. This research should lead to better models that can predict the fate of similar materials for which less hard data are available

Principle Sources of Exposure and Exposure Routes Due to Life Cycle Stages Beyond Manufacturing of a Commercial Product Which Includes the ENM

- The four principle sources of exposure and exposure routes that are applicable to assessing exposures due to manufacturing and incorporation are also relevant to assessing life cycle exposures.

ENV 3. Determine the Factors Affecting the Environmental Transport of Nanomaterials

Background

The 2008 NNI EHS Strategy named the following research priorities for determining factors affecting transport of nanomaterials in the environment:

- Key physico-chemical properties affecting transport
- Key transport processes
- Development of predictive tools

Discussion Summary

Participants agreed that the research priorities under ENV #3 are of high priority, and noted that the research topics in ENV #3 provide a high-cut view of the elements needed to understand how ENMs move through the environment. However, participants did provide more specificity to these research priorities and some timing adjustments relative to the timing noted in the NNI 2008 Research Strategy document. In general, it should be noted also that separating this research on environmental transport from the ENV #4 research on environmental transformation is difficult to do due to the interrelated nature of the two research areas. Second, there is a fundamental near-term need for the development of analytical methods for detection and measurement of both as-manufactured and transformed ENMs in environmental matrices which are sufficiently sensitive to distinguish ENMs from other materials/chemicals in those matrices.

Key Physico-Chemical Properties Affecting Transport

- There is a near-term need for standardized materials and procedures for evaluating the transport and transformation potential of ENMs
- There are near- to medium-term needs in the following areas:
  - The influence of size of the ENM on transport potential should be more fully investigated.
  - The effects of ENM surface coatings and surface chemistry need to be better understood relative to their impacts on transformation and fate of the ENM
  - The factors that determine the affinity of an ENM for a particular environmental matrix need to be addressed in more detail.

Key Transport Processes

- A short- to medium-term need was identified by participants: the effects of ENM surface coatings and surface chemistry should to be better understood relative to their impacts on transformation and fate of the ENM
- Two areas for pursuit in the medium- to longer-term were identified by participants:
  - Colloid science should be examined to determine its utility in predicting ENM partition coefficients and subsequent environmental distributions.
  - Affinities of ENMs for different surfaces should be evaluated to see if this information can be used to predict ENM distributions.
- Long-term research should include characterizing ENMs released from solid commercial product matrices into which they are incorporated and understanding the biodegradation of ENMs.

Development of Predictive Tools

- A medium- to longer-term area for investigation is to determine if the affinities of ENMs for different surfaces can be used to predict ENM distributions.

- A longer-term need is to develop models that can predict ENM–macromolecule interactions, and partitioning of ENMs in environmental and other matrices (such as sewage sludge).

Other Recommendations

- Two different research approaches were advocated by participants for transport and transformation research: a top-down approach and a bottom-up strategy:
  - For the top-down strategy, consider releases from the full life cycle of an ENM, and pursue more of an ecosystem-level approach for a top-down understanding of effects and fate. Since hazard evaluations cannot be done without adequate exposure information, and exposure evaluations are not anchored to risk if hazards are unknown, a balanced progression between exposure and effects' research is needed.
  - The bottom-up strategy would begin with partitioning first, then address transfer between phases, followed by unsteady state and then non-equilibrium state research. The simpler chemistry of attachment efficiencies for ENMs should be examined, followed by disaggregation and subsequent effects on transport. The effect of biological surfaces on attachment and bioavailability should then be considered. Transformations and finally numerical models for nanoparticle-macromolecule interactions should then be developed.
- An understanding of effects of colloids, surface chemistry, and stability in test media is required to support valid toxicity models.

ENV 4. Understand the Transformation of Nanomaterials under Different Environmental Conditions

Background

The 2008 NNI EHS Research Strategy named the following research priorities for understanding the transformation of nanomaterials in the environment under different conditions:

- Key physico-chemical properties affecting transformation
- Key transformation processes
- Development of predictive tools

Discussion Summary

The general conclusions for research needed for environmental transformation were similar to those reached for ENV #3 on environmental transport. Participants agreed that the research priorities under ENV #3 are of high priority, and noted that the research topics immediately above provide a high cut view of the elements needed to understanding how ENMs move through the environment. However, participants did provide more specificity to these research priorities and some timing adjustments relative to the timing noted in the NNI 2008 Research Strategy document. In general, it should be noted also that separating this research on environmental transformation from the ENV #3 research on environmental transport is difficult to do due to interrelated nature of the two research areas. Second, there is a fundamental near-term need for the development of analytical methods for detection and measurement of both as-manufactured, and transformed ENMs in environmental matrices which are sufficiently sensitive to distinguish ENMs from other materials/chemicals in those matrices.

Key Physico-chemical Properties Affecting Transformation

- Short-term needs for this research area included:
  - Increased research with a focus on identifying and testing standard parent ENMs should be a priority.
  - Research that would lead to nomenclature and metrology associated with ENM transformation products could assist in medium- and longer-term research in this area.
- Short- to medium-term needs included:
  - Not only should ENM key physico-chemical characteristics (including size, shape, surface chemistry and coatings) be addressed in the near term, but also the effects of environmental factors on transformation

    - should also be considered (redox environment, sunlight, biological effects).
- Medium- to long-term needs were identified:
    - Improved detection methods are needed for tracking and metabolism/transformation of ENMs. Well-characterized and labeled ENM standards should be developed for use in research.
    - In order to make some ENMs such as carbon nanotubes more biodegradable, functional groups that would lead to biodegradability should be identified.

Key Transformation Processes

- Short- to medium-term needs included the following:
    - The long-term release rates of ENMs from nano-based products is an area requiring further investigation, perhaps through the use of accelerated aging studies.
    - The transformations of products containing the ENMs also need to be better understood so that this knowledge can be coupled with data related to release of the ENMs from the product matrices.
- Medium- to long-term needs included development of a categorization system applicable to nanomaterial transformations in the environment. If nanomaterials could be grouped in accordance with their potential to transform, those that transform could be further categorized into those which mineralize, detoxify, become activated, etc.
- Develop predictive tools. The long-term goal of the work on environmental transformation is to develop predictive models based on functional relationships between physico-chemical parameters that can be measured in the lab, and the potential for an ENM to be transformed and/or transported in the environment. Such models should also indicate the potential for environmental impacts from transformation/degradation products.

Other Recommendations

Laboratory data on transformation potential of ENMs should be converted into units such as half-life that are usable by regulators.

Soil and rhizosphere effects on transformation of ENMs are under-studied.

ENV 5. Evaluate Abiotic and Ecosystem-Wide Effects

Background

The 2008 NNI EHS Research Strategy named the following research priorities for evaluating abiotic and ecosystem effects of nanomaterials:

- Population
- Community
- Ecosystem and abiotic effects

Discussion Summary

Participants found that the research topics above provide a high-cut view of the elements needed to elucidate understandings of environmental effects beyond those observed through testing done on individuals of a species (as noted under ENV #1). In order to move forward in this research area, however, there is a fundamental need for material standards, instrumentation, and environmental measurements capabilities to be enhanced for detection of ENMs in environmental matrices and biological tissues and fluids. Second, there is a near-term need to develop inventories of production and use information that include locations of major production, use and disposal facilities; information from this should be used to assist in exposure modeling efforts aimed at understanding which ecosystem components and species are most likely to be exposed to ENMs and their transformation products. Third, more work is needed in the near term on terrestrial species, as compared with aquatic species; for both terrestrial and aquatic environments, keystone species in food webs and sediment/soil-dwelling organisms should be given higher priority. Fourth, surrogate biomarkers such as stress response genes and alternative endpoints associated with control systems (such as the endocrine and nervous systems) should be targeted in the appropriate species. Additional specificity for research topics in this area have been suggested by participants, as noted below, organized according to the research priorities noted in the 2008 NNI Research Strategy.

Effects at the Population Level

- To approach population-level effects, it may be useful to examine dietary exposure to ENMs and subsequent effects on bioenergetics of appropriate receptor species since such effects on bioenergetics can be linked to population-level effects such as reproductive and locomotion-related outcomes.

Effects at the Community Level

- Research focused more on soil and sediment communities may be appropriate, considering that these form the bases of ecosystems, and fate information indicated that ENMs may concentrate in these systems.
- Additional communities which are representative of broader potential impacts should also be considered, such as those involved in key biochemical, photosynthetic, respiration and geochemical pathways.

Ecosystem, and Abiotic, Effects

- In order to understand the relevant exposures, fate and key environmental species impacted, selected ecosystem-level studies, such as those in microcosms and mesocosms, need to proceed more quickly in the near term than as suggested in the 2008 NNI EHS Research Strategy.
- Direct field measurements could also be initiated in the nearer term, with more complete field studies then occurring in the longer term.

Other Recommendations

Industry is an important stakeholder and should be invited to participate in the pooling of resources and in research coordination.

Establishing priorities for research on specific ENMs should be important in order to reduce the need in the short term for research on all ENMs; government, industry, academia, and the public sector should be included in the prioritization process.

3. Summary of Findings and Recommendations: Research Needs for Instrumentation, Metrology, and Analytical Methods (IMA)

This chapter reports the workshop participants' major findings and recommendations pertaining to the five Instrumentation, Metrology, and Analytical Methods Research Needs described in the 2008 NNI EHS Research Strategy. Following each research need is a short description of its research priorities from the strategy, followed by a summary of the participants' discussion points for that research need.

IMA 1. Develop Methods to Detect Nanomaterials in Biological Matrices, the Environment, and the Workplace

Background

The 2008 NNI EHS Research Strategy indicated the following research priorities:

- Develop methods to detect nanomaterials in biological matrices, the environment, and the workplace.
- Evaluate scope and suitability of technologies to quantify nanomaterials across biological media indicative of exposure.
- Develop common, commercially available samplers for measuring mass concentrations of nanoparticles in air (indoor and outdoor).
- Develop instruments to measure nanomaterials in water.
- Develop samplers for personal monitoring of nanomaterials and biomarkers indicative of exposure.

Summary of Panel Report

Participants in the discussions on Instrumentation, Metrology, and Analytical Methods Research Need (IMA) #1 had both general findings, and ones more specific to the research priorities noted in the 2008 NNI EHS Strategy document. The recommendations for this research need are organized by the research priority areas noted in the 2008 NNI EHS Research Strategy.

Evaluate scope and suitability of technologies to quantify nanomaterials across biological media indicative of exposure

- Methods to measure ENMs in environmental matrices are the most immediate need, and underpin the majority of challenges associated with nanomaterials in the environment. This recommendation is in agreement with the NNI 2008 EHS Research Strategy.
    - Efforts should focus on distinguishing ENMs from background matrices such as naturally occurring colloidal particles, and on detecting low concentrations of ENMs likely to be present in these matrices. Methods developed for colloid work may be applicable.
    - The characterization needs should be prioritized: concentration, size, and surface charge may be more important than other

parameters. In addition, characteristics that contribute to predictive model building should be pursued.

- Measurement of transformation products of ENMs and methods for tagging ENMs and their transformation products for enhanced detection were identified as medium-term needs.
- Aggregates and agglomerates formed from ENMs in environmental matrices should also receive additional attention.

Develop common, commercially available samplers for measuring mass concentrations of nanoparticles in air (indoor and outdoor)

- Identifying exposures in the workplace to decrease potential worker risk is a principal industry focus and need at this time. The measuring equipment is currently research grade, cumbersome, and has a high associated cost. Methods need to be developed that allow for real-time nanoparticle measurements down to 2 nms.

Develop instruments to measure nanomaterials in water

- ENM detection in aqueous samples should be given a higher priority than that noted in the NNI 2008 EHS strategy document.
    - Sample extraction and shipping and handling methods need further development.
    - Low concentrations of ENMs in water will necessitate methods that are applicable to larger sample volumes

Develop samplers for personal monitoring of nanomaterials and biomarkers indicative of exposure

- The need for portable, rapid personal air monitoring devices for worker exposure estimation is a high priority.

Other Recommendations

- There is an immediate need for methods to monitor releases of ENMs from production sites.
- It is necessary to prioritize ENMs to use effectively the limited resources that are currently available.
- As the number of ENM manufacturing sites increase, there is a corresponding need to establish a program or process to document releases from such sites, as well as procedures to remediate a release.
- Life cycle approaches should be used to determine the ultimate fate of ENMs in the environment.
- An accessible database that catalogues research results and knowledge gaps for IMA #1 topics and other broader EHS results, would benefit the ENM research community.

IMA 2. Understand How Chemical and Physical Modifications Affect the Properties of Nanomaterials

Background

The 2008 NNI EHS Research Strategy named the following research priorities for understanding how chemical and physical modifications affect the properties of nanomaterials:

- Evaluate solubility in hydrophobic and hydrophilic media as a function of modifications to further modeling of biological uptake
- Understand the effect of surface function on mobility and transformations in water

Summary of Panel Report

Participants primarily focused on the effects of surface function on the transport and transformation of nanomaterials in the environment. Below are the participants' key findings on Instrumentation, Metrology, and Analytical Methods Research Need #2.

- There is a need to understand the impact of surface chemistry and coatings on transformation rates.
    - Agglomeration is affected by Van der Waals forces and repulsive forces, but with coatings osmotic repulsions and elastic repulsions must also be considered.
- There is a need to understand how natural processes and inputs such as humics, oxidation, and other organic matter or biological processes impact the properties of nanomaterials and how that in turn affects agglomeration, deposition, etc.

IMA 3. Develop Methods for Standardizing Assessment of Particle Size, Size Distribution, Shape, Structure, and Surface Area

Background

The 2008 NNI EHS Research Strategy named the following research priorities for developing methods for standardizing assessment of particle size, size distribution, shape, structure, and surface area:

- Develop automated microscopic methods for the rapid screening of nanomaterials.
- Evaluate correlation of microscopic methods with other size-measurement techniques.
- Evaluate or modify microscopic and mass spectrometric approaches for determination of shape and structure of nanomaterials.
- Explore methods beyond isothermal adsorption for nanomaterial surface area determinations.

Summary of Panel Report

While the specific application of interest will determine what parameters need to be measured and the degree of accuracy, characterization at a minimum must establish particle size and morphology. It is imperative that the protocols used for preparation and analysis have no effect on the morphology of the particles. Below are the participants' key findings on Instrumentation, Metrology, and Analytical Methods Research Need #3, organized by the 2008 NNI EHS research priorities listed above.

Develop automated microscopic methods for the rapid screening of nanomaterials

- Development of such systems is critical for real-time monitoring in the manufacturing environment.
- Although optical microscopy and spectroscopy may be adequate for characterization of particles in the 100 nm size range, improved measurement methods for particles less than 50 nm are critical.

Evaluate microscopic methods with other size-measurement techniques

- Methods that may have sufficient particle number sensitivities for the characterization of size and number distribution include differential light scattering, analytical ultracentrifugation, ion mobility classification, scanning tunneling microscopy, atomic force microscopy, and small angle scattering using X-ray or neutron sources.
- Accurate correlations of electron microscopy with other size-measurement techniques, such as differential light scattering or field flow fractionation are critical.
- In addition, separation techniques, such as liquid chromatography, size-exclusion chromatography, capillary electrophoresis, field flow fractionation, or microfluidic techniques, may be applicable to the determination of the size-distribution of ENMs.

Evaluate or modify microscopic and mass spectrometric approaches for determination of shape and structure of nanomaterials; explore methods beyond isothermal adsorption for nanomaterial surface area determinations

- Both of these areas from the 2008 NNI EHS Research Strategy were mentioned, but were not assigned any priority or discussed further.

Other recommendations

- A database of physical properties of ENMs would be of value.
- The government should consider fostering the development of consortia to bridge the gap for instrument development.
- The government should consider fostering the development of consortia with instrument manufacturers, researchers, and ENM producers/suppliers to develop sector-specific instrumentation for nanometrology.
- A roadmap for instrumentation development, similar to the "International Technology Roadmap for Semiconductors", may guide technology development and assist instrument manufacturers in providing measurement tools within a reasonable timeframe.
- Invest in integrated computational methods to develop predictive and assessment tools for nanometrology.
- Real-time process development, quality control, and ENM monitoring and control during the manufacture of ENMs are needed.

IMA 4. Develop Certified Reference Materials for Chemical and Physical Characterization of Nanomaterials

Background

The 2008 NNI EHS Research Strategy named the following research priorities for developing certified reference materials for chemical and physical characterization of nanomaterials:

- Develop materials to support exposure assessment approaches, fundamental research, and instrumentation.
- Develop materials to support applied toxicology and hazard identification.

Summary of Panel Report

There are currently few documentary or reference material standards for nanomaterial properties that are directly relevant to environmental, health, and safety research. Of the approximately 65 currently available nanoscale reference materials, only 15 are likely to have environmental, health, and safety relevance. Standards that are currently available often have limited applicability or lack sufficient validation; this situation is widely recognized as a substantial barrier to progress in EHS research. There are additional complications due to other factors such as the wide range of ENMs and properties under consideration, and the inherent instability of many engineered nanomaterial formulations.

Participants did not distinguish between the two research areas noted in the 2008 NNI EHS Research Strategy in making their recommendations, but rather provided input applicable to both research areas. Their additional findings on Instrumentation, Metrology, and Analytical Methods Research Need #4 are noted below.

Reference Materials

- The availability of certified ENM reference materials (whose characteristics are specified by accepted methods) could contribute significantly to meeting nanoEHS research needs and should be developed in the near- to mid-term.
- Certain criteria and specific reference materials should be pursued as a first priority.
  - Since reference materials cannot be developed for every possible material type, matrix or property measurement, the role of reference materials should be to provide benchmarks, primary measurement validations, and calibrations that would enable interlaboratory comparisons and increase overall confidence in EHS results. In order to do this, the specific materials and minimum sets of properties for certified reference materials must be better defined by the research and regulatory communities.
  - Reference materials development efforts could focus initially on those ENMs that have the greatest potential impact on the environment or human health, based on production volume, widespread use in products, and known or potential hazards.
- Certified reference materials, and the documentary standards for them (procedures, protocols, guides to practice) should be developed simultaneously, with one underpinning the other.
- The longer-term stability of certified reference ENMs in liquid needs to be addressed to ensure described property values and measurands are maintained.

Other Recommendations

- There is an immediate need to standardize physical and chemical characterization criteria for publication of EHS data.
- Certification bodies, including government and nongovernment organizations, are needed to collaborate with academia.
- It would be advantageous to establish a national or international system for interlaboratory comparisons. Such a system would enable: (1) confidence in property measurements of prototype reference materials; (2) validation of properties of new materials; and (3) proficiency testing of participating laboratories through measurements of a common material.

IMA 5. Develop Methods to Characterize a Nanomaterial's Spatio-Chemical Composition, Purity, and Heterogeneity

Background

The 2008 NNI EHS Research Strategy listed the following research priorities for developing methods to characterize a nanomaterial's spatio-chemical composition, purity, and heterogeneity:

- Evaluate scope and suitability of techniques to assess purity and batch-to-batch production of nanomaterials
- Develop method for 3D chemical characterization at 1 nm resolution

Summary of Panel Report

At the nanoscale, single defects and slight changes to surface dimensions and composition can dramatically influence reactivity; hence proper characterization of spatial composition is critical. Changes to nanomaterial composition are dependent on a number of factors such as the properties of raw materials used in the manufacturing process and nanosynthetic methods both of which need to be rigorously defined. Observing, correlating, and understanding structure and function at the nanoscale is essential to developing reproducible ENMs. New instrumentation is needed with improved resolution and sensitivity, increased speed of data acquisition and data reduction, and new or integrated measurement approaches. Analytical tool capability must move from static measurements to dynamic real-time measurements at the 1 picogram measurement level, and the ability to characterize multiphase systems will be critical. Below are the additional participants' key findings on Instrumentation, Metrology, and Analytical Methods Research Need #5, organized by the research priorities noted from the 2008 NNI EHS Research Strategy.

Evaluate scope and suitability of techniques to assess purity and batch-to-batch production of nanomaterials

- This general area of research was viewed as critical, and panelists made general comments regarding its priority and implications for instrumentation development immediately above.

Development of methods for 3D chemical characterization at 1 nm resolution

- Accurate 3D visual, chemical, and physical characterization methods are important in both the manufacture of ENMs and in environmental research.
- Environmental research requires instrumentation to determine elemental composition, location, and chemical state of all atoms in nanostructures in three dimensions and the ability to predict the resulting properties of a nanostructure. Since ENM physical properties are known to depend on size, large-scale bulk measurements of physical and chemical properties may not adequately reflect properties at the nanoscale.
  - New instrumentation will require the development of corresponding standards and calibrations.
  - Panelists specifically recommended the development of methods for 3D chemical characterization at 1 nm level of resolution.
- Barriers to development of three-dimensional measurements include spatial and spectral resolution and specificity, data acquisition speed and throughput, synthesis of three-dimensional information from two-dimensional data sets, merging of data from different metrology tools, and measurement artifacts.

Other Recommendations

- An atomic-scale modeling program should be promoted.
- A coordinated, consortium-style organization is needed for developing instrumentation and methods.

Appendix A: Workshop Agenda

Tuesday, October 6, 2009

PLENARY SESSION

8:00 **Welcome**—Travis Earles (White House Office of Science & Technology Policy)

8:15 **Charge to Participants**—Phil Sayre (EPA)

8:30 ***How to make decisions under conditions of uncertainty in nanotechnology from your perspective***—Dianne Poster (NIST)

Industry Perspective—David Arthur (Southwest Nanotechnologies)

Insurance Perspective—John Monica (Porter, Wright, Morris, & Arthur LLP)

States' Perspective—Leonard Robinson (California EPA)

Public Stakeholders' Perspective—J. Alan Roberson (American Water Works Association)

EPA Titanium Dioxide Case Study: A Perspective—David Andrews (Environmental Working Group)

9:45 BREAK

10:00 **Case Scenario**—Paul Westerhoff, Arizona State University

Responses to the case scenario: Current assessment of state of the science, gap analysis, and future priorities

Environmental Effects—Richard Handy (University of Plymouth, UK)

Fate & Transport—Mark Wiesner (Duke University)

Instrumentation, Metrology, and Analytical Methods—Hendrik Emons (European Commission Joint Research Centre, Institute for Reference Materials and Measurements)

11:00 **Science in the Round (table discussions)**—Rebecca Klaper (Great Lakes WATER Institute)

- What is your perspective on the #1 need in nanotechnology EHS research?
- Table reviews of perspectives from plenary speakers and case scenario
- Needs not covered by the EHS strategy

12:00 Charge for afternoon technical sessions—Dianne Poster (NIST)

12:15 LUNCH

1:30 **CONCURRENT TECHNICAL SESSIONS**

Panel 1: Fundamental interactions of nanomaterials with organisms. Basic science principles and application to needs of regulatory agencies

Moderator: Paul Westerhoff (Arizona State University)

Panelists/Presenters: Steve Diamond, William Johnson, Rebecca Klaper, Rajan Menon, Robert Tanguay

Panel 2: Transport of nanomaterials in the environment

Moderator: John Gannon (DuPont)

Panelists/Presenters: Dan Herr, Jae-Hong Kim, Greg Lowry, Alex Star, Paul Tratnyek, Ron Turco, Mark Wiesner

Panel 3: Measuring and predicting levels of exposure of nanomaterials for various species in the environment

Moderator: John Cowie (American Forest & Paper Association)

Panelists/Presenters: Don Baer, Gary Casuccio, Shaun Clancy, Horacio Espinosa, Greg Meyers, Mike Postek

Panel 4: Developing standard measurements to allow comparisons across experiments

Moderator: Alan Roberson (American Water Works Association)

Panelists/Presenters: Hendrik Emons, Howard Fairbrother, Vince Hackley, Steve Wilson

Panel 5: How environmental exposures occur and change under different environmental conditions

Moderator: Dave Andrews (Environmental Working Group)

Panelists/Presenters: Pedro Alvarez, William Ball, Richard Handy, Lisa DeLouise, R.D. Holbrook, Ann Miracle, Keith Swain

5:00 **Public Comment Period**

Wednesday, October 7, 2009

PLENARY SESSION

8:00 **Welcome**—Phil Sayre (EPA)

8:15 **Report Out from Sessions 1, 2, 3, 4, and 5**—Session Moderators / Selected Panelists

9:10 **Charge** for Breakout Sessions—Dianne Poster (NIST)

9:15 **CONCURRENT TECHNICAL SESSIONS**

Panel 6: Transformation in the organism and in the environment: What do we measure and how do we develop testing strategies to measure impacts of particles that may be transformed over time in the environment?

Moderator: Paul Westerhoff (Arizona State University)

Panelists/Presenters: Lisa DeLouise, Dan Herr, Rebecca Klaper, Jae-Hong Kim, Ann Miracle, Robert Tanguay

Panel 7: Transformation of nanomaterials in the environment

Moderator: John Gannon (DuPont)

Panelists/Presenters: Howard Fairbrother, Greg Lowry, Alexander Star, Paul Tratnyek, Ron Turco, Mark Wiesner

Panel 8: Developing methods to detect nanomaterials and determine exposure routes

Moderator: Dave Andrews (Environmental Working Group)

Panelists/Presenters: Steve Diamond, William Johnson, Rajan Menon, Keith Swain

Panel 9: Developing standards for nanomaterial properties

Moderator: John Cowie (American Forest & Paper Association)

Panelists/Presenters: Don Baer, Gary Casuccio, Shaun Clancy, Hendrik Emons, Vince Hackley, Greg Meyers, Mike Postek, Steve Wilson, Horacio Espinosa

Panel 10: How environmental exposures occur and change under different environmental conditions

Moderator: Alan Roberson (American Water Works Association)

Panelists/Presenters: Pedro Alvarez, William Ball, Richard Handy, R.D. Holbrook

12:15 LUNCH

CLOSING SESSION

1:15 **Report Out from Sessions 6, 7, 8, 9, and 10**—Session Moderators / Selected Panelists

2:15 **Synthesis Session**

Discussion of the most important research needs based on technical sessions on Days 1 and 2, and how the identified research priorities compare to the priorities and timing of the NNI EHS Research Strategy

3:45 **Public Comment Period**

4:00 **Closing Remarks**—Dianne Poster (NIST) and Phil Sayre (EPA)

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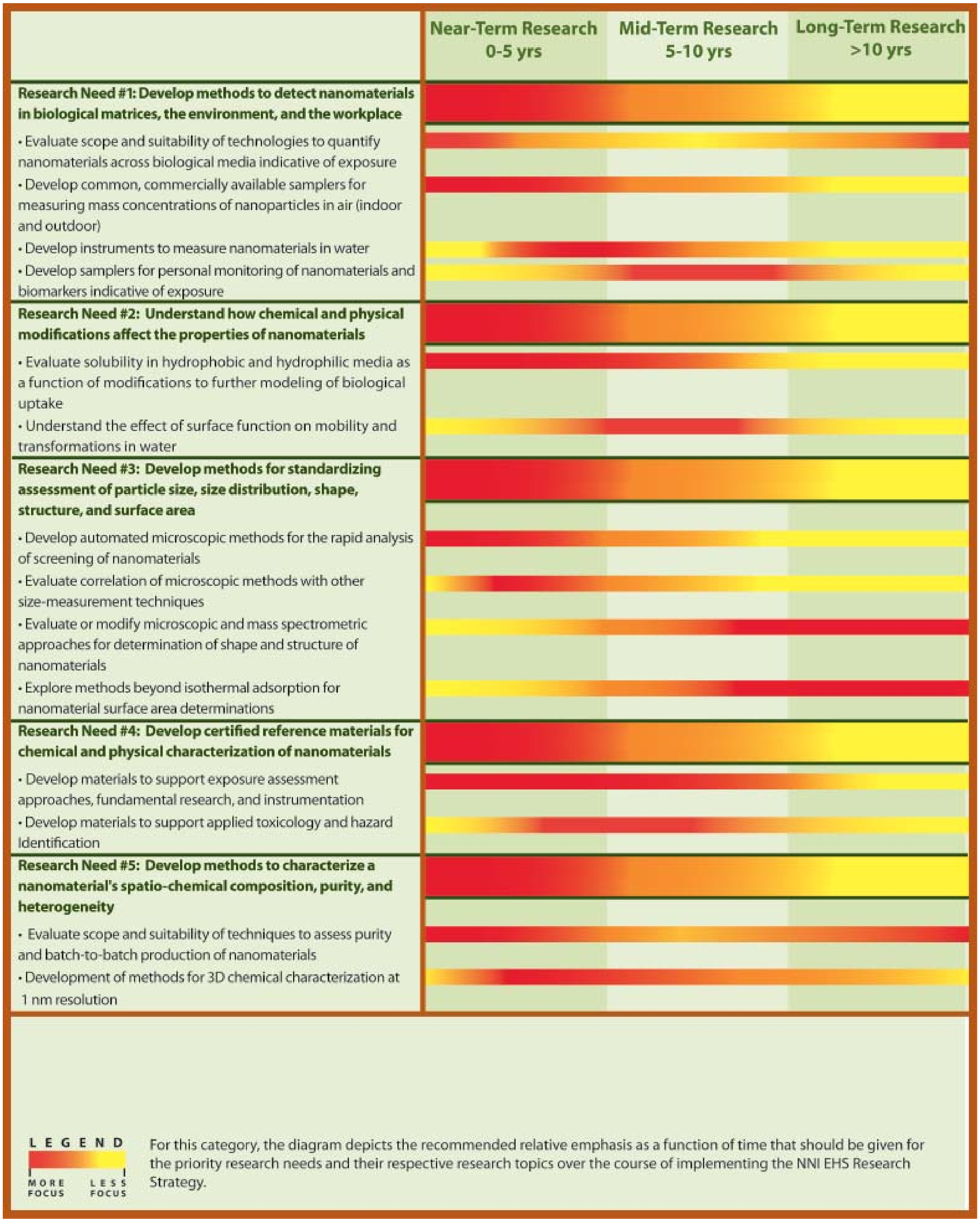
Appendix C: Environmental and IMA Research Needs



Near-Term Research 0-5 yrs
Mid-Term Research 5-10 yrs
Long-Term Research >10 yrs
Research Need #1: Develop methods to detect nanomaterials in biological matrices, the environment, and the workplace
• Evaluate scope and suitability of technologies to quantify nanomaterials across biological media indicative of exposure
• Develop common, commercially available samplers for measuring mass concentrations of nanoparticles in air (indoor and outdoor)
• Develop instruments to measure nanomaterials in water
• Develop samplers for personal monitoring of nanomaterials and biomarkers indicative of exposure
Research Need #2: Understand how chemical and physical modifications affect the properties of nanomaterials
• Evaluate solubility in hydrophobic and hydrophilic media as a function of modifications to further modeling of biological uptake
• Understand the effect of surface function on mobility and transformations in water
Research Need #3: Develop methods for standardizing assessment of particle size, size distribution, shape, structure, and surface area
• Develop automated microscopic methods for the rapid analysis of screening of nanomaterials
• Evaluate correlation of microscopic methods with other size-measurement techniques
• Evaluate or modify microscopic and mass spectrometric approaches for determination of shape and structure of nanomaterials
• Explore methods beyond isothermal adsorption for nanomaterial surface area determinations
Research Need #4: Develop certified reference materials for chemical and physical characterization of nanomaterials
• Develop materials to support exposure assessment approaches, fundamental research, and instrumentation
• Develop materials to support applied toxicology and hazard Identification
Research Need #5: Develop methods to characterize a nanomaterial's spatio-chemical composition, purity, and heterogeneity
• Evaluate scope and suitability of techniques to assess purity and batch-to-batch production of nanomaterials
• Development of methods for 3D chemical characterization at 1 nm resolution
LEGEND
MORE FOCUS
LESS FOCUS
For this category, the diagram depicts the recommended relative emphasis as a function of time that should be given for the priority research needs and their respective research topics over the course of implementing the NNI EHS Research Strategy.

Appendix D. Panel Reports: Discussion Notes for the Nanomaterials and the Environment (ENV) Research Needs

ENV 1. Understand the Effects of Engineered Nanomaterials in Individuals of a Species and the Applicability of Testing Schemes to Measure Effects

Findings on the State of the Science

While recognizing that the list was not comprehensive, participants reviewed the approximately 74 standardized protocols used by the U.S. EPA Office of Chemical Safety and Pollution Prevention (OCSPP)[1] and the OECD for assessing adverse effects of chemicals on environmental species. Many of these protocols have been used for some time for traditional chemical assessments and are harmonized through bodies such as the OECD. The EPA has reviewed the ecological effects protocols for their adequacy in assessing nanomaterials (Diamond *et al*. 2009[2]). Among its findings were the following:

Methods are needed to standardize current toxicity assays such as those used by regulatory agencies so the characteristics and behavior of nanomaterials in variable media as they relate to dosing of test organisms are understood and controlled. This will involve better characterization of nanoparticles that are in test media, more consistent methods of dosing that will result in stable concentrations in test media, clarifications in terminology (since most of the protocols are explained within the context of soluble chemicals), adjustments made as a result of gaining better knowledge on modes of action of various engineered nanoparticles, and possible adjustments to dose metrics. Dose metrics need to be better identified for nanomaterials because the traditional mass metric for chemicals may not be relevant for all nanomaterials (the most relevant metric may vary relative to the material, receptor, and route of administration).

While these limitations to the approximately 74 OECD/EPA OCSPP protocols exist, the apical endpoints targeted and species used are likely appropriate for nanomaterial evaluation, such that experiments become comparable among laboratories.

In addition, other organizations are also taking steps in this direction. As noted on the website of the International Alliance for NanoEHS Harmonization (http://www.nanoehsalliance.org/), "Understanding the biological impacts of nanoparticles on human health and the environment is important to secure the safe and responsible world-wide implementation of nanotechnology. Technical differences of opinion on toxicological impacts of nanoparticles have arisen in the literature, and most scientists are increasingly aware of the challenges of achieving reproducibility. Confusion arising from these differences can lead to undeserved negative public perception of nanotechnology, without basis in scientific fact. On the other hand, if genuine hazards are identified, this confusion could lead to the warnings of scientists being ignored. The priority is to create a trustworthy, and trusted, co-operation between scientists where results are simultaneously and carefully checked in blind studies, in different laboratories around the world."

Test Protocols: Dosing and Dose–Response, Relationships

- In aquatic matrices, the current paradigm of chemical pollutant testing may not translate for nanomaterials. Existing pollutant testing protocols include terminologies (e.g., "dissolved") that are not sufficient for engineered nanomaterials. The preparation and delivery of engineered nanomaterials in these tests are critical, because exposures may be changing over time due to instability of engineered nanomaterials.
  - Examples were given where TiO_2 agglomerates in media alone but not when organisms are present.
  - Another example was for fullerene (C_{60}), where the preparation methods have varied over the past several years and the subsequent materials being tested can be very different, despite starting with similar pristine materials.

1 Formerly the Office of Prevention, Pesticides, and Toxic Substances (OPPTS)

2 Please note that references are provided in Appendix E, by section.

    - Effects of water quality (e.g., dissolved organic matter, pH, ionic strength) all affect the aggregation state of engineered nanomaterials.
- Endpoints are all mass-based (e.g., EC50 values) and may not be suitable for nanomaterials where surface area or reactive surface sites may be critical.
    - The methods have not been validated for many details; one given was effect of light sources (sunlight versus fluorescent lamps) while studying TiO_2.
    - All effects levels are based upon mass concentrations, as opposed to surface area or other measurements that may be more appropriate for engineered nanomaterials. In at least one study (Christensen *et al*. 2010.). There was not a consistent dose–response for effects of nanosilver of different diameters based upon mass concentrations. However, once normalized to surface area, then a uniform dose–response effect was observed.
    - At the simplest level, engineered nanomaterials do exhibit dose–response relationships in many conventional pollutant-testing experimental platforms. However, because of so many other variables, perhaps nanomaterials offer the opportunity to establish a new paradigm for testing potential pollutants.
    - The fact that nanomaterial properties transform over time during their exposure to biological test models, and within the environment, creates a significant analytical and interpretative challenge.
    - To what degree do environmental transformations of engineered nanomaterials need to be understood (just for as-manufactured forms, versus transformations of engineered nanomaterials degradation or derivative products after release to the environment or incorporation into consumer products?).
    - If biological testing is not required, perhaps we can require industries (*ed. note*: responsibility will likely be shared between the U.S. government and industry), at a minimum, to report surface chemistry, particle size distributions in test media, surface area, charge, solubility, and a few other critical parameters of concern for nanomaterial environmental fate.
- Currently the field lacks even the most common acceptance of consistent terminology and measurement methods for these parameters or for how to describe the state of dose conditions applied in biological tests.
    - Many current biological testing protocols focus on the hazard (outcome) rather than the mechanism or model of action. Which should be applied for nanomaterials? For example, oxidative stress seems to be one important mode of action for carbonaceous nanomaterials, so should a reactivity parameter representing this mode of action be pursued?
    - Additionally, genomic studies are starting to identify modes of action and biomarkers that may indicate when and how a nanomaterial causes an effect. Because nanomaterials are foreign bodies, natural cell responses (e.g., the immune system) may be triggered as one of the primary responses to particles.
    - Likewise, nanomaterials can have similar physical dimensions as the food of some organisms, so does the presence of nanomaterials affect feeding behavior?
- Use more complex testing systems for evaluation of nanomaterials. More complex test systems such as microcosms and mesocosms potentially offer more representative environments than beakers and growth media for understanding the complex and simultaneous fates and biological effects.
    - While there are regulatory protocols for such tests, they are not frequently used.
    - However, in the case of nanomaterials, several researchers noted that a concept that fundamental studies of the potential hazards from nanomaterials should be guided by a view of how nanomaterials behave in environmental systems. This view evolved from the use of mesocosms that simulated river systems, which were dosed with two types of titania or silver citrate nanomaterials (Miracle *et al*. 2009). Nanomaterials were

dosed into the mesocosms at concentrations expected to occur based upon life cycle assessments and/or reports near the LC50 of target organisms (*Daphnia*). Nanomaterials were "overwhelmed" by natural particulates, organics, and ions in a relatively clean river water (7 mg/L of suspended solids). To describe the polydispersity of nanomaterials, size fractionation (50,000 Dalton which is ~5 nm) was employed. Bioaccumulation was evaluated after a 24-hour pulse exposure to nanomaterials and 24-hour depuration. The two different forms of titania exhibited different transformations, especially in size, and potential bioaccumulation. However, very little of the citrate nanosilver settled out in comparison to the "white snow" aggregates from titania. Specific data were presented, but the conclusion was that toxicity will be different in natural settings than in standard tests, and different in static tests than in conditions influenced by natural fluid hydraulics.

- High-Throughput Screening: In addition to evaluation of standard protocols and the use of more complex test systems as noted above, the need for high-throughput testing was also identified as critical because of thousands of nanomaterial variants likely to rapidly be seen in industry.
  - For such high-throughput testing, a focus on "early responses" rather than later responses, which lead to specific pathologies or disease, may be desirable.
  - High-throughput testing was seen as a means to be able to build safer materials if we had sensitive systems to avoid undesirable outcomes. Testing early stages of biological life (e.g., fish embryo development) may be optimal because a wide range of developmental endpoints are well known, readily evaluated, and take days to weeks rather than weeks to months or multigenerational studies (Harper *et al*. 2008). Embryonic development may be superior to single-cell studies because these *in vitro* platforms fail to consider cell–cell interactions, which occur in all tissues, and that cells provide a limited number of responses compared to more complex assemblages of cells. Specifically, the use of zebra fish embryo may be a good choice because:
    - many organs are similar to mammals
    - the full genome is known
    - each embryo can be individually cultured
    - small quantities of test materials are required
    - the early-stage development is very rapid (6- to 120–hr. exposures to nanomaterials give meaningful data)
- The sequence of such studies would involve the following steps: Exposure – tissue dose – biologically effective dose – early response – late response – pathology/disease.
- Early responses were put forth as being more sensitive and more likely to occur than later responses of disease or toxicity.
- If a nanomaterial is toxic, it must influence activity of a molecular pathway that would ultimately lead to toxicity.
- While early responses (biomarkers, developmental endpoints) don't always lead to undesirable or predictable adverse outcomes, such connections are becoming more commonly established.
- If we design materials to avoid adverse early responses, it is highly probable that later responses would also be avoided, even if the direct connectivity between the two has not yet been developed.
- Early responses are often easier to measure at lower, more environmentally relevant concentrations, and avoid shifts in toxicity mechanisms that can occur when nanomaterials are exposed to cells or organisms at unrealistically high doses. For the zebra fish embryos evaluated, most nanomaterials tested so far exhibit very little adverse "early responses," which were monitored by morphology (malformations, circulation, heartbeat, developmental progression, viability) and behavior.

Overall, several needs exist to implement high-throughput screening, including:

1. Disseminate results efficiently, which could be facilitated by a central government agency.
2. Reduce randomness of assessments through selection of test materials, organisms, etc.
3. Create a data-sharing infrastructure that would allow comparative analysis using shared datasets.
4. Develop predictive behavioral models.
5. Test the reliability and accuracy of predictive models.

Detailed Research Recommendations

Many of the detailed research recommendations flow from comments above on the state of the science:

- Heat diagrams and text (Appendix C) assume engineered nanomaterials research needs should mirror approaches for other pollutants. The group believed that acute toxicity tests do not provide all information and need to be supplemented with tests that focus on modes of action. What other mechanisms do regulatory protocols miss? What is the mode of action protocols that should be targeted for aquatic receptors?
- What are the proper endpoints to study? Perhaps high-throughput testing should be sought to evaluate early responses. Early responses using sensitive models (zebra fish embryos) were discussed.
- Key limitations to existing regulatory testing protocols involve terminology. This is not only for what engineered nanomaterials are, but what do terms like dose, preparation of standards, etc., mean for engineered nanomaterials. How do we define polydispersity during exposures?
- Interaction of disciplines is critical to assess exposure, visualization, toxicity, and modes of action.

Short-Term Research Needs

- Validate existing test protocols for ENMs.
- Key limitations to existing regulatory testing protocols as noted by Diamond *et al*. (2009) involve terminology and engineered nanomaterials exposure preparation, application, and quantification (over 55 test methods for pollutants currently exist, e.g., sunlight versus fluorescence lamps with TiO_2).
- Confirm measurements by at least two techniques to detect nanomaterials in biological media.
- Mass-based endpoints may not be suitable for measuring dose–response relationships for all ENMs. What are the appropriate dose metrics for various classes of ENMs?
- How valid are traditional toxicology studies given that engineered nanomaterials transform in reality? The NNI document does not contrast static versus pulse inputs and consequences for transformations in engineered nanomaterials during the test.
- Create a government clearinghouse of all toxicity testing conducted to date. Several standard organisms exist, but industry and regulators may need different research than what researchers are trying to address.
- Standardize global test protocols (harmonization).
- Continue traditional testing protocols, but move towards understanding mechanisms of action under environmentally relevant dosing scenarios.
- Autopsy every environmental compartment in systems impacted by engineered nanomaterials in current use, and conduct detailed biological testing for compartments where nanomaterials are found.

Mid-Term Research Needs

- Short-term exposures at high dosages may not be realistic. A move towards low-dose exposure tests as well as microcosms at realistic environmental levels or reported LC50's may be more realistic. Microcosms are encouraged. Use environmental "view" to guide fundamental studies.
- Transformations matter.
- Aggregation, photolysis, natural organic matter (NOM), biodegradation—multiple/sequential processes—affect biological effects.
- Conceptual models can help (hydroxylated engineered nanomaterials behave differently than nonfunctionalized models).

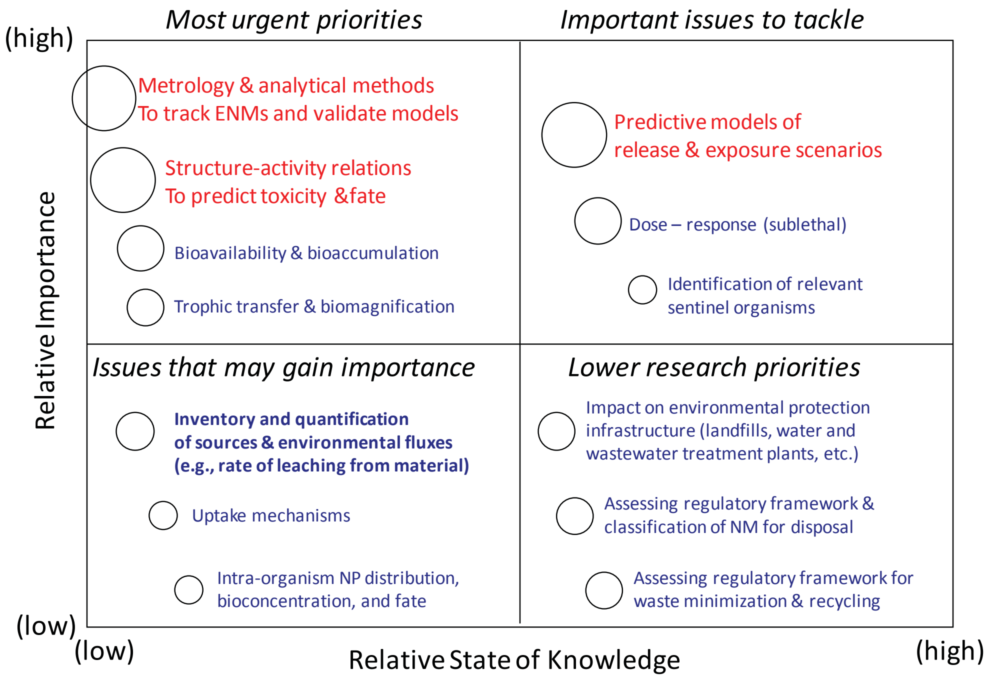


Figure C.1. Towards eco-responsible nanotechnology (Alvarez *et al*. 2009).

- Identify biomarkers or "early response" or "sensitive" endpoints that could be tracked. The examples using genomics were well received.
- Consider applications of more complex test systems such as microcosms and mesocosms to better understand the complex interactions of ENM fate and effects parameters.

ENV 2. Understand Environmental Exposures through Identification of Principle Sources of Exposure and Exposure Routes

Findings on State of the Science

- Little data are available for releases from manufacturing sites for either nanomaterials themselves or for the sites that incorporate nanomaterials into final commercial products.
- Tools available to track nanomaterials as they are released from such sites are limited but should be developed further, by examining their fate in waste streams, in order to gain a foothold on the fate of nanomaterials when released.
- Less is known regarding life cycle exposures beyond the manufacturing steps; this research should also be pursued, in conjunction with fate/transformation studies and, eventually, modeling efforts.

Detailed Research Recommendations

- In regard to NNI research focused on identification of possible environmental sources of engineered nanomaterials—such as those associated with manufacturing and incorporation and the bioaccumulation of engineered nanomaterials in environmental receptors—this panel noted that they are both high-priority work (see Figure C.1). Further, as noted in figure C.1, there is a need to support instrumentation and metrology methods, such the labeling of nanoparticles, so they can be tracked in the environment; this need is particularly apparent for carbon-based particles such as carbon nanotubes.
- In general, at least three different approaches could be considered to better understand the sources of manufactured nanomaterials through the life cycle: (1) generation of an inventory of the production and use of nanoparticles within defined media/geographical locations; (2) research to determine typical nanoparticle waste production patterns due to production, use, and disposal; and (3) temporal and multimedia sampling of release and exposure sites. It is believed that chronic exposures in ecosystems via soils/sediments deposition and entry into food chains are important to examine for both

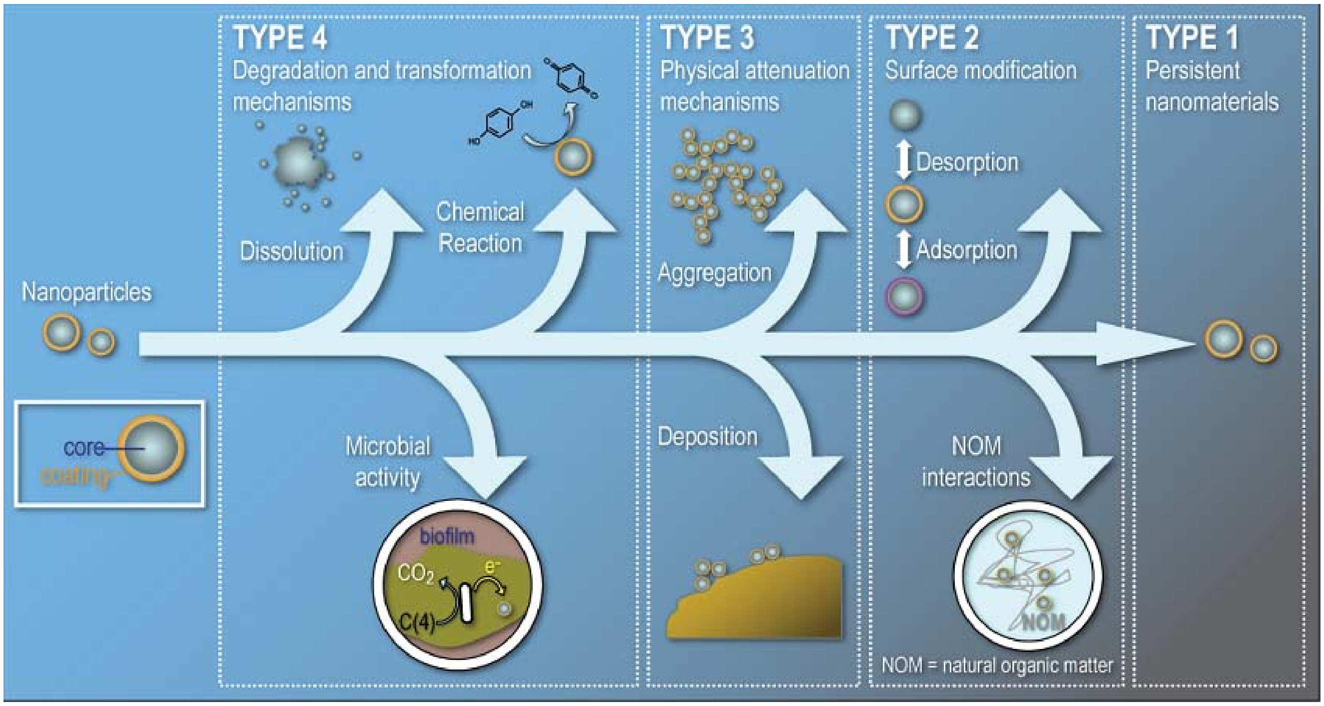


Figure C.2 Nanoparticle modifications in the environment (Alvarez *et al*. 2009).

environmental receptor effects and human health effects. Additionally, it may be possible to identify and possibly monitor surrogate parameters at such sites.

- Following on the comment above regarding waste production patterns, it is believed that nanomaterials are likely to be present in the solids of waste water treatment plants through production, use, or disposal. Early research should focus on the initial fates of nanomaterials as they enter the waste stream, since the flow of the materials through waste streams is not well understood. This will lead to better models to predict the fate of materials, for which less hard data are available (as noted in Figure C.1).
- Exposures to nanomaterials are influenced by transformation of those materials prior to release to, and after release in, the environment. Dissolution, microbial or abiotic redox reactions, aggregation, deposition, and at the very least, acquisition or loss of coatings, will change their mobility, bioavailability, reactivity, and toxicity. For the case of carbon nanomaterials, for example, recent studies have begun to provide insights as to how surface oxidation can dramatically affect carbon nanotube (CNT) interactions with each other, with other dissolved substances, and with naturally occurring suspended and stationary phases in the environment. Potential nanomaterial exposures, such as those resulting from release of fullerenes, could be better understood if materials were classified according to specific transformation pathways (Figure C.2). Research should be directed at which material is the most relevant to track in the environment (with regard to the materials' life cycle and bioavailability): the parent material, or the environmentally modified product whose structure will dictate its movement in the environment. Some of this may be predicted from the intrinsic characteristics of the original starting material.
- One of the most important ways to mitigate risk is to minimize exposure. In this context, it is important to understand and control issues of engineered nanoparticle weathering and release from products, the effects of engineered nanoparticle modifications during weathering and transport, and properties that control and affect engineered nanoparticle transport through environmental media.
- A second way to mitigate risk is to engineer nanoparticles in a way that removes the features that make them hazardous without getting rid of what makes them useful (such as through surface modifications of some nanoparticles). The panel

agreed that this approach may not be especially fruitful in many cases, given that the goals of utility and risk may sometimes require properties that are in direct conflict, and that engineered properties (such as surface modifications) can sometimes be altered during release and transport.

- Another means of risk mitigation may be to couple the second approach above (minimization of adverse effects) with a controlled plan for collection, reprocessing, and reuse (true recycling and continued reuse rather than "down-cycling" and ultimate environmental release, i.e., application of the "cradle-to-cradle" concept to nanotechnology).

ENV 3. Determine the Factors Affecting the Environmental Transport of Nanomaterials

Findings on the State of the Science

- The most immediate need, and perhaps the fundamental underpinning for most challenges associated with nanomaterials in the environment, will be to develop analytical tools that will allow for measurement of manufactured nanomaterials in environmental matrices.
- Furthermore, these analytical tools will need to enable differentiation of manufactured nanomaterials against background levels of both incidental and naturally occurring nanomaterials. As with conventional chemicals, to understand the transport, it is essential to first look at the nanomaterial from a life cycle perspective.
- Understanding all of the potential entry points into the environment, along with applications and uses, will be the first steps toward helping us understand transport of nanomaterials in the environment.
- We will continue with a very limited understanding of the transport and fate of nanomaterials in the environment until we have the critical analytical tools for detection and measurement in the environmental matrices of interest.

When considering the fate of nanomaterials in the environment, transport and transformation are inseparable topics. Environmental interactions change the fates of all materials, especially nanoparticles. Detection is based on separation schemes, so transformation will change our ability to separate for detection. Therefore, the challenges of detecting transported nanomaterials will be linked to their transformation potential.

It is important not only to look at the size of the nanomaterial, but also to consider that unique properties may arise as size decreases.

Special emerging, size-dependent properties: change the size, you change the property. Examples:

- Size-dependent properties: size of quantum dots affects color
- Iron oxide nanoparticles: there is a very sharp increase in their capacity to adsorb arsenic when the size goes below 20 nm

As the size of iron oxide nanoparticles goes from 300 nm to 20 nm, the arsenic adsorbed per surface area is constant, i.e., surfaces of nanoscale vs. conventional arsenic are the same. Below 20 nm, however, the iron oxide particles are able to adsorb much more arsenic; therefore, there is a size-dependent nanoscale effect. So, quantum effects like adsorption and reactivity may change with size. Size also determines how nanoparticles interact with cells (Chithrani and Chan 2007).

There is a need to consider all sources—manufactured, incidental, and natural nanomaterial sources, i.e., what the relative contributions are from natural, incidental, and manufactured sources to the exposure of a given material in a given situation.

When nanoparticles are released to the environment, four types of nanoparticle modifications are possible in the environment, all essentially happening at same time:

- Biodegradation and chemical transformation mechanisms: dissolution, chemical reaction, and microbial activity
- Physical attenuation mechanisms: aggregation and deposition
- Surface modification : desorption, adsorption, and natural organic matter (NOM) interactions
- Persistent nanomaterials

All transport is affected by aggregation state of the material and by deposition of those materials onto surfaces. Factors affecting aggregation and deposition for colloids also hold for nanomaterials:

- Size of nanoparticle.
- Energy input: flocculation process and energy input influence the size of engineered nanomaterials.

Chemical Properties	Nanomaterials (e.g. C_{60})	Nanomaterials (proposed)
Vapor Pressure (P^{sat})	~0	N/A
Aqueous solubility (χ)	~0	Agglomeration state, Dispersion stability
Octanol-Water Partitioning (K_{ow})	Very high (low for metal oxides)	Interfacial behavior (deposition)
Environment Properties		
f_{oc}, compartment volumes	?	Ionic strength, ionic composition, pH, mixing, f_{OC}, mineral surface

Figure C.3. Properties needed to assess the distribution of environmental pollutants. (G. Lowry, "Elucidating the Physicochemistry of NP Attachment to Surfaces: Implications for Environmental Transport," October 6, 2009, presentation at NNI workshop).

- It is less understood how NOMs or organic acids that attach to these nanoparticles surfaces affect aggregation and deposition and, therefore, transport. These are kinetics processes, not thermodynamic processes, so exposure is critical.
- Most particles have coatings: how do surfactants, polymers, or polyelectrolytes affect aggregation and deposition and therefore transport?
- Biological modifications by extracellular polymeric substances (EPS): we can modify particles with EPS but do not have good handle on how this affects transport.

Research has shown that C_{60} properties change as a result of aggregation; chemical reactivity also changes.

For chemicals, if we know vapor pressure, water solubility, and Log K_{ow} of the chemical and something about the environment—f_{oc} (the mass fraction of soil organic carbon content, compartment, and volumes)— then we could say something about how they are distributed in the environment at equilibrium, and this gives us a good indicator where to look them. This is what we need to do for nanomaterials, but the question is, what are these properties that we need to look at for nanomaterials? If we look at nC_{60}, vapor pressure, water solubility, and Log K_{ow}, these are not likely the key properties. It can be postulated that we should look at agglomeration state and stability of a dispersion, rather than water solubility; these parameters may tell us something about where these particles (materials) may end up. Rather than Log K_{ow}, perhaps we can consider interfacial behavior (deposition). For environmental components—ionic strength, ionic composition, pH, mixing, f_{oc}—mineral surface may be factors to consider. Whether or not this list is correct is debatable, but it is the current hypothesis of the Center for Environmental Implications of NanoTechnology (CEINT) at Duke University (see Figure C.3).

Attachment efficiency refers to deposition or sticking of nanoparticles to surfaces and varies with how particles stick/attach to biological surfaces. Attachment efficiency is essentially the number of collisions that result in attachment. Depending on what the value is, you can get different types of structures.

Most nanomaterials have coatings that provide dispersion stability, functionality, targeting capabilities, and biocompatibility. Coatings dominate the interaction energies between particles. When we look at collision between particles, we consider Van der Waals forces (attraction) and repulsion (electrostatic) forces. But with coatings, we must also consider osmotic repulsions (which means you can't push a lot of charge into a small area) and elastic repulsions (you can't push a lot of mass into a small area), which are strong repulsive forces resulting in agglomeration.

What makes nanomaterials useful (i.e., novel properties) are often the same things that may pose risks. If our goal is to accept and manage the risk of nanomaterials, then the best way to do that may be as follows:

1. Focus on hazard: focus on something less hazardous, e.g., substitute benign nanomaterials for a more hazardous nanomaterial.
2. Keep the functionality but change the material.
3. Modify the material: tune out the toxicity of given material. A lot of things change when you tune out toxicity, so it is difficult to tie properties of nanomaterials to effect.

Focusing on exposure, green chemistry does play a role in tuning nanomaterials to reduce bioavailability, to reduce/engineer mobility and persistence, much as we engineer coatings on drugs to control timed release. Make nanomaterials green by design, i.e., materials that are safe from synthesis on. Design them to biodegrade at end of product life.

Detailed Research Recommendations

Two possible approaches for determining the factors affecting environmental transport of nanomaterials were discussed:

Approach 1

- Look at releases from each part of the life cycle.
- Look at possible sources that also come from incidental and natural sources.
- Look at the properties of nanomaterials, including what they do, their function, how they were transformed in nature, or how we, perhaps, engineered their transformation; look at the modified properties and how they affect the distribution concentrations and, ultimately, their effects.
- Most of the research strategies tend to be bottom-up—cellular- up to population-level. Suggest looking at ecosystem effects, to do a top-down approach.
- You cannot do hazard without exposure or exposure without hazard. A balanced progression between exposure and effects (research) is needed.

Approach 2

- Start small and deal with partitioning first; this is what we did with organics.
- Look at equilibrium partitioning behavior, which may be a function of the attachment parameter.
- Then deal with rates of transfer between phases and where they end up going; then [deal with] the unsteady state or non-equilibrium state.
- Need to get some chemistry into the attachment efficiency, particularly for nanoparticles.
- Need to understand how pH, ionic strength, etc., affect attachment.
- Need to start thinking about disaggregation—how easily nanoparticles disaggregate will also affect how they transport in the environment.
- Need to understand attachment to biological surfaces—bacteria, plant roots, etc.—which may affect bioavailability considerably.
- Need to understand transformations that affect engineered nanoparticle aggregation and deposition: redox reactions, biological interactions, and condensation of organic matter from atmosphere or from solution will modify your particle.
- Numerical models for nanoparticles—macromolecule interactions and under what conditions and what conformations do they form.

Short-Term Research Needs

Analysis and characterization

- Development of analytical methods for detection and measurement of nanomaterials, coated nanomaterials, and/or transformed nanomaterials in environmental matrices (air, water, soil, sediments, sludge, etc.).
- Research on standard materials and procedures for evaluating transformations of nanomaterials.
- Need to think about chemical composition of nanomaterials, e.g., certain CNTs have metal catalysts residuals. It's important to characterize nanomaterials down to the parts-per-billion or parts-per-trillion level so we can avoid blaming nanomaterials when impurities/residuals cause a problem.

Facilitated transport

- Facilitated transport of nanomaterials of and by other materials:
  - Smaller or larger particles may facilitate transport of nanomaterials.
  - Nanomaterials can facilitate transport of other chemicals that you may not want to move (e.g., toxic contaminants): if you move clay, then you also move all of the nanoparticles attached to it.

Short-Term to Mid-Term Research Needs

Physico-chemical properties/surface chemistry/coatings

- Need to understand how nanomaterials will change in size and properties after we put them into the environment.
- What is the impact of surface chemistry on the transport/transformation of nanomaterials?
- What are the relative affinities of nanomaterials for key environmental fates/compartments/environmental matrices, and are these affinity sites size-dependent?
- Do surface coatings affect partitioning behavior: how do they do it, to what degree, can you predict it?
- What is the ultimate fate of the coatings? We know that coatings play a huge role in how they move around the environment and what impact they have on human toxicity; understanding the fate of coatings is critical for understanding transport and transformation:
    - What is the fate of the coating on nanomaterials, and how do their transformations affect transport?
    - The same nanomaterials with different surface chemistry/coatings may behave differently in the environment.
- Initial size as we make them is important, but actually, what we need to focus on is the transitional size or changes in environmental properties as nanomaterials get transferred through the environment.

Colloids

- What is the real relationship between transport of nanomaterials and colloids?
- The history and evolution of colloid science is a starting point for moving forward. We cannot forget what we already know, and we do not want to reinvent the wheel if nanomaterials truly behave as colloids.
- Where does conventional colloid science stop, and considering that these are active objects, how does that change the particles' transport?
- Can what we know about colloid science be used to predict these partition coefficients, which can then be used to predict the distribution of nanomaterials in the environment?

Nano-effects

- Is there a nano-effect on transport? If so, at what size does it occur?

Testing

- What will be the appropriate screening tests to predict toxicity, environmental fate, and environmental effects of these materials?

Mid-Term to Long-Term Research Needs

Surface chemistry

- Can distribution of nanomaterials in the environment be predicted from understanding the relative affinities of the nanomaterials for different surfaces, like distribution coefficients?

Colloids

- Can what we know about colloid science be used to predict these partition coefficients, which can then be used to predict the distribution of nanomaterials in the environment?

Stability

- What is the stability of nanoparticles in the environment? Are they the same as manufactured and released, or do they change with time in the environment?
- Transformation over time: does it affect physico-chemical behavior of nanomaterials over time?
- What is the impact of transformation on surface chemistry of nanomaterials on the subsequent transport of the nanomaterials (including coatings and organic matter)?
- Does the toxicity of nanomaterials change as they move through the environment and undergo transformation?
- Some nanomaterials have the potential to change repeatedly in the environment. It is possible that the nano-effects will also change with the particles' inherent properties.

Dose/Exposure

- There is a need to understand what is a significant dose of nanomaterials in the environment.
- What kind of working dose should we have in terms of exposure studies?

- What is the actual dose of nanomaterials that will cause effects in the environment? How can we quantify that?

Long-Term Research Needs

Modeling

- Similar to predicting partitioning of organic materials, can we predict partitioning of nanomaterials?
- Predictive models are a longer-term goal; we need to ensure that we do them well.

Stability

- Is a particle that is manufactured and incorporated into a solid matrix such as plastics the same as what is released from the matrix over time?
- Need to understand factors that control persistence—can we design biodegradability into nanomaterials?

ENV 4. Understand the Transformation of Nanomaterials under Different Environmental Conditions

Findings on the State of the Science

Similar to transport of nanomaterials in the environment, the most immediate need will be to develop analytical tools that will allow for detection and measurement of potential transformation/degradation products in environmental matrices.

- When considering the fate of nanomaterials in the environment, transport and transformation are inseparable topics. Environmental interactions change the fate of all materials, especially nanoparticles. Therefore, movement of nanomaterials via intra-media or intermedia transport will impact transformation potential as well as transformation rates.
- Overall, a limited number of studies have been performed on disparate systems. There is a paucity of available data, so the current state of the science offers very limited insight into the potential for transformation/degradation of nanomaterials.
- Results have clearly shown that environmental transformation is possible and that the properties of environmentally aged nanomaterials can differ from "as-released" nanomaterials.

Figure C.4 gives an overview of the potential environmental fates of fullerenes and fullerols.

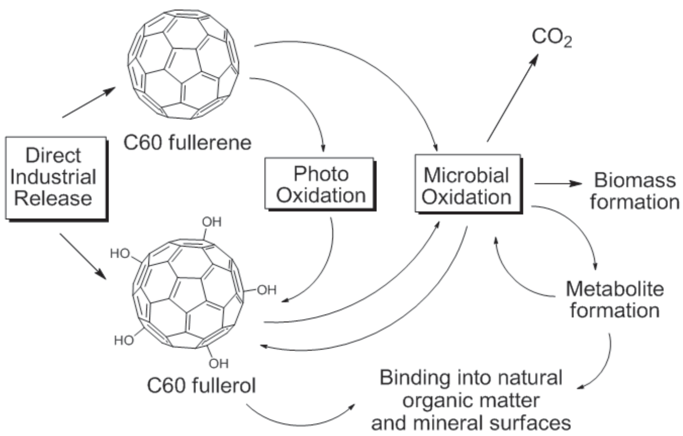


Figure C.4. Overview of potential environmental fate of C60 fullerenes and fullerols (Schreiner *et al*. 2009).

Detailed Research Recommendations

Approaches to consider for understanding the transformation of nanomaterials under different environmental conditions:

- Consider transport and transformation together.
- Consider all types of transformation together.
- Consider kinetics and products of transformation.
- Distinguish mass- and surface-normalized kinetics.
- Do not presume true nano-size effect on reaction kinetics in solutions.
- Consider that material/solution effects can be equal to or greater than nano-size effects.
- Need to understand the transformation of nanomaterials under different environmental conditions.
- Need to measure impacts of nanoparticles that may be transformed over time in the environment: This is essential information for decision making regarding handling, disposal, and management of nanoscale materials in commerce, manufacturing, and the environment.

Short-Term Research Needs

Analysis & characterization

- Need to measure and characterize the starting materials (metrology, analytical methods)
- Develop analytical methods for detection and measurement of transformed nanomaterials in environmental matrices—air, water, soil, sediments, sludge, etc.
- Do research on standard nanomaterials and potential transformation products
- Need to categorize or classify transformation products:
  - Determine how to classify transformation products of nanomaterials
  - Develop nomenclature and metrology associated with transformation products of nanomaterials

Short-Term to Mid-Term Research Needs

Physico-chemical properties/surface chemistry/coatings

- How do chemical characteristics or surface chemistry/coatings of nanomaterials affect their transformation potential or rate?
- Corollary: Can we take advantage of this to create nanomaterials that are not so persistent?
- Need to understand how surface chemistry (including coatings) and environmental factors (redox environment, sunlight, and biology) affect the potential for transformation and the rate of transformation.
- Nanomaterials come in lots of shapes, sizes, etc., so it is important to know which variables matter and which do not (e.g., for CNTs, length, diameter, chirality, surface chemistry, humidity, exposure time). Need systematic studies where one variable is controlled. It is also good to know what doesn't matter.
- What are the kinds of transformations, changes in surface chemistry and properties, etc., that will potentially influence toxicity, mobility, etc.?

Exposure conditions and impact on transformations

- There is a need to well-define exposure conditions, then understand how those exposure conditions lead to changes in materials properties, degradation rates, and environmentally relevant behavior (toxicity, mobility).
- As a function of exposure, need to understand how different or well-defined exposure environments relate to potential for transformation, e.g., exposing samples to artificial lighting in a lab environment vs. exposing samples to the full solar spectrum of natural light.
- To what extent do the exposure conditions change the properties of the particles from those we started off with? You can clearly modify the properties of the nanoparticles based on exposure.

Releases from nano-products

- Need balance of real-world and fundamental research, i.e., release from nano-products (e.g., composites) vs. looking at just pristine nanomaterials. Most nanomaterials are not present in isolation but are part of larger systems (e.g., CNTs in polymer composites, etc.).
- Need to understand long-term release rates of nanomaterials from nano-products.
- Need for accelerated exposure studies: studying environmental aging and transformations in the environment is likely to be very slow, so difficult to do in a lab environment. As a model for accelerated exposure studies, the NIST integrating sphere[3] can simulate one year of aging in one week. Most nanomaterials will be in a product, so releases will likely be very slow.
- Need to understand the transformations of products containing nanomaterials and impact on release rates.

Mid-Term to Long-Term Research Needs

Transformation mechanisms

- What are the key transformations that we would expect of nanomaterials in the environment?
- Can we somehow categorize these transformations (such as using the chart in C.5.) to help better understanding of mechanisms

3 An "accelerated ultraviolet weatherability testing device"; see http://nist.gov/el/facilities_instruments/integrating_sphere_fac.cfm

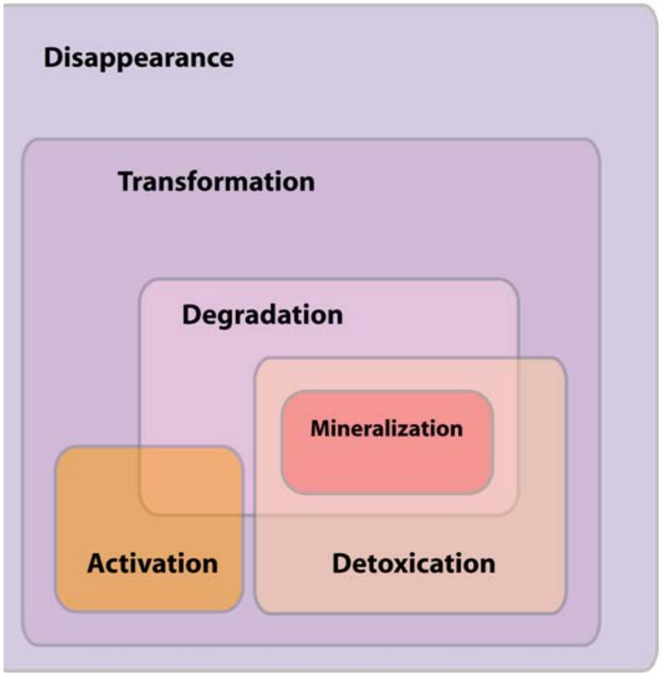


Figure C.5. Transformation vs. other changes (Paul G. Tratnyek, "Transformation of Nanomaterials in the Environment: The Challenge of Kinetics," October 7, 2009, presentation at NNI workshop).

and categorization of transformations of nanomaterials?

- Figure C.5 shows other possible outcomes for nanomaterials.
- What are the factors that control persistence of nanomaterials?
- What are the enzymatic processes that control fullerene degradation?
- What are the processes that control the stability of coats on metal oxides and their subsequent dissolution?
  - Need better detection methods (^{13}C / ^{14}C) for tracking and metabolism studies. ^{14}C is expensive and dangerous, i.e., poses health risks
  - ^{13}C does not share all of ^{14}C's issues, but tracking it is a bit more problematic.
  - If the Federal Government could provide well-characterized radio-labeled nanomaterials, then this would accelerate the advancements in the research.
- What functional groups need to be incorporated into CNTs to make them biodegradable?
- Or is it possible to incorporate certain groups that can be activated later and make them biodegradable?

Implications:

- Priority issues arise at all levels.
- Priority/significance depends on context.
- More consideration of the relationships between these levels.
- Need to match unique properties of CNTs with controllable biodegradation
  - Posed question: Can we synthesize biodegradable molecules or can we activate the biodegradation property after the disposal of nano-product containing CNTs?
  - Can we synthesize biodegradable CNTs by design or perhaps activate the property at the end of the nanotubes' product cycle?

Long-Term Research Needs

Predictive tools

- Importance of scientific rationalization—develop functional relationships: Can we take the kind of metrics that we can measure in the lab such as size, shape, chemical composition, surface chemistry, surface composition, particles in solution, aggregation state, and can we relate them to exposure and potential for transformations?
- Need for predictive tools to determine what are the:
  - Physico-chemical and environmental properties that control the persistence?
  - Transformation potentials of nanomaterials?
  - Transformation pathways of nanomaterials?
  - Transformation products of nanomaterials?
  - Environmental impacts from potential transformation/degradation products?
  - Environmental effects from potential transformation/degradation products?
- In addition to developing the needed predictive tools, we need to use rational engineering to engineer out the negative effects.
- We need to develop a nomenclature for degradation products of nanomaterials.

- How do we convert laboratory values into something such as half-life that can be used by regulators?
- We need to focus on soil and rhizosphere interactions as key research priorities; these systems are currently under-studied. We need to look at persistence in soils.
- How do we close the gap between environmental chemists and toxicologists?
  - We know that pristine nanomaterials will not degrade vs. oxidized forms that do degrade, yet the toxicologist tests pristine nanomaterials.
  - Do toxicologists test coated nanomaterials or just the pristine non-coated nanomaterial?
- Can the expected nanomaterial transformations be determined from a chemical process-based classification rather than a media base (e.g., subject to phototransformation, biodegradation, etc. vs. persistence in air, water, soil, etc.)?
- We need to do some thinking about the need for "activation" for initiating transformation/degradation.
- Abiotic process activates and then biodegradation follows.
- Lastly, but importantly, release rates from nanoproducts are among the least studied and yet most important variables in many risk assessment models.

ENV 5. Evaluate Abiotic and Ecosystem-Wide Effects

Findings on State of the Science

- In order to understand ecosystem-wide effects, it is important to understand sources (production/use/disposal), pathways, and key environmental receptors. Source, pathways, and receptor exposures are influenced by the fate and transformation of nanoparticles. For example, oxidized CNTs may have increased stability in water allowing for greater transport, and allow for increased sorption of metals and other non-organic compounds or decreased extent of uptake of nonpolar organic phases. These and other related studies of carbon nanomaterials in these respects were reviewed by Chen *et al*. (2010).
- There are little data on population, community, ecosystem, and abiotic effects in the literature. Limited data exist from a handful of studies on aquatic ecosystems due to mesocosm and similar studies, and some data are available on individual microorganisms and microbial communities on exposure to nanoparticles.
- Mesocosm studies are being utilized to understand the toxicity and uptake of nanomaterials in complex aquatic systems. System-wide effects can be studied currently in such experiments, yielding important information on relative species sensitivities, appropriate methodologies for dosing, and relative toxicities of different nanoparticles. Clams and amphipods showed a much greater intake of titanium dioxide nanoparticles in flow through systems than in static systems. Acute toxicity was not observed in the titania or silver nanoparticle systems using Columbia River water. In microbial communities there was a species shift after 14 days exposure to nano-silver which was not seen in the ionic silver control group.
- Research results on C_{60} and nC_{60} compounds and their toxicity to bacteria indicated that bacteria are key to the biodiversity of some systems, and a disruption of microbial activity could lead to widespread disruption of agriculture and/or nutrient cycles.
- Bacteria could also be useful indicators of toxicity to higher-level organisms. For some nanoparticles such as silver, it appears that they partition from the water column into the sediments within a matter of weeks after introduction into the water column which elevates concerns for sediment deposition and adverse effects in sediment dwellers. However, with some estuarine systems at least, silver nanoparticles seem to have little adverse effect on microbial communities within the sediments. Estuarine microcosms exposed to gold nanoparticles indicated that gold was concentrated in sediments, and tissues of grass shrimp and snails, 12 days after release of gold to the water column.
- Less data are available for terrestrial systems than for aquatic systems. Within the literature on terrestrial systems, even less are available on avian species than mammalian species

(due in part to the tests already done on mammalian species for purposes of human health assessment), and the least data are present for plants. Most of the data on both mammalian and plant species are from tests on individuals only. On the terrestrial side, little is known about nanoparticle effects on nutrient and water cycling, or on biomass production.

Testing Scale & Complexity: Research Towards Use of Tests Systems More Representative of Ecosystems

- Microcosms and/or mesocosms potentially offer more representative environments than beakers and growth media for understand the complex and simultaneous fate and biological effects.
- Several researchers noted that a concept that fundamental studies of the potential hazards from nanomaterials should be guided by a view of how nanomaterials behave in environmental systems.
- This view evolved from the use of mesocosms simulating river systems that were dosed with two types of titania or silver citrate nanomaterials. Nanomaterials were dosed into the mesocosms at concentrations expected to occur based upon life cycle assessments and/or reports near the LC50 of target organisms (*Daphnia*). Nanomaterials were "overwhelmed" by natural particulates, organics and ions in a relatively clean river water (7 mg/L of suspended solids). To describe the polydispersity of nanomaterials, size fractionation (50,000 Dalton which is ~ 5 nm) was employed. Bioaccumulation was evaluated after a 24 hour pulse exposure to nanomaterials and 24 hour depuration. The two different forms of titania exhibited different transformations, in size and potential bioaccumulation. However, very little of the citrate nanosilver settled out in comparison to the "white snow" aggregates from titania. Specific data were presented, but the conclusion was that toxicity will be different in natural setting than standard tests; and between static tests and conditions influenced by natural fluid hydraulics.

Detailed Research Recommendations

- In order to understand which ecosystems are likely to be most impacted, a better understanding of certain source/fate information should be pursued in the near term. Sources of nanomaterials from both production facilities and from life-cycle perspectives (use, disposal, etc.) should be identified (as noted in the 2008 NNI EHS Research Strategy). Also, inventories of production and use information, which include locations of major production, use and disposal, should be identified by GPS positions. Incentives to provide this type of information need to be developed. Such information should be used for exposure modeling efforts. Differences between nanomaterial behavior in the laboratory versus the environment should be better understood in the near term, and correlated with appropriate in-lab dosing methods (static, pulsed, or continuous flow protocols). Additional physico-chemical oriented research should be put to use in the medium term: receptors (and surrogate biomarkers such as stress genes) should be identified based on the near-term findings noted in this paragraph, and the effects of the environment on nanoparticles should be better understood (both physico-chemical effects, and biological effects).
- With regard to species tested, there should be more work done on terrestrial species as compared to aquatic systems, with more work on terrestrial plants relative to terrestrial animals. Plant effects could focus on effects associated with relevant exposures such as canopy and root functions. In the medium term, better methods for imaging nanoparticles in plant tissues are needed. For both terrestrial and aquatic environments, keystone species in food webs, and sediment/soil-dwelling organisms should be given higher priority. Species which differ in some instances from those currently tested in standard regulatory protocols such as those of the OECD would provide a better understanding of the effects across a more representative cross section of communities exposed. Selection of species may be guided in part by findings from higher-level testing noted in items directly below. Endpoints tested may need to concentrate more on control systems such as those associated with the endocrine, immune, and nervous systems. Testing for adverse effects should consider not

only the parent nanoparticle, but also consider transformation products and other toxic chemicals associated with nanoparticles in the environment. Whole effluent testing may be useful to better understand adverse effects of nanoparticles within a realistic background.

- To approach population-level effects, it may be useful to examine dietary exposure and effects since bioenergetics of aquatic receptors can be linked to individual and population level effects. Receptors such as fish may preserve growth at the expense of other functions such as locomotion and reproduction: for example, increasing dietary copper concentrations lead to decreased swimming activity and increased oxygen consumption in trout (Campbell, Handy, and Sims 2002).
- For community-level effects, research focused more on soils and sediments may be appropriate considering that these form the base of the ecosystem, that fate information indicates that nanoparticles may concentrate in these systems, and that there are some indications that particle size is inversely correlated with adverse effects in some bacteria (Neal, *Ecotoxicology* (2008) 17:362–371). Additional communities representative of broader potential impacts should be considered such as those involved in key biochemical, photosynthenthetic, respiration, and geochemical (nitrogen, water) pathways.
- Given the current lack of understanding regarding the fate and effects of nanoparticles, ecosystems should be examined for adverse effects noted above sooner than that recommended in the 2008 NNI document through microcosms and/or mesocosms. Direct field measurements could also be done in the nearer term. The range of effects noted above should be examined, along with fate/transformation considerations and food-chain effects. Longer-term studies would include more complete field studies. All of these findings should feed back into modelling of both effects and exposure/fate of nanoparticles and their transformed products.
- Inventory databases of production and use information should be developed, and coupled with nanoparticle properties of interest (including persistence, toxicity, transformations, and phase distributions).
- Industry should pool resources and coordinate research to address the range of commercial materials and endpoints in question. Mechanisms for industry/academic partnerships should be developed to leverage resources.
- Priorities should be developed for research needs that would reduce the need to have every major nanoparticle exhaustively researched prior to commercialization.
- Research should be advanced that focuses on developing nanomaterials that are designed from the start with an eye not only toward efficacy, but also with minimal potential for adverse effects.

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Appendix E. Panel Reports: Discussion Notes for the Instrumentation, Metrology, and Analytical Methods (IMA) Research Needs

IMA 1. Develop Methods to Detect Nanomaterials in Biological Matrices, the Environment, and the Workplace

Findings on the State of the Science

- Participants agreed that the most immediate need, and perhaps the fundamental underpinning for most challenges associated with nanomaterials in the environment, will be to develop analytical tools that will allow for measurement of manufactured nanomaterials in environmental matrices. However, the focus needs to include more activity on elucidating methodologies for the detection of nanomaterials in the environment. If we are delayed in developing our understanding of how nanomaterials behave in the environment, then our ability to assess exposure will be limited, and this will limit our ability to conduct suitable risk assessments. Notably, the need for the appropriate analytical tools for detection in environmental matrices is a high-priority item for our understanding of nanomaterials in the environment.

Participants largely agreed upon the following:

- Most of the emphasis for this research need is currently focused on the characterization of nanomaterials in tissues, cells, or at subcellular levels. Some of this emphasis should be redirected towards an environmental focus.
- There has been minimal emphasis on developing analytical methods for measuring nanomaterials in environmental media (water, soil) because of complications from background matrices and likely low concentration levels that will be present under realistic exposure or release scenarios.
- While advanced nanoscale material sampling and analysis instrumentation is available for air-phase sampling, instrumentation may not be as portable, rapid, or standardized as desired for certain air-phase sampling applications such as that needed for monitoring of specific workers as they move through a manufacturing facility.
- Furthermore, these analytical tools will need to enable differentiation of manufactured nanomaterials against background from both incidental and naturally occurring nanomaterials. We will continue with a very limited understanding of the transport and fate of nanomaterials in the environment until we have the critical analytical tools for detection and measurement in the environmental matrices of interest. While there are numerous reviews of potential instruments and analytical schemes, evidence of applying them for nanomaterials in environmental matrices are limited. The tables below summarize the current state of science.
- Environmental interactions change the fate of all materials, especially nanoparticles. Detection is based on separation schemes so transformation will change our ability to separate for detection. Therefore, the challenges of detecting transported nanomaterials will be linked to its transformation potential.

Detailed Research Recommendations

- Add quantum dots to EPA's lists as they facilitate detection and are in many commercial products.
- Determine if we are asking the right questions related to what we need to measure to access exposures. The analysis of ultrafines in air is ahead of water, and experience with air suggests asking the proper question(s) before proceeding with method development for other environmental matrices (e.g., lung deposition).
- Conduct occurrence surveys and require monitoring of potential releases.
- Build and populate a globally accessible database with knowledge maps.
- Focus on the traits necessary for model building.
- Validate methods: Ensure that research test result and methods are shared in an easily accessible manner. Ensure that measurement methods are developed and validated.

- General goal: Establish mileposts such as a nanoparticle standard or particle labeling with a completion date.

Short-Term Research Needs

- Industry should have methods to measure pristine engineered nanomaterials they produce and be able to find them in environmental matrices.
- Methods are needed soon to monitor possible releases of engineered nanomaterials from production facilities and commercial products.
- Methods exist from natural colloid work for separation and concentration of engineered nanomaterials that can be linked with detection (e.g., flow field fractionation/inductively coupled plasma mass spectroscopy (FFF/ICP-MS)) but need development and application to environmental and biological matrices.
- Confirmation of measurements in biological media by n >1 techniques.
- Testing whether "reactions" can be used to amplify engineered nanomaterial detection.
- Transformation of certain engineered nanomaterials *would* most likely occur once released into the environment; monitoring needs to be figured out.
- Testing how environmentally relevant concentrations of engineered nanomaterials can be detected or separated from naturally occurring colloids? Engineered nanomaterials become "overwhelmed" by naturally occurring materials even in clean water (e.g., 7 mg/L TSS).
- Prioritization of characterization needs: concentration, size, surface charge may be more important than other parameters.
- Validation of separation techniques (e.g., FFF, ultra filtration) when engineered nanomaterials are non-spherical (stars, sheets, tubes).
- Interaction of disciplines is critical.
- Engagement with manufacturers to bring industrial hygiene air samples to market. The big challenge is characterizing the particle stream.
- There is a strong need for analytical tools.
- Need to know what to measure to move forward with assays.
- How do toxicity characteristics compare to what we know about other chemicals?
- Characterization of aggregates.
- Shape factor detection.
- Determination of characteristic(s) that dominate toxicity.
- Connection of nanoparticles to agglomerations, and larger particles.
- What can we learn about ultrafine particles from other industries such as mining?
- Should our focus be on nanoparticles?
- Agglomerates and nano-objects are adhering to surfaces.
- Do we focus on dispersal of nanoparticles?
- Do we look at the product containing the engineered nanoparticle, the agglomerates/aggregates of the nanoparticles, or the nanoparticle singlets?
- Can we break apart an agglomerated material to get a calculable measurement for the bulk material?
- Shape is a factor in looking at agglomerates.
- There are two levels of uncertainty between the primary and the aggregates. There is a bigger degree of uncertainty with the aggregates.
- If different instruments and methods produce different measurements of the same thing, how do we decide which is accurate? Which do we use?
- Development of analytical methods (measurement and characterization) for detection and measurement of nanomaterials, coated nanomaterials, and/or transformed nanomaterials in environmental matrices such as air, water, soil, sediments, sludge, etc.
- Research on standard materials and procedures for evaluating transformations of nanomaterials.
- Consider chemical composition of nanomaterials, e.g., certain CNTs may have metal catalysts residuals. It is important to characterize nanomaterials down to the parts per billion (ppb) or parts per trillion (ppt) level so we can avoid blaming nanomaterials if impurities/residuals cause a problem.

- Need to understand how nanomaterials will change in size and properties after possible environmental release and then how to measure and detect the nanomaterials that have undergone these changes.
- What is the ultimate fate of the coatings? We need analytical tools that will allow for detection and differentiation of the transformation of coated nanomaterials to non-coated nanomaterials to metabolites and/or transformation products in environmental matrices.
- Notably, the same nanomaterial with different surface chemistry/coatings may require different detection methods in the various environmental matrices.
- Some engineered nanoparticles have been show to change in the environment along with their properties. Initial size as we make them is important for detection, but we also need analytical methodologies for detection of nanomaterials as they undergo transitional size changes and we also need detection methods to account for changes in environmental properties as nanomaterials get transferred through the environment.
- Need detection and characterization tools to address the following question: Is a particle that is manufactured and incorporated into solid matrix, e.g., plastics, the same as what is released from the matrix over time?

Mid-Term Research Needs

- Will industry be required to understand the transformations of engineered nanomaterials in the environment? How does this compare with chemical pollutants?
- Will need to document / monitor releases from existing / new facilities.
- Would tagging help identify the vectors?
- There is controversy that tags will render nanomaterials ineffective. Will this tool be helpful?
- A marker: unknown how to achieve it.
- Would tagging be different for carbon and metal-based materials? For engineered versus naturally occurring nanomaterials?
- How do you mark them or even know they're being made? They're proprietary.
- Using the isotope is probably the best we can do to tag CNTs.
- Hazard investigations will catch up in ten years, but how to demonstrate that there is no carbon nanotube in the product waste stream? No practical methods currently exist. The practical implications of having no method: There are products backed up at EPA awaiting this kind of testing. Also, no standard exists, which regulators would probably like to have.
- Need to look at combinatorial hazards.
- Need better detection methods (^{13}C / ^{14}C) for tracking nanomaterials and their metabolites in the environment.
- ^{14}C very expensive; need to mitigate potential hazards.
- ^{13}C does not have the same issues as ^{14}C, but tracking it is a bit more problematic.
- If the (Federal) government could provide well-characterized radio-labeled nanomaterials, this would accelerate the advancements in the research.

Long-Term Research Needs

- Document / monitor releases from existing / new facilities.
- Assuming by 2015 that adequate methods for engineered nanomaterials detection and characterization in air, water, and soil exist,; standards for validation of methods and exposure data should be available.
- Who is funding and/or doing this research now to reach these goals?
- If a site were contaminated by nanomaterials, what would be needed?
- In order to remediate, need to know what was at a site.
- Use forensics to define the extent of a "plume."
- Determine if engineered nanomaterials are present as the pristine material or as transformed materials.
- Remedial investigation may include fate and transport models; some companies may develop

fate and transport of pristine engineered nanomaterials before major events occur.

- Perform feasibility study; may need to draw upon basic science (fungal degradation of hydroxylated materials).

IMA 2. Understand How Chemical and Physical Modifications Affect the Properties of Nanomaterials

Findings on the State of the Science

All transport is affected by the aggregation state of the material and by deposition of that material on to surfaces. Factors affecting aggregation and deposition for colloids also hold for nanomaterials.

- Size of nanoparticles.
- Energy input—flocculation process and energy input influences size of engineered nanomaterials.
- Less understood is how natural organic matter or organic acids that attach to these engineered nanoparticle surfaces affect aggregation and deposition and therefore transport. These are kinetics processes, not thermodynamic processes so exposure is critical.
- Most particles have coatings—how do surfactants, polymers, polyelectrolytes affect aggregation and deposition and therefore transport?
- Biological modifications by EPS—we can modify particles with EPS but do not have good handle on how this affects transport.
- Research has shown that C_{60} properties change as a result of aggregation. Chemical reactivity will change as well.

Most nanomaterials have coatings that provide dispersion stability, functionality, targeting capabilities, and biocompatibility. Coatings dominate the interaction energies between particles. When we look at collision between particles we consider Van der Waals forces (attraction) and repulsion (electrostatic) forces, but with coatings we must also consider osmotic repulsions (which means you cannot push a lot of charge into a small area) and elastic repulsions (cannot push a lot of mass into a small area) which is a strong repulsive force resulting in agglomeration.

As an example, Figure D.1 presents the chemical functionalization of CNTs. Understanding the potential impact of these modifications on the

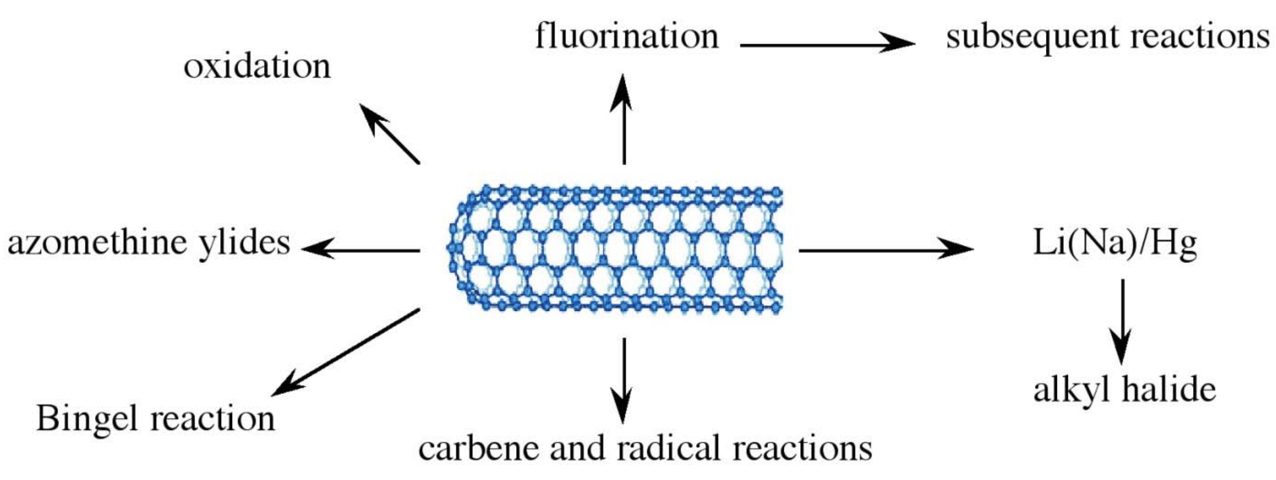

Figure D.1. Chemical functionalization of CNTs (Alexander Starr, presentation at the October 7, 2009, NNI workshop).

fate and effects of CNTs in the environment is a fundamental research need.

Detailed Research Recommendations

Short-Term to Med-Term Research Needs

Surface chemistry/coatings

- What is the impact of surface chemistry/coatings on the transport of nanomaterials in the environment?
- Do surface coatings affect partitioning behavior? How they do it, to what degree, can you predict it?
- How do chemical characteristics or surface chemistry/coatings of nanomaterials affect their transformation potential or rate?
- Corollary: Can this be used to create nanomaterials that are not so persistent?
- How does surface chemistry (including coatings) and environmental factors (redox environment, sunlight, and biology) affect the potential for transformation and the rate of transformation?
- What is the ultimate fate of the coatings? What is the impact they have on human toxicity?
- What is the fate of coatings on nanomaterials and how do their transformations affect transport, mobility, and toxicity?
- Same nanomaterial with different surface chemistry/coatings may behave differently in the environment.

Stability

- What is the impact of transformation on surface chemistry of nanomaterials on the subsequent transport of the nanomaterials? Including coatings and organic matter.
- Some nanomaterials are likely to change repeatedly in the environment along with their properties. Will the nano-effects change also in the environment if the nanomaterials change?

IMA 3. Develop Methods for Standardizing Assessment of Particle Size, Size Distribution, Shape, Structure, and Surface Area

Findings on the State of the Science

Participants found that EHS is an area where there is definite need for fundamental scientific research that will be beneficial for all nanomanufacturing sectors. The development of a traceable measurement infrastructure and metrology will assist in determining the fate of nanoparticles to be tracked from their point of production, including as raw materials, to their end of use and disposal, either in raw form or in a product. This requires cross-cutting EHS efforts focused on research and development of instrumentation and analytical methods applicable to the nanoscale size-regime.

Characteristics include purity, particle size and distribution, shape, crystal structure, composition, surface area, surface chemistry, surface charge, surface activity, and porosity. However, the application ultimately determines what needs to be measured and to what degree of accuracy. Characterization at a minimum must establish particle size, and morphology. It is imperative to establish that those protocols used to either prepare or analyze the material have no effect on the morphology of the particles.

- Many challenges exist for the determination of the shape, structure, and surface area of particles associated with nanomaterial production.
- Conventional electron microscopy is not fast enough to provide population statistics necessary to sufficiently characterize the structure of nanomaterials.
- Methods for rapid analysis of screening of nanomaterials are lacking for the measurement of the particle size and particle-size distribution of nanomaterials.
- Development of analytical tools for the automated characterization of nanoparticles by electron-beam analysis methods is needed.
- Improved measurement methods for particles that are less than 50 nm are critical. Optical microscopy and spectroscopy may be adequate for nanomaterial characterization at less than 100 nm resolution using super-resolution optical microscopy.
- Methods that may have sufficient particle number sensitivities for the characterization of the size and number distribution of nanoparticles include differential light scattering, analytical ultra centrifugation, ion mobility classification, scanning tunneling microscopy, atomic force

microscopy, and small angle scattering using X-ray or neutron sources.

- Development of automated microscopic methods for the rapid analysis and screening of a large number of nanomaterials is critical for systems for real-time monitoring in the manufacturing environment.
- Accurate correlations of electron microscopy with other size-measurement techniques, such as differential light scattering or field flow fractionation, are critical for scientists in the field.
- In addition, separation techniques, such as liquid chromatography, size-exclusion chromatography, capillary electrophoresis, field flow fractionation, or microfluidic techniques, may be applicable to the determination of the size-distribution of manufactured nanoparticles preferred for manufacturing or process control.

Detailed Research Recommendations

Entirely new metrology tools will be required to meet the needs of emerging nanotechnologies. Currently available equipment in most cases is at the limits of resolution, and much greater metrology capabilities will be required for every area from laboratory research to commercial-scale manufacturing. Metrology is a key enabling technology for the discovery, development, and manufacture of emerging nanomaterials and systems, citing specific needs in real-time analytical and characterization tools, standardization, and informatics.

Specifically, this research need seeks to:

- Provide rapid, statistically valid, standardized methods for measuring particle size, size distribution, shape, structure, and surface area of nanomaterials.
- Develop instrumentation for automated and real-time process development, scale-up, and control; for quality control; and for EHS monitoring and control.
- Develop methods for the rapid analysis/screening of nanomaterials.
- Develop miniaturized instruments for process monitoring and control.
- Define standard operating procedures for the synthesis of nanomaterials and sample preparation procedures for measuring, handling, and storing nanomaterials.
- Evaluate correlation of microscopic methods with other size measurement techniques.
- Establish and populate a database of physical property data for nanomaterials.
- Develop predictive multi-scale models to discover new materials based on physical property data and reference materials, and for process development, control, and predicting product performance and life cycle.
- Evaluate or modify microscopic and mass spectroscopic approaches for determination of shape and structure of nanomaterials.
- Explore methods beyond isothermal adsorption for nanomaterial surface area determination.
- Extrapolate from existing measurement techniques/methods from other fields/industries.
- Develop a method for measuring nanoparticles in aqueous solutions.
- Invest in integrated computational methods to develop predictive and assessment tools for nanometrology.
- Foster the development of consortia co-funded by government and industry tasked to bridge the gap for the development of sector-specific instrumentation for nanometrology.
- Develop a technology roadmap for nanotechnology for instrumentation and metrology similar to the current *International Technology Roadmap for Semiconductors* (http://www.itrs.net) to guide technology development and assist instrument manufacturers in providing measurement tools within a reasonable lead time.

IMA 4. Develop Certified Reference Materials for Chemical and Physical Characterization of Nanomaterials

Findings on the State of the Science

According to participants, there are currently few documentary or reference material standards for nanomaterial properties that are directly relevant to environmental, health and safety research. Of 65 currently available nanoscale reference materials as compiled by BAM [2] only 15 are likely to have environmental, health, and safety relevance. Additional reference materials are in the development pipeline at the National Institute of Standards and Technology (NIST), an agency within the U.S. Department of Commerce (see Table 4). Other reference material projects are in the preproduction research phase, and are therefore not identified in Table 4.

List of Abbreviations Used in Table 4

NIST, National Institute of Standards and Technology, Department of Commerce, USA

IRMM, Institute for Reference Materials and Measurements, Joint Research Centre, European Commission

AQSIQ, General Administration of Quality Supervision, Inspection and Quarantine, China

JSR, JSR Corporation, Japan (STADEX brand series)

C, certified reference material

R, not identified as certified reference material

Standards that are currently available often have limited applicability (e.g., reference materials for particle size measurement) or lack sufficient validation (e.g., documentary standards without corresponding interlaboratory evaluations). This situation is now widely recognized as a substantial bottleneck to progress in the accurate assessment of the EHS risks posed by engineered nanomaterials.

Table 4. Status of Nanoscale Reference Materials Relevant to EHS Applications

List of currently available nanoscale reference materials*

RM Name	Description	Nominal Size (nm)	Source	Type	Status
SRM 1963a	polystyrene latex spheres	100	NIST	C	available
SRM 1964	polystyrene latex spheres	60	NIST	C	available
RM 8011	gold nanoparticles	10	NIST	C	available
RM 8012	gold nanoparticles	30	NIST	C	available
RM 8013	gold nanoparticles	60	NIST	C	available
SRM 2483	raw SWCNT soot		NIST	C	in production
RM 8282	purified SWCNT "Bucky paper"		NIST	C	in production
RM 8281	purified, length sorted SWCNTs	800, 400, 150	NIST	C	in production
SRM 1898	titanium dioxide powder	22	NIST	C	in production
IRMM 304	silica nanoparticles	40	IRMM	R	available
GBW 12011	polystyrene latex spheres	61	AQSIQ	R	available
SC-0030-A	polystyrene latex spheres	29	JSR	R	available
SC-0050-D	polystyrene latex spheres	48	JSR	R	available
SC-0055-D	polystyrene latex spheres	55	JSR	R	available
SC-0060-D	polystyrene latex spheres	61	JSR	R	available
SC-0070-D	polystyrene latex spheres	70	JSR	R	available
SC-0075-D	polystyrene latex spheres	76	JSR	R	available
SC-0080-D	polystyrene latex spheres	80	JSR	R	available
SC-0100-D	polystyrene latex spheres	100	JSR	R	available

Sources: V. Hackley, NIST, and German Federal Institute for Materials Research and Testing (http://www.nano-refmat.bam.de/en/)

The situation is further complicated by other factors such as:

- Limited resources for funding of standards research;
- Wide range of engineered nanomaterials and properties under consideration;
- Numerous sources supplying engineered nanomaterials for research and development purposes;
- Inherent instability of many engineered nanomaterials formulations; and
- Time and effort required to develop, optimize, and validate methods for property characterization and to certify reference materials. Some progress has been made in the past two years toward prioritization of standards and measurement needs, but broad consensus and lack of specificity are still slowing progress.

Detailed Research Recommendations

- Hazard and exposure effects of engineered nanomaterials are dependent on physico-chemical properties of the materials (e.g., size, shape, surface charge and composition, stability). Certified reference materials are required to ensure accurate and precise measurements of such properties required for exposure assessment and toxicology and other hazard identification.
- Reference material development efforts should focus initially on those engineered nanomaterials that have the greatest potential impact on the environment and human health, based on volume production, widespread use in products, and known or potential hazards. Reference materials cannot possibly be developed in a manner that would permit every possible research need, material type, matrix, or property measurement to be directly addressed in the near- to mid-term time period. Instead, the role of reference materials, and perhaps more generally "standards," should be to provide benchmarks, primary measurement validations and calibrations, to enable interlaboratory comparisons, and to increase the overall confidence level associated with nanoEHS research findings. To accomplish these objectives, the specific materials and properties for certified reference materials must be better defined and prioritized by the research and regulatory communities.
- Minimum physical and chemical characterization criteria should be established for the publication of nanoEHS data in peer reviewed journals and publicly accessible databases. These minimum criteria should furthermore serve as a priority list for development of standards for engineered nanomaterial property characterization. The development of reference material standards for nanoEHS requires collaborations between researchers across disciplines, e.g., chemists, physicists, and toxicologists.
- Research need IMA4, by virtue of the term "certified," is generally considered to be the purview of national and international bodies such as the national metrology institutes. However, these bodies should increase their level of collaboration with academia, industry, and other governmental and non-governmental organizations in order to leverage limited resources and to better define and address the specific needs of the EHS research and regulatory communities.
- So-called artifact standards (e.g., certified reference materials) and documentary standards (e.g., procedures, protocols, guides to practice) should be developed in unison, with one supporting or underpinning the other.
- The need for certified reference materials is greatest in the near- to mid-term and is viewed as a bottleneck area slowing overall progress of nanoEHS research and the accurate assessment of the potential hazards associated with engineered nanomaterials. Progress is being made on reference material standards, but there is a need to accelerate the efforts and to develop reference materials that are specifically designed to support EHS research and assessment. This will increase both public and regulatory confidence in reported findings of EHS studies, and enable advances in all nanoEHS areas.
- Engineered nanomaterials are often inherently unstable with respect to their dispersion within liquid matrices or their chemical structure, composition, or phase over time. This is a

substantial challenge for the development of certified reference materials, which require long-term stability of their assigned property values or measurands.

- Sustainable metrological traceability should be an objective for the long term. In the short term, emphasis should be placed on the harmonization and validation of measurement procedures in conjunction with the development of crucial reference materials that are well characterized and facilitate both method development and quality control for engineered nanomaterial property measurements.
- It would be advantageous to establish a national or international system for interlaboratory comparisons. Such a system would enable: (1) confidence in property measurements of prototype reference materials; (2) validation of properties of new materials; and (3) proficiency testing of participating laboratories through measurements of a common material.

IMA 5. Develop Methods to Characterize a Nanomaterial's Spatio-Chemical Composition, Purity, and Heterogeneity

Findings on the State of the Science

At the nanoscale, single defects and slight changes to surface dimension and composition can dramatically influence reactivity; hence, proper characterization of spatial composition is critical. This research need seeks methods to characterize an ENM's spatial composition, the identification of possible defects or impurities, and batch-to batch variation in nanomaterial production or biological activity.

- Evaluate scope and suitability of techniques to assess purity and batch-to-batch production of nanomaterials
- Development of methods for 3D chemical characterization at 1 picogram level.

The production of nanomaterials poses unique challenges to raw material specification, purity, and quality control not typically encountered when manufacturing materials of larger dimensions. Currently, the ability to synthesize nanomaterials with reproducible defect control, purity, and structure is limited. Purifying nanomaterials after they are produced is extremely difficult and expensive; it is far easier to control these parameters on the front end of the process. In many cases impurities are carried through a particular process unaltered and, in some instances, concentrated during the process. The final product may be complex matrices of intractable materials, which do not easily lend themselves to analysis.

- A critical need is to understand the raw material requirements to ensure the quality of nanomaterials produced for specific applications.
- Applications for nanomaterials are often very sensitive to impurities and have narrower tolerances than applications in commodity markets.
- A number of real-time techniques must be developed and implemented to accelerate synthesis of nanomaterials with predetermined structure, function, and purity.
- Nanosynthetic methods must be rigorously defined to provide a reference for both laboratory researchers and manufacturers similar to small molecule compendia that are used by the synthetic community today.
- Another key area often overlooked for addressing EHS and measurement issues is the development of atomic-scale modeling efforts with respect to nanomaterials. These approaches can provide fundamental insight into their stability, an important issue in sample handling and processing. Atomic-scale modeling efforts may also assist with determining surface and material interactions. In addition, physico-chemical properties of nanomaterials may be computed using atomic-scale models. These results could enhance, advance, and guide experimental characterizations of the materials. Computational efforts are necessary to provide fundamental information for nanometrics and method development for nanomaterial suppliers
- Accurate three-dimensional visual, chemical, and physical characterization at the nanoscale is essential to understanding structure-property relationships in nanomaterials. Physical properties are known to depend on size, particularly at the size scales considered for nanomaterials. Therefore, large-scale

bulk measurements of physical and chemical properties may not adequately reflect properties at the nanoscale.

- Observing, correlating, and understanding structure and function at the nanoscale is essential to developing reproducible nanomaterials by design. To do this, analytical tool capability must move from static measurements to dynamic, real-time measurement. Chemical, physical, and temporal properties at the nanoscale must be monitored as reactions occur and as systems evolve (including living systems). Accurate and precise three-dimensional characterization tools providing this capability are essential to the advancement of R&D in fundamentals and synthesis, manufacturing, and modeling as well as commercial production. New analytical tools are needed to evaluate nanomaterials with a spatial resolution of 0.1 nm and analyze high throughput in real time that are easy to use.
- A critical need is to develop advanced methods and instrumentation (hardware and software) to provide chemical and physical properties and structural information in real time, at the 1 picogram measurement level. These systems will need to have integrated imaging, spectroscopy, and scattering capabilities to provide the array of information necessary to characterize nanomaterial features and behavior across relevant scales. Development of these capabilities will evolve into a new instrument capable of generating a real-time, three-dimensional map of both chemical and physical properties.
- A coordinated consortium-type organization is critical to the development of instrumentation and methods. A committed public-private partnership of instrument vendors, national laboratories, academics, and industrial technologists is needed to develop measurement systems. This partnership could be patterned after SEMATECH, the successful semiconductor industry association that helped to develop the International Technology Roadmap for Semiconductors (ITRS). The ITRS is another successful model that should be emulated.
- Three-dimensional visual, chemical, and physical characterization at the nanoscale is essential to understanding structure-property relationships in nanomaterials. Physical properties are known to depend on size, particularly at the size scales considered for nanomaterials. Therefore, large-scale bulk measurements of physical and chemical properties may not adequately reflect properties at the nanoscale.

Detailed Research Recommendations

It is a priority that instrumentation be able to determine the elemental composition, location, and chemical state of all atoms in a nanostructure in three dimensions, and the ability to understand and predict the resulting properties of the nanostructure be developed. This requires both laboratory research and nanomanufacturing. For nanoscale characterization of chemical composition and structure, new measurement capabilities will be required. The instrumentation that emerges to meet these needs will require standards and calibrations for the underpinning metrology that cannot be provided by existing metrology. In addition, the processing of data will need to be integrated with the measurement process to a far greater degree than is currently done. Some of this data processing will build on modeling and simulation of the measurement process itself, and some will require merging of data from multiple measurements into a single representation. New instrument development is needed to address improved resolution and sensitivity, increased speed of data acquisition and data reduction, and new or integrated measurement approaches. Although many of the barriers to existing metrology systems and approaches can be overcome through evolutionary advances, other nanoscale characterization needs will require significant breakthroughs which are not expected to occur without a focused effort. In particular, the ability to characterize multiphase systems on the nanoscale will be critical to the assessment of a broad range of systems and will require metrologies that go well beyond what has been developed for periodic, ordered, or uniformly flat materials. The key challenges and barriers for nanocharacterization of the chemical composition, purity ahomogeneity include:

- Multiphase capability
- Merging of data from multiple measurements
- Standards and calibration

- Resolution, sensitivity, and speed

The ability to examine complex structures in three dimensions is another key barrier that must be addressed if characterization on the nanoscale is going to be capable of addressing the structures that have been envisioned. The key challenges and barriers for nanocharacterization of the three-dimensional structure include:

- Spatial and spectral resolution and specificity
- Data acquisition speed and throughput limitations
- Synthesis of 3D information from 2D datasets
- Merging of data from different metrology tools
- Measurement artifacts

Sample preparation remains a pivotal question for both first surface and transmission measurements. Measurement strategies that enable data taken using one tool to be merged with complementary data taken from a separate tool will be required to extract full characterization of many of the structures that are anticipated. To make measurements at this dimensional and compositional level, quality control capabilities will need to be developed to ensure that the physics associated with nanostructures do not contribute to artifacts in the measurement system. The key challenges and barriers for nano-characterization sample preparation and handling include:

- Inability to extract information about 3D arrangement of atoms
- Manipulation of particles
- Non-destructive sample sectioning

Instrument development effort should focus on techniques that will have the greatest effect on existing needs. Along these lines, three topics were identified to overcome barriers to viable nanocharacterization methods. These topics are summarized below:

- 3D characterization of individual nanostructures: characterization of the structure, function, and chemistry of nanostructures. This includes developing tools and techniques that will allow a detailed characterization of three-dimensionally complex nanostructures.
- Speed of characterization: increased speed of characterization to enable productivity improvements, high-throughput and dynamic time-resolved capabilities for an improved understanding of nanomaterials.
- Interface characterization: characterization of the chemical and physical properties of interfaces at the nanoscale. Techniques would include identification of atomic and structural characteristics as well as composition, defects, and anomalies.

IMA References and Selected Readings

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Appendix F. List of Acronyms

BAM	(German) Federal Institute for Materials Research and Testing
CNT	carbon nanotubes
EHS	Environment(al), health, and safety
ENM	Engineered nanomaterial
ENV	Environment category of nanotechnology-related research needs
EPA	U.S. Environmental Protection Agency
EPS	Extracellular polymeric substances
IMA	Instrumentation, metrology, and analytical methods category of nanotechnology-related research needs
ISO	International Organization for Standardization (and associated standards)
LCA	Life cycle analysis
NEHI	Nanotechnology Environmental and Health Implications Working Group
NSET	Nanoscale Science, Engineering, and Technology Subcommittee of the National Science and Technology Council's Committee on Technology
NIST	National Institute of Standards and Technology
NOM	natural organic matter
NNCO	National Nanotechnology Coordination Office
NNI	National Nanotechnology Initiative
NSET	Nanoscale Science, Engineering, and Technology Subcommittee of the National Science and Technology Council's Committee on Technology
OCSPP	Office of Chemical Safety and Pollution Prevention
OECD	Organisation of Economic Co-operation and Development
R&D	Research and Development

National Science and Technology Council; Committee on Technology
Subcommittee on Nanoscale Science, Engineering, and Technology

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