Nanomaterials and Human Health & Instrumentation, Metrology, and Analytical Methods

Report of the National Nanotechnology Initiative Workshop
November 17–18, 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 are 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 of the new tools and methods required for the research that will enable risk analysis and assist in regulatory decision making.

About this Report

This document is the report of a workshop held November 17–18, 2009, the third in a series of four workshops on nanotechnology environmental, health, and safety issues sponsored by the NSET Subcommittee to inform the NNI's long-range planning efforts for the EHS research. Any ideas, findings, conclusions, and recommendations presented in this report are those of the workshop participants. This report 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 Human Health & Instrumentation, Metrology, and Analytical Methods

Report of the National Nanotechnology Initiative Workshop

November 17–18, 2009, Arlington, Virginia

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

Workshop Co-Chairs

Dianne Poster
National Institute of Standards and Technology

Sally Tinkle
National Institutes of Health

Report Editors

Laurie Locascio
National Institute of Standards and Technology

Sally Tinkle

Series Editor

Liesl Heeter
National Nanotechnology Coordination Office

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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 Human and Environmental Exposure Assessment Workshop a reality and to write and produce this report.

Workshop Organizing Committee:

- Sally Tinkle, Co-Chair (NIH/NIEHS)
- Dianne Poster, Co-Chair (NIST)
- Carolyn Cairns (Consumers Union)
- David Castner (University of Washington)
- Charles Gause (Luna Innovations)
- William Kojola (AFL-CIO)
- Amit Kulkarni (GE Research)
- Steve Roberts (University of Florida)

The committee planned, organized, and ran this workshop, and wrote and reviewed the report chapters.

Workshop Presenters: Don Baer, Alison Elder, Martin Fritts, Charles Geraci, David Grainger, Vicki Grassian, Eric Grulke, Tom Kalil, Andrew Maynard, Scott McNeil, Nancy Monteiro-Riviere, Günter Oberdörster, Michele Ostraat, Martin Philbert, Richard Pleus, Joel Pounds, Justin Teeguarden, and Kim Williams shared their expert perspectives with workshop participants on the state of the science in nanotechnology-related human health and instrumentation, metrology, and analytical methods and, in many instances, contributed their comments to the final report (for affiliations see Appendix B).

AAAS Fellows: Corey Cohn, Robert Rivers, and David Tobias took substantive notes in the breakout sessions, contributing to the drafting of this report.

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 organizing committee and handled workshop logistics along with Halyna Paikoush. Marlowe Epstein, Patricia Foland, Geoff Holdridge, Pat Johnson, Lapedra Tolson, and Ken Vest assisted at the workshop. Lapedra Tolson designed the website and workshop branding, Liesl Heeter was series editor of the report, Kristin Roy formatted the report, and Pat Johnson copyedited it.

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 November 17–18, 2009, workshop, held in Arlington, Virginia. The substance of the workshop depended upon the thoughtful engagement of the speakers, moderators, and participants whose presentations and discussions at this workshop provide the foundation for this report.

Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and workshop participants and do not necessarily reflect the views of the United States Government or the authors' parent institutions.

Preface

Nanotechnology, as an enabling technology, offers potential new solutions for critical scientific, industrial, and commercial challenges through the engineering of application-specific nanoscale materials. As with all emerging technologies, the benefits of nanotechnology must be weighed against potential health and environmental hazards associated with their development, use, and disposal. The National Nanotechnology Initiative (NNI) identified addressing environmental, health, and safety (EHS) impacts as a critical component of the U.S. goal to be the world leader in nanotechnology.

The benefits of the field of nanotechnology, and public acceptance of nanotechnology-enabled products and solutions, will depend on a reliable scientific capability to assess and manage potential hazards to human health and the environment. This requires the coordinated efforts of scientists of many disciplines coming from a variety of organizations, namely, the Federal Government and its public–private partnerships with academia, industry, and public health advocates. The immediacy of the need for responsible and sustainable development of engineered nanomaterials cannot be overstated. To this 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 implemented an adaptive management plan for the NNI's 2008 *Strategy for Nanotechnology-Related Environmental, Health, and Safety Research*, an important component of which was holding public workshops on the state of the science.

This document summarizes discussions that occurred during the Nanomaterials and Human Health & Instrumentation, Metrology, and Analytical Methods Workshop, held November 17–18, 2009. This third in a series of four NNI EHS workshops was organized through a multisector planning team model that included representation from academia, industry, public health advocacy groups, and NEHI. The workshop was convened to determine the state of environmental, health, and safety science for engineered nanomaterials in human health and relevant instrumentation and metrology and to identify gaps and barriers in the research needs explicated in the NNI's 2008 EHS Research Strategy. The proceedings from this workshop will inform the NSET Subcommittee and its NEHI Working Group in the adaptive management process that guides the continued refinement of 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 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.

Sally S. Tinkle
Co-Chair
NSET Subcommittee

Travis M. Earles
Co-Chair
NSET Subcommittee

E. Clayton Teague
Director
NNCO

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 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/publications-resources:

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 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 strategy.

Table of Contents

Executive Summary

The examination of potential human health responses to nanomaterials, and the instrumentation, metrology, and analytical methods required to reliably assess those effects, are integral components of a risk assessment and risk management framework. Knowledge of human health hazards is the first stage of risk evaluation and risk mitigation; however, nanotechnology is a young, diverse field, and data on the human health effects of and exposure to engineered nanomaterials are limited. This paucity of data in this area hinders identification of potential health concerns and advancement of the safety and health guidelines that are needed to ensure the responsible development of nanotechnology. Therefore, building this critical environmental, health, and safety (EHS) knowledge base—a knowledge base of research findings for risk assessment that supports timely risk prevention and mitigation—is essential to the future of nanotechnology.

As research on an emerging technology develops, analysis of scientific progress against the goals of a strategic plan is essential to maintain the relevancy and utility of the plan. The U.S. National Nanotechnology Initiative (NNI) laid out its first EHS research strategy in the 2008 document, *Strategy for Nanotechnology-Related Environmental, Health and Safety Research* ("NNI EHS Research Strategy").[1] In 2009, the Nanotechnology Environment and Health Implications (NEHI) Working Group of the Nanoscale Science, Engineering, and Technology (NSET) Subcommittee of the National Science and Technology Council initiated an adaptive management process to assess the research goals and needs outlined in the 2008 NNI EHS Research Strategy against the state of the science and to identify gaps and barriers to further progress. Four public workshops were held to evaluate the five major research need categories outlined in the 2008 EHS plan (see "About the 2009–2010 NNI Series of EHS Workshops and Report," p. iv) . The research need catogory "instrumentation, metrology, and analytical methods" is essential to EHS research in general and to both the environment and the human health categories, especially; therefore, this category was assessed as part of the workshops on environment (workshop 2, held October 6–7, 2009) and on human health (workshop 3, held November 17–18, 2009).

The planning team for the NNI Workshop on Nanomaterials and Human Health and Instrumentation, Metrology & Analytical Methods (http://www.nano.gov/events/meetings-workshops/humanhealth) identified three overarching EHS research need areas into which the 5 human health research needs and 5 instrumentation, metrology, and analytical methods research needs were assigned. These areas, designated for workshop breakout session discussions, were:

1. Characterization of engineered nanomaterials
2. *In vitro* research and related instrumentation and metrology
3. *In vivo* research and related instrumentation and metrology

This workshop report reflects this tripartite structure and provides an assessment in these areas of the state of the science, overarching themes and cross-cutting issues, and recommendations to address critical research gaps and barriers in these areas. These recommendations are intended to improve

1 NSET/NSTC, Washington, DC, 2008; http://www.nano.gov.

the coordination of the Federal nanotechnology EHS research agenda and to maximize the utility of research findings for risk assessment.

The breakout sessions focused specifically on addressing the questions, "how far has nanoEHS research come?" and "where do we go from here?" Participants identified research progress, gaps, and barriers, proposed timelines for completing the research, and developed milestones for each of the ten research needs. Participants were also asked to comment specifically on the clarity, appropriateness, and completeness of the existing research needs and the feasibility of the timelines. This exercise was patterned after the format for the existing research needs and the heat diagrams, or graphical timelines, in the 2008 NNI EHS Research Strategy (see Figure 3, p. 18, and Figure 5, p. 24, of that strategy). Because the strategy included an analysis of research performed in 2006, workshop participants were asked to evaluate research in progress since 2006 and to establish timelines over the next 15 years. For these reasons, the timelines presented in this report span 2007–2022.

Special workshop sessions on data-enabled predictive modeling for nanotechnology EHS research and on vignettes describing real-world experiences with engineered nanomaterials were used to inform discussions throughout the workshop.

After collecting and analyzing the information produced during the two-day workshop, the participants identified a number of notably consistent themes across the three breakout session topic areas. Overarching themes generally describe broad biological data or instrumentation needs or related observations.

The research needs in the human health and instrumentation areas are generally different and are reported separately; however, the one theme that cuts across these areas is the need for appropriately comprehensive and standardized physico-chemical characterization procedures for engineered nanomaterials.

In addition to overarching themes, there were a number of recommendations expressed across breakout sessions. These recommendations described the need to improve the efficiency or the success of environmental, health, and safety research through better coordination.

The intent of these recommendations is to improve the coordination of the Federal nanotechnology environmental, health, and safety research agenda, and to accelerate and maximize the utility of research findings for risk assessment in a timeframe that supports risk prevention and mitigation. As with the overarching themes, these recommendations are divided according to human health and instrumentation and metrology recommendations.

This report integrates the comments, opinions, and ideas put forth during the workshop, and its contents will be used to help guide the next iteration of the NNI EHS strategy. Ultimately, the report aims to inform the NNI, Federal agencies, and other parties working on nanotechnology about the critical nanotechnology environmental, health, and safety research needs and to facilitate collaborative research across the U.S. Federal Government.

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 ((available at 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 in the EHS community.

Since the workshop, the International Standardization Organization (ISO) has adopted core terminology, including specific definitions for *engineered* and *manufactured* nanomaterials: see ISO/TS 80004-1:2010, available at http://cdb.iso.org/.

1. Introduction

Background

The examination of potential human health responses to nanomaterials, and the instrumentation, metrology, and analytical methods required to reliably assess those effects, are integral components of a risk assessment and risk management framework. Knowledge of human health hazards is the first stage of risk evaluation and risk mitigation; however, nanotechnology is a young, diverse field, and data on the human health effects of and exposure to engineered nanomaterials are limited. This paucity of data in this area hinders identification of potential health concerns and advancement of the safety and health guidelines that are needed to ensure the responsible development of nanotechnology. Therefore, building this critical environmental, health, and safety (EHS) knowledge base—a knowledge base of research findings for risk assessment that supports timely risk prevention and mitigation—is essential to the future of nanotechnology.

Recognizing the importance of this research area, the Nanotechnology Environmental and Health Implications (NEHI) Working Group identified human health and associated instrumentation, metrology, and analytical methods as two of five priority categories for nanotechnology-related EHS research. The November 2009 workshop addressed both priority categories of EHS research.

About the Workshop

To carry out its adaptive process for managing the EHS strategy of the National Nanotechnology Initiative (NNI), the NSET Subcommittee implemented a series of four public workshops under the auspices of its NEHI Working Group. The aims of the workshops were to evaluate the 2008 NNI EHS Research Strategy's five categories of priority research needs: (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.[1] NIH, in recognition of its role as the coordinating agency for human health, and NIST, as coordinating agency for instrumentation, metrology and analytical methods, played leading roles in organizing the NNI Workshop on Human Health, and Instrumentation, Metrology, and Analytical Methods. The workshop was structured as an open forum that would bring stakeholders together to discuss the state of the science for human health and instrumentation/metrology research, identify gaps between the state of the science and the research needs outlined in the NNI EHS Research Strategy, determine barriers to advancing the research, and build dialogue and facilitate collaborations to achieve the EHS research goals.

The workshop was organized by a multisector planning team composed of representatives from industry, academia, nongovernmental organizations (NGOs), public health advocacy groups, and the NEHI Working Group. It was held on November 17–18, 2009, in Arlington, Virginia, with more than 180 scientists and other stakeholders from national and international government, industry, labor, and other sectors participating in person. An additional 70 viewers joined from other locations through the webcast plenary sessions. The ten EHS research needs set out in the 2008 NNI EHS Research Strategy—five each from Human Health and from Instrumentation,

1 NSET/NSTC, *National Nanotechnology Initiative Strategy for Nanotechnology-Related Environmental, Health, and Safety Research* (NSET/NSTC, Washington, DC, 2008; http://www.nano.gov).

Metrology, and Analytical Methods—were examined through the lenses of 3 overarching research areas that were the main topics of the six breakout sessions (three on Day 1, three on Day 2, with specific questions and follow-up tasks for each session):

1. Characterization of engineered nanomaterials
2. *In vitro* research and related instrumentation and metrology
3. *In vivo* research and related instrumentation and metrology

Three keynote presentations served as catalysts for general open-floor discussions by the workshop participants and provided state-of-the-science overviews for each of the three research areas. The keynote presentations were:

- Characterization of Engineered Nanomaterials—Dr. Eric Grulke, University of Kentucky
- Biological *In Vitro* Interactions of Engineered Nanomaterials—Dr. David Grainger, University of Utah
- Biological *In Vivo* Interactions of Engineered Nanomaterials—Dr. Martin Philbert, University of Michigan

Abstracts of these talks are provided in Appendix C.

Following the keynote presentations, three concurrent breakout sessions took place. During the first day, breakout sessions focused on a thorough examination of the research needs assigned to each session. Specific technical questions for each of the breakout sessions helped focus discussions. Special attention was paid to progress that had been achieved on each research need and to identification of missing elements.

To keep the workshop grounded in practical research questions, three case studies were presented on the second morning:

- Challenges to Making Exposure Measurements—Dr. Charles Geraci, National Institute of Occupational and Environmental Health Science
- Obstacles to Characterization—Dr. Donald Baer, Pacific Northwest National Laboratory
- Challenges to *In Vitro* Experimental Design: The Alliance for NanoEHS Harmonization Experience—Dr. Allison Elder, University of Rochester

Discussions on Day 2 of the workshop centered on the timeline for each research need, the rationale for that timeline, and milestones to evaluate progress.

Additional information that contributed to the integrated examination of the Human Health and the Instrumentation, Metrology, and Analytical Methods research needs included a working lunch with talks on "Nano-Informatics: Data-Enabled Predictive Modeling for NanoEHS," by Dr. Justin Teeguarden (Pacific Northwest National Laboratory), and Dr. Martin Fritts (NCI Nanomaterials Characterization Laboratory), and the White House and Congressional perspectives on nanotechnology environmental, health, and safety research by Mr. Travis Earles (Office of Science and Technology Policy) and Dr. Dahlia Sokolov (House Science & Technology Committee).

All plenary presentations and the public comment period were webcast to facilitate broader public participation.

About the Report

This report summarizes and discusses the principal findings of the presentations and discussions that took place during the November 2009 NNI Workshop on Nanomaterials and Human Health, and Instrumentation, Metrology, & Analytical Methods. This report is the main output of the workshop; however, additional materials related to the workshop are available on the workshop website http://www.nano.gov/events/meetings-workshops/humanhealth/.

The workshop report's structure reflects the grouping of the 5 human health and the 5 instrumentation, metrology, and analytical methods research needs into the three overarching research areas, characterization, *in vitro* research, and *in vivo* research. The findings from the breakout sessions on these three topics, along with those of the predictive modeling lunch session, are found in Chapter 2. Chapters 3 and 4 present the overarching themes and the key recommendations of the workshop, respectively. Supporting information is provided in the appendices: the workshop agenda (Appendix A), a list of workshop participants (Appendix B), abstracts of the three keynote talks (Appendix C), detailed recommendations and comments on the ten research needs (Appendix D), public comments (Appendix E), and a list of acronyms (Appendix F).

2. Breakout Session Summaries

Characterization of Nanomaterials

State of the Science

New or modified analytical tools and methodologies to characterize engineered nanomaterials have been developed over the last few years, but these technologies are still insufficient to fully characterize all engineered nanomaterials in their relevant milieu for risk assessment purposes. Specific tools and methods are being developed to understand the influence of surface properties and surface modifications on the behavior of nanomaterials, as well as the effects of these properties and modifications on their biological fate. Additional tools have been developed to visualize engineered nanomaterials in cells and tissues, although the resolution and limits of detection could be improved.

Additionally, characterization of engineered nanomaterials, an integral component of environmental, health, and safety assessment, needs to be evaluated across the materials' life cycles, from research, development, and manufacturing, through use and disposal or recycling. The physical and chemical properties of engineered nanomaterials frequently change as the microenvironment or use context changes, thus modifying the potential for and the route of exposure. It is also necessary to define the exposure context. External exposure occurs when an individual or species encounters engineered nanomaterials in air, water, or soil, and internal exposure occurs when engineered nanomaterials have entered into or translocated through biological spaces in the body. This distinction is necessary because the methods for quantification and characterization of internal and external exposure of engineered nanomaterials are context-specific.

Gaps and Barriers

The ability to link the physico-chemical properties of engineered nanomaterials to the biological responses they invoke *in vitro* and *in vivo* is critical to the responsible development of nanotechnology-enabled products and devices. Although novel characterization techniques are being developed, they are not cost-effective, not always sensitive to actual environmental and use conditions, and not easily accessible to everyone working with nanomaterials. A standardized toolkit consisting of various techniques enabling complete characterization of most engineered nanomaterials is absolutely necessary to accelerate progress in this area. Obtaining a greater understanding of how key variables, such as sample preparation, storage, temperature, solvents, and other parameters, affect engineered nanomaterial properties will be critical to this effort, as will be the development of analytical tools and methods to characterize an engineered nanomaterial's spatiochemical composition, purity, and heterogeneity in pristine conditions, as a function of shelf life, and throughout the life cycle.

A substantial body of work exists to address these needs, but major issues remain, such as quality control, sensitivity, reliability, and reproducibility of methods. It would be beneficial to establish and validate methods for analysis, quantification, characterization, and commutability through cooperative interlaboratory comparison of pristine (as-manufactured) engineered nanomaterials to these materials in their use context. The techniques should also be available to international users.

Finally, there is also a strong need to create a database of reference engineered nanomaterials that could contain much of this information. As analytical tools and methods are developed, good communication and coordination across the field and with standards-making bodies such as ASTM International and the International Organization for Standardization (ISO) is critical.

Priorities and Next Steps

A "high priority" timeline should be constructed for basic and applied research objectives in the near term (2007–2012) and medium term (2012–2017)—timeframes in this report are an extension or revision of those in the 2008 NNI EHS Research Strategy—across all of the prioritized characterization research needs. The effort should focus on development of cost-effective, rapid assessment tools and consensus-derived experimental protocols that provide reproducible data and that are versatile enough to use in research, development, manufacturing, and use conditions. This work is essential for developing the capability to perform risk assessment on nanomaterials in multiple exposure contexts. The new NNI EHS Research Strategy needs to revise existing research needs and develop new instrumentation and metrology priorities that address these gaps and barriers.

In Vitro Research and Related Instrumentation and Metrology

State of the Science

Significant *in vitro* studies of engineered nanomaterials are underway that use different models of the environment, pharmacology, toxicology, medicine, and developmental biology. The ultimate goal of *in vitro* testing is to provide a simple, accessible, convenient, predictable, and reliable understanding of how engineered nanomaterials interact with biological systems. The primary advantage of this approach is that adverse effects can be predicted for the purpose of protecting human health and the environment without involving costly and ethically complex testing in animals or in whole organisms.

To date, most studies have consisted of procuring engineered nanomaterials from various sources and evaluating them in a series of bioactive, enzymatic, or cell-culture-systems assays to establish the structure–activity response and to identify potential toxic effects. In many prior *in vitro* studies, the engineered nanomaterials were often poorly characterized prior to experimentation, and the absence of the physico-chemical characterization data necessary to compare results among research studies severely limits their utility. Results from studies of the same engineered nanomaterial vary greatly, due in part to different synthesis chemistries, sample purity, purification techniques, methods of material administration, and methods to perform the assays.

Cell-based assays are generally designed to qualitatively understand the mechanisms of biological response to a test material. Some of the most common effects studied include cell adhesion and material internalization, intracellular processing and trafficking, changes in cell signaling such as production of cytokines and reactive oxygen species, cell viability, and test-material-induced changes in cell phenotype. Additional effects include protein-engineered nanomaterial interactions and kinetics, material aggregation, and opsonization, all with the potential to alter the surface chemistry of the engineered nanomaterial. Engineered nanomaterials have been shown to skew the results of many commonly used *in vitro* assays through assay inhibition, interference with redox activity, or optical absorption. Engineered nanomaterials may also change their physico-chemical state in assay systems by, for example, forming aggregates or agglomerates that confound the results by altering the intrinsic toxicity observed for the same nanomaterial in a nonaggregated state. Little is known about aggregation of engineered nanomaterials in commercial applications, when aggregation decreases toxicity, and which aggregation states, if any, are the most relevant to human exposure pathways.

Gaps and Barriers

In reviewing the state of the science for *in vitro* studies of engineered nanomaterials, several themes have emerged as primary challenges and immediate priorities. There is a significant need for comprehensive, meaningful, and standardized methods to characterize the nanomaterials under study, to understand their fate in standard cell-based

and other biological and environmental assays, and to appropriately represent and measure dose and other factors important in characterizing the cellular and molecular pathways the tests are designed to evaluate.

There are confounding influences of cell culture conditions, including the stability of test materials in the culture system. Cells in simple cultures do not necessarily represent the targeted *in vivo* phenotypes and are often not validated for such equivalence. Additionally, cell monocultures cannot replicate an *in vivo* response that involves many interacting cell types. Short-term cell cultures cannot reliably duplicate aspects of either acute or chronic materials exposure, and widely varying cell culture conditions (e.g., different media, culture times, or exposure conditions) confound the material–cell interactions under study. While several of these issues are unique to engineered nanomaterials and their behavior in cell cultures, others are not. For example, concerns about proper interpretation of results obtained by superdosing cell cultures to levels much higher than may occur *in vivo*, and the relevance of the biological response invoked to the human response, have been debated for conventional chemical compounds as well as engineered nanomaterials. Focusing on the unique issues and challenges that engineered nanomaterials bring to traditional *in vitro* assays will be important to establishing their value in future research.

Validation studies to establish the relevance of *in vitro* experiments to *in vivo* observations are critical to the development of reliable *in vitro* test methods. These studies should include the determination of a realistic dose, or dose metric, for *in vivo* engineered nanomaterial exposures, and the translation of that metric to an appropriate dose for *in vitro* experiments. Additional studies should establish procedures for the dispersion of engineered nanomaterials in test systems, assessment of the aggregation/agglomeration state in the assay, the effect of serum and the microenvironment on bioavailability, and biological persistence. The development of a systematic process to better understand the differences in reported biological, environmental, and toxicological effects due to perceived differences in dose, route of administrations, cell line fidelity and control issues, materials chemistry, contamination, shape, or other aspects of the testing system would provide for higher decision confidence to validate the correlations reported *in vitro* to *in vivo*.

Standard reference nanomaterials to correlate test results across the scientific community would also improve the reliability and reproducibility of the data. Lastly, improved detection tools are needed to detect, identify, and characterize engineered nanomaterials and their interactions with complex biological systems, both *in vitro* and *in vivo*.

Priorities and Next Steps

Many individuals believe commercialization of nanotechnology-enabled products is proceeding more rapidly than EHS research, and information on use is needed to focus screening studies on those engineered nanomaterials most likely to generate significant exposures and to pose the greatest hazard to human health and the environment. The selection of these high-priority nanomaterials should drive the near-term development of the instrumentation and measurement tools.

The research described above is essential for developing new *in vitro* capabilities and validating *in vitro* to *in vivo* correlations—all of which are essential for engineered nanomaterials risk assessment. Near-term research should focus on developing consensus-generated *in vitro* experimental protocols that are reliable, reproducible, rapid, and cost-effective.

Other Considerations

Formal systems are needed to define dose metrics that are more relevant to engineered nanomaterials than to mass alone. Although there is agreement that characteristics such as surface area, particle number, shape, and charge are important, there remains very little progress in formalizing standard methods for incorporating these characteristics into the definition of dose to ensure that the scientific community complies with these standards when reporting *in vitro* toxicity tests.

Coordinating scientific understanding is needed of the mechanisms of action that enable the extrapolation of *in vitro* findings to *in vivo* systems through high-throughput screening tests and the development of predictive models. Such an effort will depend on the ability of the nanotechnology EHS scientific community to understand how critical material properties change with size or with

aggregation, and how surface functionalization affects biological response.

In Vivo Research and Related Instrumentation and Metrology

State of the Science

In vivo research using animal models provides a critical linchpin between *in vitro* studies and the human response, and should provide reliable capabilities to predict adverse properties of engineered nanomaterials early in materials development. A limited number of studies have been conducted in many aspects of *in vivo* research, including studies on the relationship between engineered nanomaterial properties and uptake by various exposure routes, assessment of the human body burden, and the absorption, distribution, metabolism, and excretion of engineered nanomaterials. Some progress has been achieved in identifying or developing *in vivo* models to predict human responses to engineered nanomaterials. Methods contributing to model improvement include protocols, procedures, and instrumentation to disperse and suspend engineered nanomaterials in test systems, to visualize some engineered nanomaterials in biological matrices, and to assess several critical nanoscale physical and chemical properties in biological matrices.

The development of *in vivo* models to predict human response to engineered nanomaterial exposures is essential. Some progress has been made in developing appropriate methods to suspend and administer engineered nanomaterials and in developing methods and analytical tools to visualize and assess nanoscale physical and chemical properties of some engineered nanomaterials in biological matrices. Research is also underway to determine the mechanisms of biological response to engineered nanomaterials at the molecular, cellular, and tissue levels, although much remains to be done.

Gaps and Barriers

Initial efforts in *in vivo* research across nearly all of the instrumentation and metrology and human health research needs should be leveraged to address the following gaps and barriers:

For engineered nanomaterials, *in vivo* studies are severely hampered by the lack of appropriate instrumentation, metrology, and analytical methods to support *in vivo* research. Furthermore, the existing human health and metrology research needs, as outlined in the 2008 NNI EHS Research Strategy, should be revised to reflect at times more focused and at other times more expansive objectives (see Appendix D). New research priorities should also be established, such as developing new instrumentation for *in vivo* measurement, generating methods that correlate exposure and toxicity with measures of risk, devising standard units of measure appropriate for engineered nanomaterials, and developing methods to measure toxicologically relevant physico-chemical characteristics of engineered nanomaterials.

Substantial future work is needed to relate nanomaterials' physico-chemical properties to uptake and exposure routes and to determine the relationship of acute and chronic exposure to body burden. This research should include mass balance studies in healthy and in susceptible populations. Considerable research is also needed to evaluate the degree to which *in vivo* models predict human response and to develop computational models that predict engineered nanomaterial effects. For this effort to be valuable, there must be an extensive database of studies that follow standardized characterization procedures on priority engineered materials.

New immediate priorities should include determining the consistency and characterization of test materials, dosimetry, developing useful animal models, standardizing *in vivo* toxicity studies, and developing reference materials. Medium-term projects should assess structure–property activity relationships, identify vulnerable populations, and determine ecological effects.

Additional barriers to advancing *in vivo* research objectives include insufficient funding and practical difficulties; for example, some Federal Government agencies are unable to sponsor applied research that will immediately impact risk assessment.

Priorities and Next Steps

In vivo research provides important data for risk assessment, especially the assessment of new entities, such as engineered nanomaterials. The research needs

for human health and instrumentation metrology should be revised to integrate the unmet research needs identified in the gaps and barriers section of this *in vivo* report. Physico-chemical characterization of nanomaterials in biological matrices and the linkage of reliable *in vivo* models to *in vitro* assays are essential first steps in developing sound scientific data for risk assessment.

Nano-Informatics Special Session: Data-Enabled Predictive Modeling for NanoEHS

Nano-informatics and predictive modeling were not detailed in the 2008 NNI EHS Research Strategy. Therefore, the workshop planning team included a dialogue about the use of informatics and predictive modeling in the workshop to understand the research needed to provide these critical components to the nanotechnology community.

State of the Science and Future Directions for Predictive Modeling

Predictive modeling is the process by which a statistical model is created or applied to selected data to predict the probability of an outcome. For nanotechnology environmental, health, and safety research, the participants assembled a well-known, but necessary list of predictive modeling activities: hazard assessment based on engineered nanomaterial characteristics, risk assessment models, exposure assessment models, and dosimetry models. Dosimetry modeling was considered the most mature area for a viable and useful modeling effort. The participants proposed archiving the existing major respiratory-tract dosimetry models for particles within a database and using these models to produce dosimetry-based adjustments to reference levels. As the science evolves, structure–activity models could be added and used in conjunction with dosimetry models to further improve the predictive capability of the models for nanotechnology. Large structure–activity databases for bulk-phase materials already exist, and such an effort for nanoscale materials could be conducted iteratively to ensure feedback from the larger environmental, health, and safety community on their requirements, suggestions for use cases (e.g., including particle reactivities), candidate materials, data analysis and curation, and suggestions for improvements.

Research Needs and Challenges for Nano-Informatics

Models are only as good as the data behind the model, and they require data that use standardized approaches and characterization techniques. The need for the development of and access to databases of standard protocols to characterize engineered nanomaterials and assess their toxicity was considered a high priority. Acquisition of data to populate a publicly accessible database was identified as the initial hurdle, and some participants supported mandating the submission of Federally funded research results to this database. Additional challenges were presented, including investments in curating databases, quality assessment and control, development of a flexible database structure that will evolve along with the types and sources of data, and identification of a standard minimal set of metadata for entry into the database. Participants from the predictive modeling community introduced the idea that databases should include models for analyzing data as well as predictive models. The databases should include data, metadata, search and pattern recognition tools, query mechanisms, and the means for sharing data.

3. Overarching Themes for Human Health and IMA Research Needs

Over two days, participants discussed the state of the science, data gaps, and emerging trends regarding the research needs for human health and for instrumentation, metrology, and analytical methods (IMA) identified in the 2008 NNI EHS Research Strategy. The first day focused on "how far we have come"; the second day focused on "where do we go from here." Through this process, research progress, and gaps and barriers were identified, a timeline was established, and milestones were developed. After collecting and analyzing the information produced during the two-day workshop, the participants identified a number of notably consistent themes across the three breakout sessions.

Overarching themes are topics and discussions that occurred in more than one of the concurrent breakout sessions. Overarching themes generally describe broad biological data or instrumentation needs or related observations. The needs in the human health and instrumentation areas are generally different and are reported separately below. However, the one theme that cuts across these areas is the need for appropriately comprehensive and standardized physico-chemical characterization procedures for engineered nanomaterials.

Overarching Themes for Human Health

1. **The assessment of surface and bulk physico-chemical properties of engineered nanomaterials.** The physico-chemical properties are the elemental boundaries that define and differentiate each material from all others. Discussions and priorities within this theme covered a number of aspects of engineered nanomaterial physico-chemical characterization for the purpose of assessing health impacts and include:
    a. Standardizing methods to characterize physico-chemical properties of engineered nanomaterials and determine how these properties change with time and environment
    b. Achieving interlaboratory reproducibility, developing comparable structure–activity relationships, and relating exposure and other variables to a particular experimental or commercially available material
    c. Using a subset of physico-chemical properties to characterize dose and exposure
    d. Characterizing the interactions of engineered nanomaterials with biological systems in terms of the physico-chemical properties of the engineered nanomaterial to ultimately understand which physico-chemical properties, or combinations of properties, support maximum benefit/minimal risk for design and hazard screening
2. **The need to include sensitive or susceptible populations.** Differences in risk from exposure to engineered nanomaterials in these populations compared to the general population have not yet been determined. The meaning of "sensitive populations" appears to be adequately defined by current toxicological risk assessment guidelines (e.g., the developing fetus, the elderly, or multigenerational effects).
3. **The need to determine the most appropriate expression of dose.** Traditionally, toxicologists define dose as the mass or concentration of a chemical. Laboratory studies show that the mass of a nanomaterial may not be the most accurate way to evaluate health effects. Several studies suggest that surface area or particle number may

be more accurate. All groups noted the urgent need to define relevant dose metrics to improve data interpretation.

4. **The need to understand human health impacts of engineered nanomaterials.** In toxicology, fundamental understanding of molecular and cellular pathways and the kinetics of absorption, distribution, metabolism, and excretion (ADME) is critical to assessing potential hazards of chemicals to the body. This theme was noted as equally critical for engineered nanomaterials, especially for those mechanisms that may be a unique response to materials at the nanoscale.
5. **The need to reemphasize the basic tenets of the scientific process in managing the complexities encountered in the study of engineered nanomaterials.** High-quality scientific data are essential to make accurate assessments of potential hazards of engineered nanomaterials in the human body and the environment. This theme includes and reemphasizes the need for proper surface and bulk physico-chemical characterization of the engineered nanomaterials, careful experimental design, reliable methods of analysis, and reproducible test results.
6. **Different amounts and types of information are required for different levels of hazard and risk assessment and should be proportional to the impact of uncertainty on risk.** The importance of defining thresholds for data requirements is increasing as more nanoscale materials are entering the marketplace.
7. **The need to develop accurate, reliable, and reproducible toxicology assays that are effective in assessing potential health hazards.** There is an increasing need to develop and validate *in vitro* assays that predict the hazards of engineered nanomaterials and reduce the overall utilization of *in vivo* assays (due to both time and cost). Although this is the goal at present, it is not possible to rely exclusively on *in vitro* assays for hazard characterization. The interest in developing *in vitro* toxicological assays in concert with the characterization of physico-chemical properties is based on the thought that it will lead to better assays for computer modeling, as well as to the redesign of engineered nanomaterials for safer, more biocompatible characteristics. Attention will also need to be paid to the data needs for development of computer models.

Overarching Themes for Instrumentation, Metrology, and Analytical Methods

1. **The need for instrumentation and methods to assess surface and bulk physico-chemical properties of engineered nanomaterials.** Instrumentation, metrology, and analytical methods for EHS assessment were grouped into the following overarching themes:
    a. A toolkit of techniques to measure physico-chemical properties that are simple, robust, reliable, cost-effective, and applicable to a wide range of engineered nanomaterials. Although instrumentation exists for measuring many physico-chemical characteristics of engineered nanomaterials, many of these methods are either too specialized or too expensive to be useful to the broad community involved in nanotechnology EHS research.
    b. A toolkit of techniques and instrumentation to measure engineered nanomaterials in various matrices, media, and mixed media. Many of the current measurement techniques and instrumentation have been developed for use in air or in vacuum and are not applicable to measurement in appropriate biological or environmental media, or for *in vitro* or *in vivo* monitoring.
    c. A toolkit of techniques and instrumentation to measure transformations in engineered nanomaterials in relevant media and matrices (*in vitro* and *in vivo*). As noted, many of the current measurement techniques and instrumentation are not well suited for measurement in relevant biological or environmental media and matrices, and those that are suitable in those media often do not exhibit the required sensitivity or specificity to measure transformations (physical or chemical) in the engineered nanomaterials.
    d. A toolkit of techniques and instruments to assess engineered nanomaterials across their life cycles (in product formulations, through product use, through disposal), including transformations.

2. **The need for instrumentation and methods to distinguish between naturally occurring and engineered nanomaterials.** Many of the instrumentation and techniques used to characterize and identify engineered nanomaterials cannot be used to differentiate between naturally occurring and engineered nanomaterials in the same sample.
3. **The need for instrumentation and methods with high sensitivity to detect engineered nanomaterials in very dilute samples.** Instrumentation to detect low levels of engineered nanomaterials is required in many cases, for instance, in surveillance modes in water systems or in air.
4. **The need for instrumentation to measure the protein corona in biological media.** New information in recent years has demonstrated the relevance of the protein coating (corona) on the engineered nanomaterial to its possible fate and distribution. Instrumentation exists to determine the protein coating in bulk samples but may not be sensitive enough to analyze the protein corona in smaller samples or on a single particle.
5. **The need for reference nanomaterials in matrices and other well-characterized study materials.** The need for certified reference nanomaterials was acknowledged in previous reports, and many prioritized materials are currently in development. However, this workshop pointed out the need for other types of nanomaterials to benchmark studies that include reference nanomaterials that are supplied in various relevant matrices and nanomaterials that are not certified at the highest level ("gold standard"), but that are characterized at a lower level as study materials to expedite their dissemination.

4. Recommendations

In addition to overarching themes, there were a number of recommendations expressed across breakout sessions. The intent of these recommendations is to improve the coordination of the Federal nanotechnology EHS research agenda, and to accelerate and maximize the utility of research findings for risk assessment in a timeframe that supports risk prevention and mitigation. As with the overarching themes, these recommendations are divided according to human health and instrumentation and metrology recommendations.

Recommendations for Nanotechnology and Human Health

1. **Continue the research focus on specific materials rather than on classes of engineered nanomaterials.** Participants recognized that at this point in the development of our knowledge base, the uniqueness of each engineered nanomaterial—that is, the physico-chemical properties inherent in the nanomaterial's engineered purpose and their possible health effects—make it difficult to develop generalized classes of engineered nanomaterials. It is recommended that there should be a continued focus on specific materials until such time as the science provides sufficient information to allow for research on classes of engineered nanomaterials. Efforts should continue to identify common mechanisms and find relationships between physico-chemical properties that would support engineered nanomaterial classification schemes.
2. **Establish specifically identified, prioritized research needs that are focused on protecting "human health."** Participants believed that the focus of the research strategy—to protect human health—should be strengthened. This could be accomplished by placing explicit emphasis in documents, such as requests for proposals, to ensure such a focus by the research community.
3. **Develop a document that identifies critical research paths to achieve the goals of the NNI EHS Research Strategy.** Requiring researchers to define critical paths by which their projects would support engineered nanomaterial risk assessment is essential to identifying those projects that will contribute significantly to specific priorities in the NNI EHS Research Strategy. Two important factors affecting research priorities are (1) establishing general principles and mechanisms of engineered nanomaterial–biological interaction, and (2) developing information needed for near-term risk assessment and regulatory decision making. The prioritization of specific research topics is difficult unless the overall goal of the NNI EHS Research Strategy has been clearly articulated and the balance between basic and applied research has been discussed. The acquisition of data for risk assessment is further complicated because some Federal Government agencies' missions do not allow the funding of applied research—that is, research that is directed primarily toward a specific, practical question or objective.

   Participants indicated that the current organization of NNI environmental, health, and safety research priorities makes it difficult to see the whole picture. For example, research related to human health is covered in both the Nanomaterials and Human Health category

and the Human and Environmental Exposure categories of research needs. This creates opportunities for important topics to be left out (e.g., epidemiological studies) and leads to redundancies in topics that are difficult to resolve into a single, clear research priority (e.g., some of the instrumentation and metrology needs). Perhaps the overall organization of the current NNI EHS Research Strategy should be revisited.

4. **Understand the relationship of knowledge thresholds and knowledge feedback loops to the NNI EHS Research Strategy, and identify procedural roadblocks and their impact on the pace of science.** This step is urgently needed to conduct risk assessment and make sound regulatory decisions. Examples of knowledge thresholds include: "What is the threshold amount of scientific data needed to insure the protection of public health and the environment?" and "What constitutes insufficient information to make public health decisions?"

   Specific challenges discussed by participants include:

   a. Confidential business information should be protected in a manner that still permits the conduct of research to protect public health and the environment.

   b. High standards of peer review for newly published research should be upheld. Participants pointed out that some journals have different levels of rigor for peer review, such as the level of detail for experimental methods and the use of positive and negative controls.

   c. A minimal set of characterization criteria should be required in peer-reviewed publications. This requirement would allow the identification of the engineered nanomaterial being tested and provide the opportunity to verify experimental results.

   d. Participants strongly recommended the publication of negative results from experiments, which are as important as positive results. Currently there is a bias toward presenting research that only demonstrates measured responses.

5. **Define the minimal data set for risk assessment.** The risk assessment process requires scientific data for hazard identification, toxicological evaluation, exposure assessment, and risk characterization. Agency guidelines that define the minimal amount of data sufficient to conduct a risk assessment should be considered when developing the next NNI EHS Research Strategy.

6. **Provide more detail in the NNI document regarding research needs.** The granularity of the research strategy provided thus far in the 2008 NNI EHS Research Strategy should be enhanced.

Recommendations for Instrumentation, Metrology, and Analytical Methods

There was considerable discussion regarding the following recommendations related to the Instrumentation, Metrology, and Analytical Methods research needs area:

1. **Make highly specialized equipment more accessible and cost-effective**. Workshop participants recommended that some national effort be focused on making highly specialized equipment more accessible and cost-effective for the average researcher. It was widely recognized that the lack of access to instrumentation with which to fully characterize engineered nanomaterials and/or the lack of access to experts with knowledge of useful instrumentation was holding back progress in nanotechnology and nanotechnology-related environmental, health, and safety research. The workshop participants also recognized the significant difficulty of achieving this recommendation.

2. **Develop central facilities for engineered nanomaterials characterization**. Participants recommended that the United States develop and support central facilities for engineered nanomaterials characterization as one solution to the problem of access to appropriate and cost-effective instrumentation and expertise.

3. **Develop standard methods to help guide instrument developers.** With the development of standard methods for measuring and characterizing engineered nanomaterials, instrument manufacturers will be able to better

target the development of new products and services to address those specific measurement needs.

4. **Develop and maintain central databases and data-sharing resources covering all aspects of environmental, health, and safety research.** This need ranges across a number of research elements, from reference materials to instrumentation, to protocols, to outcomes, etc. Central resources for data sharing are imperative to move this field forward. Without coordinated databases and data-sharing efforts, research will be much less efficient and less effective, and significant research dollars will be wasted.

5. **Develop a strategy for communication and coordination in standards development.** Standards development activities (both reference materials development and documentary standards development) should be better communicated and coordinated to better serve the nanotechnology research community. The development of conflicting documentary standards would be detrimental to progress of the field and lead to more confusion and frustration by industry, stakeholders, and governments.

Appendix A. Workshop Agenda

Tuesday, November 17, 2009

7:30 Registration & Continental Breakfast

8:30 – 12:00 ***Morning Session***

Introductions, Dianne Poster, National Institute of Standards and Technology

Welcome and Expectations for the Workshop

Clayton Teague, Director, National Nanotechnology Coordination Office
Sally Tinkle, National Institute of Environmental Health Sciences

Plenary Session, Chair, Steve Roberts, University of Florida

Three presentations to set the stage for the workshop, identifying the critical issues and providing common knowledge and language:

–Eric Grulke, University of Kentucky, *Characterization of engineered nanomaterials*

–David Grainger, University of Utah, *Biological* in vitro *interactions of engineered nanomaterials*

–Martin Philbert, University of Michigan, *Biological* in vivo *interactions of engineered nanomaterials*

Charge to Breakouts, Sally Tinkle, NIEHS

12:00 ***Lunch*** (on your own)

1:30-4:15 ***Concurrent Breakout Sessions***

Participants will probe the state of the science and identify gaps and emerging trends as they relate to the research needs identified in the Federal NNI EHS strategy.

- Characterization of engineered nanomaterials
- *In vitro* research and related instrumentation and metrology
- *In vivo* research and related instrumentation and metrology

Session 1: Characterization (with assigned IMA and Human Health research needs)

Co-Chairs: Amit Kulkarni, GE Global Research, and Scott McNeil, Nanotechnology Characterization Laboratory

I1. Develop methods to detect nanomaterials in biological matrices, the environment, and the workplace

I2. Understand how chemical and physical modifications affect the properties of nanomaterials

I3. Develop methods for standardizing assessment of particle size, size distribution, shape, structure, and surface area

I4. Develop certified reference materials for chemical and physical characterization of nanomaterials

I5. Develop methods to characterize a nanomaterial's spatiochemical composition, purity, and heterogeneity

H2. Develop methods to quantify and characterize exposure to nanomaterials and characterize nanomaterials in biological matrices

- **Session 2: In vitro – biological – associated instrumentation** (with assigned IMA and Human Health research needs from the 2008 NNI EHS Research Strategy)

Co-Chairs: Carolyn Cairns, Consumers Union, and Andrew Maynard, Woodrow Wilson Center for International Scholars

I1. Develop methods to detect nanomaterials in biological matrices, the environment, and the workplace

–Is this important to understanding hazard to human health?

–Will we get the data we need to complete this research need (e.g., do we have the tools we need)?

I2. Understand how chemical and physical modifications affect the properties of nanomaterials

H1. Understand the absorption and transport of nanomaterials throughout the human body/cells

H3. Identify or develop appropriate *in vitro* [and *in vivo*] assays/models to predict *in vivo* human response to nanomaterials exposure

H5. Determine the mechanisms of interaction between nanomaterials and the body at the molecular, cellular, and tissue levels

- **Session 3: In vivo – biological – associated instrumentation** (with assigned IMA and Human Health research needs from the 2008 NNI EHS Research Strategy)

Co-Chairs: Bill Kojola, AFL-CIO, and Richard Pleus, Intertox

I1. Develop methods to detect nanomaterials in biological matrices, the environment and the workplace

–Is this important to understanding hazard to human health?

–Will we get the data we need to complete this research need (e.g., do we have the tools we need)?

I2. Understand how chemical and physical modifications affect the properties of nanomaterials

H1. Understand the absorption and transport of nanomaterials throughout the human body

H2. Develop methods to quantify and characterize exposure to nanomaterials and characterize nanomaterials in biological matrices

H3. Identify or develop appropriate [*in vitro* and] *in vivo* assays/models to predict *in vivo* human response to nanomaterials exposure

H4. Understand the relationship between the properties of nanomaterials and uptake via the respiratory or digestive tracts or through the eyes or skin, and assess body burden

H5. Determine the mechanisms of interaction between nanomaterials and the body at the molecular, cellular, and tissue levels

4:30 – 5:00 ***Invited Presentation***

Introduction, Travis Earles, Office of Science and Technology Policy

The White House Perspective on Nanotechnology Health and Safety ,Tom Kalil, Deputy Director for Policy, Office of Science and Technology Policy

5:00 Reception

Wednesday, November 18, 2009

7:30 Registration & Continental Breakfast

8:30 – 10:30 ***Plenary Session***

Welcome & Logistics for the Day, Dianne Poster, NIST

Report-Outs from Session Rapporteurs, Chair, Heather Evans, NNCO

Case Studies, Chair, Carolyn Cairns, Consumers Union

Vignettes about real-world experiences help to inform the discussions in the breakout sessions:

–Chuck Geraci, NIOSH, *Exposure Measurements*
–Don Baer, Pacific Northwest National Laboratory, *Characterization Obstacles*
–Alison Elder, University of Rochester, *International Alliance for NanoEHS Harmonization*

Charge to breakouts, Sally Tinkle, NIEHS

Break

10:30-12:15 ***Concurrent Breakout Sessions***

Framework Strategy Analysis Discussion

Sessions build upon the previous day to identify solutions for gaps and barriers, establish a timeline for the research needs, and develop milestones

Session 4: Characterization

Co-Chairs: David Castner, University of Washington, and Vicki Grassian, University of Iowa

Session 5: In vitro – biological – associated instrumentation

Co-Chairs: Charles Gause, Luna Innovations, and Nancy Monteiro-Riviere, North Carolina State University

Session 6: In vivo – biological – associated instrumentation

Co-Chairs: Steve Roberts, University of Florida, and Joel Pounds, PNNL

12:30 ***Working Lunch*** (lunch provided)

Introductions, Amit Kulkarni, GE Global Research

Nano-Informatics: Data-Enabled Predictive Modeling for nanoEHS:
Martin Fritts, NCL, and Justin Teeguarden, PNNL

1:45-3:00 ***Closing Session***

Public Comments, Facilitator, Bill Kojola, AFL-CIO

Report-Outs & Summary, Chair, David Castner, University of Washington

Summary of the thoughts from the three breakout sessions, and audience comments on research needs and framework strategy

Introduction, Charles Gause, Luna Innovations

Congressional Remarks on Nanotechnology Health and Safety, Dahlia Sokolov, Staff Director, Subcommittee on Research and Science Education, House Committee on Science and Technology.

Next Steps & Final Thoughts

Looking to the Future, Sally Tinkle, NIEHS, and Dianne Poster, NIST

Appendix B. Workshop Participants*

**affiliations as of November 2009*

Eric Ackerman, Sandia National Lab

Parag Aggarwal, FDA

Norris Alderson, NNI/Food and Drug Administration

Fredric Arnold, U.S. Environmental Protection Agency

Don Baer, Pacific Northwest National Laboratory

Lajos Balogh, Nanomedicine: Nanotechnology, Biology and Medicine

Mark Banash, Nanocomp Technologies Inc

Brenda Barry, American Chemistry Council

William Bauer, Idaho National Laboratory

Vitalius Benokraitis, WTEC

Andrew Berglund, NIST Center for Nanoscale Science and Technology

Fred Blosser, NNI/CDC-NIOSH

Sara Brenner, U. of Albany College of Nanoscale Science & Engineering

Erica Brown, Assoc. of Metropolitan Water Agencies

Betty Bugusu, Institute of Food Technologists

Ambika Bumb, National Cancer Institute

Kristin Bunker, RJ Lee Group

Gavin Burdge, BMT Designers and Planners

Carolyn Cairns, Planning Team/ Consumers Union

Chris Cannizzaro, NNI/State Department

Chris Carroll, U.S. Army CHPPM

Janet Carter, Occupational Safety and Health Administration

Patricia Casano, GE – Corporate Environmental Programs

Marcia Cash, AeroSolver

David Castner, Planning Team/ University of Washington

Gary Casuccio, RJ Lee Group

Jonathan Chen, Antimicrobials Division, OPP, U.S. EPA

Hung Cheung, Dr. Cheung/ OEM Advisor, LLC

Matthew Cho, NNI/Navy and Marine Corps Public Health Center

Shaun Clancy, Evonik

Megan Clark

Mark Clayton, U.S. EPA

Corey Cohn, AAAS Fellow/Office of Science, U.S. Department of Energy

Ryan Costello, ATSDR/Division of Regional Operations

Charles Cropper

Benita Dair, FDA

Jennifer Decker, National Research Council of Canada

Jeffrey DePriest, DTRA

Kapal Dewan, FDA

Timothy Dole, EPA OPP Antimicrobial Division

Travis Doom, Arizona State University

Pat Durbin, Branch Health Clinic Washington Navy Yard

Travis Earles, NNI/OSTP

Kathleen K. Eggleson, University of Notre Dame

Alison Elder, University of Rochester

Brian Englert, U.S. EPA

Marlowe Epstein, NNCO

Joy Erdman, Chief of Naval Operations, Safety Liaison Office

Heather Evans, NNCO

Kenneth Farmer, TSI Incorporated

Patricia Foland, WTEC

Steve Freiman, Freiman Consulting

Lisa Friedersdorf, nanoSTAR Institute, University of Virginia

Martin Fritts, Nanotechnology Characterization Laboratory

George Gamota, ITRI Inc.

Charles Gause, Planning Team/ Luna Innovations

Charles Geraci, NNI/NIOSH

Margaret Glass, Association of Science-Technology Centers

Peter Goering, U.S. FDA

Christin Grabinski, Air Force Research Laboratory

David Grainger, University of Utah

Vicki Grassian, University of Iowa

Eric Grulke, University of Kentucky

Gary Guggolz, U.S. Government Accountability Office

Bill Gulledge, American Chemistry Council

Vince Hackley, NIST

Nicole Harkin, U.S. Government Accounting Office

Liesl Heeter, NNCO

Lori Henderson, NNI/NIH

Robin Henderson, DOE

Angela Hight Walker, National Institute of Standards and Technology

Geoff Holdridge, NNCO

Mark D. Hoover, CDC-NIOSH

Nina Horne, UC Berkeley

Matthew Hull

Pat Johnson, NNCO

Olcay Jones, WRAMC

Barbara Karn, EPA

Olayiwola Kazeem, University of Glasgow

Jong Sung Kim, The University of Iowa

Frederick Klaessig, Pennsylvania Bio Nano Systems LLC

Bill Kojola, Planning Team/AFL-CIO

Li Ni Komatsu, FDA

Eleni Kousvelari, Sandia National Lab

Amit Kulkarni, Planning Team/GE Global Research

Silvia Lacerda, FDA

William LaFountain, USAF/ASC

David Lai, U.S. EPA

Rebecca Lally, University of California Irvine

James "Jay" Larson, Department of Energy; Office of Science

Rachel Layman, Luna Innovations Incorporated

June Liang, CVM/FDA

Laurie Locascio, NIST

Aaron Lovell, Inside EPA's Risk Policy Report

James Luo, NIBIB/ NIH

Martha Marrapese, Keller and Heckman LLP

Daniel Marsick, NNI/U.S. Dept of Energy

J Patrick Mastin, NIEHS

Krish Mathur, NNI/U.S. Department of Education

Heidi Maupin, NNI/Army Research Lab

Jean-Marie Mayas, The MayaTech Corporation

Andrew Maynard, Woodrow Wilson Center

Bill McArthur, Dept of Energy

William McDevitt, U.S. Army Environmental Command

Scott McNeil, NCI, SAIC-Frederick

Heather Meeks, DTRA

Kalman Migler, National Institute of Standards and Technology

Dennis Mikel, EPA

Nancy Miller, NNI/Office of the Director, NIH

Dragan Momcilovic, FDA

Nancy Monteiro-Riviere, North Carolina State University

Hallie Morgan, U of Albany College of Nanoscale Science and Engineering

Jeff Morris, NNI/U.S. EPA

Matthew Morris, RBC Wealth Management

Joseph Moslow, Roswell Park Cancer Institute

Li Mu, FDA

James Munhall, Nanosight Ltd

Srikanth Nadadur, NIEHS/NIH

Ritu Nalubola, Food and Drug Administration

Elisabet Nalvarte, University of Missouri-Kansas City

Madeleine Nawar, U.S. EPA

Tinh Nguyen, NIST

Van Nguyen, NNI/DOE Office of Science

Janet Normandy, U.S. Dept of Energy

Günter Oberdörster, University of Rochester

Michele Ostraat, RTI International

Halyna Paikoush, NNCO

Ben Palmer, ConocoPhillips

Nicholas Panaro, Nanotechnology Characterization Laboratory

Karen Peterson, NIBIB/ NIH

Martin Philbert, University of Michigan, School of Public Health

Joseph Pickel, Oak Ridge National Laboratory

Richard Pleus, Intertox

Dianne Poster, Planning Team/ NNI/CEQ/NIST

Viji Potula, NIOSH/CDC, Atlanta

Joel Pounds, Pacific Northwest National Laboratory

George Redmond, NCI

Rebecca Reznik-Zellen, University of Massachusetts Amherst

Penelope Rice, FDA/CFSAN/ OFAS/DFCN

Kensley Rivera, U.S. Department of Energy

Robert Rivers, AAAS Fellow/ Clinical Proteomic Technologies for Cancer

Pat Rizzuto, Daily Environment Report/BNA

Steve Roberts, Planning Team/ University of Florida

Welford Roberts, U.S. Air Force Surgeon General's Office of Modernization

Andrew Salamon, PerkinElmer Corp.

Jeffery Schloss, NNI/National Human Genome Research Institute, NIH

Paul Schulte, NNI/NIOSH

Loretta Schuman

Robert Shelton, WTEC

Kristinba Sherry, Inside OSHA

Timothy Simon, USACHPPM

James Slawski, Federal Aviation Administration

Clifford Smith, GE Healthcare

Christina Spring, CDC-NIOSH

Scott Steele, University of Rochester

Ansalan Stewart, OS/HHS

Bruce Stockmeier, Argonne National Lab

Carol Stroebel, CEHN

Pushpa Tandon, National Cancer Institute

Jenny Tao, U.S. EPA

Clayton Teague, NNI/National Nanotechnology Coordination Office

Justin Teeguarden, Pacific Northwest National Laboratory

Treye Thomas, NNI/U.S. CPSC

Scott Thurmond

Sally Tinkle, Planning Team/NNI/ NIH/NIEHS

David Tobias, AAAS Fellow

Lapedra Tolson, NNCO

Andrew Torres, GE Global Research

Thomas Umbreit, FDA

Ken Vest, NNCO

James Votaw, Wilmer Cutler Pickering Hale & Dorr

Paul Wambach, NNI/Department of Energy

Stephanie Watson, NIST

Greg Weatherman, AeroSolver

Robert Wiacek, Pixelligent Technologies, LLC

Kim R. Williams, Colorado School of Mines

Steve Winiecki, Galson Laboratories

Brenda Yamen, OSHA

Shen-yi Yang, U.S. EPA

Junjie Yin, FDA

Appendix C. Abstracts of the Plenary Presentations

Characterization of Engineered Nanomaterials for Human Health Studies

Eric Grulke, Chemical and Materials Engineering, University of Kentucky

Engineered nanomaterials have attracted much interest for their novel physico-chemical properties and potential technological applications. In contrast to the rapid advances in research and manufacturing of nanomaterials, examination of the occupational and environmental, health, and safety of nanomaterials (nanoEHS) lags behind. NanoEHS research is essential to the responsible expansion of the materials portion of the nanotechnology industry (*1, 2*). This work requires accurate and thoughtful characterization of nanomaterials, careful design of the toxicological experiments, and the correlation of physico-chemical properties of engineered nanomaterials to their interactions with relevant biological systems (*3, 4*).

A major engineered nanomaterial toxicology study has been initiated by the Organisation for Economic Co-operation and Development (OECD). Its Working Party on Manufactured Nanomaterials (WPMN) focuses on a priority list of representative engineered nanomaterials and has established a set of 15 endpoints for physico-chemical properties and materials characterization *(5)*.

One interdisciplinary study has identified the following elements needed to assess human health hazards of nanoparticles: extensive physico-chemical characterization, the capacity for macromolecular perturbation, the potential for unintended transport of toxic molecules, translocation of the nanoparticles, their agglomeration state, and their chemical composition (*6*).

This talk will address the mapping of physico-chemical property evaluations to these information elements, and the mapping of analytical tools to the physico-chemical properties. The result is a suite of analytical tools that help characterize nanoparticles for nanoEHS toxicity testing. The initial set includes nanoparticle morphology, composition, surface properties, and aqueous dispersion properties. Examples of property measurements for many of the OECD priority list of engineered nanomaterials are used to illustrate analytical challenges. Some potential research "gaps" associated with nanomaterial characterizations for nanoEHS research are identified.

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In Vitro Considerations

Nanotoxicity Assessment: All Small Talk?

David Grainger, University of Utah

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In the past decades, materials engineered into nanotechnology have moved from an exotic research pursuit to inclusion in hundreds of mainstream consumer products with nanomaterials markets exceeding billions of dollars annually. Many medical innovations under assessment now claim benefits from nanotechnology. Drug delivery systems by their very nature are part of these international pursuits with several nanophase formulations approved for human use, and dozens more nano-therapeutics in clinical trials. With this nano-phase invasion of new materials and products into nearly every aspect of life comes increasing calls for prudent assessment of new safety and exposure risks: published health assessments and toxicological studies of nanosystems are growing at exponential rates annually. It is often argued that human exposure to environmental non-engineered, natural sources of nanomaterials is by far and away the most significant exposure to nanomaterials, persisting throughout the human presence on earth. Certainly, the human physiome must have developed tolerance to constant nanoparticulate exposure from diverse natural sources. By contrast, deliberate acute exposure to human-made nanosystems is a relatively recent phenomenon with no histories yet substantial enough to provide clear or broad-sweeping safety or hazard assessments. One argument in this regard is that synthetic nanomaterials with specific engineered properties (i.e., applied surface coatings, specifically controlled size ranges, dopants, drug inclusion, optical, redox, and electronic properties) concentrated within products as a single species, and distributed into the ecosystem (i.e., disposal into the food chain and ecosystem) or dosed directly to patients are unique to humans at this time. Human tissues and organs have not witnessed such materials or properties. Certainly, in this case of nano-medicines and therapeutics, humans have no evolutionary experience with nano-engineered systems in acute dosing regimens. These arguments about possible "exotic nanomaterials toxicity" have brought about a flurry of concerns from particulate toxicologists in defense of humans as both public stakeholders of substantial government speculations in this enterprise, and possible unwitting victims of adverse nanotechnology impacts (*1,2*).

Engineered nanomaterials have all the traits that should raise eyebrows with regard to health assessments of any particulate: novelty in both form and function, unique chemistry and physics by design, complex interactions with biological and environmental milieu, biopersistence (both within the organism and within the food chain), ready dispersibility and possible bioaccumulation, tissue penetration, and/or irreversible biochemical and materials activities. These types of properties have history in case studies of toxicities resulting from newly introduced substances (*3*). Like these case studies, nano-engineered materials are already in consumer markets, and in some cases, the risks of such exposure personally and environmentally are unknown or poorly understood. The global compendia of nanomaterials safety data to date raise few certainties either pro or con. One can find almost any result published for a given nanomaterial: from "overt toxicity" to "no observable toxicity" (*4–7*). Hence, a classic "cup is either half-full or half-empty" analogy with regard to nanotechnology's promise exists where exaggerated commercial benefit forecasts and motivations are offset by equally extreme, doomsday adverse human health impact scenarios. Public health policy mandates and responses are inconsistent: few are willing to risk political or policy careers influenced by diverse stakeholders without compelling evidence. Enter nanotoxicology (*8*).

Many current nanosystems under development comprise nanoparticles as fundamental building blocks. Specialized nanoparticle chemistries (e.g., metals, ceramics, and carbon allotropes) are produced in metric tons annually for commercial ventures. Interestingly, more and more size- and treatment-dependent properties are reported to distinguish nanomaterials of a given chemistry. Gold nanoparticles unreactive to catalytic reactions in the 40-nm size regime are found to be very reactive chemical catalysts in the Au55 cluster size

due to distinguishing oxide chemistry (*9*). While virgin carbon nanophases (e.g., C_{60}, multi-wall and single-wall nanotubes) are not readily water-dispersible or stable as colloids without aggregation, many adsorbates and etching processes alter this nanomaterial surface to provide aqueous stability. However, most of these important modifications used both *in vitro* and *in vivo* for this popular carbon-based materials set are not reported nor distinguished. Nonetheless, these may very well be critical for understanding their bioavailability and behaviors in aqueous biological test systems. The development of modern particle toxicology, mostly focused on respiratory adverse events historically, has dominated environmental health and is heavily influencing the current thinking for nanotoxicology as well. Nanomaterials size overlap with well-studied inhalable ultrafine particles (diameter 0.1 μm) sourced from urban air pollution provides a convenient, immediately accessible comparison and some initial basis for toxicity concerns. Ultrafine particles from air pollution are long-known to increase morbidity and mortality from pulmonary and cardiovascular causes with both long-term and immediate effects (*10–13*). Moreover, inhaled ultrafine (nanometer range) particles in rodents distribute beyond the lung and can cause both pulmonary and systemic inflammation and promote blood coagulation within minutes to days of exposure (*14–16*). To date, human studies, limited to acute exposures, measuring both pulmonary and systemic inflammatory endpoints, have been inconsistent, perhaps in part attributable to variable particle sources (*17–20*). Nanomaterials toxicology is being developed within a known model historically addressing the toxicology of metal fumes, radioactive and nuisance dusts, rat lung particle overloads, silica, asbestos and synthetic fibers, and more recently air pollution particles (*21, 22*). Given the confounding aggregation phenomena known for these nano-systems under "real" conditions (*23*), atmospheric chemistry, advanced colloid science, and even virology will contribute to understanding and distinguishing nanoparticle toxicology in model *in vitro* and *in vivo* systems.

Significantly, toxicological information compiled for nanomaterials must also consider actual human exposure levels. Any particulate material, nano-sized or larger, is expected to produce adverse effects at high enough doses in a given dosing context. Unfortunately, dosing or exposure amounts for various different nanomaterials applied in toxicological studies both *in vitro* and *in vivo* are frequently unrealistically excessive for most any plausible actual exposure scenario. Dosing is also delivered to model experimental systems as a bolus (i.e., unrealistically high dosing rate), with very limited relevance to actual human exposures except in certain extreme cases, some certainly including bolus nanomedicine dosing. In fact, drug delivery systems constitute a special case of nanomaterials exposure where drug vehicle and therapeutic chemistry and characterization data are well-documented, and dosing is carefully controlled in both formulation and administration. This represents an opportunity to exploit in tool-kit development for safety and efficacy testing. A third general concern with model nanomaterials studies is the lack of ability to control or even know the physical state of the nanomaterial introduced or residing within environmental or biological model systems (23). Many nanomaterials must use surface modification with coatings or surfactants to impart aqueous solution-phase stability or deliberately alter bioavailability. However, not all aqueous solutions with these nano-materials are comparable. Colloidal stability has always been complex when multiple surfactants are present. Presence of food, environmental or serum proteins, lipids, fatty acids, fungal or bacterial remnants ubiquitous to real samples will alter nano-phase material physical presentation to model test systems (*24*), complicating and confounding both the measurement and interpretation of mechanisms in the resulting host reactivity, toxicology, or, in the case of nanomedicines, therapy. That these solution-phase aggregation differences might produce rather profound contrasting results reported in the literature for similar nanomaterials remains an open issue. This also has important implications for behaviors of nano-phase drug delivery systems *in vivo*.

The most commonly observed failure in the biomaterials translational development spectrum in general is the noted disconnect between *in vitro* and *in vivo* performance that fails to predict actual host response. This is likely true for nanomaterials deployed *in vivo* as well, including imaging agents

and drug delivery systems. Hence, developing high-throughput, sensitive, reliable, and predictive *in vivo* preclinical screening models to elucidate aspects of biocompatibility and bioperformance for nanomaterials is critical.

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In Vivo Considerations

Martin A. Philbert, School of Public Health, University of Michigan

The best possible characterization of pristine test articles provides the investigator with no more than an excellent starting point for the analysis of the biological effects of nanomaterials. *In vitro* tests enable the identification of plausible biochemical mechanisms by which biological sequelae may proceed. However, unlike small and diffusible molecules, nanometer-scaled materials and particles are prone to opsinization in the internal compartments of the body and more generally to adsorption of macromolecules (*1,2*). Inherent increases in hydrodynamic radius induced by adsorption of biological macromolecules may be a result of surface charge, the presence of targeting ligands, the chemical identity of the material, and other considerations, and may mask those properties from biological systems. Therefore, as pointed out by Dawson and others (*3,4*), the bio–nano interface changes abruptly and, perhaps, irrevocably upon first contact with biological fluids. Relatively more is known about the interactions between nanoparticles of different types and proteins (*5–8*).

This simple consideration leads to a number of biologically relevant questions *in vivo* that pertain to the absorption, distribution, biological alteration/dissolution of nanomaterials, and elimination from the body. It is not apparent that the following parameters have been addressed in the currently available literature and may provide valuable inputs into a data-rich predictive informatics approach to safety. For the purposes of discussion, these considerations have been lumped into two major questions, i.e., physical and biological sequelae, respectively:

1. Since size has been shown to be a major influence on the biological effects of some nanomaterials (*9–11*), what effect does interaction of nanomaterials with biological macromolecules have on:
    a. Dissolution properties of metal-containing nanoparticles in situ (*12*)?
        i. How do we measure kinetics of dissolution, i.e., ion production, *in situ*?
    b. Polydispersity of the material?
        i. Is there a general shift to a larger hydrodynamic radius?
        ii. Are all components of a polydisperse material equally affected by biological coating(s)?
    c. Dynamic change of the macromolecular interactions?
        i. Are there dynamic changes in the nanomaterial corona that affect its biological activity?
            1. Are some materials passivated/activated by macromolecular coatings?
            2. 2. Where might this passivation/activation occur?
        ii. What is our current understanding of the role of the bioactivity of nanomaterials?

    It is important to note here that the realm of biological macromolecules includes (but may not be limited to) micronutrients, hormones, signaling moieties, immunogens, persistent hydrophobic environmental contaminants, and so on.
2. What does this passivation/activation do for the PK/PD and TK/TD profiles of the nanomaterial?
    a. Do surface nanomaterial–macromolecular interactions induce sequestration of nanomaterials into specific biological compartments?
        i. Interstitial
            1. Long-term stabilization and formation of storage/aging complexes
            2. Localized loss/gain of tissue function
                a. e.g., Liver – Induction of P450 and other metabolic systems
                b. e.g., Inflammatory response followed by stimulation of fibroblast proliferation
        ii. Reticuloendothelial/immune system
        iii. Parenchyma
        iv. Blood/lymphatic system

v. Translocation potential of nanomaterials between tissue compartments, tissues, and organ systems (*13,14*)

vi. Penetration of subcellular compartments and alteration of function, e.g., mitochondria damaging (*15,16*) and partially protective (*17*)

This brief listing of mechanistic and descriptive studies provides a point of departure from which other investigations may be designed. Many will require the development of new techniques and/or the adaptation of existing techniques and technologies that will provide for quantitative and kinetic analysis of the nano–bio interface and, ultimately, biological effect.

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Appendix D. Research Needs Assessments and Recommended Changes

As breakout sessions focused on "how far nanoEHS research has come" and "where do we go from here," participants identified research progress, gaps, and barriers; proposed timelines for completing the research; and developed milestones for each of the ten research needs. Participants were asked to specifically comment on the clarity, appropriateness, and completeness of the existing research needs and the feasibility of the timelines. This exercise was patterned after the format for the existing research needs and the heat diagrams, or graphical timelines, in the 2008 NNI EHS Research Strategy (see Figure 3, p. 18, and Figure 5, p. 24). Because the 2008 NNI EHS Research Strategy included an analysis of research performed in 2006, workshop participants were asked to evaluate research in progress since 2006 and to establish timelines that spanned the next 15 years. For these reasons, the timelines presented encompass 2007–2022.

As a final note, the information presented here reflects the output of the breakout session process and the writing team's efforts to faithfully present the discussions as they occurred. When information was not supplied by the participants, we have indicated "No information provided." Additionally, responses are sometimes cryptic, reflecting the flow of the discussions and the notes provided to the writing team.

The format for this section is:

- Research Need: the *original* text for each research need as written in the 2008 NNI EHS research document. The overarching research needs are labeled H1–H5 for Human Health and I1–I5 for Instrumentation, Metrology, and Analytical Methods. The bullet points are labeled a, b, etc.
- Group responses to the charge questions.
- Analysis of individual bullets: recommendations for changes in the overarching research need (H1–5 or I1–5) and each bullet point (H1a, H1b, I1a, etc.) or addition of new bullet points. Each of these sections contains the original bullet and, where stipulated, the revised bullet, the suggested timeline, milestones to assess progress, and rationale for the milestones. Participants labeled the timelines using yellow to indicate low priority research, orange for medium priority, and red for high priority. Green is used to show good progress has been made in achieving the particular research need.

HUMAN HEALTH

H1. Understand the absorption and transport of nanomaterials throughout the human body

H1a. Interaction of nanomaterials with exposure organ, including relationship of exposure to uptake

H1b. Sequestration of materials in the exposure organ

H1c. Metabolism or biological transformation of materials

H1d. Translocation out of the exposure organ

H1e. Mechanism of transport through the body

H1f. Sequestration of material in secondary organs

H1g. Excretions routes

Group Responses to Charge Questions

Is research occurring on this research need?

Ongoing research was considered by component areas within the need to understand absorption and transport. The importance of systematic mass balance studies was noted for each of the component areas.

Exposure

More accurate determination of human exposure levels is needed so that *in vivo* and *in vitro* experiments more accurately mimic human exposures. More studies of nanomaterials have been conducted using an inhalation exposure route than using dermal, oral, and intravenous routes, and more fundamental information from pharmacology and clinical medicine could be useful in assessing intravenous and oral exposures. For example, few studies have employed oral exposure and assessed transport of nanomaterials across gastrointestinal epithelial and endothelial barriers. For all exposure routes, there are limited studies on the fate of nanoparticles after absorption using mass balance analysis.

Uptake

Most current research focuses on cellular uptake and processing, is fairly mechanistic, and could be more quantitative. Determining the properties of nanomaterials that drive cellular uptake and processing and the rate at which these processes proceed is an important area that needs more focused study. Several studies focus primarily on pulmonary exposure and subsequent effects; however, these studies were considered insufficient to support generalization of uptake mechanisms across diverse nanomaterials. Additional areas requiring focused research include transpulmonary distribution of nanoparticles and systematic mass balance studies on absorption, distribution, metabolism, and excretion (ADME).

Sequestration

Coordinated integration of imaging and analytical methods will allow a distinction to be made between nanomaterials and their constituent elements/chemicals and will enhance systematic mass balance analysis over the time course from exposure to sequestration, as well as analysis of biological response. Stable radioactive isotopic tracing approaches should be employed to enhance sensitivity and selectivity of the imaging and analytical tools.

Metabolism

Identifications of dose–response or structure–activity relationships and the impact of nanoparticles on normal cell function are limited. Little information is currently available on the effect of nanomaterial surface functionalization on metabolism, intracellular and intra-organ dissolution of nanomaterials into their component elements/chemicals, and biologically mediated changes in the material properties. More progress is needed to identify generalized principles of nanomaterial interaction with biological systems,

protein opsonization, and nanomaterial dissolution. The differences in experimental results using *in vitro* and *in vivo* models and the relationship of data from these experimental systems to human exposures needs to be determined so that *in vitro* and *in vivo* data can be interpreted meaningfully. It is important to help researchers understand the relative value of increasing dose or using a specific assay.

Do the bullets under each research need adequately define and provide sufficient granularity for this research need? Is anything missing?

Additional cross-cutting areas cited for study include mechanisms of transport through the body, translocation out of the primary exposure organ to secondary organs, and sequestration in secondary organs. Concern was expressed that there are no studies of susceptible populations—specifically, the effect of genetic polymorphisms or the effect of compromised health due to acute or chronic disease on ADME of nanoparticles. Examples of susceptible populations include loss-of-function diseases (renal disease and diabetes), inflammatory diseases (endotoxemia/sepsis, colitis, and multi-organ failure), and chronic diseases (cardiovascular disease and asthma), as well as susceptible populations such as children, pregnant women, and the senior population. The use of animal models for susceptible populations and compromised health status was considered important for comprehensive understanding of adsorption and transport of nanomaterials.

Participants noted there are ADME guidelines in place in the pharmaceutical industry for nanomaterials-based clinical therapeutics and asked if those should not be guiding this environmental, health, and safety analysis.

Are there tools, methods, or other research topics that need to be developed to complete this research need?

Critical barriers to this work include the metrology challenge of quantifying nanomaterials in relevant media, the poor resolution of nanomaterials in tissues, the need for more quantitative methods of dosimetry, and the lack of human-exposure-driven choices of materials to study. There is a paucity of information on the commercial uses of nanomaterials and on the availability of industry standards.

Recommended Revision of Overarching Research Need

Original overarching research need H1:

Understand the absorption and transport of nanomaterials throughout the human body.

Revised overarching research need H1:

Understand the absorption and transport of nanomaterials throughout the human body in healthy individuals and in susceptible populations. Susceptible populations include, but are not limited to, individuals at risk of altered adsorption, distribution, metabolism, and elimination of nanomaterials, as well as changes in individual risk due to genetics, lifestyle, age, existing illness, or other factors.

Analysis of Individual Bullets and Recommended New Bullet

H1a

Original bullet H1a: Interaction of nanomaterials with exposure organ, including relationship of exposure to uptake.

Timeline

2007-2012	2012-2017	2017-2022
Low	High	

Rationale

- Current progress is very limited, and there is a need for more data.
- Understanding these interactions is essential for risk assessment and extrapolation.

Milestones to demonstrate progress:

- Research on different nanoparticles with more than ten publications per particle type by 2022
- Use of adsorption and transport data in over 30 risk-assessment settings

H1b

Original bullet H1b: Sequestration of materials in the exposure organ.

Revised bullet H1b: Understand sequestration of materials in the exposure organ, translocation out of the exposure organ, mechanisms of transport through the body, sequestration of materials in secondary organs, and excretion routes.

Timeline

2007-2012	2012-2017	2017-2022
Low	High	

Rationale:

- These data will allow scientists and risk assessors to evaluate *in vivo* human toxicity with an appropriate toxicological context.

Milestones:

- Understanding general principles of sequestration, translocation, and excretion for nanomaterials

New bullet: Understand cellular uptake and processing, including solubility in cells and tissues, uptake mechanisms and kinetics, and intracellular transport mechanisms and kinetics.

Timeline

2007-2012	2012-2017	2017-2022
Medium		Low

Rationale:

- These activities can be correlated with physico-chemical features to enable safe design and hazard screening.

Milestones:

- Effective techniques and instrumentation available for classes of commercially relevant nanomaterials with priority given to those most relevant to materials with most exposure-intensive applications
  - Ability to track priority nanomaterials in cells/tissue
  - Highly reproducible assays that employ different cell types and functioning
  - Widely available quantitative instrumentation and methods to measure dose *in situ*
  - Readily available cellular and acellular model systems
- Establishment of a five-year program to produce usable datasets, instrumentation, and effective cell assays for priority nanomaterials

H1c

Original bullet H1c: **Metabolism or biological transformation of materials.**

Revised bullet H1c: Understand metabolism, biological transformation, and biokinetics of nanomaterials in the exposure organ and secondary organs.

Timeline for metabolism and transformation

2007-2012	2012-2017	2017-2022
	High	Medium

*Stripes indicate variable opinion as to low vs high near-term priority

Timeline for biokinetics

2007-2012	2012-2017	2017-2022
Medium		High

Rationale:

- There is a critical need to understand general principles of metabolism and transformation. In general, we know in which tissues and cell types these materials commonly sequester following exposure, but their long-term fate (e.g., if they degrade, dissolve, or transform) is not known.
- Understanding metabolism and biotransformation of nanomaterials was considered by a number of participants to be more important than the mechanism of uptake because, regardless of how nanomaterials enter the cell, toxicity will be closely related to dose (accumulation, duration of exposure), metabolism, and transformation rates. Additionally, the route of exposure and mechanism of uptake of nanomaterials into major tissues, especially lung and skin, are being established.
- Studies on biokinetics—that is, quantitative determination of dose and time course of migration into and between tissues—is important but should follow studies on cellular transformation and metabolism. This time offset would allow researchers to focus biokinetic analysis on materials most likely to accumulate in organs and tissues and require timely risk assessment and risk management.

Milestones:

- Establishment of a 5-year program to produce usable datasets, instrumentation, and effective assays for nanomaterial assessment, including
  - effective, quantitative techniques and instrumentation available to assess classes of commercially relevant nanomaterials—that is, materials with the highest likelihood of exposure-related application
  - ability to track priority nanomaterials in cells and tissues
  - high level of reproducibility for, and availability of, cellular and acellular assays with different phenotypic and functional parameters
- Use of sequestration, metabolic, and biokinetic data in a computational model

Note: Original bullets (H1d: Translocation out of the exposure organ, H1e: Mechanism of transport through the body, H1f: Sequestration of material in secondary organs, and H1g: Excretions routes) were discussed in the response to the breakout questions, but were not called out for further comment by any of the breakout groups. Instead, the content of these bullets was incorporated into the revised overarching research need.

H2. Develop methods to quantify and characterize exposure to nanomaterials and characterize nanomaterials in biological matrices

H2a. Determine relevant measurement parameters for each class of nanomaterials in a simple exposure matrix and in a simple biological matrix.

H2b. Determine appropriate parameters for sampling and analysis.

H2c. Establish methods for quantification and characterization.

H2d. Determine relevant measurement parameters for each class of nanomaterials in complex exposure matrices and in complex biological matrices.

H2e. Validate methods for each exposure route.

H2f. Develop biomarkers for exposure.

Group Responses to Charge Questions

Is research occurring on this research need?

There is general agreement that some research is occurring in each component of this research need, although no area is adequately advanced or completed. Translation of research to hazard and risk assessment is just beginning.

Do the bullets under each research need adequately define and provide sufficient granularity for this research need? Is anything missing?

There is a need for greater clarity in almost all bullet points, including the overarching research need. For example, methods to enable quantification and characterization of both internal and external exposure are not available.

The terminology for exposure needs to be clarified. External exposure refers to the quantity/number of nanoparticles inhaled, ingested, or applied to the skin; this is frequently just called "exposure." Internal exposure refers to the particles that enter and move through the body and interact with biological matrices; this is also called "dose."

There is not a clear distinction between simple and complex matrices and why characterization needs would/should be different.

Are there tools, methods, or other research topics that need to be developed to complete this research need?

Tools and methods are needed to detect and quantify nanomaterials in biological materials directly or in preserved samples. To be useful, they need to be rapid and cost-effective. It is also necessary to

- Determine critical measurands for nanomaterials in both internal and external exposure matrices (exposure and dose matrices) as well as key influences for each measurand, e.g., sample preparation, storage, temperature, and solvents/solutions.
- Establish and validate instrumentation and methods for quantification, characterization, and commutability. A commutable reference material is one for which measurements will have similar values to authentic samples when evaluated by more than one analytical method. Commutability is a method-specific characteristic.

The wording "classes of nanomaterials" is not clearly understood by stakeholders and should be used carefully when attributing a biological outcome beyond the scope of the specific material used in the study. Additionally, there was a suggestion to change the term nanomaterial to nanoparticle, as it seems to more accurately describe the dose-related issues under consideration. The group recognizes the importance of terminology, and there was consensus to substitute recognized, accepted terminology as it develops.

Recommended Revision of Overarching Research Need

Overarching research need H2:

Develop methods to quantify and characterize exposure to nanomaterials and characterize nanomaterials in biological matrices.

Revised overarching research need H2:

Develop methods to enable the quantification and characterization of nanomaterials in biological matrices and exposure matrices (e.g., air, water, food).

Analysis of Individual Bullets and Recommended New Bullet

H2a and H2d

Original bullet H2a: Determine relevant measurements parameters for each class of nanomaterials in simple exposure matrix and in simple biological matrix.

Original bullet H2d: Determine relevant measurement parameters for each class of nanomaterials in complex exposure matrices and in complex biological matrices.

Revised bullet H2a and d: Determine which physico-chemical properties of nanomaterials to measure in biological matrices and exposure matrices and develop measurement methods that are rapid and cost-effective.

Timeline

2007-2012	2012-2017	2017-2022
	Medium	Low

*Stripes indicate variable opinions among breakout groups as good progress versus medium priority

Rationale:

- These tools and methods will enable mechanistic studies and *in vitro* and *in vivo* localization studies, as well as research to determine changes in particle properties that are associated with their interactions with biological matrices.
- Collaboration between toxicology and pathology will be important so that characterization methods are applicable to multiple nanomaterials and diverse biological specimens.
- There has been more progress on this than on other research needs; therefore, given limited resources, this should be a lower priority.

Milestones:

- The ability to follow a nanomaterial throughout the entire *in vivo* absorption, distribution, metabolism, and excretion process
- New methods disseminated to and adopted by the research community

H2b

Original bullet H2b: Determine appropriate parameters for sampling and analysis.

Revised bullet H2b: Determine critical measurands for nanomaterials in both exposure- and dose-relevant matrices and identify key influences for each measurand (e.g., sample preparation, storage, temperature, solvents/solutions) to enable measurement uncertainty analysis.

Timeline

2007-2012	2012-2017	2017-2022
High		

Rationale:

- Recent studies highlight the influence of factors such as endotoxin, oxidation state, and residual catalyst on research results.

Milestones:

- Identification of key measurands for specific nanomaterials (perhaps start with list from OECD) determined through case studies for biological matrices (e.g., tissues and organs) and exposure matrices (e.g., air, water, food)
- Determination of key influences for specific exposure matrices as determined through well controlled and documented studies (maybe round-robins)

H2c

Original bullet H2c: Establish methods for quantification and characterization.

Revised bullet H2c: Refine and optimize existing methods and refine or develop methods to characterize and quantify exposure and dose for all exposure routes, including potential biomarkers of exposure. The methods should be rapid and cost-effective.

Timeline:

2007-2012	2012-2017	2017-2022
High		

Rationale:

- There is more research on airborne exposures than on the other exposure routes.
- Airborne exposure data have not been verified for multiple nanomaterials or real-world exposures.

Milestones:

- Identification of key measurands for specific nanomaterials (perhaps start with list from OECD) determined though case studies for biological matrices (e.g., tissues and organs) and exposure matrices (e.g., air, water, food)

New bullet: Develop methods to visualize nanomaterials in biological matrices.

Timeline

2007-2012	2012-2017	2017-2022
	Medium	Low

*Stripes indicate variable opinion among breakout groups as good progress versus medium priority

Rationale:

- Methods for visualization were defined as photoluminescence, radioactive labeling, scanning electron microscopy, and transmission electron microscopy.
- *In vivo* imaging helps to distinguish nanomaterials from constituent biological elements so that biological response and kinetics can be correlated correctly.

- There has been more progress than on other research needs, and although much still needs to be done, given limited resources, this should be a lower priority.

Milestones:

- Ability to follow a nanomaterial throughout the entire *in vivo* absorption, distribution, metabolism, and excretion process
- New methods disseminated to and adopted by the research community

H2e

Original bullet H2e: Validate methods for each exposure route.

Revised bullet H2e: Establish and validate instrumentation and methods for quantification, characterization, and commutable/comparable (interoperable) measurements of nanomaterials.

Timeline:

2007-2012	2012-2017	2017-2022
Medium		Low

Rationale:

- There has been some early progress, such as that occurring within the International Alliance for Nanomaterials Harmonization.
- A formal government-sponsored effort that incorporates research gaps noted in several previous bullets would significantly enhance the research capabilities of the nanotechnology community.

Milestones:

- Establishment of plans for detailed (government-sponsored) round-robin tests
- Establishment of traceability to the International System of Units (SI) and validation through international comparison

H2f: These bullets were combined into H2c

Original bullet H2f: Develop biomarkers for exposure.

Revised bullet H2f: Develop biomarkers for nanoparticles.

H3. Identify or develop appropriate *in vitro* and *in vivo* assays/models to predict *in vivo* human responses to nanomaterials exposure

H3a Validate *in vitro* and *in vivo* test methods.

H3b Determine appropriate methods to suspend and administer nanomaterials.

H3c Develop methods to assess the nanoscale physico-chemical properties in biological matrices.

H3d Develop new test methods as testing gaps emerge.

H3e Develop high-throughput screening technologies.

H3f Evaluate the degree to which *in vitro* and *in vivo* models predict human response.

H3g Translate research data into computational models to predict toxicity *in silico*.

Group Responses to Charge Questions

Is research occurring on this research need?

There is some research on all aspects of this research need. The traditional toxicological endpoints (mortality and impaired activity) should be utilized and validated for nanomaterials.

Do the bullets under each research need adequately define and provide sufficient granularity for this research need? Is anything missing?

The bullets provide necessary granularity for the research need; however, the bullet "Develop new test methods as testing gaps emerge," was considered unnecessary because this action is part of the scientific process.

Are there tools, methods, or other research topics that need to be developed to complete this research need?

Developing the ability to predict biological effects of nanomaterials in exposed humans from *in vitro* tests was recognized as an iterative process, starting with the documentation and validation of existing methods in a manner that can be widely shared among researchers. Expanding this knowledge base with new methods developed for broader classes of nanomaterials will then enable the development of high-throughput screening tests and predictive models that relate nanoscale physico-chemical properties to biological impacts in various biological systems. Accelerating this transition will require a systematic approach that allows for simultaneous development of new methods for imaging nanomaterials in biological matrices, administering and suspending nanomaterials in tissues and cell cultures in ways relevant to real-world exposure pathways, and translating results to computational models. The group identified a need to catalog findings from existing assays so researchers can share results and understand effective methods and those that are limited.

There is also a need for information on nanomaterials in development or production to track methods for characterizing each material and to translate knowledge into good practices. New tests are emerging, but translation to risk management methods is limited. Whenever new tests are developed, it is important that researchers understand the contribution of the test to risk assessment and risk management efforts. There was considerable discussion of the need to establish guidelines for the inclusion of minimum criteria for characterization and biological analyses when results are published and to evaluate the role of peer review and documentation of all work, including that which is not reproducible.

Confidential business information (CBI) was also discussed as a meaningful barrier to progress. The need for a reporting system that enables sharing of critical information regardless of CBI status was discussed.

Analysis of Individual Bullets and Recommended New Bullet

Overarching research need H3:

Identify or develop appropriate *in vitro* and *in vivo* assays and models to predict *in vivo* human responses to nanomaterials

H3a

Original bullet H3a: Validate *in vitro* and *in vivo* test methods.

Revised bullet H3a: Develop and apply a process to validate and verify *in vitro* and *in vivo* test methods.

Timeline

2007-2012	2012-2017	2017-2022
	High	Medium

*Stripes indicate variable opinion among breakout groups as low versus high priority

Rationale:

- Criteria and protocols for test validation need to be well defined.
- Without validated tests, the ability to make inferences about exposure risk in humans is severely hampered; thus, this is considered a priority, and as results are published and validated methods are implemented, research funding could taper off.
- This research bullet would also assist researchers and industry in understanding when a materials is "go/no go" for further development and to know what tests need to be performed to assist them in making that determination.
- The current OECD and NIOSH guidelines should be examined to see if there is some overlap in the tests needed to evaluate risk and benefit.

Milestones:

- Reproducible and reliable test methods made available to the nanotechnology community
- Validation of hazard identification methods in several different animal systems
- Validation of hazard identification models and methods against human responses

H3b

Original bullet H3b: Determine appropriate methods to suspend and administer nanomaterials.

Timeline

2007-2012	2012-2017	2017-2022
	High	Medium

*Stripes indicate variable opinion among breakout groups as low versus high priority

Rationale:

- No appropriate and widely accepted suspension and administration methods were identified.
- This is an important variable for test reproducibility and study of relevant exposures.
- This is also considered a high-priority research area.

Milestones:

- Publication of peer-reviewed articles and guidance documents that detail appropriate methods across all exposure routes

H3d and H3e

Original bullet H3d: Develop new test methods as testing gaps emerge.

Original bullet H3e: Develop high-throughput screening technologies.

Revised bullet H3d and H3e: Develop new test methods and high-throughput screening technologies.

Timeline

2007-2012	2012-2017	2017-2022
	Low	

*Stripes indicate variable opinion among breakout groups as low versus medium priority

Rationale:

- This is a long-term and low-priority objective. It will require significant work to develop high-throughput screening technologies that will reliably replace *in vivo* systems.
- This goal may be unrealistic for the current timeline, but some foundation work should begin in the near term.

Milestones:

- Development of initial components of a high-throughput screening technology whose endpoints are applicable to *in vivo* systems

H3f

Original bullet H3f: Evaluate the degree to which *in vitro* and *in vivo* models predict human response.

Timeline

2007-2012	2012-2017	2017-2022
Low		

Rationale:

- This is a long-term and low-priority objective. There have always been anatomical and physiological differences between animal models and humans that make translating research findings difficult (e.g., lung tissue of a mouse versus the lung tissue of a human), although, at the molecular level, pathways often function similarly.
- It will require significant work to translate data on nanomaterials from animal models to humans.
- To successfully achieve this goal, initial efforts should focus on endpoints that are known to be similar in animal models and humans.
- *In vivo* models are more applicable to humans than *in vitro* models.

Milestones:

- Identification of molecular pathways in animal models and humans that respond similarly following nanomaterial exposure
- Identification of organ system responses in animal models and humans that respond similarly following nanomaterial exposure

H3g

Original bullet H3g: Translate research data into computational models to predict toxicity *in silico*.

Timelines

2007-2012	2012-2017	2017-2022
Low		

Rationale:

- This objective is long-term and low priority for use in *in vivo* systems. This is considered a very long-term research need because significant foundational research needs to be accomplished to identify those variables necessary to developing models.
- Computational models should include characterization, exposure, dose, and response, and should extrapolate animal data to humans.

Milestones:

- An initial *in silico* model that incorporates characterization, dose, and response data from animal models and predicts toxicity in a test set of nanomaterials
- A more sophisticated *in silico* model that can predict the effects of nanomaterial exposure in human subjects

New bullet: Catalog current research findings, including null results.

Timeline

2007-2012
High

Rationale:

- Development of applications and their commercialization is underway. A catalogue of existing positive and null findings is critical to expedite the research and development process.

Milestones:

- None specified

H4. Understand the relationship between the properties of nanomaterials and uptake via the respiratory or digestive tracts or through the eyes or skin, and assess body burden

a. Characterize the physico-chemical properties of the major classes of nanomaterials by exposure route.

b. Determine the relationship of acute exposure/uptake to body burden by class of nanomaterial.

c. Determine the relationship of chronic exposure/uptake to body burden by class of nanomaterial.

Group Responses to Charge Questions

Is there research occurring on this research need?

There is some limited research on this need, but these studies lack technical specificity on characterization and exposure, such as physico-chemical characteristics of the nanomaterials, transformation of nanoparticles at the interface between the cell and the nanomaterial, and mechanisms of cellular uptake.

There has been strong attention to inhalation exposure, but this research is biased by high dosing loads and use of intratracheal instillation, a method that instills materials directly into the lungs, thus bypassing the upper respiratory tract. Research is also needed to characterize the effect of lung surfactants and other biomaterials at the lung epithelial barrier that have the potential to coat nanomaterials and alter their biological behavior. This lack of realism in inhalation studies does not provide the data needed for risk assessment. A similar gap exists for nanomaterial interactions with cells of the intestinal tract and skin.

Do the bullets under each research need adequately define and provide sufficient granularity for this research need? Is anything missing?

The following bullets need to be added:

- Improve characterization of nanomaterials at exposure organ interfaces, such as lung epithelia and stomach lining, and improve identification of subsequent mechanisms of uptake and absorption, distribution, metabolism, and excretion.
- Examine uptake and absorption, distribution, metabolism, and excretion in disease models, such as compromised lungs (asthmatics), abraded skin, or diseases of the gastrointestinal tract.
- Evaluate nontraditional routes of uptake such as ear and eye.

Are there tools, methods, or other research topics that need to be developed to complete this research need?

There is also a need for more complex *in vitro* models that more closely mimic tissues and organ systems, new *in vivo* models that more closely resemble disease states, and new analytical techniques to assess physico-chemical status of nanoparticles in complex biological media. Additional studies are needed on mechanisms of cellular uptake by cell type.

Instrumentation or techniques are needed to characterize nanomaterials from their native state through dosing, and both short- and long-term exposures. Finally, there is a need to improve the research paradigm and tools for acute studies so that they will better identify the need for, and inform the design of, chronic studies.

Analysis of Individual Bullets and Recommended New Bullet

Overarching research need H4:

Identify or develop appropriate *in vitro* and *in vivo* assays/models to predict *in vivo* human responses to nanomaterials exposures.

H4a

Original bullet H4a: Characterize the physico-chemical properties of the major classes of nanomaterials by exposure route.

Revised bullet H4a: Characterize nanomaterials at exposure organ interfaces and identify subsequent mechanisms of uptake and absorption, distribution, metabolism, and excretion.

Timeline

2007-2012	2012-2017	2017-2022
High		

Rationale:

- Uptake is the most important step in toxicokinetics. There is some progress in understanding this phenomenon, but it is limited to certain groups of nanoparticles, such as carbon nanotubes and metal oxides.
- A specific nanoparticle may not be representative of all nanoparticles within a class. It would be difficult, if not impossible, to extrapolate from studies on one material to another. Therefore, this is a high-priority area.

Milestones:

- Development of methods to characterize exposure and uptake at the cell-nanoparticle interface
- Determination of a set of units for characterization
- Characterization of nanoparticles by uptake pathway *in vitro* and exposure route *in vivo*
- Development of animal models for organ-specific absorption
- Definition of classes of nanomaterials consistent with ISO and OECD definitions

H4b

Determine the relationship of acute exposure/uptake to body burden by class of nanomaterial

Timeline

2007-2012	2012-2017	2017-2022
High	Medium	High

Rationale:

- This is a high-priority over the next five years, because there are many products in the market without available health effects information.
- Standard protocols are critical to gathering data for safety assessment and for informatics and modeling.

Milestones:

- Literature review to see if there is sufficient data for risk assessment
- Development of protocols and models for acute exposure, that is, exposure of animals for 24 hours or less
- Determination of the physico-chemical properties of the nanoparticles that are the most easily absorbed by each route of exposure
- Nomination of particles determined to have the highest likelihood of adverse health effects in humans for immediate chronic studies and risk assessment analysis

H4c

Determine the relationship of chronic exposure and uptake to body burden by class of nanomaterial.

Timeline

2007-2012	2012-2017	2017-2022
High		

Rationale:

- This is a high priority because not much is known about chronic exposure, but it is anticipated that the potential for adverse effects of chronic exposure could be significant.
- People are already being exposed to products containing nanoparticles.

Milestones:

- Development of protocols and models for chronic exposure that employ low doses and repeated exposure over extended periods of time
- Determination of the physico-chemical properties of the nanoparticles that are the most easily absorbed by each route of exposure
- Pilot epidemiological studies

New bullet: Examine nanomaterials uptake in disease models mimicking compromised lungs, abraded skin, or diseases of the gastrointestinal tract.

No timeline, rationale, or milestones were provided; however, the NNI will consider this recommendation when developing the next iteration of the NNI EHS strategy.

H5. Determine the mechanisms of interaction between nanomaterials and the body at the molecular, cellular, and tissular levels

H5a. Identify mechanism through which nanomaterials interact with fundamental, protective biological response pathways.

H5b. Identify mechanism by which nanomaterials disrupt protective pathways and cause adverse health effects.

H5c. Determine the relationship of dose, physical, and chemical properties to protective versus adverse responses.

H5d. Validate *in vitro* biological responses in animal models.

H5e. Determine the relationship of biological response in animal models to human response.

Group Response to Charge Questions

Is research occurring on this research need?

The status of research and need for new studies in this area are thought to vary considerably among different toxicological endpoints, exposure pathways, and classes of nanomaterials, making it difficult to draw general conclusions.

Considerations cited by participants when assessing the status of current research include the following:

- There has been a significant amount of fundamental research, usually *in vitro* studies, and extrapolating results from *in vitro* to *in vivo* must be done carefully. In some cases, there is no connection between *in vivo* and *in vitro* models.
- If we have limited resources and want to do *in vivo* first, how does one determine what needs to be tested? FDA ranks products and devices by likelihood of exposure and induction of cancer. FDA does not perform an entire battery of tests for each material it examines, only those deemed necessary and appropriate. For example, if a nanomaterial is not going to be biopersistent, a targeted subset of toxicity tests could be performed. FDA has a critical path process: can this be applied to nanoEHS?
- Current Good Laboratory Practice (GLP) standards should be modified and expanded for use with nanomaterials. Following the development of such GLP standards, this information should be widely disseminated and implemented.
- A report that assessed nanotechnology environmental, health, and safety research from 2004 to 2008 was published in the UK in 2009. Of the projects studied, more than half did not meet the editors' criteria for inclusion or were duplicates (Institute of Occupational Medicine, EMERGNANO: A review of completed and near completed environment, health, and safety research on nanomaterials and nanotechnology [IOM Report TM/09/01, 2009; http://www.safenano.org/Uploads/EMERGNANO_CB0409_Full.pdf]).
- Consider creating risk classes for nanomaterials instead of using a safety analysis report framework.
- Fundamentally, can we use the results from the testing of nanoparticles in medicine in risk assessment for environmentally relevant nanomaterials?

What are the current gaps in knowledge?

Two significant research challenges are the identification of the matrices incorporating nanomaterials and the standardization of dosimetry.

At present, it is not clear if the appropriate dose metric against which to evaluate biological response is surface area, mass, or some other physical or chemical parameter of a nanomaterial. From a risk assessment perspective, mass is still the predominate metric used to estimate risk; however, other physico-chemical parameters might become useful. Standardization was seen as critical, particularly with respect to characterizing dose and correlating dose employed in *in vitro* models with the dose employed in *in vivo* systems. There is also concern that the consideration of alternative metrics for dose is a "red herring" that diverts attention from other critical research questions.

The need for analyzing toxicity across the life cycle of a material was highlighted, and the analysis should include the effect of aging of the nanomaterial on toxicity. Additional research needs include multiple exposure studies, acute and chronic exposure studies, and translation studies to create an anatomical and physiological "bridge" between *in vitro* and *in vivo* studies. Heightened susceptibility to nanomaterials during sensitive time periods was identified, including fetal exposure across the placental barrier, exposure of children during developmental windows, and elderly individuals who may have alterations in their adsorption and excretion parameters due to aging or to existing disease. The need to assess both direct and indirect consequences of exposure to nanomaterials was noted, as was the need to identify appropriate animal models.

Ecologists are concerned that the funding and the focus of nanomaterials EHS research are on the impact on humans within the environment, and research on the environment itself is limited. The effect of nanomaterials on the flora, fauna, and food chain should be assessed, as should point-source apportionment for exposure.

What type of studies should there be?

Studies should focus on oral ingestion, not only dermal or inhalation exposure routes, and the exposure studies should mimic real-world experiences.

Research should examine fundamental biological processes, including the immune system, as well as chronic exposure, chronic toxicity, and sequestration of materials *in vivo*.

Studies on biological mechanisms are of varying importance, depending on the type of nanomaterial, likelihood of exposure, and exposure pathways. There is a need to establish criteria to define knowledge thresholds and knowledge feedback loops to enable researchers to determine when studies indicate that new studies are needed to correct or validate preexisting information.

A prioritization schema was offered that included the following comments:

- Scientific considerations
  - Perform exposure assessment studies that examine a mechanism or pathway response to exposure.
  - Determine the relationship of dose, physico-chemical properties to protective versus adverse responses.
  - Determine the relationship of biological response in animal models to human response and an expansion of all animal models used.
  - Create a knowledge network: bring academia to industry to accelerate the discovery process.
  - Develop decision matrices that enable a systematic approach to prioritizing research.
  - Foster a greater consensus among researchers with respect to the sterility issue of *in vitro* assays and criteria for determining when data indicate a particular approach is completely invalid, when it requires adjustment, and when it is considered acceptable for predictive toxicology.
  - Define a minimum data set for risk-assessment purposes.
- *In vitro* and *in vivo* models
  - Anchor *in vitro* assays to a pathological endpoint; cytotoxicity is not an endpoint.
  - Perform *in vitro* assays in batteries of cell types; co-cultures may improve *in vitro* testing.
  - Validate *in vitro* biological responses in animal models.
  - Develop new *in vitro* systems that recapitulate the biology, especially for complex systems such as the skin and kidney.
  - Support appropriate flexibility in the judicious use of live-model testing for all applications.
- Study vulnerable populations and multigenerational effects; perform developmental toxicity to look at varying risk groups.
- Mandate the reporting of primary data to peer-reviewed journals, particularly for negative results.
- Consider information gathering and disclosure policies that require manufacturers to disclose nanomaterial ingredients.

Analysis of Individual Bullets and Recommended New Bullets

Overarching research need H5:

Determine the mechanisms of interaction between nanomaterials and the body at the molecular, cellular, and tissular levels.

Note: original bullets are out of sequence to accommodate the new bullets and place assays/validation needs before biology.

H5d

Original bullet H5d: Validate *in vitro* biological responses in animal models.

Timeline

2007-2012	2012-2017	2017-2022
Medium	High	Medium

Rationale:

- *In vitro* test systems have to be validated before experimental observations can be applied to *in vivo* models.
- Animal models should include standard rodent models and models with larger phylogenetic ranges, such as zebra fish, birds, nonrodent mammals, and invertebrates.

Milestones:

- Identification of a core set of *in vitro* model systems that are translatable to *in vivo* responses
- Standardization of *in vivo* toxicity models that perform reliably with an increasing variety of test materials and that will form the foundation for a reference database

New bullet: Create consensus-based, reliable tools, techniques, and approaches for toxicity assessment.

Timeline

2007-2012	2012-2017	2017-2022
High	Medium	Low

Rationale:

- This aspect of the research is recognized as a critically important iterative process that should be initiated immediately.

Milestones:

- Demonstration of accurate *in vitro/in vivo* correlations across many different models
- Demonstration/validation of assay and model reliability across multiple labs
- Generation of reproducible toxicity quality assurance/quality control (QA/QC) protocols
- Capability to produce high-throughput methods

New bullet: Create consensus-based, reliable tools, techniques, and approaches to establish valid dose–response relationships.

Timeline

2007-2012	2012-2017	2017-2022
Low	High	Medium

Rationale:

- There has been some activity to develop tools and approaches to understand dose response, but it has been diffuse and needs more focus now.
- A large burst of activity is needed, followed by continuous, moderate ongoing activity.

Milestones:

- Establishment of lower, more sensitive limits of detection with good reliability and accuracy across several experimental systems
- Determination of reliable assays through interlaboratory comparisons
- Capability for high-throughput analysis
- Validation of *in vitro/in vivo* correlations

H5a and H5b

Original bullet H5a: Identify mechanism through which nanomaterials interact with fundamental, protective biological response pathways.

Original bullet H5b: Identify mechanism by which nanomaterials disrupt protective pathways and cause adverse health effects.

Revised bullet: Identify mechanisms through which nanomaterials interact with and/or perturb protective pathways, thus leading to adverse health effects.

Timeline

2007-2012	2012-2017	2017-2022
High		

Rationale:

- There is a critical need for reliable and reproducible methods that will support mechanistic research.
- The predictive value of mechanistic research requires a clear understanding of the dose–response relationship.

Milestones:

- Establishment of reliable and reproducible assays that are correlated with *in vivo* outcomes

New bullet: Determine mechanisms of toxicity.

Timeline

2007-2012	2012-2017	2017-2022
Low	High	Medium

Rationale:

- Reliable data sets do not currently exist at the level needed to ascertain mechanisms. This will take some time to develop and collect.

Milestones:

- Identification of reliable experimental models that compare well across systems and labs
- Understanding of fundamental mechanisms of toxicity at molecular, cellular, tissue, and organism levels
- Theoretical models that predict experimental outcomes
- Reliable dose–response forecasting
- Established structure/property/toxicity relationships
- Enabled *de novo* material design that maximizes benefit and minimizes risk

H5c

Original bullet H5c: Determine the relationship of dose, physical, and chemical properties to protective versus adverse responses.

Revised bullet: Determine the relationship of physico-chemical properties to exposure dose and to protective and adverse responses.

Timeline

2007-2012	2012-2017	2017-2022
High		Medium

Rationale:

- Understanding of the relationship of physico-chemical properties to biology is critical to defining exposure and target-organ dose and disposition.

Milestones:

- A well-defined matrix incorporating dose–response relationships and correlating the properties of a nanomaterial to its biological response
- Consistency in dosing, route of administration, matrix effects on nanomaterials, relevance, establishment of range, life cycle, vulnerable populations, and ecological effects

H5e

Original bullet H5e: Determine the relationship of biological response in animal models to human response.

Timeline

2007-2012	2012-2017	2017-2022
Low	Medium	High

Rationale:

- Extrapolation from *in vitro* responses to *in vivo* responses to human responses is critical to understanding nanomaterials, although it is currently not feasible.

Milestones:

- Establishment of an *in vitro* model that can be extrapolated to potential human response

INSTRUMENTATION, METROLOGY, AND ANALYTICAL METHODS

I1. Develop methods to detect nanomaterials in biological matrices, the environment, and the workplace

I1a Evaluate scope and suitability of technologies to quantify nanomaterials across biological media indicative of exposure.

I1b Develop common, commercially available sampler for measuring mass concentrations of nanomaterials in air (indoor and outdoor).

I1c Develop instruments to measure nanomaterials in water.

I1d Develop sampler for personal monitoring of nanomaterials and biomarkers indicative of exposure.

Group Responses to Charge Questions

Is research occurring on this research need?

With regard to the detection of nanomaterials in biological and environmental matrices, there is a sufficient amount of research being done to develop analytical methods and instrumentation. However, much of the current work is focused on proof of concept rather than directed toward the development of instrumentation that can help to address environmental, health, and safety concerns in the short term. Additionally, although progress has been made in this area over the past few years, most of the techniques seem to be focused on measuring nanomaterials in air rather than in other complex biological or environmental media. It is noted that technologies are still not sufficient to provide practical answers, particularly with respect to nanomaterials in complex media.

With regard to the detection of nanomaterials in the workplace, information obtained to date from the workplace is gained largely through the adaptation of existing technologies focused on simple detection. Because of limitations in these technologies, controlling exposure (e.g., containment/disposal) processes is a critical element in maintaining a safe workplace.

Some of the biggest obstacles to the development and implementation of appropriate instrumentation and methods to detect nanomaterials in biological matrices, the environment, and the workplace include:

1. Different techniques may be required for each different nanomaterial in a single medium; there is no "one size fits all" solution.
2. Different technologies and instrumentation may be suitable for only one type of medium (e.g., air).
3. Although many different characterization methods are available, more guidance is needed to help researchers determine how to select and implement the most appropriate techniques and measurement protocols for a given medium and material of interest.
4. For some media and materials, some existing methods are flawed, specifically with respect to dose characterization at the cellular level.
5. There are no standard techniques and protocols for routine interrogation. (ISO TC 229 may be the place to provide guidance in this realm.)
6. Existing methods focus on simple detection and ignore biological activity. Biological activity may be the most important parameter, but it may also not be elucidated thoroughly enough to allow the design of accurate and adequate tests.
7. There is a need for greater sensitivity of nanomaterial detection in highly diluted situations.
8. Methods may be required to distinguish between natural and engineered nanomaterials.

This described research is considered of low intensity, but urgent. The group identified a number of procedural barriers in this area that are critical to accelerating the development of meaningful research that will have

actionable outcomes for engineered nanomaterial risk assessment and management. In particular, strategies are required to make using specialized equipment more cost-effective and accessible across the research community. Participants recognized that, increasingly, the barriers to obtaining the fundamental details needed for meaningful scientific findings are not technology, but the cost and accessibility of specialized equipment.

Do the bullets under each research need adequately define and provide sufficient granularity for this research need? Is anything missing?

Each bullet will be addressed individually below; however, a few major issues arose in the discussion of the bullets in this research need. In general, it was recommended that the bullets need to consider:

1. The entire life cycle of an engineered nanomaterial. The engineered nanomaterial life cycle consists of the research and development stage, manufacturing stage, consumption or usage stage, and waste or recycle stage. Exposure to nanomaterials could happen in all stages, although it is possible that the engineered nanomaterial will be in different forms in these stages.
2. The need for the development of appropriate detection methods for nanomaterials in commerce (e.g., the same forms and chemistries as in commercial applications).
3. The need for development of appropriate detection methods for transformations that occur in the nanomaterial in product formulations, through product use, or during exposure. This requires the detection of the material in various matrices and media relevant to possible exposure.
4. The need for methods to relate exposures used in *in vitro* models with those used in *in vivo* tests, and those actually occurring in industry. As biomarkers for detection are developed, it is important to validate the biomarker as a true indicator of the biological effect of the nanomaterial.

Are there tools, methods, or other research topics that need to be developed to complete this research need?

The instrumentation requirements for this research need are high. To completely address this research need requires the development of a toolkit of techniques that are inexpensive, reliable, repeatable, robust, sensitive, and routine (i.e., not labor-intensive). These techniques must be applicable to a wide range of engineered nanomaterials in various matrices, media, and mixed media, and be applicable across the entire life cycle of the material (including transformations). The development of standard methods may help guide instrument developers to build the necessary equipment to meet these needs.

In the case of workplace exposure, there is a need for air samplers that are able to evaluate not only nanomaterial mass, but also shape, size, size distribution, environmental interactions, dispersion, etc.

Analysis of Individual Bullets and Recommended New Bullets

Overarching Research Need I1:

Develop methods to detect nanomaterials in biological matrices, the environment, and the workplace

I1a

Original bullet I1a: Evaluate scope and suitability of technologies to quantify nanomaterials across biological media indicative of exposure.

Revised bullet I1a

- Evaluate technologies to quantify nanomaterials across biological media indicative of exposure.
- Develop methods to quantify engineered nanomaterials across biological media indicative of exposure.
- Evaluate and apply characterization technologies that are appropriate to measure nanomaterials *in vitro*.
- Develop sensitive methods to detect a range of "as-manufactured" engineered nanomaterials and determine nanomaterial physico-chemical properties in appropriate media.

Timeline

2007-2012	2012-2017	2017-2022
High		Medium

Rationale:

- One cannot control what cannot be measured, so this is considered a critical goal and a high priority.
- Lack of these techniques and standards would slow economic and commercial development.
- Although there is progress in this area, the lack of standards techniques and protocols and minimum data requirements has greatly limited the effective communication of findings and the feasibility of comparing and reproducing study findings and of interpreting results accurately. Delayed progress in this area would severely limit the utility of new research findings and hamper efforts to reach consensus on implications of new findings.
- R&D and manufacturing are the initial stages in the life cycle of engineered nanomaterials. This need is a basic requirement for understanding the nanomaterial in these early stages.
- This is extremely important from a workplace exposure standpoint, especially during the manufacturing stage of the engineered nanomaterial.

Milestones:

- A standardized toolkit consisting of various techniques that enable complete characterization of most engineered nanomaterials
- A standardized toolkit consisting of various techniques that enable complete characterization of most engineered nanomaterials in biological media
- Guidance to support the selection of appropriate measurement/assessment techniques for a given medium and material
- Development of indicative exposure routes/levels
- Development of exposure limits

I1b and I1c

Original bullet I1b : Develop common, commercially available samplers for measuring mass concentrations of nanomaterials in air (indoor and outdoor).

Original bullet I1c: Develop instruments to measure nanomaterials in water.

Revised bullets I1b and I1c:

- Develop methods to quantify engineered nanomaterials in the environment (air, soil, wastewater, etc.).
- Develop common, commercially available samplers, instrumentation, and techniques for measuring mass concentrations of nanomaterials in air (occupational and environmental).

  (*Note*: The group recommending this change questioned the validity of this bullet point with regards to the work and research being performed. Currently, there are more traditional industrial hygiene tools for measuring mass, and these tools are likely to be applicable to nanomaterials. Additionally, it is not clear whether the designation of mass is the appropriate measurement with the current level of research available. Regarding the designation of indoor versus outdoor, the group suggested that "occupation versus environmental" would be more appropriate.)
- Improve accessibility to and cost of instrumentation for *in vitro* characterization and quantification.

Timeline

2007-2012	2012-2017	2017-2022
High		Medium

Rationale:

- The development of samplers, instrumentation, and techniques will be critical in the short term for performing an accurate analysis of the nanomaterial in question.
- This is important from an environmental exposure standpoint, especially during the usage and waste/recycle stage of the engineered nanomaterial.
- Accurate measurement of nanomaterials is a high priority for risk assessment and exposure control.
- Lack of these techniques and standards would slow economic and commercial development.

Milestones:

- Development of a standardized toolkit consisting of various techniques that enable complete characterization of most engineered nanomaterials in the environment
- Development of methodologies to correlate measurements to exposure/toxicity
- Laboratories with affordable and timely access to appropriate instrumentation

I1d

Original bullet I1d : Develop tools for personal monitoring of nanomaterial exposure and biomarkers indicative of that exposure.

Revised bullet I1d

- Develop samplers for personal monitoring of nanomaterial and biomarkers indicative of exposure. (*Note*: This group believed that this bullet item could be removed, as it is addressed in the above revised bullets.)
- Use *in vitro* methods to develop biomarkers indicative of exposure.
- Develop samplers for personal and air monitoring of engineered nanomaterials in workplace environments.

Timeline

2007-2012	2012-2017	2017-2022
High	Medium	

Rationale:

- This is extremely important from a workplace exposure standpoint, especially during the manufacturing stage of the engineered nanomaterial.
- The priority given to this research need will depend on the commercial status of the different classes of nanomaterials, with the highest priority given to types of nanomaterials in use or near commercialization and those applications that have the greatest risk of leading to human and/or environmental exposure

Milestones:

- Sensitive samplers to detect engineered nanomaterials in workplace environments, followed by personal samplers
- Biomarkers and other validated methods to accurately characterize exposure in *in vitro* models relevant to real-world exposures are available for all nanomaterials that are in commercial use or proposed for commercial development

New bullet 1: Develop standard units for measurement appropriate for nanomaterials.

Timeline

2007-2012	2012-2017	2017-2022
High		Medium

Rationale:

- Instrumentation that is useful for measuring nanomaterials in water (e.g., light scattering, ultraviolet-visible spectroscopy) already exists. However, standard methodologies for the collection of nanomaterials and correlation of measurements to dose/toxicity are needed to make sure that these techniques or newly developed techniques are relevant.

Milestones:

- Standard methods for collection of nanomaterials
- Methods to correlate nanomaterial measurements to dose/toxicity

New bullet 2: Develop methods to differentiate between natural nanomaterials and engineered nanomaterials.

Timeline

2007-2012	2012-2017	2017-2022
Low	Medium	High

Rationale:

- Better understanding is needed of all nanomaterials to help risk assessment and risk mitigation.

Milestones:

- Methods to distinguish between natural and engineered nanomaterials

New bullet 3: Development of instrumentation (all-in-one device/universal capabilities) for commercial methods.

Timeline

2007-2012	2012-2017	2017-2022
Low	High	Medium

Rationale:

- The correlation of measurements to dose/exposure/response and toxicity is needed immediately to provide context for the accurate characterization of nanomaterials.
- Common and commercially available instrumentation is not needed until after such correlations are developed, so its timeframe is delayed.

Milestones:

- None provided

New bullet 4: Develop methodologies to correlate measurements to exposure/toxicity.

Timeline

2007-2012	2012-2017	2017-2022
High		Medium

Rationale:

- None provided

Milestones:

- None provided

I2. Understand how chemical and physical modifications affect the properties of nanomaterials

I2a Evaluate solubility in hydrophobic and hydrophilic media as a function of modifications to further modeling of biological uptake.

I2b Understand the effects of surface function on mobility and transformation in water.

Group Responses to Charge Questions

Is research occurring on this research need?

Some research is ongoing in all areas related to understanding how physico-chemical modifications affect the properties of nanomaterials; however, our knowledge and our ability to determine these effects and their impact are still evolving. Participants generally recognized this research need as fundamentally critical to accurately predicting nanomaterial toxicity; therefore, this work should be a high priority.

Do the bullets under each research need adequately define and provide sufficient granularity for this research need? Is anything missing?

The current bullets do not adequately define the research need. Each bullet will be addressed individually below and additional bullets are recommended; however, a few major issues arose in the discussion of the bullets in this research need.

In general, it was recommended that the bullets should consider the following:

1. The wording of the bullets seems too simplistic for engineered nanomaterials. For example, solubility and dispersibility are easily confused terms, and dispersibility may be the more appropriate term. Surface function does not capture all relevant information about engineered nanomaterials as surface chemistry and surface structure should be used to clearly define engineered nanomaterial properties.
2. It is important to determine how the environment and media affect the engineered nanomaterial physico-chemical properties.
3. It is necessary to characterize the engineered nanomaterial after purposeful chemical and physical modifications to determine if the modifications produced the desired results.
4. There is a need to understand how the engineered nanomaterial's crystal planes control its reactivity.
5. It is also necessary to understand how engineered nanomaterial physico-chemical properties change during the life cycle of the material and how to analyze engineered nanomaterials in complex environments and/or matrices.

Additionally, the research in this area should be more appropriately focused toward commercial and exposure-relevant substances and findings that can be directly used to assess risk. In this regard, research to emphasize the understanding of how chemical and physical modifications specific to well-characterized engineered and/or

environmentally produced nanomaterials affect their reactions with human tissue and impact cell toxicity. Emphasis was placed on identifying key properties most relevant to biological endpoints.

Are there tools, methods, or other research topics that need to be developed to complete this research need?

The instrumentation requirements for this research need are high. New tools and validated methods are needed for dynamic analysis, surface area measurement, surface mapping measurement, property and property distribution measurement, and measurement of changes of materials *in vivo*. Additionally, new modeling tools are needed to support molecular modeling and modeling of biological uptake. New approaches, tools, systems and models are also needed to more effectively triage nanomaterials according to anticipated toxicity for relevant dosimetry.

Analysis of Overarching Research Need

Overarching research need I2:

Understand how chemical and physical modifications affect the properties of nanomaterials.

Revised overarching research need I2:

- Develop methodologies for measuring physico-chemical characteristics (parameters) that include composition, surface chemistry, surface area, charge, size, shape, solubility/dispersibility, and agglomeration/aggregation.
  (*Note*: This list should be consistent with the ISO TC-229 physico-chemical characterization list with the intention of understanding how these parameters might affect toxicological outcomes [e.g., structure–activity relationships]).
  - Determine if purposeful surface modifications result in the desired properties.
  - Understand how deliberate chemical and physical modifications of engineered nanomaterials and their adventitious modified forms affect their reactions with molecules, cells, and tissues, with a focus on desired health assessment outcomes.

Timeline

2007-2012	2012-2017	2017-2022
Progress	High	Medium

Rationale:

- Equipment/technology is being developed for analyzing nanomaterial properties, but it is not widely available due to cost constraints.
- Progress has been made in recent years on understanding the influence of surface characteristics during manufacturing processes.
- There is a strong need for new *in situ* techniques, which will be a long-term challenge.

Milestones:

- Development of facilities for advanced nanomaterial characterization
- Development of equipment that is affordable for individual researchers
- Development of new instruments, methods, and advanced capabilities to allow *in situ* characterization
- Development of new standardized techniques for surface characterization
- Development of database(s) and data-sharing tools

Analysis of Individual Bullets and Recommended New Bullets

I2a

Original bullet I2a: Evaluate solubility in hydrophobic and hydrophilic media as a function of modifications to further modeling of biological uptake.

Revised bullet I2a:

- Evaluate solubility, dispersibility, and aggregation/agglomeration in relevant media as a function of modifications of physico-chemical characteristics to model the bioavailability of the nanomaterial.
- Evaluate engineered nanomaterial physico-chemical properties in hydrophobic and hydrophilic media as a function of modifications to refine models of biological uptake.

Timeline

2007-2012	2012-2017	2017-2022
High		Medium

Rationale:

- Current research is addressing these properties in relevant media, but increased effort is required to understand these properties *in vivo*. The result for *in vivo* systems will likely be very different than for *in vitro* and model cell-free systems.
- Biological matrices are complex.
- There will always be new nanomaterials and new formulations.
- Complete biological studies take a long time.

Milestones:

- Development of improved studies for characterization of these properties in model systems
- Development of new methods for characterization of these properties *in vivo*
- First-principles survey of common nanomaterials assessed against biological media
- Partition coefficients determining solubility in hydrophobic versus hydrophilic environments
- Database development and data-sharing for mining of composite data

I2b

Original bullet I2b: Understand the effect of surface function on mobility and transformation in water.

Revised bullet I2b:

- Evaluate how surface chemistry and structure affect overall nanomaterial properties, and evaluate the effects of dispersant media on the surface properties of engineered nanomaterials.
- Understand the effect of the parameter of surface function of nanomaterials on mobility and transformations in water and physiologically relevant media (air, blood, etc.).

Timeline

2007-2012	2012-2017	2017-2022
High		Medium

Rationale:

- The number of nanotechnology-enabled products on the market is increasing. Some waste may end up in surface as well as groundwater; therefore, this is also a long-term life cycle issue.
- Experimental designs to understand life cycles are complex and, therefore, associated with longer timelines.
- There is minimal research currently on nanomaterials in groundwater, wastewater, and air pollution because of limited resources; however this is a priority research area.

Milestones:

- Creation of a database of surface properties and effects on mobility
- Development of methods to study these properties in water and physiological media
- Development of correlating results to predict these interactions *in vivo*

New bullet 1: Evaluate and characterize the surface coverage of nanomaterials for proteins (surfactants and other molecules) and the affinity and dynamics of these protein coronas in physiologically relevant media as they relate to surface characteristics and modifications.

Timeline

2007-2012	2012-2017	2017-2022
Low	High	Medium

Rationale:

- This has been recognized as important, as the surface properties dictate interactions *in vivo* (e.g., fate and clearance). Few research projects are currently being performed on surface characteristics; more are needed.
- Given the analytical complexity of this research, the development of sufficient data to understand these biological phenomena will require a concerted effort by researchers.

Milestones:

- Development of available techniques for static observation of protein adsorption
- Development of new techniques for dynamic studies
- Perform studies of protein adsorption first in model systems, then *in vitro*, then *in vivo*

New bullet 2: Create consensus-based, reliable tools, techniques, and approaches for toxicity assessment.

Timeline

2007-2012	2012-2017	2017-2022
High	Medium	Low

Rationale:

- This aspect of the research is recognized as an iterative process, but one that is critically important to address immediately.

Milestones:

- Demonstration of accurate *in vitro/in vivo* correlations across many different models
- Demonstration/validation of reliability across various labs
- Development of the capability to produce high-throughput methods
- Generation of reproducible toxicity quality assessment/quality control protocols

New bullet 3: Determine mechanisms of toxicity.

Timeline

2007-2012	2012-2017	2017-2022
Low	High	Medium

Rationale:

- Valid data sets do not currently exist at the level needed to ascertain mechanisms; collection will take time.

Milestones:

- Fundamental understanding of toxicity at the molecular, cellular, tissue, and organ system levels
- Theoretical models that predict experiments
- Experimental models that compare well across systems and labs
- Enabled *de novo* materials design
- Reliable dose–response forecasting
- Established structure/property/toxicity relationships

New bullet 4: Create consensus-based, reliable tools, techniques, and approaches to establish valid dose–response relationships.

Timeline

2007-2012	2012-2017	2017-2022
Low	High	Medium

Rationale:

- Although there has been some activity, it is not focused enough. A high burst of activity is needed to get started, followed by continuous, moderate activity.

Milestones:

- Low thresholds of detection and reliable accuracy across many experimental systems
- Capability for high-throughput analysis
- Validated *in vitro/in vivo* correlations
- Reliable interlaboratory comparisons

I3. Develop methods for standardizing assessment of particle size, size distribution, shape, structure, and surface area

I3a. Develop automated microscopic methods for the rapid analysis of screening of nanomaterials.

I3b. Evaluate correlation of microscopic methods with other size measurement techniques.

I3c. Evaluate or modify microscopic and mass spectrometric approaches for determination of shape and structure of nanomaterials.

I3e. Explore methods beyond isothermal adsorption for nanomaterial surface area determinations.

Group Responses to Charge Questions

Is research occurring on this research need?

This is a valid research need with work in progress on the development of standardized methods by ISO and ASTM International. It is important to assess the current status of this work and then perform a gap analysis to

see where standardized methods are needed. For the methods being developed, it is necessary to keep in mind the importance of the media in which the engineered nanomaterial exists (soil, air, water, etc.).

Do the bullets under each research need adequately define and provide sufficient granularity for this research need? Is anything missing?

Each bullet will be addressed individually below; however, a few issues arose in the discussion of the bullets in this research need:

1. There are good methods for larger particles using automated microscopic methods, but for nanomaterials, the methods are expensive and tedious.
2. ASTM International and ISO are developing scanning electron microscopy (SEM) and scanning tunneling microscopy (STM) methods, but they are not automated.
3. There is a need to correlate microscopic methods with other techniques, not only for size, but also for the shape of the engineered nanomaterial. There is recent interest in using computational methods to evaluate size and shape.
4. Other techniques beyond microscopic and mass spectrometric approaches are needed for determination of shape and structure of engineered nanomaterials.
5. It is necessary to include surface area, structure, size, effects of aggregation and agglomeration, particle size distribution curves, effect of sonication, etc.

In general, there is a limited effort to standardize techniques, and there is inadequate communication of these efforts across the field. NIST is developing a website with links to organizations developing standards and techniques. Attention should also be given to sample preparation associated with a particular method, as it can greatly influence the measurements.

Are there tools, methods, or other research topics that need to be developed to complete this research need?

In general, there is still a need for more responsive, affordable, and validated instrumentation and methods to address this research area.

Analysis of Individual Bullets and Recommended New Bullets

Overarching Research Need I3:

Develop methods for standardizing assessment of particle size, size distribution, shape, structure, and surface area.

Original bullet I3a:

Develop automated microscopy-based methods for the rapid analysis and screening of nanomaterials.

Timeline

2007-2012	2012-2017	2017-2022
High		Medium

Rationale:

- Technological barriers are high, and limited historical data exist.

Milestones:

- Commercially available automated scanning electron microscopy (SEM) and transmission electron microscopy (TEM) software to detect presence of nanomaterials in a sample (i.e., for screening applications)
- Affordable analysis

I3b

Original bullet I3b: Evaluate correlation of microscopic methods with other size measurement techniques.

Revised bullet I3b: Evaluate correlation of microscopic methods with other size, shape, and surface area measurement techniques.

Timeline

2007-2012	2012-2017	2017-2022
High		Medium

Rationale:

- Standard reference material has been developed that enables progress.

Milestones:

- Identification of which standard materials are to be used
- Complete laboratory analysis with identified standard materials
- Field study validation of laboratory results
- Measurements performed to determine comparability of results between microscopy methods and other measurement techniques

I3c

Original bullet I3c: Evaluate or modify microscopic and mass spectrometric approaches for determination of shape and structure of nanomaterials.

Revised bullet I3c: Modify, as needed, approaches for determination of shape, structure, size, surface area, and agglomeration state of engineered nanomaterials based on previous testing efforts.

Timeline

2007-2012	2012-2017	2017-2022
Medium	High	Medium

Rationale:

- This research need is dependent on work that is currently in progress but not yet completed.

Milestones:

- Determination of modification requirements from field evaluations
- Development of more responsive and affordable methods and equipment

13d

Original bullet I3d: Explore methods beyond isothermal adsorption for nanomaterial surface area determinations

Timeline

2007-2012	2012-2017	2017-2022
Medium		Low

Rationale:

- The BET (Brunauer, Emmett, Teller) method for measuring specific surface area via gas adsorption is available, but other methods need to be defined and developed as necessary.

Milestones:

- Evaluation of the current status of existing technologies
- Identification of potential alternatives

New bullet 1: Standardize/validate methods of engineered nanomaterial sampling and characterization in various media (e.g., water, air, soil, biological media) and consider sample preparation, laboratory equipment, general area, personnel, and real-time/direct reading and indirect reading approaches.

Timeline

2007-2012	2012-2017	2017-2022
High		Low

Rationale:

- Results are often conflicting and standardization increases reproducibility. Progress is underway in identifying standardized materials, which facilitates the initiation of this effort.

Milestones:

- Identification of existing standardized methods
- Performance of gap analysis

I4. Develop certified reference materials for chemical and physical characterization of nanomaterials

I4a. Develop materials to support exposure assessment approaches, fundamental research, and instrumentation.

I4b. Develop materials to support applied toxicology and hazard identification.

Group Responses to Charge Questions

Is research occurring on this research need?

There is research occurring to develop certified reference materials at NIST and these efforts are integrated into the research community's efforts to support assessment approaches, fundamental research, and instrumentation.

Do the bullets under each research need adequately define and provide sufficient granularity for this research need? Is anything missing?

Each bullet will be addressed individually below; however, a few major issues arose in the discussion of the bullets in this research need.

1. There is a strong need for a database on reference nanomaterials. The National Institute of Biomedical Imaging and Bioengineering (NIBIB) along with the National Institute of Environmental Health Sciences (NIEHS) and the National Cancer Institute (NCI) will start a project to build a nanomaterials registry that will include reference materials (about 1.5 years to get online). All are encouraged to go to the site and provide information.
2. It is difficult to list physico-chemical properties for a reference material since the size, charge, agglomeration state, etc., will change depending on the media that it is in. It may then be necessary to develop reference materials in appropriate matrices or media as well as in a simpler form.
3. Dropping the word "certified" from "reference material" would facilitate a quicker release of the material for researchers to use. "Well-characterized" materials are what are necessary to serve the community.

Are there tools, methods, or other research topics that need to be developed to complete this research need?

Tools are needed to characterize nanomaterials in pure form and in various matrices. These tools are also required in other research needs.

Analysis of Individual Bullets and Recommended New Bullets

Overarching Research Need I4:

Develop certified reference materials for chemical and physical characterization of nanomaterials.

Revised Overarching Research Need I4:

Develop reference materials for chemical and physical characterization of nanomaterials.

I4a

Develop materials to support exposure assessment approaches, fundamental research, and instrumentation.

No timeline, rationale, or milestone was provided.

I4b

Original bullet I4b: Develop materials to support applied toxicology and hazard identification.

Revised bullet I4b: Develop materials to support applied toxicology and hazard identification; develop positive and negative nanomaterial controls for toxicity testing.

No timeline, rationale, or milestone was provided.

New bullet 1: Evaluate current databases on nanomaterials and, if there is a need, develop a database that includes physico-chemical properties, a list of literature that includes the use of a nanomaterial, and nanomaterial purchasing options.

No timeline, rationale, or milestone was provided.

New bullet 2: Evaluate what types of materials are needed to support the testing community: materials may be certified reference materials, reference materials, well-characterized materials, positive and negative control materials, materials in matrices, etc.

No timeline, rationale, or milestone was provided.

I5. Develop methods to characterize a nanomaterial's spatiochemical composition, purity, and heterogeneity

I5a. Evaluation of scope and suitability of techniques to assess purity and batch-to-batch production of nanomaterials

I5b. Development of 3D chemical characterization at one-nanometer resolution.

Group Responses to Charge Questions

Is research occurring on this research need?

There is great deal of research going on to address this need, but the major issue is repeatability. There are many concerns about creating uniform, pure compounds repeatedly.

Do the bullets under each research need adequately define and provide sufficient granularity for this research need? Is anything missing?

Each bullet will be addressed individually below; however, a few major issues arose in the discussion of the bullets in this research need:

1. Methods for nanoparticles do not extrapolate to systems.
2. How do we establish quality control around sample preparation and sampling techniques?
3. A high level of coordination and collaboration among stakeholders is needed.
4. Simple and cost-effective methods for the characterization of engineered nanomaterials are needed.

Are there tools, methods, or other research topics that need to be developed to complete this research need?

As with many of the other research needs, simple and cost-effective methods and tools are required for the characterization of engineered nanomaterials. Additionally, different tools maybe needed at each point in the life cycle of the engineered nanomaterial.

Analysis of Overarching Research Need

Original Overarching Research Need I5:

Develop methods to characterize a nanomaterial's spatiochemical composition, purity, and heterogeneity.

Revised Overarching Need I5:

Develop techniques to measure the "significant" and "impactful" properties of nanomaterials through their life cycles, including sample preparation protocols.

Timeline

2007-2012	2012-2017	2017-2022
High	Medium	

Rationale:

- Research progress is just beginning for this need, but it is essential to start work immediately. Progress will depend on the identification of critical properties. Although the process may never end, the intensity of activity should be able to decrease.

Milestones:

- Set of critical characteristics identified and prioritized
- Development of methods (detailed and simple) to measure the specific characteristics
- Development of simplified robust instruments for similar measurements
- Development of appropriate sample preparation protocols

Analysis of Individual Bullets and Recommended New Bullets

Original bullet I5a: Evaluate scope and suitability of techniques to assess purity and batch-to-batch production of nanomaterials

No timeline, rationale, or milestone was provided.

I5b

Original bullet I5b: Development of 3D chemical characterization at one-nanometer resolution.

Revised bullet I5b: Develop methods for 3D chemical characterization at one-nanometer resolution appropriate to the specific nanomaterials.

Timeline

2007-2012	2012-2017	2017-2022
Progress	High	Medium

Rationale:

- Excellent progress has been made in some areas, including microscopy. New methods with increased resolution of a wider range of properties are needed. Gentle methods for probing unstable nanomaterials are essential. Additional methods for obtaining these measurements in situ are important.

Milestones:

- Advancement and application of *in situ* electron microscopy
- Development of optical methods with improved resolution in realistic environments
- Development of new types of sensors to measure surface functionality in "real" conditions

New bullet 1: Further develop coordination and collaboration among stakeholders to collect information.

Timeline

2007-2012	2012-2017	2017-2022
High		

Rationale:

- There are apparent limitations to sharing information about important materials, critical properties, and their applications. There are no robust communication tools for sharing information on critical instrument needs, for discussing the value of specific methods, and for highlighting important progress. Improving these techniques needs to be an ongoing effort.

Milestones:

- Establishment of new communication sites and tools, list servers, etc.
- Development of protocols for measurement and sample preparation, reference materials for testing methods, and latest reliable data identifying important materials properties
- Development of better mechanisms to share methods and expertise that are useful for studying nanomaterials

New bullet 2: Develop appropriate, rapid, low cost, and robust methods to characterize engineered nanomaterials for industrial and field applications.

Timeline

2007-2012	2012-2017	2017-2022
Medium	High	Low

Rationale:

- There needs to be activity now, but the essential parameters are not yet known. This research need is high priority, but significant progress can best occur when critical properties are established and some level of agreement is reached.

Milestones:

- Identification of essential properties, and development of robust test methods for specific properties and materials

Appendix E. Public Comment

Carol Stroebel

Children's Environmental Health Network

Thank you for the opportunity to comment.

The Children's Environmental Health Network is a national organization whose mission is to promote a healthy environment and to protect the fetus and the child from environmental health hazards. The world in which today's children live has changed tremendously from that of previous generations, including a phenomenal increase in the substances to which children are exposed. Every day, children are exposed to a mix of chemicals, most of them untested for their effects on developing systems. The Centers for Disease Control and Prevention's National Human Exposure Report has amply demonstrated that such chemicals often are ubiquitous, appearing in the vast majority of blood and urine samples taken at random from the general population in the U.S. Many of these are readily passed across the placenta to the fetus or to the infant via breast milk.

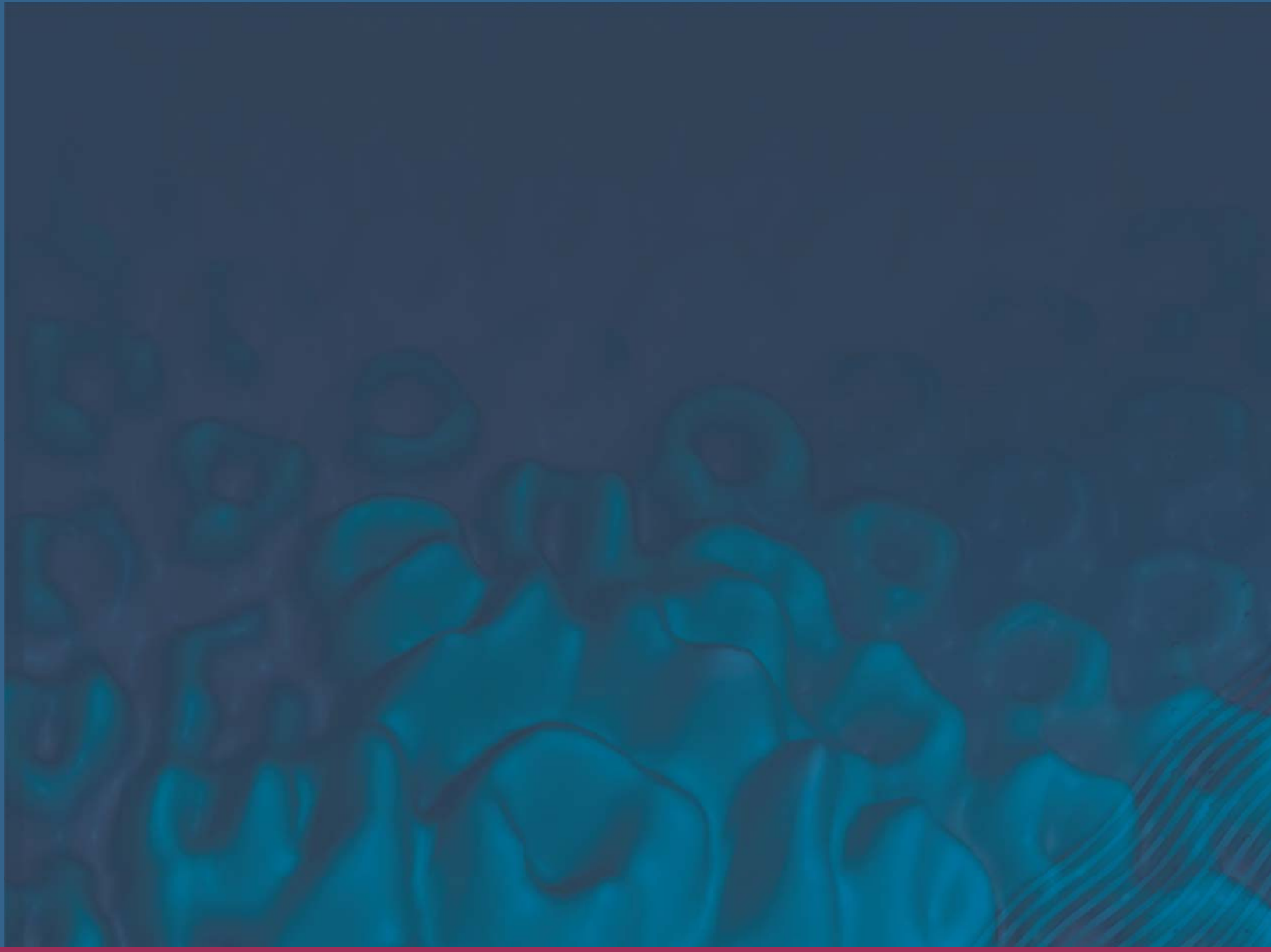
And, as indicated at the workshop, today's children are now being exposed to nanomaterials, about which we know even less.

In general, children can be more susceptible and more vulnerable than adults to toxic chemicals for a variety of reasons, such as the following:

- Children are growing. Pound for pound, children eat more food, drink more water and breathe more air than adults. Thus, they are likely to be more exposed to substances in their environment than are adults.
- Children have higher metabolic rates than adults and are different from adults in how their bodies absorb, detoxify and excrete toxicants.
- Children's systems, including their nervous, reproductive, digestive, respiratory, and immune systems, are developing. This process of development creates periods of vulnerability. Exposure to toxicants at such times may result in irreversible damage when the same exposure to a mature system may result in little or no damage.
- Children behave differently than adults, leading to a different pattern of exposures to the world around them. For example, they exhibit hand-to-mouth behavior, ingesting whatever substances may be on their hands, toys, household items, and floors. Children play and live in a different space than do adults. For example, very young children spend hours close to the ground where there may be more exposure to toxicants in dust, soil, and carpets as well as low-lying vapors such as radon, mercury vapor or pesticides.
- Children have a longer life expectancy than adults; thus, they have more time to develop diseases with long latency periods that may be triggered by early environmental exposures, such as cancer or Parkinson's disease.

Appendix F. List of Acronyms

AAAS	American Association for the Advancement of Science
ADME	absorption, distribution, metabolism, and excretion
CDC	Centers for Disease Control and Prevention
CPSC	Consumer Product Safety Commission
DOC	Department of Commerce
DOD	Department of Defense
DOE	Department of Energy
EHS	environmental, health, and safety
EPA	Environmental Protection Agency
FDA	Food and Drug Administration (HHS)
HHS	Department of Health and Human Services
ISO	International Organization for Standardization (and associated standards)
NCI	National Cancer Institute (NIH)
NEHI	Nanotechnology Environmental and Health Implications Working Group of the NSET Subcommittee
NGO	nongovernmental organization
NIEHS	National Institute of Environmental Health Sciences (NIH)
NIH	National Institutes of Health (HHS)
NIOSH	National Institute for Occupational Safety and Health (CDC)
NIST	National Institute of Standards and Technology
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
NSF	National Science Foundation
OECD	Organisation for Economic Co-operation and Development
ORNL	Oak Ridge National Laboratory (DOE)
PNNL	Pacific Northwest National Laboratory (DOE)
R&D	research and development
SBIR	Small Business Innovation Research program (across several U.S. Government agencies)
USDA	U.S. Department of Agriculture
WTEC	World Technology Evaluation Center



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

National Nanotechnology
Coordination Office

4201 Wilson Blvd.
Stafford II, Rm. **405**
Arlington, VA **22230**

703-292-8626 phone
703-292-9312 fax

www.nano.gov