Highlights of Recent Research on the Environmental, Health, and Safety Implications of Engineered Nanomaterials

Overview

Nanotechnology involves harnessing the unique properties of materials at the nanoscale to enable innovation. Nanotechnology has an established role in fields as diverse as electronics, energy, environmental remediation, and medicine. Addressing potential nanotechnology-related environmental, health, and safety (nanoEHS) issues is essential to the safe and responsible development of nanomaterials and nanotechnology-enabled products—a key goal of the U.S. Government's National Nanotechnology Initiative (NNI) [*1*]. While considerable progress has been made in characterizing the potential risk posed by engineered nanomaterials (ENMs), research and development of products and devices containing nanomaterials (nanotechnology-enabled products, or NEPs) continues at a rapid and accelerating pace. The evolving applications of nanotechnology require continuously refining and advancing ways to detect, measure, and assess ENM behavior in settings that reflect realistic workplace, consumer, and environmental exposures in order to develop effective management strategies. Furthermore, by ensuring that a robust scientific framework is available for evaluating nanomaterial applications, nanoEHS research promotes productivity in advanced materials and manufacturing.

Well-coordinated nanoEHS research is thus essential to establishing the public confidence and regulatory certainty needed for the commercial success of NEPs. The NNI's nanoEHS activities are coordinated through the Nanotechnology Environmental and Health Implications (NEHI) Working Group of the Nanoscale Science, Engineering, and Technology (NSET) Subcommittee of the National Science and Technology Council, and by the NNI Coordinator for EHS Research. Including additional nanoEHS-related activities associated with the NNI Nanotechnology Signature Initiatives (www.nano.gov/signatureinitiatives), the total NNI nanoEHS investment for fiscal year (FY) 2016 is estimated at approximately $150 million, accounting for about 10% of the overall NNI investment [*2*].

NNI agencies continue to be guided by the 2011 NNI EHS Research Strategy [*3*]. The strategy aims to ensure responsible development of nanotechnology and identifies the following six core research categories:

- Nanomaterial Measurement Infrastructure
- Human Exposure Assessment
- Human Health; Environment
- Risk Assessment and Risk Management Methods and
- Informatics and Modeling.

NNI agencies participating in NEHI have individually and collectively undertaken a range of activities to address the six research areas. These research categories support the goal of creating a comprehensive knowledge base for evaluating the potential risks of nanotechnology, and ultimately for enabling effective and broader risk management options where necessary. The following select examples represent important milestones and new knowledge gained from recent nanoEHS research, and updates the 2014 Progress Review on the 2011 EHS Research Strategy [*4*]. This information is merely a small sample of NNI-supported research in the peer-reviewed literature and in publicly available agency documents.

Nanomaterial Measurement Infrastructure: Facilitating Reliable Assessments of Exposure and Hazard

The NNI's 2014 review of progress in implementing the 2011 NNI EHS Research Strategy emphasized that a well-developed measurement infrastructure (instrumentation, protocols, standards, reference libraries, and models) is critical to delivering accurate, precise, and reproducible measurements of the quantity, behavior, and fate of ENMs and NEPs [*4*].

NNI agencies have led research on new techniques and protocols aimed at overcoming limitations in detecting and measuring exposure to NEPs. Researchers at the National Institute of Standards and Technology (NIST) have developed instrumentation and methods capable of detecting ENMs in complex media (e.g., composites and conjugates) and in consumer products. NIST published the first protocol for single-particle inductively coupled plasma mass spectrometry (sp-ICP-MS), a promising technique for simultaneously estimating particle size and mass [*5*]. This technique has enabled researchers to detect ENM concentrations that are four orders of magnitude lower than previous limits [*6*]. NIST has also prepared and validated new nanocytotoxicity assays [*7*, p.16].

Nanoparticle size considerations are especially important in determining the potential for demonstrating undesirable effects, and thus accurate measurements of a particle's volume are essential [*8*] . NIST researchers are combining scanning electron microscopy and atomic force microscopy techniques to produce a more accurately measured 3D shape (Figure 1), leading to more precise volumetric estimates.

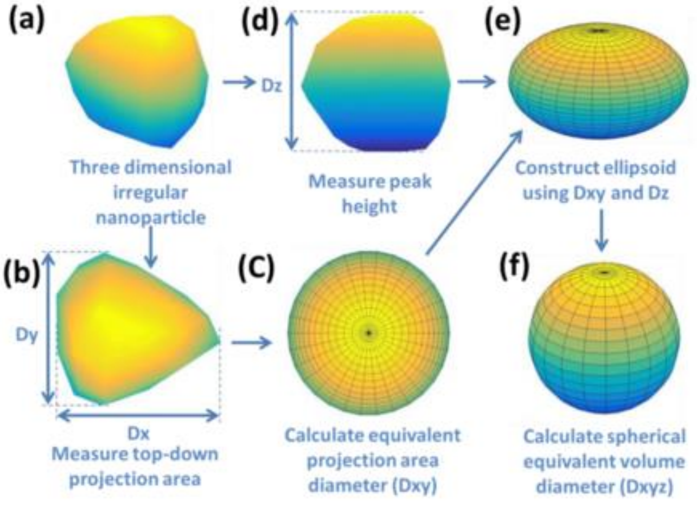

Figure 1. The combination method for determining nanoparticle volume involves measuring both the width and length using top-down SEM imaging to get a diameter reading (a, b, c), measuring the height using AFM (d, e), and then combining the readings to calculate the volume (f).
Image credit: NIST [8].

Polymer-nanoparticle composites are an area of active research due to the widespread applicability of these materials. For example, quantum dots are often encapsulated in polymers to serve as biologically relevant probes, but there have been few experimental studies that directly quantify and characterize nano-polymer adsorption mechanisms. Continued advances in using nanoparticle complexes in disease diagnostics and tumor imaging rest on accurate characterization of their form and behavior. The National Institutes of Health (NIH) and the National Science Foundation (NSF) have supported research employing x-ray photoelectron spectroscopy and sum frequency generation together as the basis for characterization of any generic nano-polymer complex [*9*]. This methodology holds the potential to also benefit the wider nanoparticle surface engineering and biomedical research fields.

The Engineer Research and Development Center (ERDC) of the U.S. Army Corps of Engineers has invested considerable effort in investigating the behavior of NEPs in natural systems (e.g., simulated fresh and marine waters). ERDC has prepared and published several scientific operating procedures (SOPs), including procedures for dispersion and dissolution testing of nanosilver (nanoAg) in a laboratory environment, a general method for abrading materials, and a method for quantifying nanomaterial release from ENM-containing products [*10*–*12*].

A number of analytical procedures have been developed to enhance the ability to detect nanomaterials. New protocols consider physical characteristics, distribution in environmental compartments (e.g., water, soil, etc.) across the particle life cycle, and unique nanoscale properties to provide a nanoparticle fingerprint. For example, research supported by the Environmental Protection Agency (EPA) used surface redox reactivity as a key surrogate for detecting the presence of nanoparticles (NPs) in complex matrices, including environmental waters, serum, urine, and dissolved organic matter at concentrations as low as parts per billion (ppb) or ng/mL [*13*].

Human Exposure Assessment and Human Health: From the Workplace to Personal Care Products

Over the past few years, there has been a significant shift in the research on potential human and environmental exposures from nanotechnology-based consumer products. The research focus has moved beyond the methods and tools for fundamental laboratory studies on pristine, as-manufactured, ENMs towards those needed to evaluate exposure potential under conditions that more closely represent real-world scenarios, as documented in a 2015 NNI and Consumer Protection Safety Commission (CPSC) workshop report [*14*]. For example, NNI agencies are actively supporting the assessment of particle release from nanotechnology-enabled manufacturing, formulation, and processing activities. To address the introduction and use of ENMs in the construction industry, the National Institute of Occupational Health and Safety (NIOSH) has demonstrated the effectiveness of ventilation in reducing exposure to nanotechnology-enabled construction materials, using mechanical abrasion to simulate release from aging and worn products [*15*]. NIH and NSF have supported extramural research on the thermal decomposition of polymer matrices containing nanomaterials [*16*]. That research noted minimal but detectable release of nanoparticles, particularly when inorganic nanofillers were involved, and identified how thermoplastic polymer layers influence the size and morphology of released aerosols.

The comparison between bulk materials and their nanoscale counterparts, and the generation of non-engineered, unintentional nanoparticles from composite materials, have important consequences for exposure assays [*17*]. In several cases, including the release of copper from treated timber, or of silver from toys, food/milk storage containers, and cleaning products, nanomaterial-specific considerations were not deemed necessary for a risk evaluation because exposure was related primarily to ionic forms [*18*, *19*]. The U.S. Army Corps of Engineers investigated the release of nanoAg from commercial inkjet printers and found that exposure was ionic in nature and therefor nanomaterial-specific scrutiny was likely not required [*20*].

Identifying and measuring worker exposures during the manufacture or formulation of ENMs is an important area of focus for NIOSH. In 2016, NIOSH updated its Nanomaterial Exposure Assessment Technique Tool (NEAT 2.0) [*21*]. NEAT identifies tasks that can result in the emission of nanoparticles into the surrounding air, facilitating a comprehensive assessment of exposures during processes and job tasks. Task-based filter samples are then used to confirm the presence of these materials (Figure 2). NEAT 2.0 adds a stronger emphasis on time-integrated, filter-based sampling.

Figure 2. Nanomaterial workplace assessment. Here a NIOSH field team member sets up integrated filter-based samples that are used to confirm the presence of nanoparticles. Image credit: NIOSH (www.cdc/niosh-science-blog/category/nanotechnology).

NIOSH has published a summary of exposure assessments conducted at 14 carbonaceous nanomaterial manufacturer or user sites [*22*]. These visits are part of the more than 100 site investigations conducted since 2006 evaluating exposures to a wide variety of nanomaterials. Over the years, there has been a notable shift to larger-volume uses of nanomaterials and growing usage in advanced manufacturing processes. Accordingly, NIOSH has funded studies investigating airborne particle exposure from additive manufacturing (AM), including polymer feedstocks and metal AM. NIOSH funded some of the early research that focused on characterizing the emissions from polymer-based 3D printers [*23*]. NIOSH also supported the development of a microwave-assisted acid-digestion process designed to improve the recovery of TiO_2 for quantitative analysis, facilitating a more accurate assessment of occupational exposure to airborne TiO_2 [*24*].

Linking exposure to human health outcomes is an important objective in EHS assessment. NNI agencies' intramural and extramural research support is laying the foundation for occupational exposure assessment in epidemiological studies [*25*] and establishing biomarkers for materials such as cerium dioxide (a fuel additive) and multiwall carbon nanotubes [*26–28*]. Research focused on industry-relevant ENMs (e.g., laser printer-emitted engineered nanoparticles) and their effect on the epigenome (those chemical elements attached to the genome that are not part of the DNA sequence) has been funded by NIOSH and NSF. *In vivo* studies using mouse models have validated *in vitro* assays, facilitating the move away from animal testing [*29*, *30*]. NIOSH scientists published a critical review on the cancer risk to workers exposed to airborne carbon nanotubes (CNTs) or carbon nanofibers (CNFs) during the production and use of these materials [*31*]. The review updated and expanded the data behind a 2014 International Agency for Research on Cancer deliberation, and generally affirms the conclusions of the original evaluation that there was inadequate or limited evidence of carcinogenicity for most types of CNTs and CNFs at this time. The study pointed to evidence gaps requiring further research; specifically, investigation of possible associations between *in vitro* and early-stage *in vivo* events that may be predictive of lung cancer or mesothelioma, and systematic analysis of dose–response relationships across materials.

Research has also been addressing knowledge gaps about NEP interaction with immune cells involved in mammalian allergic responses. Aldossari et al. demonstrated that the physicochemical properties of NEPs influence the potential for induction and/or promotion of an allergic immune response [*32*]. These findings, and results from related research, indicate that properties such as intrinsic defects (e.g., the proportion of Zn vacancies in ZnO NPs) may be exploited in the design of benign ENMs without compromising their intended applications [*33*, *34*]. This research shows that ENMs can be designed to be safer without sacrificing performance.

Extramural research supported by EPA systematically evaluated four high-volume ENMs (titanium dioxide, silicon dioxide, nanoAg, and multiwall carbon nanotubes) as used in industrial and consumer product lines. Sunscreen products containing TiO_2 nanomaterials are a source of potential human exposure to nanoscale TiO_2, and the potential toxicity in dermal and retinal tissue is an area of active investigation. Intramural research conducted by EPA found that, relative to uncoated TiO_2, the aluminum hydroxide coating on the TiO_2 conferred a measure of protection, despite environmental transformation and degradation of the coating in water [*35*]. Additional life cycle assessment of consumer product exposure includes the work of Hicks et al., who developed a life cycle assessment for nanoAg-enabled textiles [*36*]. Their review concluded that the environmental impacts associated with nanoAg-enabled clothing do not require a shift in consumer behavior (e.g., fewer launderings) to balance the environmental impacts associated with incorporating the nanotechnology.

A number of agencies have worked to advance our understanding of exposure and potential human health effects of nanoparticles and NEPs in food and food contact applications. For example, in an evaluation of the migration of silver nanoparticles from commercially available polymeric food contact materials, silver nanomaterials were not detected in food simulants, and the study concluded that current U.S. Food and Drug Administration (FDA) guidance on food packaging remained applicable for these applications [*18*]. Experiments have also been performed to unravel the relationship between the physicochemical properties and the transport, absorption, biodistribution, and toxicity of foodborne TiO_2 NPs during digestion. The U.S. Department of Agriculture's National Institute of Food and Agriculture supported research to review the toxic responses to nanoscale metal and metal oxides in food products. *In vitro* cell cultures exposed to nanoscale SiO_2, TiO_2, and ZnO showed internalization of the NPs, but more research is needed to determine route(s) of distribution and potential sites of accumulation [*37*]. Furthermore, repeated exposure of cells to these metal oxides did not alter their growth patterns or render them any more susceptible to toxicity. McCracken et al. reviewed the literature on this subject, focusing on the potential effects of dosage, assay methods, and physicochemical properties on toxicological outcomes [*38*]. EPA's National Health and Environmental Effects Research Laboratory provided funding for genomic analyses that examined the links between *in vitro* treatments and *in vivo* adverse outcomes following hepatic cell exposure to TiO_2 [*39*].

Environment: NEP Transformation, Availability, and Impacts

Investigating the behavior, fate, and bioavailability of ENMs in the environment is challenging, but is important to understanding the mechanisms by which nanomaterials enter, remain in, degrade, and are transported through environmental media [*3*]. ENMs used in industrial processes and consumer products enter into the environment primarily through wastewater streams [*40*, *41*]. The passage, retention, and transformation of ENMs through water treatment facilities has implications for the form and concentration of ENMs that arrive in estuarine and marine ecosystems. The University of California's Center for Environmental Implications of Nanotechnology investigated the effect of nanoscale copper (nanoCu) on septic systems [*42*]. That study discovered that separate exposures to three types of Cu particles caused distinct (but reversible) disruptions in septic tank function, while Clar et al. found that Cu ions from Cu NPs drive microbial inhibition in waste water treatment plants [*41*].

Research to examine the ecological impact, environmental transformation, and biological fate and availability of several ENMs (fresh and aged CeO_2, ZnO, TiO_2, and Ag) in agroecosystems was funded by the EPA. ENM transport was found to be highly limited in natural soils collected from farmland and grasslands, with the majority of particles retained in the upper 3 cm of the soil profile [*43*]. A critical review of the literature on uptake, translocation, and accumulation of nanomaterials in plants identified many examples of uptake, clogging, or translocation by nanoparticles in the size range of 5 nm to 20 nm [*44*]. Plants with low transpiration, drought-tolerance, tough cell wall architecture, and tall growth displayed a lesser tendency to translocate ENMs. In the absence of toxicity, accumulation was often linearly proportional to exposure concentration. NSF and EPA funded research that recently confirmed the potential for release of nanoCu from commercial antifouling paint in water [*45*]. The study found that the amount of copper released was strongly dependent on salinity, paint surface, and drying time. Because the amount of Cu released over 180 days increased with paint drying time, consumer behavior could lessen the quantity of copper in leachates by placing boats, especially wooden ones, back in the water directly after the manufacturer's specified curing time.

Support from NNI agencies has enabled researchers at the Bodega Marine Lab to use high-throughput screening methodology to investigate the impact of chemicals and other stressors on a number of marine invertebrate taxa, including sea urchins in productive California marine ecosystems (Figure 3). Research on the uptake pathways and biokinetics in the earthworm (*Lumbricus rubellus*) demonstrated that at realistic exposure concentration, nanoscale and dissolved forms of ZnO had no distinguishable differences in uptake [*46*].

Figure 3. Purple sea urchins. Image credit: National Oceanographic and Atmospheric Administration (NOAA) (www.photolib.noaa.gov/).

Risk Assessment and Risk Management Methods

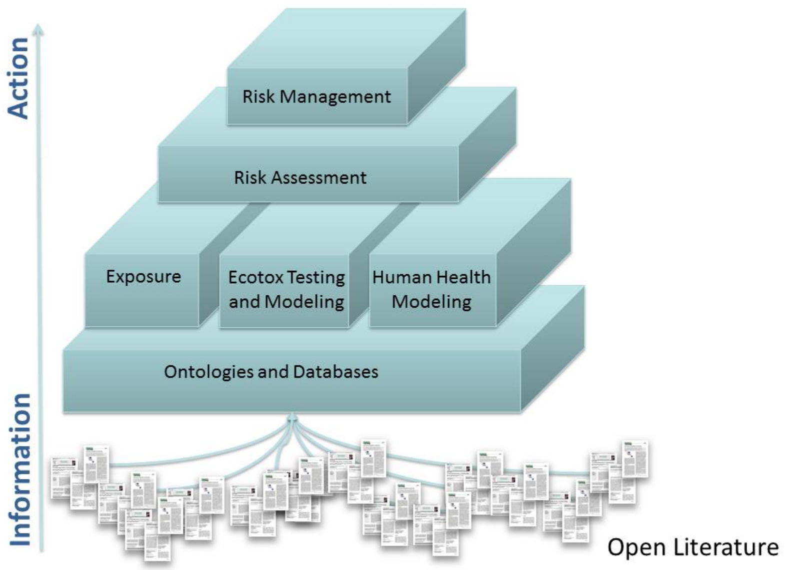

Figure 4. Graphic depicting the integration of risk assessment and risk management in safety evaluation. Image credit: M. Hoover (NIOSH) and C. Hendren (Duke University).

Risk assessment involves the application of analytical tools, data, and expert knowledge to the evaluation of potential exposure to humans and the environment, and the hazards that such exposure might cause. Scientific knowledge of nano implications may be derived from multiple sources, including life cycle assessment and risk analyses. Each tools has unique but complementary methodological approaches that provide unique outputs on nanomaterial risk [*47*, *48*].

Risk management methods for nanotechnology identify and implement strategies to address potential hazards [*3*]. Risk assessment and risk management methods thus integrate information from the previously discussed areas of measurement, exposure assessment, human health, and the environment [*4*], illustrated graphically in Figure 4.

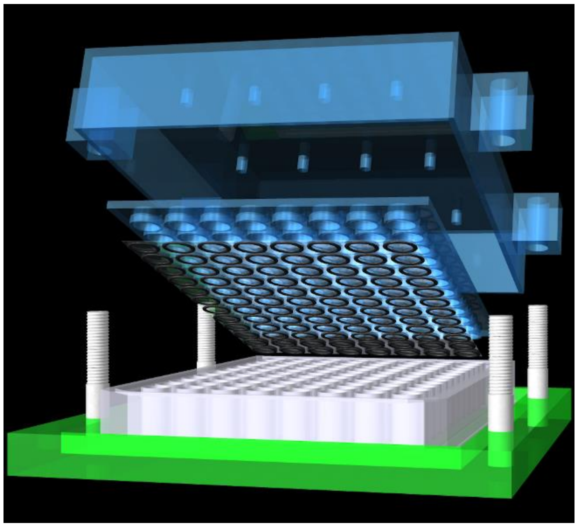

Figure 5. Graphic showing inverted cell culture assembly developed to test buoyant nanoparticles [51]. Image credit: Center for Nanotechnology and Nanotoxicology at the Harvard School of Public Health.

To this end, NNI researchers have participated in critical reviews that have identified information gaps and refined research priorities. For example, researchers funded by NIST and NSF reviewed research trends in nanoEHS risk assessment [*49*, *50*]. NNI agencies (NSF and NIH) have funded research that created new techniques to improve estimation of *in vitro* hazard screening, accounting for under-evaluated features of certain nanoparticles such as buoyancy. Accounting for buoyancy uncovered dose-dependent effects of polypropylene on human macrophages [*51*] (Figure 5). Several NNI agencies (NIH's National Institute of Environmental Health Sciences, NSF, EPA) have funded work that advanced hazard characterization of single-wall CNTs (SWCNTs), including investigations of the effects of electronic properties and chirality on SWCNT toxicity in mammalian systems. Chirality is related to the geometry of the molecule, and the reactivity, behavior, and potential adverse effects of a chemical may be influenced by the chirality of the molecular components.

However, Wang et al. found that various chiral forms of SWCNTs showed no significant differences in their hazard potential to rodent lungs [*52*]. They argue that their findings suggest there is no need to use chirality of SWCNTs as an independent category for regulatory purposes.

Building on an extensive body of work in environmental risk assessment, investigators are now able to construct ENM-specific species sensitivity distributions (SSDs). An SSD is a cumulative probability distribution of a chemical's toxicity effects. The SSD uses toxicity testing of species to extrapolate to a community level of risk associated with a specific toxicant. Based on SSDs for 10 ENMs, investigators supported by NSF and EPA concluded that size, formulation, and the presence of a coating can alter toxicity, and thereby corresponding SSDs [*53*]. That analysis also found few statistical differences were observed between SSDs of an ENM and its ionic counterpart (Figure 6).

NNI agencies have supported the continued development of alternative testing strategies (ATS) for nanomaterials. ATS utilizes mechanism-based *in vitro* assays and *in silico* predictive tools for expedited screening of the hazard potential of chemical substances [*54*]. Twenty-first century risk assessment is moving toward methods based on an understanding of cellular response pathways that, when triggered by a chemical substance, could initiate key biological events that lead to adverse outcomes at the individual or population level. Investigators have developed case studies demonstrating this approach based on dermal and inhalation exposures [*55*].

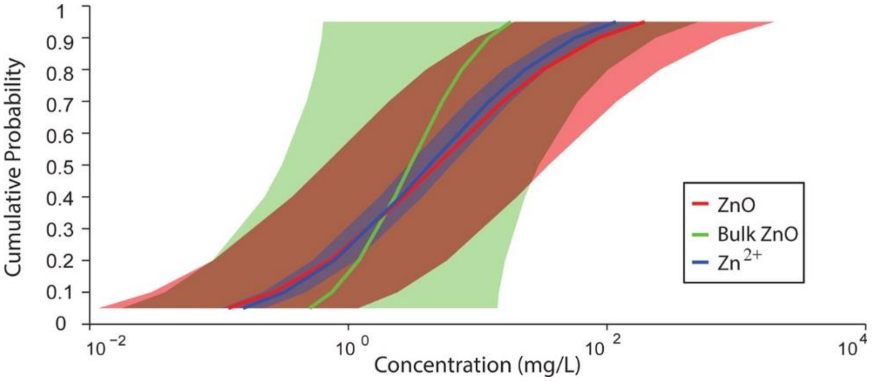

Figure 6. A comparison of the species sensitivity distributions (SSDs) of bulk, ionic, and nanoscale Zinc. The shaded region in the color corresponding to each curve shows the 95% confidence interval. The cumulative probability distribution of values from toxicity assays of the nanoscale forms overlaps with the distribution of values of the bulk and ionic versions. Image credit: Garner et al. [53].

A number of platforms to enhance nanoEHS risk assessment, including tier-based risk assessment and value of information (VoI) frameworks, have been developed by ERDC researchers. VoI provides guidance for practitioners and/or risk assessors to understand the characterization, release potential, hazard, and environmental fate/transport of nanomaterial-enabled technologies [*47*, *56*]. CPSC and ERDC have collaborated on using this tool to evaluate military-relevant and consumer product case studies. ERDC's achievements in the risk assessment area allow stakeholders to concentrate on collecting the most relevant data, thus accelerating technology deployment while addressing potential risk [*56*, *57*]. ERDC's work and collaborations with other agencies such as CPSC and NIOSH will thus contribute to the framework for prioritizing chemical risk, a requirement under the revisions to the Toxic Substances Control Act [*58*].

Informatics and Modeling

An interagency nanoinformatics (creation, storage, and access) infrastructure, and a strategy for expanding this infrastructure, is central to meeting the NNI's nanoEHS goals [*4*]. The NNI's Nanotechnology Knowledge Infrastructure (NKI) Nanotechnology Signature Initiative supports development of this informatics infrastructure [*59*], and NNI agencies continue to support the maintenance of federally-funded databases and the development of advanced modeling and simulation tools. The Nanomaterial Data Curation Initiative (NDCI), a project of NIH's National Cancer Informatics Program Nanotechnology Working Group, is exploring critical aspects of data curation within the development of informatics approaches to understanding nanomaterial behavior [*60*]. This work reflects the growing interest in developing data repositories and tools for integrating and interrogating complex nanomaterial datasets. Data curation is important in addressing uncertainty, reproducibility, and interoperability, and represents an important path to addressing key challenges in the responsible development of nanomaterials and other emerging technologies.

Models and *in silico* approaches have been central to characterizing and predicting NEP hazard profiles and in prioritizing risk evaluations. Biokinetic models have continued to evolve, incorporating nanotechnology-specific, non-equilibrium dynamics in agglomeration, sedimentation, and dissolution processes [*61*]. Classic quantitative structure activity relationship (QSAR) models for nanomaterials (nanoQSARs) are efficient across many nanomaterial categories (organic, inorganic, metals, etc.).The Department of Energy has funded research comparing classical 2D QSARs for nanomaterials with 3D nanoQSARs. The study concluded that 3D nanoQSARs were applicable for organic nanomaterials [*62*]. NIH-supported research has demonstrated that computational models can be employed to guide the design of surface-modified nanomaterials with the desired biological and safety profiles, advancing the development of nanoQSARS. EPA and international agencies have supported studies using computational approaches to validate nanoQSARs that led to the building and validation of QSARs as a toxicity screening mechanism for a suite of 83 surface-modified CNTs [*63*].

Communicating Evaluations and Incorporating EHS Knowledge into Best Practices

In order for the knowledge gained through EHS research to be applied, research results and the current state of the science need to be shared with the broader community. NNI agencies have held workshops and prepared and disseminated a number of reports and critical reviews of various aspects of nanoEHS research. NIOSH prepared and submitted for public comment a Current Intelligence Bulletin (*Health Effects of Occupational Exposure to Silver Nanomaterials*) and published *Building a Safety Program to Protect the Nanotechnology Workforce: A Guide for Small to Medium-Sized Enterprises* [*64*, *65*]. ERDC disseminated select SOPs for the preparation, characterization, release fate, and hazard of nanomaterials at www.labtube.tv/channel/ERDC-Nano-EHS.

The NEHI Working Group launched its webinar series (see www.nano.gov/nanoEHSwebinars) in May 2016 with a NIOSH-sponsored webinar entitled Applying a Lab Safety Culture to Nanotechnology: Educating the Next Generation of Nano Scientists. In the fall of 2016, NEHI and the NSET Subcommittee's Nanotechnology Innovation and Commercialization Ecosystem (NICE) Working Group co-sponsored a webinar that examined insurance issues for the nanotechnology industry (see http://www.nano.gov/node/1685). The webinar series explores the state of the science in nanoEHS topics such as alternative testing strategies and nanoEHS evaluation, and how nanomaterial properties affect interactions within the human body.

Promoting international standardization enhances the quality of scientific evaluations and facilitates the global harmonization of regulatory science. FDA's National Center for Toxicological Research serves as the Executive Secretariat for the Global Coalition for Regulatory Science Research (GCRSR). The GCRSR has organized the Global Summit on Regulatory Science, an annual forum to objectively assess the regulatory implications of emerging technologies such as nanotechnology. FDA co-hosted the 2015 and the 2016 Global Summits, which focused on regulatory bioinformatics and nanotechnology, respectively.

Nurturing the nanoEHS Community

The coordinated activity of NNI agencies leverages resources and scientific knowledge in promoting the responsible development of nanotechnology. Interagency collaboration and networking is key to advancing knowledge of the fate and behavior of nanomaterials in the environment and their effects on human health, to developing predictive and computational approaches, and to the development of protocols and standards.

The NNI's support for nanoEHS research bolsters the enterprise in the wider nanotechnology and EHS communities. NNI agencies use the interagency working group forum (NEHI) as a cooperative framework to collaborate and share information, and to provide leadership in establishing the national nanotechnology EHS research agenda. NEHI members and the broader U.S. nanoEHS community have played a critical role in fostering and supporting the international Communities of Research (CORs) in nanoEHS (http://us-eu.org/communities-of-research/) to develop a more thorough understanding of the potential EHS implications of nanotechnology. These community-driven efforts have resulted in several collaborations, publications in scientific journals, webinars, and technical workshops on key topics. The examples presented here demonstrate that nanoEHS research continues to evolve, and that the growing sophistication of EHS science is progressively enabling more comprehensive safety evaluations of new and complex nanotechnology-enabled materials and products.

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