CHAPTER 4

NANOTECHNOLOGY ENVIRONMENTAL, HEALTH, AND SAFETY ISSUES

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4.1 VISION FOR THE NEXT DECADE

The environmental, health, and safety (EHS) of nanomaterials has been defined as "the collection of fields associated with the terms 'environmental health, human health, animal health, and safety' when used in the context of risk assessment and risk management" (NSET 2006, p. 2). In this chapter, the term "nano-EHS" is used for convenience to refer specifically to environmental, health, and safety research and related activities as they apply to nanoscale science, technology, and engineering. This chapter outlines the major advances in nano EHS over the last 10 years and the major challenges, developments, and achievements that we can expect over the next 10 years without providing comprehensive coverage or a review of all the important issues in this field.

Changes in the Vision over the Last Ten Years

Although exposure to engineered nanomaterials (ENMs) in the workplace, laboratory, home, and the environment is likely more widespread than previously perceived, no specific human disease or verifiable environmental mishap has been ascribed to these materials to date. Perceptions of ENM hazard have evolved from "small is dangerous" to a more realistic understanding that ENM safety should best be considered in terms of the specific-use contexts, applications, exposures, and the specific properties of each nanomaterial.

Because organic, inorganic, and hybrid materials can be produced in various sizes, shapes, surface areas, surface functionalities, and compositions, and because of their widely tunable compositions and structures that can be dynamically modified under different biological and environmental use conditions, most ENMs cannot be described as a uniform molecular, chemical, or materials species. One major conceptual advance in nano-EHS assessment has been the recognition that these dynamic material properties play a determination role in ENM conditioning, dissemination, exposure, and hazard generation at the nano-bio interface[21] (Colvin 2003; Maynard et al. 2006; Nel et al. 2006; Oberdörster et al. 2005; Seaton et al. 2010). Thus, it has become clear that since a large number of novel materials and material properties are continuously being introduced, it is imperative to develop a robust scientific platform to understand the relationship of these properties to EHS outcomes

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[21] The nano-bio interface is defined here as the dynamic physicochemical interactions, kinetics, and thermodynamic exchanges between nanomaterial surfaces and the surfaces of biological components such as proteins, membranes, phospholipids, endocytic vesicles, organelles, DNA, and biological fluids.

(Maynard et al. 2006; NIEHS 2004; National Research Council 2007; Walker and Bucher 2009; Walker and Bucher 2009). Because this knowledge generation will require time and consensus building, rational decision-making in nano-EHS is likely to be incremental. However, this process could be accelerated by implementation of high-throughput and rapid ENM screening platforms (Abraham et al. 2004; Abraham et al. 2008; George et al. 2010; Service 2008), as well as exploiting computational methods to assist in risk modeling and hazard assessment.

We have come to recognize that, because of the diverse and unique properties of engineered nanomaterials, safe implementation of nanotechnology is a multidisciplinary exercise that goes beyond traditional hazard, exposure, and risk assessment models. In addition to properties research, the nano-EHS community requires information about the commercial uses of ENMs, their fate and transport, bioaccumulation, and lifecycle analysis, all of which demand careful coordination and incremental and adaptive decision making to guide safe implementation of nanotechnology. The need for data and information collection is now understood to be essential for researchers, producers, consumers, and regulators of ENM products to allow the formulation of adequate regulatory policy for engineered nanomaterials.

The National Science Foundation has established a research program solicitation with a focus on nanoscale processes in the environment beginning with August 2000. The Environmental Protection Agency has a research program solicitation on nanotechnology EHS since 2003, and the National Institute for Environmental Health Sciences in 2004.

Vision for the Next Ten Years

Due to the rapid pace at which nanotechnology is expanding into society via its many applications, as well as to the likelihood that significant human, animal, and ecosystem exposures are already occurring (Benn et al. 2010; Walker and Bucher, 2009; Warheit et al. 2008), it is necessary to develop an integrated, validated scientific platform for assessment of hazards, exposures, and risks at a scale commensurate with the growth of this technology. Instead of performing the nano-EHS exercise one material at a time, rapid-throughput and high-content screening platforms will emerge to survey large batches of nano-phased materials in parallel (Abraham et al. 2004; Abraham et al. 2008; George et al. 2010; Service 2008).

Thus, the vision for the next 10 years includes the discovery and development of ENM property–activity relationships, high-volume data sets, and computational methods used to establish knowledge domains, risk modeling, and nano-informatics capabilities to reliably assist decision-making. This information needs to be integrated into predictive science (Meng et al. 2009; National Research Council 2007; National Toxicology Program 2004; Walker and Bucher 2009) and risk management platforms that relate specific materials and ENM properties to hazard, fate and transport, exposure, and disease outcomes. Ensuring safe implementation of nanotechnology over the next decade also requires the development of new, sensitive analytical methodologies, tools, and accepted protocols for screening, detection, characterization, and monitoring of ENM exposure in the workplace, laboratory, home, and the environment (Hutchinson 2008; Nel et al. 2009). We also need to develop effective monitoring, containment, and nanomaterial removal methods for waste disposal systems. New data and knowledge gathering will lead to the design of safer materials and green manufacturing that could transform nanotechnology into a cornerstone of sustainability (Hutchinson 2008). Safe implementation of nanotechnology requires close cooperation between academia, industry, government, and the public, all of whom have a

stake in seeing this technology succeed for the benefit of society, the economy, and the environment.

4.2 ADVANCES IN THE LAST TEN YEARS AND CURRENT STATUS

Ten years ago nanotechnology was recognized for its enormous potential to produce revolutionary advances in electronics, low-cost solar cells, next-generation energy storage, and smart anti-cancer therapeutics, among other fields of application. The first collective efforts in nano-EHS awareness commenced early after the National Nanotechnology Initiative (NNI) was established in 2000, including organization of several workshops that addressed the environment, nanobiotechnology, and societal implications (Roco 2005); nevertheless, it required considerable time to comprehend and integrate all the scientific disciplines that are necessary to understand the possible impact of this disruptive new technology on humans and the environment. Some of the early steps in the awareness/integration process were the following:

- In 2003, the National Toxicology Program considered first tests on nanoparticles, nanotubes, and quantum dots, and Environmental Protection Agency (EPA) has the first program announcement on nano-EHS.
- In December 2004, the Nanoscale Science, Engineering, and Technology (NSET) Subcommittee of the Committee on Technology of the National Science and Technology Council published the NNI Strategic Plan for the Federal fiscal years (FY) 2006–2010 in which environmental science and technology were well represented.
- Several coordinated academic centers emerged early in the decade that began to focus on nano-EHS, such as the Center for Biological and Environmental Nanotechnology (CBEN) at Rice University and the University of California Nanotoxicology Research Training Program.

The pace of research and implementation of nano-EHS regulatory policy began to speed up by 2008, at which point the number of peer-reviewed publications addressing nano-EHS risk assessment increased rapidly, amounting to >250 papers in 2009 as compared to ~50 in 2004. Concerns about ENM safety also led to a steady increase in the number of regulatory interventions by Federal agencies, as well as an increase in the U.S. Federal budget for nano-EHS research from $67.9 million in FY 2008 to a requested amount of $116.9 million in FY 2011. (Budget considerations will be covered in Section 4.3.)

From an EHS standpoint, researchers have made some progress in developing toxicological screening for the most abundant ENMs in their primary form, and new data have emerged on the importance of several material properties that may pose a hazard at the nanoscale level (Lundqvist et al. 2008; Nel et al. 2009; Oberdörster et al. 2005; Walker and Bucher 2009). This has elicited new concerns about possible hazard, fate and transport, exposure, and bioaccumulation. The significant challenge now is the standardization, harmonization, and implementation of nano-EHS monitoring and screening, data collection, streamlined risk reduction procedures, and a coordinated governance strategy to ensure safe implementation of this technology. The imminent introduction of active nanosystems and nano-engineered devices, including integrated assemblies of multiple different nanomaterials that perform more complex functions than those of individual materials, will necessitate the development of additional nano-EHS procedures for composite materials.

Data Gathering, Monitoring, and Governance of Carbon Nanotubes

Carbon nanotubes (CNTs) are one example of an important industrial class of ENMs for which considerable nano-EHS data collection is now available (Lam et al. 2004; Liu 2007; Mercer et

al. 2008; Poland et al. 2008; Porter et al. 2010; Shvedova et al. 2003; Shvedova et al. 2005). CNT inhalation exposure in the workplace is a potential concern, as a result of the widespread use of CNTs in manufacturing, their high volume of production, and ready aerosolization by activities such as packaging, dispensing, vortexing, acting, grinding, and vessel transfer. Extensive current CNT production and distribution capabilities, together with expanding product and user bases, have led to a significant increase in the number of studies and guidance procedures. Several acute toxicity studies with rodents that have been completed since 2003 support some likelihood that certain types of single- and multiwalled CNTs pose hazards to the lung or mesothelial surfaces under experimental exposure conditions (Lam et al. 2004; Liu 2007; Mercer et al. 2008; Poland et al. 2008; Porter et al. 2010; Shvedova et al. 2005). One scenario is the potential for CNTs to induce granulomatous airway inflammation or interstitial fibrosis in the alveolar region of the lung, depending on the dispersal state of the carbon nanotubes. Another possible hazard emerging from these studies is granulomatous inflammation in the mesothelial lining after peritoneal instillation in mice. This could be a precursor to mesothelioma, as demonstrated in disease outcome in p53 knockout mice exposed to CNTs (Takagi et al. 2008).

While there is no clinical evidence to date that CNT exposure is responsible for pulmonary fibrosis or mesothelioma in humans, the U.S. National Institute of Occupational Safety and Health (NIOSH) has concluded after its review of multiple rodent studies that the collective evidence points to the possibility that human CNT exposures in the workplace may indeed pose a hazard to the human lung (Figure 4.1). NIOSH set up a new generation of airborne particle counters to monitor and quantify airborne CNTs in the workplace (further reviewed in Section 4.8.5). Not only did the occupational surveys demonstrate significant airborne levels in response to specific workplace procedures, but that monitoring could also establish limits of detection (LOD). Utilizing animal lung burden assessments and extrapolation of those data to humans using alveolar epithelial surface area ratios, NIOSH has established an exposure limit and demonstrated that control measures such as ventilation, respirators, and HEPA filters can effectively decrease workplace exposure to below the LOD. NIOSH has also published guidelines for worker safety and recommends that companies working with ENMs implement a safe risk management program as outlined in Section 4.8.5. The NIOSH risk management scheme for CNTs is outlined in Figure 4.1.

It is important to emphasize that the generic NIOSH guidelines for CNTs do not imply that all CNT formulations are harmful. There is a burgeoning literature demonstrating in animal studies that CNTs can be functionalized and used safely as imaging and drug delivery agents (Kam et al. 2005; Kostarelos 2008; Porter 2007). Thus, it is important to distinguish the properties of CNTs in their as-prepared states (e.g., carbon allotrope with substantial surface-adsorbed contaminants and associated synthetic by-products) from their purified and functionalized forms, which appear to be more benign.

In step with scientific developments and occupational guidelines, CNTs have also come under increased scrutiny from the EPA. In October 2008, the EPA issued a formal notice of the agency's interpretation of the inventory status of CNT under the Toxic Substances Control Act (TSCA), and announced a plan to enforce that interpretation, beginning in March 2009. EPA's position is that CNTs are not equivalent to graphite or carbon allotropes for TSCA purposes, and therefore it is illegal for companies to import or manufacture CNTs in any amount for non-exempt commercial purposes until after a TSCA pre-manufacture notice (PMN) for the CNT has been submitted to EPA and the 90-day review period has expired (U.S. EPA 2008).

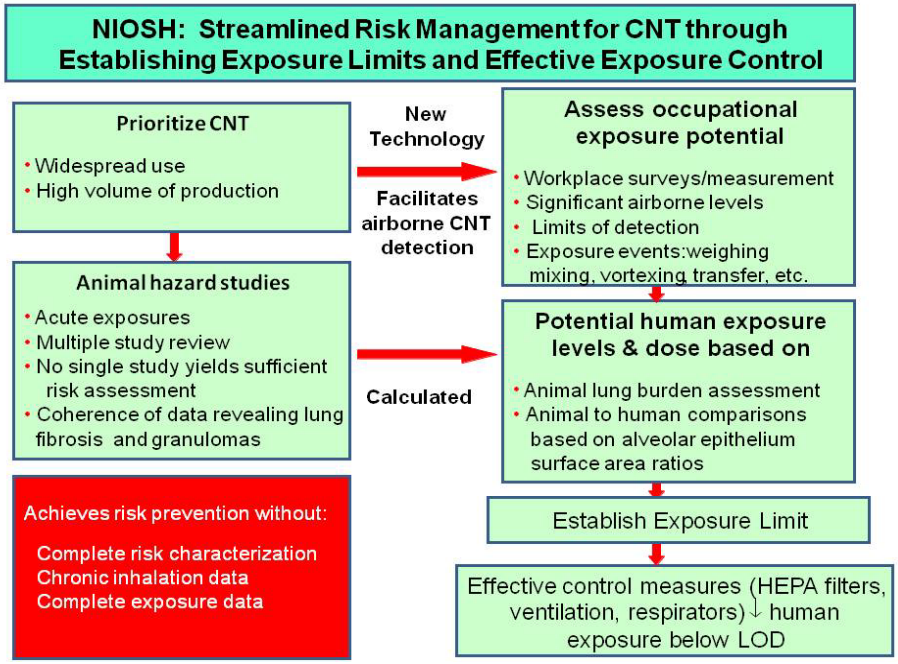

Figure 4.1. NIOSH scheme for streamlined risk management for CNTs (courtesy of A. Nel).

Data Gathering, Monitoring, and Governance of TiO_2, ZnO, and Silica Nanoparticles

The CNT example is just one of a number of ENM decision-making approaches emerging from data gathering. Titanium dioxide (TiO_2), zinc oxide (ZnO), and silica nanoparticles also represent mature, relatively well-characterized materials in terms of available information and readiness of regulators to address risk and hazard concerns (Lux Research 2007; Warheit et al. 2006; Xia, Kovoshich, Liong, Maädler et al. 2008; Zhang et al. 2002). For instance, TiO_2 has been used as a pigment for decades and has been studied in its nano-particulate form since the 1980s. Not only is there an extensive literature, but NIOSH has established effective risk management strategies for TiO_2 practices in the workplace. These guidelines have been made available through portals like NIOSH's report and website, *Approaches to Safe Nanotechnology* (NIOSH 2009),and DuPont and Environmental Defense Fund's *NANORisk Framework* report and website (2007; see also Section 4.8.4). Moreover, extensive research into the use of TiO_2 and ZnO in sunscreens and cosmetics has demonstrated that the actual consumer risks are low, even prompting the nongovernmental organization the Environmental Working Group, previously critical of nanoparticle use in sunscreens, to make a statement that, "many months and nearly 400 peer-reviewed studies later, we find ourselves drawing a different conclusion and recommending some sunscreens that may contain nano-sized ingredients" (EWG 2009). While there remain a number of unanswered questions about the end-of-life risk of TiO_2, there is no evidence that the spread of these particles to water treatment systems or the environment pose any greater risks than the more widespread micron-scale pigment-grade materials. Currently, nano-structured TiO_2 is still officially regarded as "potentially harmful" to the environment (Kahru and Dubourguier 2010). It should be clarified that the end-of-life risk for nano-ZnO may be different from that of TiO_2, as it is regarded in the literature as being "extremely toxic" in the environment (Kahru and Dubourguier 2010). EPA's current inventory approach is that new nanoscale forms of TiO_2 and ZnO are not considered new chemicals requiring reporting under Section 5 of TSCA (EPA 2008). However, EPA is developing a Significant New Use Rule

(SNUR) to require reporting and filing a 90-day PMN for new nanomaterials based on existing chemical substances.

Data Gathering, Monitoring, and Governance of Nanostructured Silver

Researchers and regulators are looking more closely at nano-silver, because it is one of the most commonly cited ENMs in "nano"-branded products. Because products containing nano-structured silver often make pesticidal claims for antimicrobial activity, EPA has been evaluating nano-silver under its Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) statute (7 U.S.C. §136 *et seq*.; U.S. EPA 1996). From a toxicological perspective, most of the concern is not directed as much to the apparent modest risk to workers and consumers as to the hazard potential in the environment, especially for aquatic life forms (Asharani et al. 2008; Mueller and Nowack 2008).

Policymakers from around the world have indicated that insufficient data have emerged to implement rational changes to existing frameworks for risk management of chemicals and nanomaterials. After a relatively long period of inactivity, national and international governments have begun to collaborate and are now more proactive on the regulatory front. Major regulatory activities include more deliberate data-gathering efforts, global standardization, and coordination of risk assessment to enable regulatory agencies to formulate policy. Examples include the data collection programs and risk management best practices initiatives from organizations such as the Organization for Economic Co-operation and Development (OECD)[22] and the International Organization for Standardization (ISO).[23]

A number of key additional nano-EHS advances over the past 10 years are worth mentioning here and will be discussed in more detail elsewhere, namely advances related to environmental remediation (Section 4.6.1), green chemistry (Section 4.6.1), and improved water and food safety and supplies (Sections 4.6.1 and 4.6.3).

4.3 GOALS, BARRIERS, AND SOLUTIONS FOR THE NEXT 5–10 YEARS

Develop Validated Nano-EHS Screening Methods and Harmonized Protocols that promote standardized ENM risk assessment at levels commensurate with the growth of nanotechnology

While some progress has been made in developing toxicological screening for abundantly produced ENMs, there is still a lack of standardized methods and protocols to assess and manage nano-EHS issues. This has resulted in contradictory and even irreproducible ENM hazard assessment that has sparked considerable debate on how best to conduct toxicity screening for risk assessment and regulatory purposes (Jones and Grainger 2009; Meng et al. 2009). One significant barrier to the development of validated and harmonized screening protocols is insufficient knowledge about which physicochemical properties of ENMs are relevant to transport, exposure, dose calculation, and hazard assessment. Other obstacles include lack of standardized nomenclature for nanomaterials classification, lack of standard reference materials to use as controls, the high rate at which materials with new properties are being introduced, and ongoing debate about whether *in vitro* and *in vivo* testing best constitute a valid approach to reliable, predictive hazard screening (Jones and Grainger 2009;

[22]For examples, see the OECD department website on Safety of Manufactured Nanomaterials http://www.oecd.org/department/0,3355,en_2649_37015404_1_1_1_1_1,00.html

[23]For examples, see the ISO catalog website for standards devised by its Technical Committee 229 on Nanotechnologies: http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_tc_browse.htm?commid=381983

Meng et al. 2009; Sayes et al. 2007). To address these barriers, a number of solutions are likely to emerge in the next 10 years. These include the following:

- Development of validated hazard assessment strategies and protocols that consider the correct balance of *in vitro* and *in vivo* testing, of biologically relevant screening platforms, and of high-throughput methods. Both *in vitro* and *in vivo* testing are important for knowledge generation about hazardous material properties (Donaldson et al. 2008; Meng et al. 2009; Rushton et al. 2010; Sager et al. 2007). *In vitro* studies at the molecular and cellular level allow for rapid knowledge generation, but the relevance of this screening must be carefully connected to a desired, validated toxicological outcome *in vivo* to make the screening predictive (Meng et al. 2009). This connectivity establishes the relevance of using cellular and biomolecular endpoints to collect primary screening data that can then be used to prioritize animal testing, where fewer observations are possible and mechanistic studies are difficult (see Figure 4.2). This approach could limit the extent, volume, and cost of animal testing. (Examples of the use of *in vitro* screening efforts that could be regarded as predictive of *in vivo* pathology or disease outcome are reviewed in Section 4.8.1.) Important considerations for the design of *in vitro* cellular assays include the choice of representative cell lines, their phenotypic fidelity, stability in culture, appropriate use of single- versus multi-parametric response tracking, reporting for acute versus chronic effects, use of an extensive dose range that assesses lethal and graded sub-lethal response outcomes in the linear part of the dose-response curve, and the ability to adapt high-content and rapid-throughput screening approaches to speed up and multiplex hazard data collection (Jones and Grainger 2009; Teeguarden et al. 2007). To assist these screening efforts, an important goal is to develop and validate harmonized protocols that lead to standardized testing; one example is the efforts by the International Alliance for NanoEHS Harmonization (http://www.nanoehsalliance.org), in which a number of leading international scientists seek to establish and validate protocols anticipated to become useful for toxicological testing of representative nanoparticles in round-robin experiments. Interlaboratory tests are designed to validate the reliability and reproducibility of the protocols as practiced in representative laboratories. At present we do not have databases adequately reporting and tracking data reliability and reproducibility. Yet without quantitative measures of error, uncertainty and sensitivity it is not possible to rationally design nanomaterial, or to evaluate a nanomaterial's health, safety or environmental risk.

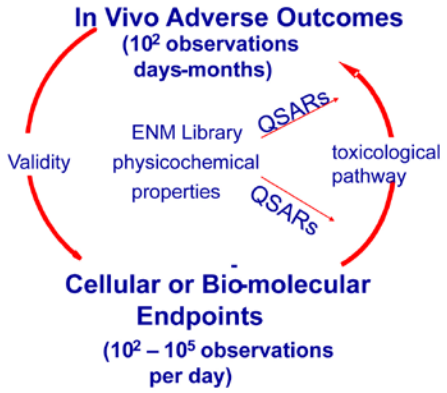

Figure 4.2. Differences in the rate of knowledge generation *in vitro* and *in vivo* show the utility of using both approaches but the necessity of validating biomolecular events *in vivo* to establish a predictive toxicological outcome (courtesy of A. Nel).

- Development of appropriate ENM dosimetry tools that go beyond the traditional mass-dose, particle number, and surface area-dose (SAD) considerations. While traditionally chemical dose levels are determined based on what the organism ingests, dosimetry for nanoparticles is often calculated based on quantities added to the exposure medium, which is conceptually incorrect. The appropriate considerations should be for the bioavailable dose at the site of injury: To relate the toxicology of ENMs to physicochemical properties that are responsible for injury, it is critical to take into consideration cationic charge, surface reactivity, redox activity, surface shedding of metal ions, dissolution chemistry and morphological changes, and the effect of chemisorbed chemical substances, stabilizers, and capping agents (Duffin et al. 2007; George et al. 2010; Monteiller et al. 2007; Xia, Kovoshich, Liong, Maädler et al. 2008; Xia, Kovoshich, Liong, Zink et al. 2008). To make valid comparisons between *in vitro* short-term mechanistic observations and *in vivo* toxicity and pathology as a result of toxicologically relevant ENM exposures, it is essential to perform dosimetry experiments in the linear region of the ENM dose-response curve. Examples of progress being made in dosimetry assessment in the field of pulmonary toxicology include the tiered assessment of cellular oxidative stress in response to abiotic and biotic oxygen radical production as well as relating SAD to pulmonary inflammation as reflected in bronchoalveolar lavage (BAL) polymorphonuclear (PMN) cell counts (Duffin et al. 2007; Monteiller et al. 2007; Oberdörster et al. 2007; Xia et al. 2006).
- *Improved technology to track the presence, fate, and transport of nanomaterials and improve exposure assessment*. Tracking, sensing, detecting, and imaging of nanoscale materials in environmental, biomedical, and biological systems require new analytical technologies that require the same level of technical sophistication as the design of ENMs. Rapid progress is foreseen in technologies that detect and characterize ENMs in aerosols, comparable to the progress discussed above for detection of CNTs in the workplace. Similar advances are being made for detection of other types of nanoparticles in the workplace. Improvements in new, sensitive instrumentation that can detect ENMs in complex biological environments are reviewed in Section 4.4. Technological requirements to assess the presence, spread, and bioavailability of ENM in complex environmental media such as agricultural products and wastewater systems are discussed in Sections 4.4 and 4.6.
- *Life cycle analysis*. An analysis of the energy consumption and materials usage throughout the value chain of ENM production, use, and disposal is essential to understand the overall environmental impact of emerging nanotechnology industries (Chellam et al. 1998). Similarly, an assessment of the wastes generated by nanotechnology production processes is needed and should include attention to waste streams coming from nanomaterial production facilities as well as conventional waste streams that may impose new pressures on environmental systems (see Section 4.8.3). This life cycle assessment of ENMs should be accompanied by a value-chain analysis that begins with estimates and projections of nanomaterials production. Such estimates are needed to obtain quantitative estimates of expected nanomaterial exposures. Important factors to be identified in evaluating potential nanomaterial exposure are the format in which nanomaterials will be present in commercial products, the potential for these materials to be released to the environment, and the transformations that those materials may undergo that affect their transport and potential for exposure.

Develop Risk Reduction Strategies that can be implemented incrementally through commercial nanoproduct data collection, regulatory activity, and EHS research directly linked to decision making

A major barrier to performing comprehensive risk characterization (Figure 4.3) is the lack of sufficient knowledge about ENM hazard, fate and transport, dosimetry, and how to perform ENM exposure assessment (Oberdörster et al. 2005). This precludes rational implementation of a mature and comprehensive risk management strategy for most ENMs. However, to mitigate perceived risk and promote widespread public acceptance of nanotechnology, it is necessary to develop safe implementation strategies using current capabilities and infrastructure that are presently at our disposal (Oberdörster et al. 2005). We can then proceed with risk reduction strategies that inform the community and the public and also help prevent unanticipated negative EHS consequences of nanotechnology implementation.

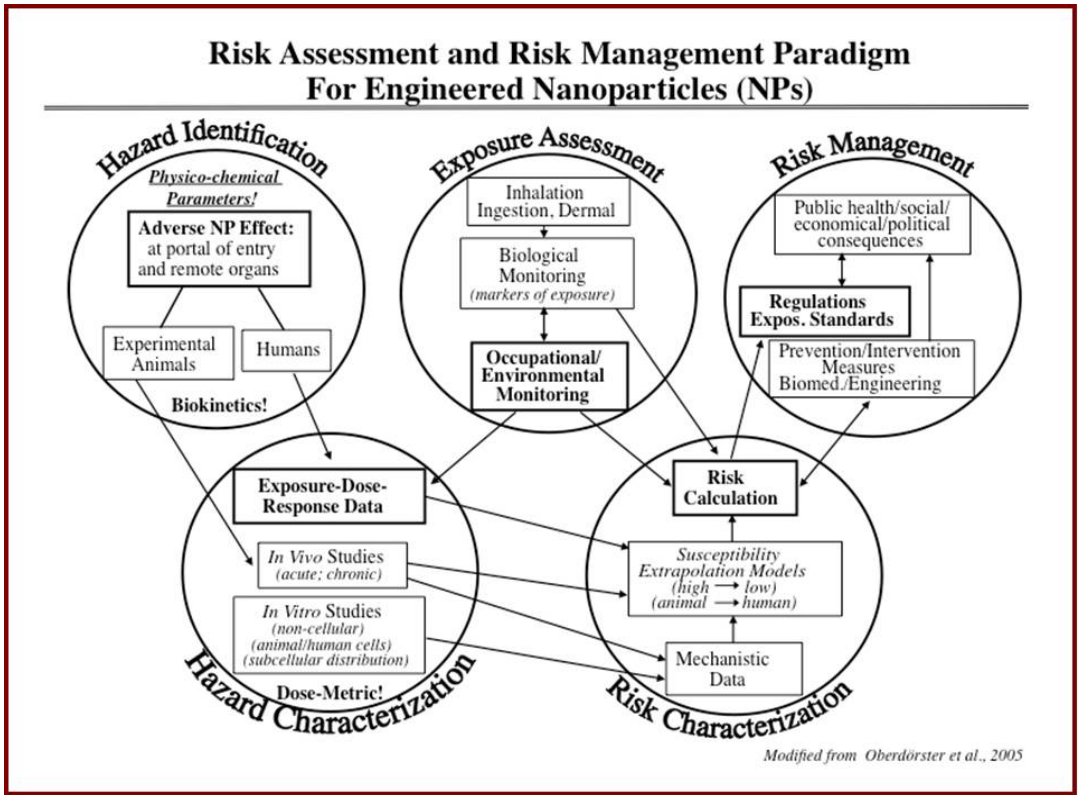

Figure 4.3. Risk assessment and risk management paradigm for engineered nanoparticles (adapted with permission from Oberdörster et al. 2005).

To manage risks associated with ENMs, commercial use data must be collected and made public to enable independent EHS researchers to conduct life cycle and exposure analyses (Chellam et al. 1998). This includes information about the chain of commerce, quantities, and types of ENMs being used in commercial applications. Although both Federal and state agencies (e.g., the California Environmental Protection Agency) have existing authorities dictating how and what data will be collected, improved NNI coordination can play a critical role in fostering the political will to collect commercial use data. Regulatory agencies worldwide are gearing up to fill the major knowledge data gaps about commercial use of nanotechnology by making changes to existing regulations or enactment of new policy to assist the data collection. Current and forecasted policies of regulatory agencies in the United

States, Canada, and the European Union (EU) appear in Table 4.1. Of particular note are the enactment of the significant new use rule (SNUR) by the U.S. EPA and the EU decision to classify specific nanomaterials as "Substances of High Concern" (SVHC) under the Registration, Evaluation and Authorisation CHemicals (REACH) regulation of the European Chemicals Agency, both of which decisions put use of specific nanomaterials under close scrutiny and regulatory procedures.

Table 4.1 Current and forecasted regulatory policies of United States, Canada, and EU regulatory agencies

Agency/Law	Jurisdiction	Current Stance	Future Prospects
Environmental Protection Agency (EPA)	U.S.	TSCA does not require registration and testing for ENMs already in its inventory, but it considers ENMs with novel molecular structures as new materials (e.g., carbon nanotubes).	Rather than labeling ENMs as new substances, the EPA is currently using tools like SNUR to restrict uses of particular nanomaterials if they are expected to present risks. TSCA reform is being considered.
Food and Drug Administration (FDA)	U.S.	FDA considers its current practices sufficient to cover NMs, but the agency will issue guidance on data to be included in submissions, including size.	Emerging scientific information suggests that certain NMs do present EHS risks. The FDA will modify its policy on a case-by-case basis.
Consumer Product Safety Commission (CPSC)	U.S.	CPSC considers its current policies sufficient for NMs until more information is known.	CPSC will consider modifications on a case-by-case basis depending upon evidence.
Occupational Safety and Health Administration (OSHA)	U.S.	OSHA considers its current policies sufficient for NMs until more information is known.	OSHA will consider modifications on a case-by-case basis depending upon evidence.
REACH (Registration, Evaluation and Authorization of Chemicals)	EU	REACH identifies chemicals by CAS registry numbers, which identify molecular structure but not particle size.	Pending new data, the European Commission through REACH may classify specific NMs as SVHC, similar to EPA's SNUR, to limit or restrict nanomaterial usage in lieu of more concrete regulations.
Canadian Environmental Protection Act (CEPA)	Canada	Through CEPA, the Canadian government in 2009 mandated that companies working with ENM must submit usage and toxicity data	Further legislation is under consideration in 2010 requiring notification of significant new activity, risk assessment procedures, and establishment of a public inventory for nanotechnology and ENMs.

Source: Adapted with permission from *The Nanotech Report* (Lux Research 2007).

EHS research should be driven by the need to make informed decisions on hazard and risk management as well as regulatory decision making. To date, U.S. interagency cooperation has not facilitated effective linkage of risk research to decision making; this disconnect has resulted in actions and strategies that do not fully address policy needs. At the moment, individual agencies are independently establishing connections between research and decision making. Similar efforts are needed at an interagency level to ensure that risk assessment and evidence-based decision making are addressed collectively. Finally, it is also

important to mention the possible contribution of *in silico* methods for risk ranking and risk modeling.

Develop a Clearly Defined Strategy for Nano-EHS Governance that is compatible with incremental knowledge generation and stepwise decision making

There are a number of divergent positions among different international stakeholders regarding regulatory policy for engineered nanomaterials, as indicated in Table 4.2, divided roughly into the positions of policymakers, business, academia, and civil society organizations (CSOs). While an integrated strategy for nano-EHS governance currently does not exist in the United States, the trend appears to be shifting from that shown in the second row of Table 4.2 to the position shown in the third row—that is, toward an across-the-board more precautionary and proactive approach to the regulation of ENMs. While putatively the best position will be evidence-based decision making, there are a number of barriers that preclude this goal, including insufficient knowledge about ENM hazard, dosimetry, exposure, and how to best perform risk assessment.

Table 4.2 Regulatory policy for ENMs among stakeholders around the world

Position/Opinion	Policymakers	Business	Researchers	CSOs
The existing regulatory situation is adequate. In the case that scientific evidence indicates a need for modification; the regulatory framework will be adapted.	+	+		
Specific guidance and standards must be developed to support existing regulations when dealing with N&N, but the existing regulatory situation is generally adequate.	++	++	++	
Regulation should be amended (on a case-by-case basis) for specific N&N, above all when a high potential risk is identified. A precautionary approach is envisaged.	++	+	++	+
The existing regulatory position is not adequate at all. Nanomaterials should be subject to mandatory, nano-specific regulations.				++

Note: CSO = civil society organizations; N&N = nanoscience and nanotechnologies. Adapted with permission from FramingNano Governance Platform (Widmer et al. 2010, p. 69).

Attributes of a desirable nano-regulatory process that most stakeholders could possibly agree upon include the following (Widmer et al. 2010):

- Responsible development of nanotechnology should be accomplished without hampering innovation and commercial growth
- Governance and regulation of nanotechnologies is a dynamic exercise that needs to be continuously adapted
- Appropriate regulation of nanomaterials requires constant implementation of state-of-the-art knowledge, methods, and monitoring
- Timely and appropriate response is needed to address the data gaps and challenges that are continuously being generated in a dynamically changing field
- Global agreement is necessary to promote commercialization

- All relevant stakeholders and the interested public must be engaged in the development of new policies and regulation of ENMs
- Cooperation between government, industry, academia, and the public is essential in developing the knowledge base required for evidence-based governance

On the basis of these principles as well as the perception that knowledge generation about essential nano-EHS domains is likely to be incremental, the goal for the next 10 years could be to follow an adaptive, iterative approach to nano-regulatory policy (left side of Figure 4.4). According to this approach we should identify current knowledge and capacity, and use the statutes and governance infrastructure currently in place, but make it more effective through coordination of data gathering and informatics efforts as well as by involving all stakeholders. This could be done by adjusting and improving the oversight procedures as the knowledge base and capacity increases. Thus, short-term approaches could include information collection, implementation of safe practices in the workplace and laboratory, use of best practices, streamlined risk management for specific ENMs (e.g., the NIOSH guidelines for CNTs), as well as augmenting current statutes to obtain more complete product information (e.g., the SNUR by the EPA or SVHC in the EU).

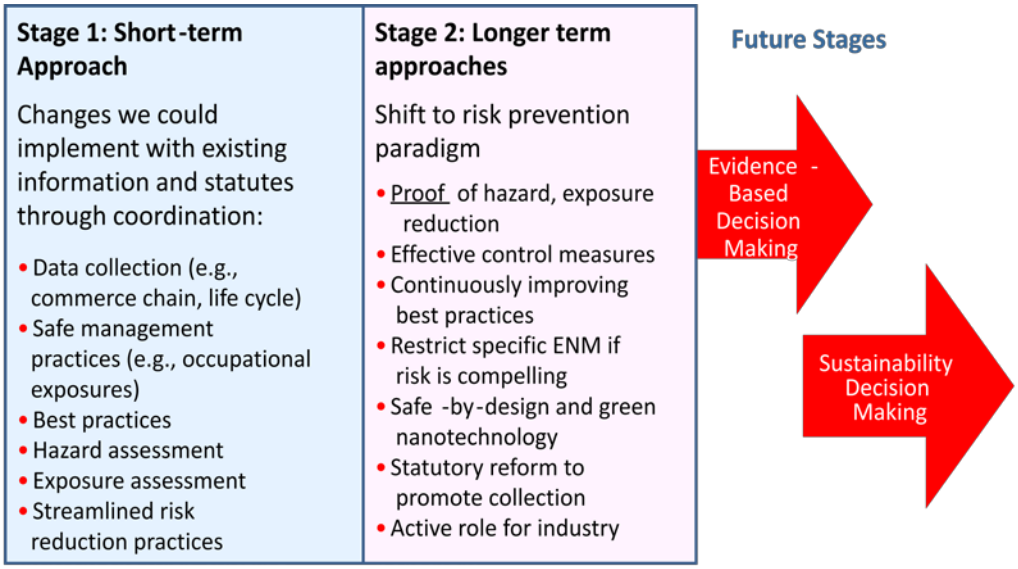

Figure 4.4. An example of an adaptive iterative approach to nano-regulatory policy that considers what can be accomplished immediately within our current framework and regulations and where we should aim to move to next as more data and information become available. This could lead us to evidence-based and ultimately sustainability-based decision making (courtesy of A. Nel).

In the long term, this approach to nano-regulatory policy should shift to a risk prevention paradigm (right side of Figure 4.4) in which the emphasis becomes the use of hazard, exposure, and lifecycle data to provide proof of risk reduction through the implementation of safe management and best practices. The long-term goal should be to utilize the information gathered through high-throughput property–activity relationships and computational analysis to develop safe design guidelines for ENM along with implementation of green manufacturing. This could ultimately evolve into evidence-based decision making and policy that promotes sustainability (see Section 4.6).

Develop Computational Analysis Methods Capable Of Providing *In Silico* Modeling of nano-EHS risk assessment and modeling

Challenging barriers to evidence-based decision making in nano-EHS include the complexity of environmental and mammalian systems, the large number of variables engineers employ to design nanomaterials, absence of critical knowledge of how to perform risk analysis, the rapid rate of expansion of nanotechnology, inability to deal with large databases, and the lack of a standardized nomenclature to codify engineered nanomaterials (Meng et al. 2009). As a result, it is apparent that the cornerstone of research in nano-EHS must be systematic, quantitative studies designed to inform and promote the use of accurate, predictive models and reliable, relevant simulations (Barnard 2009). Such models must effectively and rigorously address diverse nanomaterial types, including their dissemination and interactions with a multitude of complex and diverse environmental and biological systems. Judicious application of models that ultimately should incorporate predictive power can accelerate safe commercialization of nanotechnology throughout its innovation pipeline. Models that describe the nano–environmental interface will enable engineers to devise "safe-by-design" nanosystems and will also equip companies to design and create containment and waste treatment strategies to minimize nanomaterial exposures. Quantitative adaptive graphical and accessible simulations of nanomaterial transport, interactions at the nano–bio interface, lifecycle analysis, and risk modeling can provide information not currently obtainable from experiments. Some concepts of what these models might look like are available in cutting-edge cell and developmental biology, where virtual environments are being designed to mimic complex biological responses to various stimuli (Butcher et al. 2004).

At present there is no equivalent of the Protein Data Bank (PDB) for ENMs. The PDB serves as a repository for protein structures to archive published molecular structures and conformations for proteins cited in the published literature, and serves as a focus for annotation, curation and validation of those structures. The lack of a repository for ENM structures is critical. Correlations of nanomaterial structure with their physico-chemical, biological, toxicological and biomedical data are being performed without knowledge of the differences in structure of the ENMs in the experimental samples or of the sensitivity of the experimental results to those differences. Nanomaterial is, in general, both polydisperse and polymorphic and modeling efforts may require structural models for several different subpopulations. The Collaboratory for Structural Nanobiology (CSN http://nanobiology.utalca.cl or http://nanobiology.ncifcrf.gov) has been developed and is being used to prototype tools to construct and validate molecular models, to obtain realistic user requirements for a repository from practitioners across the disciplines relevant to nanotechnology, and to explore nanobioparticle data storage, retrieval and analysis in the context of nanobiological studies.

Although it is likely that computational models will need to be trained or fed with valid experimental input data to be valuable for predicting actual behaviors, predictive models can be used to great effect in determining the sensitivity of ENM properties to changes in their environment and structure. With the advent of new online environments, key databases are being developed by consortia such as nanoHUB.org or the caBIG(R) Integrative Cancer Research Nanotechnology Working group at the National Cancer Institute (http://www.nanoehsalliance.org/index.php). Another example of how machine-learning analysis is being used to provide predictive modeling is the framework developed by the University of California Center for Environmental Implications of Nanotechnology (UC CEIN) (Godwin et al. 2009). (This aspect is further discussed in Section 4.8.2.)

The OECD has published a set of guidelines for the validation of quantitative structure-activity relationship (QSAR) models for regulatory purposes (OECD 2007). These guidelines focus on five main concepts:

- Defined endpoint
- Use of an unambiguous algorithm
- Defined applicability domain
- Measures of goodness-of-fit, robustness, and predictability
- Mechanistic interpretation of the model whenever possible

QSAR modeling requires the computation of structural and chemical descriptors as well as large experimental databases of physicochemical properties. In contrast with QSARs for chemicals, the nano-QSAR concept is still in its early development (Puzyn et al. 2009). Due to high variability in the molecular structures and different mechanisms of action, one goal could be to group ENMs into classes and model individual classes of ENM separately. In each case, the applicability domain of the models should be carefully validated. Successful development of new nano-QSARs needs reliable experimental data and requires experimentalists to work together with the nano-informatics community. (Section 4.4 discusses the new capabilities, instruments, and tools that are required for nano-informatics.)

Develop High-Throughput and High-Content Screening as a universal tool for studying nanomaterial toxicity, ranking hazards, prioritizing animal studies and nano-QSAR models, and guiding the safe design of nanomaterials

Major barriers in the assessment of ENM hazard potential include the lack of capacity to perform safety screening on large batches of nanomaterials, lack of data on core structure-activity relationships that predict toxicity, inability to cover all of the potentially hazardous materials or material properties in a single experiment, inability to prioritize the execution of costly animal experiments, and the limitations of using single-response parameters (e.g., lethality) without considering a full range of sublethal and lethal dose-response parameters.

One possible solution to these problems is the use of high-content screening methodologies that have facilitated understanding of biological phenomena in cells as well as improved drug screening (Abraham et al. 2004; Abraham et al. 2008; George et al. 2010; Service 2008). Rapid-throughput multiparametric cellular screening recently has been shown to be an important tool for toxicology. The goal over the next 10 years is to develop and implement new screening tools to enhance the efficiency and rate at which ENM hazard profiling can be performed. A considerable amount of exploration is required to produce appropriate, dose-dependent responses at the nano-bio interface that can be used for high-throughput screening (Oberdörster et al. 2007; Teeguarden et al. 2007).

Examples of possible applications of high-content screening include assessments of toxicological injury pathways, signaling pathways, membrane damage, organelle damage, apoptosis and necrosis pathways, DNA damage, and mutagenicity (Meng et al. 2009). Rapid pathway-based cellular screening studies that utilize one or more of these endpoints allow the establishment of property-activity relationships in which material properties such as size, shape, dissolution, band-gap, surface charge, and so forth are varied to test the biological consequences (George et al. 2010). This requires the development of ENM libraries that include specific properties for testing (as outlined in Section 4.8.2), as well as microplate optical reader–based assays that rely on fluorescence, fluorescence polarization, time-resolved fluorescence, luminescence, or absorbance (Godwin et al. 2009). High-throughput or

high-content screening can also help to identify hazardous material properties that could be used for safe-by-design approaches to nanomaterials synthesis (George et al. 2010).

While much of the knowledge about current ENM cellular toxicity has been generated by single-readout cellular screening assays, the major drawback is that each assay represents only a single specific reaction to a toxic stimulus and thus is of limited predictive value. The use of multiparametric screening assays allows for the elucidation of connected molecular pathways or biochemical events (George et al. 2010). Thus, understanding the initial mechanism of injury and time-sequence information is gained. An estimation of the severity of the insult (e.g., lethal vs. sublethal) is possible through the use of dose-response relationships captured during high-throughput screening.

Cytotoxicity screening as a stand-alone exercise has several limitations, and the true toxicological significance of a cellular injury response can only be determined if it is correlated with adverse biological effects in intact organisms and animals (Jones and Grainger 2009). For the *in vitro* screening to be a truly predictive toxicological tool, the *in vitro* injury response should be directly and unequivocally connected to an *in vivo* injury response or adverse health effect. The duration and intensity of exposure (i.e., acute vs. chronic) must also be considered. Thus, *in vitro* screening assays constitute just one of multiple steps required for ENM safety assessment and validation.

Improve Safety Screening and Safe Design of Nanomaterials used for therapeutics and diagnostics

Nanotechnology has made major inroads in medicine and looks poised to transform many of nanomedicine's traditional components (see Chapter 7). The expected advances encompass improvements in the delivery of therapeutic molecules through systemic injection and locally implanted devices, contrast agents for all modalities of radiological imaging, and innovation in laboratory diagnostics and screening methodologies (Ferrari 2005; Riehemann et al. 2010). In addition to improvements over existing approaches in health care, nanotechnology offers truly transformative opportunities as a necessary enabler of key aspects of personalized medicine (Sakamoto et al. in press), regenerative medicine, and reformulation of basic tenets of biological and medical sciences (Ferrari 2010).

Nanomedicine is pervasive throughout contemporary medicine, with nanostructured drugs and contrast agents widely available in the clinic (Ferrari 2010). Since the approval of the first nanotechnology-based drugs in 1994, this sector has grown into a $6 billion market as of 2006 in the United States alone. Because of direct and deliberate human exposure in their use, the safety of nanoscale devices is of prime importance and can benefit from some of the same discovery platforms as discussed above for exploration of the nano–bio interactions leading to toxicity. There is relatively little known currently about the safety of the nanoscale devices used increasingly for drug delivery, imaging, or theranostics (McNeill 2005). There is a lack of detailed information about hazardous nanoscale properties that could require novel safety testing procedures currently not included in the traditional drug screening approaches. However, all current clinically available nanotechnology-enabled agents of therapy and imaging contrasts have obtained regulatory approval from the U.S. Food and Drug Administration (FDA) and other similar agencies worldwide. No adverse event has been reported to date for nanoparticles being used in the clinic. Similarly, there is no literature evidence of health hazards or adverse impact on personnel working in manufacturing, transportation, disposition, storage, medical administration, or dispersion of clinically used nanoparticles. Thus, current concerns about the safe design of nanomaterials largely relate to hypothetical problems that may arise for future generations of nanoscale drugs and devices.

Rationalizing the regulatory process guiding the use of ENM in nanomedicine is a major challenge. The FDA's current position is that nanostructured drugs and nanoparticulate imaging agents and theranostics can be regulated without special consideration of the nanoscale (Sanhai et al. 2005). Demonstrated safety and efficacy of the therapeutic platform is the most important requirement, and experience to date indicates that the drugs being delivered by the nanoparticles are generally much more toxic than the ENM carriers being used. (Parenthetically, it is noted that a nontoxic chemotherapeutic drug would be like a blunt surgical scalpel that would not have any efficacy against cancer.) Thus it may be argued that it is not the lack of toxicity that is the objective, but the balance between risk and benefit for the patients and the community at large (Ferrari et al. 2009). At this time, the regulatory approval pathway is the same as for any other drug or contrast agent; the position of the FDA (2007) is that intact nanoparticles as drugs or agents are to be tested as a unit rather than as a combination of individual components.

Some consumer groups and nongovernmental organizations (NGOs) question whether the regulatory processes for nanoscale therapeutics are sufficient, given the lack of comprehensive knowledge about reactions to ENM properties in the body. Moreover, the field of nanotechnology-enabled biomedicine is advancing rapidly and may yield more complicated biomedical nanostructures in the future. These uncertainties are further compounded by the observation that the FDA has not taken any specific actions with respect to monitoring ENM use in foods and cosmetics, despite perceived risks. The FDA does address the safety of drugs and devices before allowing their entering the market and their use in healthcare. On the other hand, the FDA authority for what relates to foods and cosmetics does not include a requirement for premarket authorization, but only a monitoring of post-market safety with the authority to mandate removal of unsafe products.

The FDA has been considering safe design principles for nanoparticles in medicine (Sanhai et al. 2007). Design practices have been sufficient to date to avoid undue safety risks from medical nanoparticles; however, novel design paradigms are emerging under rubrics such as "rational design of nanoparticles" (Decuzzi and Ferrari 2008; Decuzzi et al. 2009). This has yielded "design maps" to attempt to assess the biological properties of nanomaterials according to their design parameters. Among the biological properties changed by ENM redesign are the shape characteristics that allow disk-shaped nanoparticles to selectively move to the flow margins in blood vessels, firmly adhere to the vessel walls, and then slip through some vascular fenestrations where they enter cells. Although these methods were primarily intended to optimize biodistributions and therapeutic indices, it is expected that they may also be the foundational cornerstone for a rigorous, quantitative modeling exercise useful to promote nanoparticle safety. Additional research that is ongoing in many laboratories and industries worldwide is directed at the development of "safe" nanoscale vectors that can optimize delivery of therapeutic and contrast agents to intended sites in the body and then disintegrate in full without leaving any trace behind them, *in situ* or systemically. This research has to take into account accessing the body's metabolic and excretory pathways.

Consideration of Safety Assessment of increasing More Complex ENMs that are being introduced in a functionalized, embedded or composite material format

While to date most of the efforts in hazard and risk assessment have concentrated on primary ENMs such as nanoparticles, nanotubes, and nanofibers, increasingly more complex, composite, embedded, hybrid, and functionalized materials are being introduced. Such new ENMs will necessitate adaptation of study approaches and deciding which materials and

commercial products to prioritize for testing. Examples include a number of active nanostructures that are being introduced as "second-generation" nanomaterials as well as "third and fourth-generation materials" that will be obtained through guided assembly, assembly of hierarchical architectures, development of nano-composites, and organic-inorganic hybrids. In addition to the current OECD efforts that focus on high volume or high tonnage primary materials, we should expect in the next decade to see materials such as platinum/palladium nanoparticles in auto catalysts, organic-modified inorganic systems, nanostructured protective coatings, nanostructured reinforced materials, designed microstructures, nanostructured composites, nano-reinforced metallics, and nano/bio-soft-condensed matter. Thus, hazard and risk assessment tools will also have to incorporate methodology and approaches to deal with these novel material characteristics. This will necessitate studies that can assess commercial products and embedded nanoscale materials in their as-produced form as well following their disintegration, shedding or emission of ENMs in the environment or human living space. This introduces another level of complexity that initially should involve data collection about material use, lifecycle analysis, and ultimate disposal in the environment. Industry will play an important role in the data generation and research into the safety of these products. These data are important for definition of the potential exposure scenarios, from which the hazard and risk assessment approaches could evolve, such as use of environmental mesocosms for ENM deposition and aging studies, collection of wear and tear particulates from car tires or vehicular emissions on or close to freeways, looking at the release of embedded nanoscale materials during combustion, erosion, grinding, sanding of composite materials, and assessment of nanomaterial release from building materials.

4.4 SCIENTIFIC AND TECHNOLOGICAL INFRASTRUCTURE NEEDS

Development of Advanced Instrumentation and Analytical Methods for more competent and reliable ENM characterization, assessment, and detection in complex biological and environmental media

Relatively few techniques are able to interrogate nanoparticles properties directly with sufficient chemical or physical sensitivity in complex environmental, agricultural, or biological milieus, in real time, or under batch-processed analytical formats. Rapid, sensitive and definitive tools to identify amounts and types of ENMs in complex samples remain a challenge and a need. New characterization tools that directly detect small ENM amounts in "real" biological and exposure environments (i.e., tissue slices, food, environmental samples, blood) are necessary to better evaluate the dynamics of nanomaterials interactions at the biological interface for nano-EHS research (Nel et al. 2009). Examples of tools and capabilities that have recently emerged or are in development for characterizing biomaterials include the following:

- Improved tracking of cellular and tissue uptake of ENM using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Standard SEM and TEM approaches are useful for imaging electron-dense (e.g., metallic nanoparticles), but not soft materials (e.g., dendrimers and liposomes). However, several recent advances improve the utility of TEM in studying the nano–bio interface. TEM cryomicroscopy is now used routinely to image intercellular structures and unstained biomolecules at the sub-nanometer level. When combined with data processing, this technique allows the molecular topographies of single biomolecules to be visualized in conformational states that are not accomplishable with X-ray diffraction (Yu et al. 2008). Thus, it is now standard practice to obtain three-dimensional reconstructions of nanoscale biovolumes

using eucentric-tilting goniometers (Baumeister 2005; Carragher et al. 2004). Moreover, sub-Ångstrom, aberration-corrected TE[A]M instruments have been developed to directly image the volumes and surface edge atomic structures of nanoparticles using both transmission (TEM) and scanning transmission modes (STEM) (Carragher et al. 2004). STEM holds great promise in enhancing the contrast of biostructures when combined with energy-filtered TEM imaging (Carragher et al. 2004; Porter 2007). In addition, a new generation of low voltage electron microscopes are now becoming available to take advantage of the high contrast of biological materials at low energies and which permit multimode (ED, SEM, TEM, STEM) operation in a desk-top instrument (http://www.lv-em.com). This development makes it possible to place an electron microscope on a manufacturing floor as well as in the field.

- *Improved techniques to resolve nanoscale particles in very large biomaterial volumes.* One approach is correlative microscopy: using optical techniques to identify targets, transferring the sample and grid coordinates to a TEM, and automatically navigating those targets to obtain high-resolution images, while maintaining the sample in a frozen, hydrated state (Lucic et al. 2007; Sartori et al. 2007; Steven and Baumeister 2008). An alternative technique uses a dual-beam instrument—an ion beam to cut a cross-section in the bulk biomaterial, and SEM to record it. By automating the cutting and recording of the image, data can be processed to provide tomographic representations of the volume (Heymann et al. 2006). This approach has applications in the fully automated analyses of bulk materials with site-specific targeting, where the structure is recognized through the different rates of sublimation of its cellular components. This technique has been used to site-specifically remove artifact-free, thin lamellae of frozen tissue for TEM cryomicroscopy (Marko 2007).
- *New approaches to fluorescence imaging.* Fluorescently labeled nanoparticles and related imaging techniques (e.g., confocal microscopy) suffer potential problems such as label instability, altered physicochemical properties, and photobleaching from laser exposure. Ideally, novel imaging techniques are required to visualize local populations of nanoparticles at nanometer resolution in real time within cells without structural damage. A promising development is live cell confocal microscopy, which is ideal for high-resolution imaging of movement through intracellular environments, including endo-exocytosis, vesicle tracking, particle transport, and nuclear-cytosol membrane mechanisms (Stephens and Allan 2003).
- Advances in Coherent Anti-Stokes Raman (CARS) scattering now permit Raman spectroscopy to be used as a "chemical microscope", mapping the detailed structure of cells and organelles according to the chemical composition at each point in three-dimensional space. without the use of dyes. (e.g., Broadband-CARS (B-CARS) http://www.sciencedaily.com/releases/2010/10/101014121156.htm) and Femtosecond Adaptive Spectroscopic Techniques for CARS (FAST-CARS) http://www.ncbi.nlm.nih.gov/pmc/articles/PMC123198/).
- *Surface-enhanced Raman scattering (SERS).* Another technique being used increasingly for bioimaging of cells and intact animals is SERS (Qian et al. 2008), which measures the enhanced Raman scattering of molecules adsorbed onto (e.g., nanotextured) metal surfaces. With enhancement factors as high as 10^{15}, this technique is sufficiently sensitive to detect single molecules (e.g., PEGylated Au and Ag nanoparticles). Recent tumor imaging with radio-labeled single-walled carbon nanotubes (SWCNTs) suggests that SERS may be a promising molecular imaging technique in living subjects (Keren et al. 2008; Liu 2007).

In similar fashion, new characterization tools/techniques such as the following are emerging to evaluate the structure and dynamics of the environmental interface:

- *Liquid chromatography-atmospheric pressure photoionization-mass spectrometry (LC-APPI-MS).* This can be used to determine aqueous concentrations of ENMs with positive electron affinity at relatively low levels (e.g., 0.15 pg detection limit for C_{60}).
- *Spectroscopic techniques.* Techniques such as x-ray absorption fine structure (XAFS), including x-ray absorption near-edge spectroscopy (XANES) and extended x-ray absorption fine-structure spectroscopy (EXAFS), can be used in conjunction with electron microscopy to determine the chemical state and local atomic structure of inorganic ENMs and assess their chemical transformations. However, these methods often require a synchrotron beamline, which is expensive, non-routine, and often inaccessible for most needs.
- *The environmental scanning electron microscope (ESEM).* This allows a gaseous environment in the specimen chamber, whereas other electron microscopes operate under vacuum. ESEM allows imaging of wet specimens and can be useful for detecting nanomaterials in the environment. Hydrated specimens can be examined, because any pressure greater than 609 Pa allows water to be maintained in its liquid phase for temperatures above 0°C, in contrast to the SEM, where specimens are desiccated by the vacuum condition. Moreover, electrically nonconductive specimens do not require the preparation techniques used in SEM, such as the deposition of a thin gold or carbon coating.

The informatics infrastructure for nanotechnology should incorporate web-enabled websites and forums to advance collaboration in gathering user requirements for use cases employing advanced instrumentation deployed in realistic settings, instrument prototyping, and partnering in production of these new instruments which promise to rapidly advance our understanding of the behavior of ENMs in biological environments.

Development of Computational Models, Algorithms, and Multidisciplinary Resources for increasingly sophisticated predictive modeling

The importance of computational and predictive modeling in advancing the goals of nano-EHS has been noted previously. The technological infrastructure required for these developments includes new computational tools that transcend traditional analytical methods, which often assess a single material under specific use conditions. The new computational methods and tools allow forecasting (of variable materials, diverse uses, and new hazards), construction of quantitative structure–activity relationships (nano-QSARs), fuzzy logic, self-learning, neural networks, and artificial intelligence. Important nano-informatics requirements are summarized in Table 4.3.

Also needed is a systematic nomenclature to codify engineered nanostructures for computational analysis. The current lack of a coherent nomenclature confounds the interpretation of data sets and hampers the pace of progress and risk assessment. The International Union of Physical and Applied Chemistry (IUPAC) has developed a nomenclature for organic, inorganic, biochemical, and macromolecular chemistries (Gentleman and Chan 2009), and the Chemical Abstracts Service (CAS) has developed a cataloging system for reagents and new substances (Rowlett 1984). However, neither of these nomenclature systems is appropriate for nanostructures. For nanostructures, a systematic nomenclature based on material composition and nanoscale properties such as size, shape, core/surface chemistry, and solubility may be particularly relevant to nano-EHS activities.

Table 4.3 Important nano-informatics requirements

Data Collection and Curation	Tools/Methods for Discovery, Innovation, Communication, and Management	Social Dimensions to Information Sharing
lab automation for high-throughput collection	data mining	defining and addressing sociological issues
tools for literature data collection	machine learning	overcoming education and perception barriers
databases and data sharing	visual analytics	determining and establishing rational governance parameters
tracking error, uncertainty, and sensitivity in ENM data		
interoperability	semantic search and analysis	instituting terms of use
metadata standards	literature analysis	
nanomaterial property data	quality control	
ontologies	standards development	
taxonomies	open source	
open access		
ENM molecular structure	CSN	
advance instrumentation		
Collaboratories	predictive model development for risk and ENM design	

Development of Workforce Capacity Through Interdisciplinary Education and Training, particularly in the nano-EHS field, where a large number of research areas are converging

The market for nanotechnology-enabled products was estimated at $254 billion in 2009 and is projected to increase to $2.5 trillion by 2015 (Lux Research 2009). A corresponding increase in the number of individuals trained to work in the various sectors involved in the development and production of nanotechnology-enabled products is essential to maintaining a competitive edge in this area and for harvesting the benefits of this effort. As is the case for many cutting-edge areas of science and technology, the future of nanotechnology is inextricably linked to interdisciplinary education and training. To create new "smart" ENMs for medical applications, researchers must be well versed not only in materials science, chemistry, and physics, but also in biological sciences, physiology, pharmacology, and engineering.

NSF has played a critical role in building a pipeline of multidisciplinary researchers and engineers through its programs Nanoscale Science and Engineering Education (NSEE), Nanotechnology Undergraduate Education (NUE), Research Experience for Teachers (RET), and Research Experience for Undergraduates (REU). These programs allow for the development of education modules in nanotechnology that can be used in a broad range of settings—from K–12 through graduate education—and hence have the ability to impact the education of a broad spectrum of our society. The National Institutes of Health (NIH) has its T32 and R25 Institutional Research Training/Education Grants programs for emerging technologies, which have had a similar impact on interdisciplinary graduate education related to nanotechnology, including the use of a multiple principal investigator (PI)

mechanism. Funding targeted at interdisciplinary educational programs (e.g., NSF's Integrative Graduate Education and Research Traineeship program) and interdisciplinary research centers (e.g., The National Cancer Institute's Centers of Cancer Nanotechnology Excellence and NSF's Nanoscale Science and Engineering Centers) plays a critical role in enabling interdisciplinary nanotechnology education and training programs in ways that individual-investigator funding cannot.

These types of interdisciplinary education and training are absolutely essential to meet the significant challenges presented by nano-EHS as an emerging field. In the short term, the development of guidelines for safe handling of ENMs requires engagement of researchers and practitioners of both industrial hygiene and public health. Dissemination of these practices across the communities of scientists and engineers who develop and work with ENMs will require collaboration not only between industrial hygienists and nanoscience-focused researchers and engineers, but also with members of the education community. Likewise, the development of risk management practices and appropriate policy and regulatory strategies for nano-EHS will require basic researchers in the nanoscience community to engage and partner with individuals working in the fields of EHS, risk management, public policy, and law. Because toxics policy is currently under broad review in the United States, an investment in activities that foster this cross-fertilization is likely to help drive stability within the field of nanotechnology as well as to inform decision making on toxics policy.

In the long term, movement beyond risk management and toward a risk prevention strategy that embraces the concepts of inherently safer design and predictability should develop the best models for correlating physicochemical ENM properties with their biological and environmental impacts and robust decision-making tools based on these models. Such tools will require coordination of research and data collection from a broad variety of disciplines. Programs such as the Centers for Environmental Implications of Nanotechnology being coordinated from the University of California, Los Angeles (UCLA), and Duke University with funding by the NSF and EPA (Godwin et al. 2009; Wiesner et al. 2009) play a critical role in driving this agenda. (The multidisciplinary research integration in the UC CEIN is summarized in Section 4.8.2.)

4.5 R&D INVESTMENT AND IMPLEMENTATION STRATEGIES

Increase the Role of Industry in Nano-EHS R&D Funding

Sufficient Federal funding is required during the early discovery and incubation phases of nano-EHS research, but funding for nano-EHS research and development should also be shared by the private sector, where implementation of nano-EHS knowledge should lead to improved products with enhanced commercial value. Industry has a particular responsibility as a partner in establishing standardized testing and development of safe nanomaterials. Moreover, nano-EHS research will also contribute to new nanotechnology-based green technologies and innovative environmental cleanup strategies, with their intrinsic commercial value added. In order to contribute to sustainability, it is important that nano-EHS funding be implemented as an integral part of new product design and manufacturing rather than as a *post facto* add-on, safety mandate, or as an imposed cleanup cost.

Increase Federal Focus on Building an Accessible Infrastructure for Understanding ENM Toxicity

A key question at present is whether the U.S. Federal support for nano-EHS efforts is sufficient to build the capacity required for safe implementation of nanotechnology. EHS

spending in the United States amounted to 2.8%–5.4% of the total Federal spending on nanotechnology between 2006 and 2010. The FY2011 Federal budget proposes $1.8 billion spending on nanotechnology, with $117 million, or 6.6% of the total, earmarked for research on EHS considerations. It remains to be seen whether this budget allocation is sufficient to allow for the implementation of all the research and knowledge generation needed in terms of new methods development, coordinated ENM candidate screening, data collection, model development, risk assessment, and effective end-stage commercialization. It is important to consider that some of the Federal money for nano-EHS research has been allocated in the past to general ENM characterization and methods development and validation, rather than for specific research directed at understanding nanomaterial toxicology. There is currently insufficient funding for extensive research and analysis of the possible health consequences of ENM in food, agricultural products, and industrial processes such as printing. Another key investment should be in technological infrastructure and new instrumentation to address the diverse analytical needs for nano-EHS. While instrumentation and tools have been addressed in previous sections, it is important to highlight the need for shared user facilities where industry, academia, and government can coordinate nano-EHS research. While several national laboratories and academic institutions have outstanding facilities and infrastructure to conduct general or applied nanotechnology research, there are no shared use facilities for nano-EHS research. As a result, there is little or no transfer of knowledge and protocols. This has contributed to a lack of cooperation and disclosure, and guarded secrecy about nano-EHS efforts in the private sector, including in the food and cosmetics industries. Moreover, in food and agricultural research, the materials to be investigated are often "dirty" and demand dedicated equipment for analysis of composition and synthesis of what are more complex test systems.

Promote Cross-Sector Partnerships in Nano-EHS R&D Efforts

The promotion of collaborative partnerships between academia, government, and industry is essential for successful creation, design, development, and value capture of nanotechnology advancements, including widespread public acceptance. These partnerships are critical not only for harvesting knowledge but for enabling investment options by creating needs-driven knowledge. Dialogue is needed to overcome the reticence of industry to actively participate in nano-EHS efforts, particularly in the formative stages of strategic program development. In this regard, it is helpful to examine the efforts by some industry sectors and corporations that have promoted safety in nanotechnology development (examples are discussed in Section 4.8.4), as well as the reasons for selective non-participation in industrial surveys by other industry sectors and businesses. Important issues that have surfaced in surveys to date include the current lack of standardized ENM screening protocols, uncertainty about the regulatory environment, possibilities of inviting unnecessary scrutiny, cost-benefit factors, and public perceptions. Industry needs to see that government and universities are listening to and addressing these kinds of concerns.

Even as we are moving to more regulation of nanotechnology-based products as a result of knowledge gaps, it is highly desirable to establish private-public partnerships to change the dynamics of the current dialogue. The best R&D partnerships involve government, industry, and academia, each playing to its own strengths. Ideally, the data collection on the safe use of nanotechnology in commercial products and industrial processes should be a position of consensus rather than of unilateral enforcement. While it is currently still possible for industry to withhold data because of fear of disclosing confidential business information, a continuing reluctance to share information could prompt a change in the environmental statutes and laws to essentially demand disclosure—a situation not conducive to fostering

collaboration and trust. This possibility is evident in the recent U.K. House of Lords (2010) recommendation that noncompliance with respect to the use of ENMs in foods could serve as the basis for exclusion of food products from the marketplace.

An example of what may be achieved through product information disclosure is encapsulated in the proposed nano-information pyramid shown in Figure 4.5. This illustrates that the first tier of information disclosure could involve broad substance information collection for registration and documentation purposes. This could transition at the next level to material substance data sheets (MSDS) handled by producers, processors, and recyclers, which depending on the level of risk, may require at the next level product inserts or labeling that provide specific information about hazard, safe handling, disposal, and recycling (Maynard et al. 2006). Another solution is an incentive-based system in which voluntary business disclosure of nano-EHS data in collaboration with academia and government agencies would facilitate safety profiling that would make it easier to move to the marketplace as compared to when there is no safety information available. Combined public-and-private research efforts can also help to develop, optimize, and validate *in vitro* and *in vivo* safety assessment protocols. Specifically, nano-EHS consensus in food, medicine, and cosmetic safety requires continuous industry participation and assistance in policy formation.

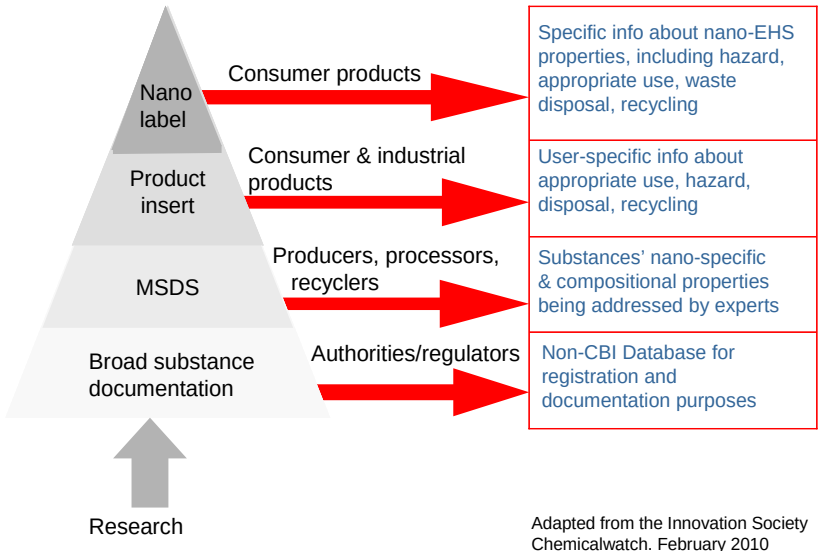

Figure 4.5. This nano-information pyramid illustrates development of an incremental information-sharing collaboration between government, academia, and industry (adapted with permission from Widmer 2010).

Private-public partnerships could also help develop the high-throughput methods, property-activity relationships, and computational methods necessary to understand any risks and hazards, and produce safer nanotechnology-based products. Not only will this promote sustainability but it will also deliver a chain of superior products capable of returning the up-front investment in nano-safety. If this kind of cross-sector interaction is established early, including dedicated funding to make it an integral part of the nanotechnology development enterprise, it will provide a precedent and strong incentive for ongoing industry participation in nano-EHS R&D. Examples of several successful private–public partnerships are highlighted in Section 4.8.4.

4.6 EMERGING TOPICS AND PRIORITIES

Role of Nanotechnology in Promoting Environmental Remediation and Sustainability, Including Through Green Manufacturing

Nanomaterials have potentially beneficial applications for future environmental remediation or as active transforming agents, sensors, and detectors. For example, iron nanoparticles can serve as powerful reductants to remove oxidized contaminants from soil and ground water as sorbents. Nanomaterials and nanodevices can be exploited for pollution prevention by functioning as components in advanced biosensors, monitors, adsorption surfaces for toxic chemicals, and new filtration membranes (Li et al. 2008; Shannon et al. 2008). Noteworthy examples include:

- Use of natural and manufactured nanostructured clays and zeolites for filtration of undesirable compounds from air or water
- Removal from groundwater of trichloroethylene (TCE) by reductive dechlorination and hexavalent chromium (Cr(VI) or Cr^{6+}) by reductive immobilization using zero-valent Fe and adsorption of nanostructured TiO_2, zeolites, nano-magnetite, or dendrimers.
- Use of polymeric membranes impregnated with silver/zeolite or photosensitive ENM to improve resistance to bio-fouling in structures in aquatic environments without the use of biocides

In addition to remediation, ENMs can help meet a growing need for point-of-use water treatment and reuse. Advancements in decentralized water treatment and reuse alleviate dependence on major infrastructure, avoid degradation of water quality within distribution networks, exploit alternative water sources for a growing population (e.g., recycled grey water), and reduce energy consumption. Future urban systems will increasingly rely on high-performance nanotechnology-enabled water monitoring, treatment, and reuse systems that target a wide variety of water pollutants, are affordable, easy to operate, and contribute toward a zero discharge paradigm, which is the ultimate goal of sustainable urban water management. Examples of ENMs that can enable this vision are summarized in Table 4.4.

Table 4.4 Example of opportunities for ENM in water treatment and reuse

Desirable ENM Properties	Examples of ENM-Enabled Technologies
Large surface area to volume ratio	Superior sorbents with high, irreversible adsorption capacity (e.g., nanomagnetite to remove arsenic and other heavy metals)
Enhanced catalytic properties	Hypercatalysts for advanced oxidation (TiO_2& fullerene-based photocatalysts) and reduction processes (Pd/Au to dechlorinate TCE)
Antimicrobial properties	Disinfection without harmful disinfection by-products (e.g., enhanced UV and solar disinfection by TiO_2andderivatized fullerenes)
Multi-functionality (antibiotic, catalytic, etc.)	Fouling-resistant (self-cleaning), functionalized filtration membranes that inactivate virus, fungal, and bacterial threats, and destroy organic contaminants
Self-assembly on surfaces	Surface structures that decrease bacterial adhesion, biofilm formation, and corrosion of water distribution and storage systems
High conductivity	Novel electrodes for capacitive deionization (electro-sorption) and low-cost, energy-efficient desalination of high-salinity water
Fluorescence	Sensitive sensors to detect pathogens and other priority pollutants

Green nanoscience aims to create and apply design rules proactively for greener nanomaterials as well as for developing efficient and reproducible synthetic strategies to

produce materials with defined composition, structure, and purity (Hutchinson 2008). As such, green nanoscience incorporates the 12 well-known principles of green chemistry in the design, production, and use of ENMs (Table 4.5).

Table 4.5 Applying green chemistry principles to the practice of green nanoscience

Green Chemistry Principles	Designing Greener NMs and NM Production Methods	Practicing Green Nanoscience
P1:Prevent waste P2:Atom economy	Design of safer NMs (P4, P12)	Determining the biological impacts of nanoparticle size, surface area, surface functionality; utilize this knowledge to design effective safer materials that possess desired physical properties; avoid incorporation of toxic elements in nanoparticle compositions.
P3:Less hazardous chemical synthesis P4: Designing safer chemicals	Design for reduced environmental impact (P7, P10)	Study NM degradation and fate in the environment; design material to degrade to harmless subunits of products. An important approach involves avoiding the use of hazardous elements in nanoparticle formulation; the use of hazard-less, bio-based nanoparticle feedstocks may be a key.
P5:Safer solvents/reaction media P6: Design for energy efficiency	Design for waste reduction (P1, P5, P8)	Eliminate solvent-intensive purification by utilizing selective nanosyntheses—resulting in great purity and nanodisparity; develop new purification methods (e.g., nanofiltration) that minimize solvent use, utilize bottom-up approaches to enhance material efficiency and eliminate steps.
P7:Renewable feedstocks P8:Reduce derivatives	Design for process safety (P3, P5, P7, P12)	Design and develop advanced syntheses that utilize more benign reagents and solvents than used in the "discovery" preparations; utilize more benign feedstocks, derived from renewable sources, if possible; identify replacements for highly toxic and pyrophoric reagents.
P9:Catalysis P10: Design for degradation; design for end of life	Design for material efficiency (P2, P5, P9, P11)	Develop new, compact synthetic strategies; optimize incorporation raw material in products through bottom-up approaches; use alternative reaction media and catalysis to enhance reaction selectivity; develop real-time monitoring to guide process control in complex nanoparticle syntheses.
P11:Real-time monitoring and process control P12:Inherently safer chemistry	Design for energy efficiency (P6, P9, P11)	Pursue efficient synthetic pathways that can be carried out at ambient temperature rather than elevated temperatures; utilize noncovalent and bottom-up assembly method near ambient temperature; utilize real-time monitoring to optimize reaction chemistry and minimize energy costs.

Note: The principles are listed, in abbreviated form, along with the general approaches to designing greener nanomaterials and nanomaterial production methods and specific examples of how these approaches are being implemented in green nanoscience. Within the figure, P*X*, where *X*=1–12, indicates the applicable green chemistry principle (Hutchinson 2008). (Adapted from Anastas and Warner 1998, p. 30, with permission from Oxford University Press.)

Green nanomaterials/processes can substitute for dangerous materials and processes shown to pose more risk. Nanotechnology-inspired production is likely to also lead to more efficient

use of materials and lower energy needs, thereby decreasing the environmental footprint. Nonetheless, the entropic penalties associated with creating order at the atomic scale set boundaries on the possible gains that are achievable by applying ENMs to solve environmental problems. For example, theoretical gains in adsorptive efficiency using nanostructured iron oxides for arsenate oxo-anion removal are more than outweighed by their necessary energy investments and associated costs when compared with conventional ferric chloride salts.

Safe-By-Design Approaches to Promote Sustainable Implementation of Nanotechnology

The awareness of safe-by-design ENM approaches is moving the nano-EHS field toward thinking about the possible proactive implications of specific applications of nanotechnology at the design and development stages, rather than waiting to reactively consider impacts until after the technology has been matured and deployed (Hutchinson 2008; Nel et al. 2009). An understanding of hazardous ENM properties is essential for safe design from a biological and lifecycle perspective. While there is no single design feature that currently fits this description, possible approaches that might contribute to this area are being identified. It is important to note that redesign of some of these properties may affect ENM performance characteristics (e.g., electrical conductivity, thermal conductivity, or magnetic properties) that are essential for technology or product development. Thus, while the potential impact on product performance must be properly explored, it is possible that certain compromises may result.

Focusing on ENM exposure control rather than on suppressing ENM intrinsic reactivity that contributes to toxicity might be a useful compromise strategy (Nel et al. 2009). Thus, risk abatement options worthy of consideration include tailored coatings that reduce bioavailability or mobility. The modern chemical industry has demonstrated that some substances can be reengineered to create safer, greener, and yet efficient products (Hutchinson 2008). Encouraging examples include the substitution of branched alkylbenzene sulfonate surfactants that cause excessive foaming in the environment, with biodegradable linear homologues (Alvarez et al. 2009). It is therefore important to discern the specific critical functionalities and physicochemical properties that make ENMs harmful, then reengineer these properties to achieve safer products.

Another route to mitigate ENM toxicity is to exploit the tendency of nanoparticles to aggregate in natural and biological media, which naturally decreases their bioavailability and possible bio-reactivity (Nel at al. 2009). Colloidal stabilizers with kinetic degradation in certain conditions allow initial ENM dispersion as desired but with a programmed loss of their dispersibility and resulting aggregation over time, controlling their nano-specific properties. Surface coating is a design feature being exploited to improve nanoparticle safety by preventing undesired bio-reactivity. For instance, TiO_2, ZnO, and Fe_2O_3 nanoparticles within cosmetic formulations (e.g., sunscreen lotions) are often coated with a hydrophobic polymer (e.g., poly[methyl vinyl ether]/maleic acid) to reduce direct contact with the human skin (Lee et al. 2007). Coating of nanoscale zero-valent iron (NZVI) with polyaspartate not only prevents particle aggregation to enhance nanoparticle mobility in contaminated groundwater so as to reach and reductively dechlorinate trichloroethylene, but it also mitigates NZVI toxicity to indigenous bacteria, enhancing their possible co-participation in the cleanup process (Kirschling et al. 2010; Xiu et al. 2010). This also suggests that artificial as well as natural coatings (e.g., dissolved natural organic matter) can be used to mitigate ENM toxicity and alter impacts on microbial ecosystem services.

An extension of this principle is the use of polymer and detergent coatings that reduce eukaryotic cell particle contact and uptake by steric hindrance. Many such coatings are environmentally labile or degradable. Thus, an initially nontoxic material may become hazardous after shedding its coating if resulting aggregation does not reliably remove it from the system. An important design feature would be to enhance the stability of coating materials or design them originally to prevent adverse biological responses. Coating nanoparticles with protective shells (i.e., core-shell systems) can also reduce the dissolution and release of toxic ions (Kirchner et al. 2005), while also providing a physical barrier against cellular uptake if undesired. Suitable shell materials include biocompatible organic or inorganic substances such as PEG-SiO_2, gold, and biocompatible polymers (Jain et al. 2005).

Altered dissolution rates and limited metal ion leaching could also be deliberately achieved by material doping (e.g., doping of ZnO with Fe_3O_4, leading to decreased cellular and zebrafish toxicities) (George et al. 2010; Nel et al. 2009). Modification of surface charge is another approach towards reducing nanoparticle toxicity (Hauck et al. 2008; Khan et al. 2007). For example, layer-by-layer coatings of polyelectrolytes on gold nanorods decrease their cellular uptake via modified surface charge and functionality. For the safe design of materials that form bio-persistent fibers (e.g., CNTs), it is important to consider aspect ratios, hydrophobicity, and stiffness (Poland et al. 2008). Chemical functionalization of short (<5 μm) multiwalled carbon nanotubes (MWCNTs) can provide stable dispersions of individual tubes in physiological media, thereby allowing their safe use as imaging and drug-delivery devices (Kostarelos 2008). Functionalization with small hydrophilic groups is a safety feature allowing the formation of stable dispersions with high excretion rates (Kostarelos 2008). Thus, ENM coatings and surface properties can produce diverse properties that enhance or diminish certain types of exposure, depending on the application, chemistry, design, and ENM properties. Identifying desired and undesired specific ENM functions and possible risks, and applying safe-by-design principles to realize these properties while mitigating risks, represent attractive objectives for this strategy.

Finally, consideration should also be given to material disposal, life cycle fate, and containment. Several priority research areas can inform the ecologically responsible design and disposal of ENMs. As a first step to understanding potential impacts resulting from incidental or accidental releases of nanomaterials and evaluating the need for ENM interception, containment, or treatment technologies, we should understand sources and the scale of potential discharges into various environmental compartments (including ENMs leaching from commercial products during their entire life cycles) (Alvarez et al. 2009). This requires having an inventory of the magnitude of ENM use within defined spatial domains and the possible flow of ENMs across domains. Quantification of potential fluxes to the environment from both point and non-point sources is also a priority that can only be accomplished after developing appropriate analytical tools or identifying sentinel species that can be monitored to detect environmental presence and pollution by ENMs.

Furthermore, ENM waste will enter protective environmental infrastructures such as sewage treatment, air filters, bag houses, and landfill liners. It is unknown how accidental or deliberate ENM releases may affect the performance of such processes (e.g., toxicity to probiotic bacteria essential in activated sludge) and how effective barrier technologies (e.g., landfill liners) would be at intercepting and containing ENMs. Knowledge about the flows of ENMs from different stages in their life cycles to waste-handling institutions will provide a basis for prioritizing research on this topic. An example of impactful research in this area appears in Section 4.8.3. Distinct properties that make ENMs so useful in a vast spectrum of products are also those that may challenge their recyclability. Specific guidelines and possibly

product labeling are needed to safely and responsibly dispose of and recycle the waste products that contain ENMs.

Role of nanotechnology in Agriculture and Food Systems, including Enhancement of Food Safety as well as ability to demonstrate that ENMs in foods are safe

Nanotechnology has an important role in creating a safer food system (Scott and Chen 2003; Srinivas et al. 2009; Tarver 2006; Weiss et al. 2006). The food supply chain can and will be affected by the utilization of nanotechnology at each point in the system along the supply chain—from production through domestic consumption (Scott 2007). While the advances and technological impacts of nanotechnology on agriculture and food systems in the past 5–6 years are limited due to its relative "newness" in this sphere, some encouraging results have been obtained in the various agri-food sectors discussed in Chapter 5. From the perspective of food safety, nanotechnology has much to offer, including:

- Carbon nanotube and surface-enhanced Raman spectroscopy (SERS) nanosensor arrays can help ensure the safety of the food supply by identifying the presence of pathogens, toxins, and bacteria, and actively eliminating their impact
- Edible nanoparticle sensors can detect food quality and safety
- DNA barcoding methods are a simple and low-cost way to detect the presence of bacteria and other pathogens in foods
- New biosensors can detect the presence of avian influenza virus
- Nano-sensing formats can be used for food packaging security, freshness, and sustainability

In order to promote and expand the role of nanotechnology in food and agriculture, it will be necessary to address the inherent safety of nanomaterials that enter the food chain as well as articulate the benefits of nanotechnology in this sector to the public. In addition to concern about the health and safety issues of possible new "nano-produced" or "nano-monitored" foods, there is a concern among some NGOs about broad social and ethical issues. One such concern is that nanotechnology will become concentrated within multinational corporations and that this could impact the livelihood of the poor. These areas of health and safety and the impact on agriculture infrastructure are currently an area of intense interest and much debate, mirroring similar concerns and issues in some previous emerging technology situations.

Some public skepticism can be influenced by factors such as a fear of novel risks, trust or lack of trust in the regulatory process, and wider social and ethical concerns. A recent study by Britain's House of Lords (2010) offers several recommendations to build public confidence and trust: (1) there should be increased research on toxicological impacts of nanomaterials, particularly in areas relating to risks posed by ingesting nanomaterials; (2) a definition of nanomaterials should be added to food legislation to ensure that all nanomaterials that interact differently with the body as the result of their small size be assessed for risk before they are allowed on the market; (3) food regulators and the food industry should collaborate to develop a database of information about nanomaterials in development to anticipate future risk needs; and (4) food regulatory agencies should create and maintain a list of products containing nanomaterials as they enter the market, to promote transparency.

Issues of perceptual risk and social and ethical concerns might be addressed with a number of steps: (1) develop a broad coalition of scientists, engineers, farmers, food processors, and manufacturers, interested NGOs, government agencies, and consumers to engage in discussions that will promote common understanding and agendas; (2) develop

comprehensive interactions with the FDA and EPA to discuss whether regulations are required; (3) develop public–private partnerships in which agricultural and food companies interact with universities, the USDA, EPA, and FDA; and (4) offer increased opportunities for the public to participate in open forums to help create an intelligent understanding of concerns and benefits.

The following key priorities have been identified for the next decade:

- Develop validated nano-EHS screening methods and harmonized protocols that promote standardized ENM risk assessment at levels commensurate with the growth of nanotechnology
- Obtain active industry participation and NGOs in nano-EHS, including hazard and risk assessment, lifecycle analysis, non-confidential product information disclosure to assess exposure scenarios, and use of nanomaterial property-activity relationships to implement safe-by-design for product life cycle strategies
- Introduce environmentally benign nanomanufacturing methods and using nanotechnology to replace commonly used processes, compounds and products with adverse effects to human health and the environment
- Develop risk reduction strategies that can be implemented incrementally through nano-EHS research, commercial nanoproduct data collection and the use of streamlined decision-making tools
- Develop high-throughput approaches, nanoinformatics and *in silico* decision-making tools that can help model and predict nanomaterial hazard, risk assessment, and safe design of nanomaterials as an integral part of new program development.
- Develop clearly defined strategies for nano-EHS governance that takes into consideration knowledge gathering and stepwise decision-making that ultimately leads to evidence-based and sustainability-enhancing decision-making.

4.7 BROAD IMPLICATIONS FOR SOCIETY

Although academia, industry, and government deal with real risk issues, the public is more prone to react to unproven perceived risks, and their views are often shaped by often-unsubstantiated reports coming from popular news media and NGOs (Pidgeon et al. 2009). As long as nano-EHS data gaps remain, threats of perceived risks, despite lack of evidence, will persist, potentially hindering market and technology development. NGOs are continuously pushing for concrete regulations (Table 4.2), and some like the Natural Resources Defense Council and Friends of the Earth continue to argue that voluntary data collection programs should be mandatory. A key issue therefore for academia, industry, and government is to effectively communicate, inform and involve the participation of the public in the dialogue on the beneficial implications of nanotechnology, the potential for risk, and what is being done to ensure safe implementation of the technology. Due to the complex and multidisciplinary nature of nanoscience and nanotechnology, knowledge transfer and public education has not been effective and needs urgent attention. Strategies for communication and education of the public are discussed in Chapter 12.

Closely associated with the issue of perceived risks is the safety of common consumer products that contain nanotechnology-based ingredients; these include such products as sunscreens, soaps, toothpastes, clothing, food, and cosmetics.[24] Greater transparency is

[24]For examples of specific nanotechnology-based products, see the Project for Emerging Nanotechnologies (PEN) consumer products inventory at http://www.nanotechproject.org/inventories/consumer.

required in disclosing the presence of nanomaterials in these products, including why their addition and use provides a better product, as well as specific technical data on their compounding and formulation. Due to perceived risks, some information about nanotechnology-based products is deliberately withheld instead of being disclosed, which in the long run could be counterproductive to credibility, transparency, perception, and image. The nano-information pyramid and proactive recommendations about how package inserts or labeling may be introduced with care and forethought could help remove such uncertainty (Figure 4.5). It is also important to explain to the public that nanotechnology can play an important role in promoting food safety, environmental remediation, better medical therapies, and product enhancements.

4.8 EXAMPLES OF ACHIEVEMENTS AND PARADIGM SHIFTS

4.8.1 Examples of predictive Toxicological Paradigms that Connect *In vitro* Hazard Assessment to *In vivo* injury in intact animals

Contact person: André Nel, University of California, Los Angeles (UCLA

Both the National Toxicology Program and the National Research Council (NRC) in the U.S. National Academy of Sciences (NAS) have recommended that toxicological testing in the 21st century evolve from a predominantly observation science at the level of disease-specific models to predictive science models focused on broad inclusion of target-specific, mechanism-based biological observations (National Research Council 2007; National Toxicology Program 2004). Predictive toxicology is an essential tool for successful drug development because it is crucial to identify and exclude new drug candidates with unfavorable safety profiles as early as possible (Rosenkranz et al. 1999). Predictive toxicology has recently been introduced to industrial chemical toxicity and is also relevant to the assessment of ENM hazard (Benigni et al. 2007). A predictive toxicological approach for ENM hazard screening could, for instance, include the assessment of injury at cellular and molecular levels as a way to predict adverse biological effects and health outcomes *in vivo* (Duffin et al. 2007; George et al. 2010; Meng et al. 2009; Monteiller et al. 2007; Rushton 2010). Evidence that such a mechanistic approach is possible emerged from the study of the adverse health effects of ambient particulate matter (Fubini 1997; Vallyathan et al. 1997; Xia, Kovoshich, Liong, Maädler et al. 2008). The physicochemical properties of ambient ultrafine particles (UFP), including their small size, large surface area, and high content of redox-cycling organic chemicals and transition metals, are instrumental in these particles' pro-inflammatory effects in cellular targets such as macrophage, epithelial, endothelial, and dendritic cells (Nel 2005). Similar responses in the lung and cardiovascular system likely play a role in the pathogenesis of inflammatory disease states such as allergic airway inflammation and atherosclerosis.

While no definitive disease processes have emerged as a result of ENM exposure in humans (Xia et al. 2009), a number of research studies have shown correlation between toxicological effects at the cellular level and organ injury at the intact animal level. Becher et al. (2001) showed good correlation between the pro-inflammatory effects (IL-6, TNF-α and MIP-2) of stone particles (e.g., quartz, feldspar, and mylonite) in macrophages and epithelial cells and their ability to generate polymorphonuclear (PMN) inflammation in the lungs of rats. Sayes et al. (2007) failed to demonstrate a correlation between the cellular and *in vivo* results when comparing carbonyl iron, crystalline silica, amorphous silica, nano-ZnO, and fine-sized ZnO in a well-designed dose-response study. This included measurement of lactate dehydrogenase (LDH) release, metabolic activity (MTT assay), and cytokine production (IL-6, TNF-α and

MIP-2) in rat lung epithelial cells and alveolar macrophages versus measurement of PMN cell count or LDH values in the BAL fluid of rats. However, upon reanalysis of the previous data set, Rushton et al. (2010) demonstrated that there was indeed a positive correlation if the particle mass was converted to SAD and the analysis performed at the steepest slope of the dose-response curve. Thus, the picture that emerged in the reanalysis was a good correlation between MIP-2 levels in cells versus the PMN response in the lung or LDH release from cells versus the PMN response in the BAL fluid.

The conclusion was that it is possible to show *in vitro*/*in vivo* predictions when using a surface area-normalized response metric. The Oberdörster laboratory (Rushton et al. 2010) independently demonstrated through cell-free and cell-based measurement of reactive oxygen species (ROS) production, LDH release, and the use of an IL-8 promoter-luciferase reporter assay that there is a correlation between *in vitro* and acute pulmonary inflammation (PMN levels) in rats being challenged by intratracheal instillation of seven distinct particle types (Au, nano-TiO_2, fine TiO_2, NH2-PS, Ag, elemental carbon, and Cu). In addition, Ken Donaldson's laboratory (Edinburgh, Scotland) has demonstrated that IL-8 production in A549 cells, exposed to a panel of low toxicity (e.g., TiO_2, carbon black) versus highly reactive quartz and metal (e.g., Ni, Co) nanoparticles, correlates with BAL polymorphonuclear cell counts in Wistar rats (Duffin et al. 2007; Monteiller et al. 2007). This group also demonstrated that the expression of the particles' SAD versus PMN counts in the BAL fluid yields a shallow dose-response curve for low-toxicity particles, whereas highly reactive materials produced a steeper dose-response curve due to a high "surface reactivity." Thus, although such predictive modeling and correlations remain at an early stage, it appears that if appropriate response metrics are chosen and corrected to appropriate dose metrics, it is possible to develop reliable scientific paradigms that allow cellular screening to predict *in vivo* hazard potential (Rushton et al. 2010).

Even if a link is established between *in vitro* and *in vivo* toxicological outcomes, human disease pathogenesis is dependent on real-life exposures at toxicologically relevant doses and distinguishes between dose-dependent acute versus chronic exposures. Fate and transport as well as exposure assessments are key ingredients that are not included in the predictive toxicological paradigm but are important ingredients for proper risk assessment. There are also chronic toxicological scenarios that involve a series of initiation and promoter events that cannot be simulated by a one-step toxicological paradigm. An example is the oncogenesis that is required to transform chronic granulomatous peritoneal inflammation into a mesothelioma in response to asbestos fibers (Poland et al. 2008; Takagi et al. 2008). Although a screening assay for "frustrated phagocytosis" in response to long and biopersistent fibers may predict chronic mesothelial inflammation, this response profiling will not shed light on the mutagenic events that are required for development of a mesothelioma. This may require another event such as p53 gene knockout to elucidate the secondary event (Takagi et al. 2008).

4.8.2 Example of the use of multidisciplinary research in the University of California Center for the Environmental Impact of Nanotechnology Leading to the establishment of Knowledge for the Safe Implementation of Nanotechnology in the Environment

Contact person: André Nel, University of California, Los Angeles (UCLA)

The mission of the University of California Center for the Environmental Impact of Nanotechnology (UC CEIN) is to develop a broad-based predictive scientific model (Godwin et al. 2009; Meng et al. 2009) premised on ENM properties and behavior that determine ENM spread to the environment, bioaccumulation, trophic transfer, and catalysis of potentially

hazardous interactions at cellular, tissue, organism, and ecosystem levels (Figure 4.6). The key components of this multidisciplinary model include the following: (1) the construction of well-characterized compositional and combinatorial ENM libraries to reflect the most abundant materials in the marketplace; (2) the fate and transport of ENM, including methods of release and physicochemical and transport properties that could lead to interactions with biological substrates; (3) biomolecular and cellular injury mechanisms that relate to bio-physicochemical interactions at the nano-bio interface (Nel et al. 2009); (4) use of injury mechanisms and bio-physicochemical interactions at the nano–bio interface to perform high-throughput screening in tissue culture cells, bacteria, yeast, and embryos; (5) use of the *in vitro* relationships to understand the possible harm to different strata or trophic life forms in freshwater, seawater, and terrestrial environments, including the identification of sentinel species to screen for ENM hazard in the environment; and (6) computational decision-making tools that utilize data capture and processing in the center for machine learning and provide a series of modeling predictions (Figure 4.6).

Predictive science as practiced at UC CEIN refers to each scientific discipline performing research that predicts or informs every other discipline what those investigators may expect to find if they utilize a common set of compositional ENM libraries as well as materials that are made to systematically vary property or property sets to study biological effects at cellular, organism and population level. An attempt is made to elucidate cellular, bacterial, yeast or embryo stress responses, including through high throughput screening, that are also relevant to whole organisms that are being studied at increasing trophic level in freshwater, seawater and terrestrial mesocosms. Fate and transport assessment as well as multi-media modeling are performed to determine how the alteration of the primary material properties in response to real-life environmental media may contribute to ENM spread, exposure, bio-accumulation and bio-processing. Computational biological and computerized decision tool are involved in data integration for purpose of hazard ranking, exposure modeling, risk profiling, and construction of property–activity relationships. These research activities are being combined with educational programs to inform the public, future generations of scientists, Federal and state agencies, and industrial stakeholders of the importance of safe implementation of nanotechnology in the environment.

Since its founding in September 2008, the UC CEIN (http://cein.ucla.edu) has successfully integrated the expertise of engineers, chemists, colloid and material scientists, ecologists, marine biologists, cell biologists, bacteriologists, toxicologists, computer scientists, and social scientists into a synergistic research program that has demonstrated the feasibility of using well-designed and well-characterized metal oxide libraries (TiO_2, CeO2, and ZnO) as well as property variations (e.g., size, shape, dissolution, and band gap tuning) to study ENM behavior in different environmental media and under different biological conditions (Keller et al. 2010; López et al. 2010; Miller et al. 2010). The implementation of this research is being facilitated by the development of protocols to harmonize particle suspension, dispersal, and initiation of experiments under freshwater, seawater, and tissue culture conditions (Ji et al. 2010). This illustrates the importance of multidisciplinary collaboration and harmonization efforts at national and international levels.

Collaborative research at the UC CEIN has identified the key material properties that lead to aggregation and sedimentation of the metal oxides in seawater, freshwater, and groundwater environments, and has also illustrated the ease with which these nanoparticles can be stabilized by capping agents under freshwater conditions, including the likelihood of inhibiting or averting spread to wastewater treatment plants and storm-water runoffs (Keller et al. 2010; Wang and Keller 2009; Wang et al. 2008). The availability of the nanoparticle

libraries has facilitated the implementation of rapid-throughput screening studies that utilize a robotized and automated high-throughput screening laboratory, epifluorescence microscopy, and reporter cell lines to perform hazard ranking and analysis of the property–activity relationships at the cellular level that may predict *in vivo* toxicity (George et al. 2010). The differential toxicity at cellular level has been further reflected by similarities and differences in the toxicity of these materials in bacteria, algae, phytoplankton, germinating seeds, sea urchins, and zebrafish embryos (Miller et al. 2010; Priester et al. 2009).

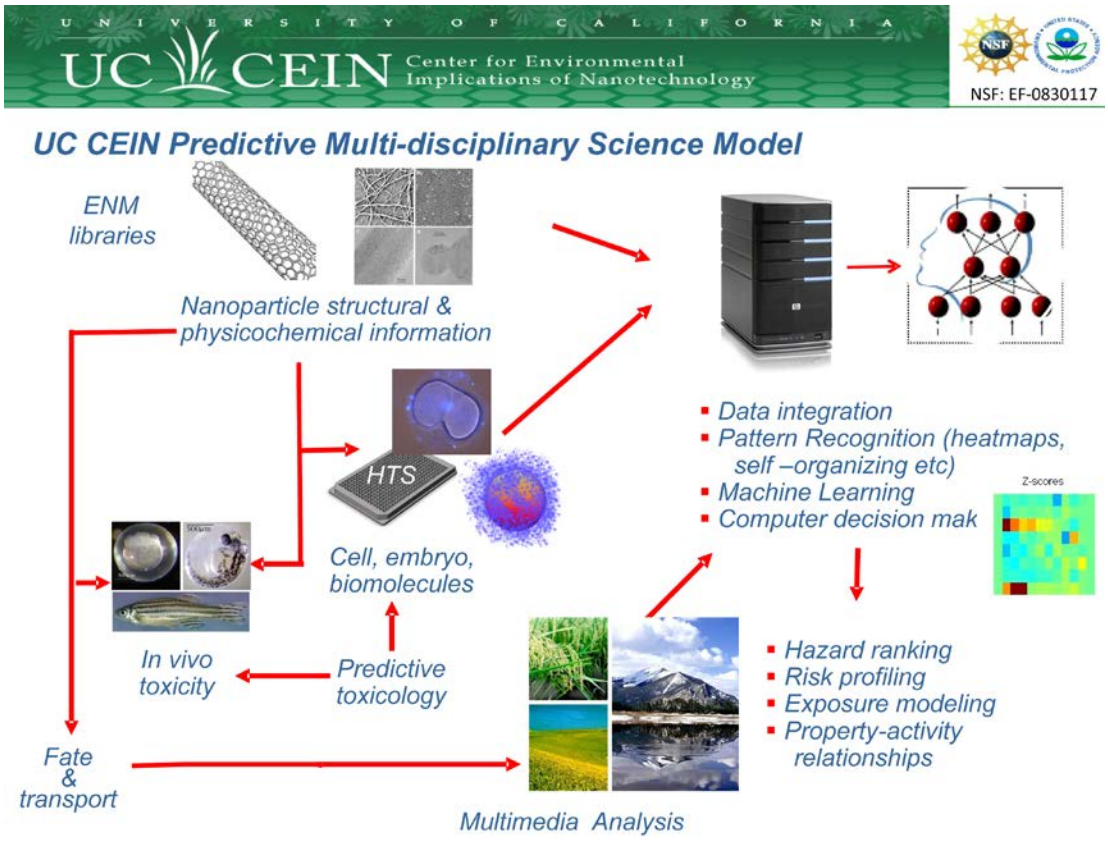

Figure 4.6. UC CEIN uses a predictive multidisciplinary model for hazard ranking and risk profiling (courtesy of V. Castranova).

There are other illustrations of the importance of the UC CEIN multidisciplinary approach to generating knowledge about nano-EHS. Mesocosm studies being carried out in collaboration with dynamic energy budget modeling have demonstrated that specific ENM properties contribute to the environmental impact at the population level and bioaccumulation at higher trophic levels in terrestrial and freshwater environments. The UC CEIN has obtained strong confirmation of the high toxicity of ZnO in primary producers in aquatic environments and could ascribe that to particle dissolution and the release of toxic Zn^{++}. This relationship was confirmed by high-throughput screening and property-activity analyses that have allowed the synthesis of less toxic ZnO nanoparticles through Fe doping (George et al. 2010). The accompanying change in the particle matrix decreased Zn^{++} shedding, thereby lowering toxicity in cellular assays, bacteria, zebrafish embryos, and rodent lungs. Another potentially useful procedure for exposure reduction involved ENM removal from the experimental aqueous systems through optimal pH destabilization, coagulant dosing, sedimentation, and ultrafiltration. This research also allowed computerized modeling to study nanoparticle

aggregation under various environmental and experimental conditions. Data capturing and analysis in the computerized expert system allow the development of novel feature selection algorithms to screen and rank nanoparticle properties to establish quantitative property–structure relationships. In summary, the integration at UC CEIN of multidisciplinary scientific platforms has been a particularly fruitful pathway to better understanding of environmental, health, and safety aspects of nanotechnology.

4.8.3 Quantitative Assessment of Environmental Exposure to Engineered Nanomaterials From Wastewater Systems

Contact person: Paul Westerhoff, Arizona State University, Tempe

Wastewater treatment plants (WWTPs) are major sources of ENM introduction into aquatic and terrestrial ecosystems. With more than 16,000 WWTPs in the United States alone that serve more than 75% of the population, WWTPs serve as interceptors of materials from residential, commercial, and industrial sources. The commercial introduction of engineered nanomaterials is already leading to a detectable footprint in sewage at WWTPs, such that it is possible to differentiate ENMs from natural colloids containing similar elements. Studies of these systems are beginning to demonstrate how properties of ENMs affect their removal from biological wastewater treatment and lead to their distribution in liquid effluent discharged to lakes and rivers or biosolid sludges that are often applied to land-based disposal sites such as agriculture crops.

Commercial products containing ENMs have been widely used for more than a decade. Titanium dioxide is an example of an ENM used for many years. Several toothpaste products that are being disposed into sewage systems were analyzed and observed to contain aggregates (200 nm–500 nm in size) of near-spherical primary TiO_2 nanoparticles (30–50 nm in size) suspended in an organic matrix (Kiser et al. 2009). Electron microscopy imaging and elemental composition were greatly enhanced by removing the organic background matrix, by applying hydrogen peroxide, and by heating to 60°C. Other larger-sized titanium materials were identified in wastewater, including angular micron-sized titanium dioxide being mined and used in paints as well as nanostructured silver." The Westerhoff group has demonstrated that products containing nanostructured silver (e.g., some fabrics, shampoos, detergents, towels, and toys) release ionic and nano-size silver during use, some of which finds its way into sewage systems (Benn and Westerhoff 2008; Benn et al. 2010). Likewise, it was demonstrated that fullerenes released from cosmetic products can be washed into sewage systems (Benn, Westerhoff, and Herckes 2010).

This experimental work helps confirm estimates that predict ENM release as part of lifecycle assessments. These models predict that TiO_2 will occur at the highest levels among several types of ENMs. Results from sampling at one WWTP are shown in Table 4.6. Overall, the facility removed nearly 80% of the influent titanium. Titanium was accumulated in the biosolids (settled bacterial materials). Titanium dioxide nanoparticles were imaged in the samples: (1) liquid effluent contained primarily nanoscale nearly spherical TiO_2 and (2) biosolids contained spherical nanoscale TiO_2, angularly shaped micron-sized TiO_2, and micron-sized sediment containing titanium, silicates, and other elements. Sampling at a dozen other WWTPs is showing similar trends and indicate that the type of wastewater treatment (e.g., fixed vs. attached bacteria, or sedimentation vs. membrane bioreactors) affects the potential to remove ENMs such as TiO_2.

Because ENMs other than TiO_2 are not yet used in high enough quantities, Westerhoff's group has developed laboratory batch experiments to compare ENM removal capabilities. Batch

sorption tests between ENMs and wastewater bacteria show that different types of ENMs exhibit different affinities for bacterial surfaces (Figure 4.7). They have shown that standard protocols of EPA's Office of Pollution Prevention and Toxics (OPPT) used to evaluate organic chemical pollutant removal during wastewater treatment are not suitable for ENMs, and new protocols are required (unpublished data). Separate long-term operational experiments that simulate WWTPs indicate that ENMs in mg/L quantities in sewage have negligible effects on the biological function (nutrient removal) of WWTPs (Wang, Westerhoff, and Hristovski 2010).

Table 4.6 Titanium concentrations across a wastewater treatment plant

Sampling Location	Titanium Concentration (µg/L)	Biosolids Concentration (µg/g-solids)
Raw sewage	180±51	
After primary settling	113±63	
After activated sludge and secondary settling	50	
After tertiary filtration	39	
Biosolids from primary settling		257
Biosolids from secondary settling		8139

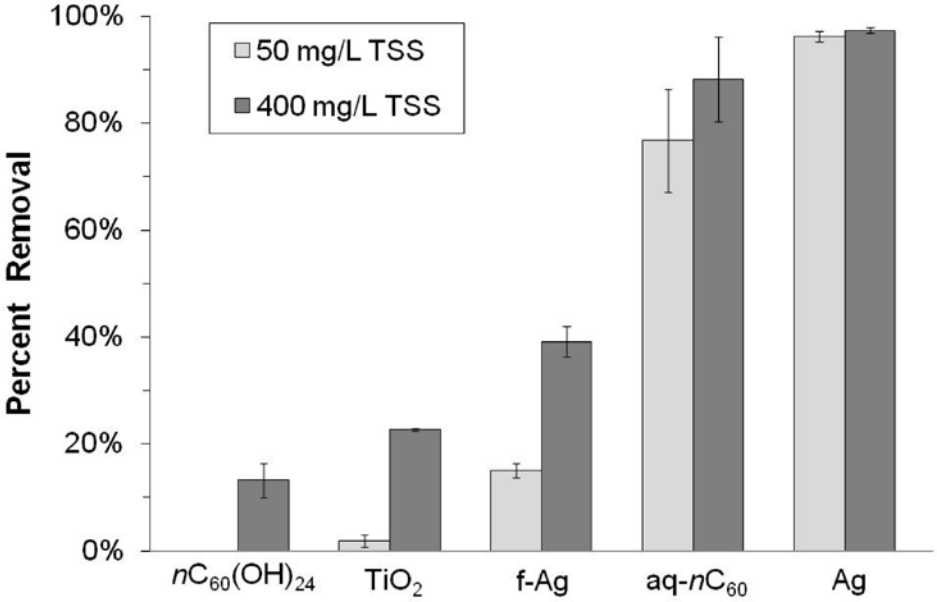

Figure 4.7. Propensity of ENMs to biosorb to wastewater bacteria (Kiser et al. 2010).

While Westerhoff's group has made significant progress in understanding the fate of ENMs during wastewater treatment and their likelihood to enter aquatic systems (river and streams), they are just beginning to understand the fate of ENMs in biosolids that may be land-applied, incinerated, or otherwise disposed. Improved analytical techniques are required to differentiate ENMs from natural or non-engineered forms of colloids of similar composition (e.g., titanium as discussed earlier, or silver from silver chloride). National reconnaissance monitoring projects should be conducted to assess current levels of ENMs in

wastewaters (raw sewage and effluents), biosolids, and rivers receiving wastewater. The beneficial effects of ENM removal at WWTPs could be greatly enhanced by understanding the fundamental interaction of ENMs of different size, charge, and composition with the surfaces of different types of bacteria (gram negative or positive, filamentous, etc.).

4.8.4 Public-Private Partnerships for Nano-EHS Awareness and Risk Reduction Strategies

Contact persons: David Grainger, The University of Utah, Salt Lake City, and Santokh Badesha, Xerox Corporation

Industries interested in commercializing emerging nanotechnologies face the usual market risks of any new product development, but these risks are compounded by the uncertainties of worker and consumer safety, unknown regulatory restrictions, and possible public backlash in the current era of disinformation and unknowns (FramingNano 2010; House of Lords 2010; Maynard et al. 2006; Pidgeon et al. 2009; Warheit et al. 2006; Xia et al. 2009). Additionally, for companies operating multinationally, there is high probability that any regulations imposed on nanotechnology's use and dissemination will be highly variable across borders (Lux Research 2007). Private-public partnerships (PPPs) can help provide both the structures and conduits for information flow to and from nanotechnology stakeholders to stymie reaction and stigma that might otherwise unfairly plague this developing industry at this early stage (ChemicalWatch 2010). A variety of PPP research models are available, including those of the U.S. domestic microelectronics organization Sematech (http://www.sematech.org/corporate), the European Union's Sixth and Seventh Framework Programmes (FP6 and FP7; Aguar and Murcia Nicolás 2008), and the U.S. government's National Institute of Standards and Technology (NIST) Advanced Technology Partnership program (NIST 2009). Nonetheless, there are currently few readily recognized or known PPPs that link governments or NGOs with private companies to jointly produce risk governance, best practices, and safety guidelines in nanotechnology and commercialization. Yet, it is likely to be in the best interests of businesses to foster an open dialogue with the various public and private constituents involved in the current discussions of the risks and benefits of nanotechnologies. The PPP mechanism is well suited to promote stakeholder interests and transparency in developing nano-EHS risk governance.

One example of a working nano-safety partnership is the DuPont and Environmental Defense NANORisk Framework (2007), an open information-gathering system to generate data to help support decisions and practices concerning the safe production and use of nanomaterials. Under development since late 2005, the pioneering program also offers guidance on how to communicate information and decision processes to key stakeholders. The intent of the Framework is to "promote responsible development of nanotechnology products, facilitate public acceptance, and support the development of a practical model for reasonable government policy on nanotechnology safety." The Framework strategy seeks to "define a systematic and disciplined process for identifying, managing, and reducing the risk of unintended consequences from engineered nanomaterials across all stages of a product's 'lifecycle'" (EDF 2008).

Significantly, as a model, DuPont's private-public partnering efforts to address questions about nanomaterials extend to other working relationships it has with NGOs, including its involvement with the OECD. Through OECD's Business Industry Advisory Committee and related activities in OECD's Working Party on Manufactured Nanomaterials, DuPont helps to provide information on potential nanotechnology-related health and environmental issues. DuPont is also involved with the American Chemistry Council Nanotechnology Panel,

providing information and recommendations to the U.S. EPA and the chemical industry on safety, health, and environmental issues and regulatory guidelines for nanomaterials. DuPont was the first company to provide product information under the EPA's voluntary Nanomaterials Stewardship Basic Program (http://www.epa.gov/oppt/nano/stewardship.html). As a member of the European Chemical Industry Association and subordinate nanotechnology working groups, DuPont is helping to develop similar industry recommendations in Europe, representing the European Chemical Industry Council on the European Competent Authority working group reviewing nanotechnology in the context of the REACH chemicals regulation. DuPont has made a commitment (DuPont 2010) to participate actively in the ISO framework (ISO 2010) for comprehensively evaluating and addressing potential environmental, health, and safety risks of nanomaterials and their applications.

DuPont has supported research at Rice University's Center for Biological and Environmental Nanotechnology and is a founding member of ICON, the International Council on Nanotechnology at Rice (http://icon.rice.edu). ICON represents industry, academia, regulatory agencies, and NGOs seeking to "assess, communicate, and reduce nanotechnology environmental and health risks while maximizing its societal benefit" (DuPont 2010).

While DuPont figures prominently as a current and past nanotechnology PPP participant and catalyst, other operational examples (e.g., ICON, NOSH, and OECD) currently foster PPPs in the nanotechnology risk-benefit dialog, and best practices are emerging. The EU FP7 Framework has recently announced a renewal of PPP targets for new research programming (see http://cordis.europa.eu/fp7/dc/index.cfm).

Generally speaking, all nanotechnology commercialization efforts should follow principles of good product stewardship and good risk management strategies in the design and manufacturing of products made with engineered nanomaterials.[25] To accommodate commercialization strategies and motives, industry response to emerging public attitudes and NGO positions on nanotechnology need to be based on facts and realism, with a rational and rapid recognition that nanotechnology as a young, dynamic field requires active, ongoing learning, rather than *post facto* reactions. Mutual stakeholder education essential to establishing public-private credibility and trust would be accelerated through open sharing of emerging experiences and data on a global basis. This is best facilitated through an open-exchange PPP mechanism that promotes active exchange of information with other industries, academia, public, and government agencies by enabling public disclosure of testing and possible risks of nanomaterials as the field and new products develop. Industry, governments, NGOs, and other stakeholders must openly collaborate to lay the proper foundation for imminent regulatory actions and to assess the potential for international voluntary agreements. To avoid backlash from relative positions of ignorance, stakeholders must be reassured that their respective concerns are considered and that private and public risk management institutions assigned to risk governance are held to accountability and articulated good practices.

It is likely that industry will advocate a system of voluntary risk governance and compliance rather than a unilaterally imposed legal regulatory enforcement (e.g., see http://www.cefic.be/en/Legislation-and-Partnership.html). Therefore, voluntary risk governance systems might best be proactively developed via a PPP mechanism to consider (1) development of standards and good practice guidelines encompassing basic research all

[25]See http://www.nanoandme.org/downloads/The Responsible Nano Code.pdf for examples of responsible risk management strategies.

the way to product testing and tracking, with methods for assessing hazards and exposure as a priority; (2) development of occupational safety guidelines, best practice scenarios, and information disclosure programs for consumers; and (3) establishment of transparent reporting processes and expectations, particularly for new data and events disclosures relevant to risk management. Nonetheless, voluntary reporting quality that assures adequate participation and transparency is difficult to achieve, and thus the desired watchdog function can be weak. Regardless of voluntary or mandatory governance, industries maintain concerns about protecting their intellectual property rights and intrinsic competitive advantage. Additionally, voluntary self-policing systems can often result in a "lowest common denominator" outcome, and as such, may not impose a sufficient incentive to those who prefer to operate outside of the voluntary system or choose not to comply.

Through PPP operations, emerging industry should try to expediently adopt preemptive, credible, and comprehensive self-regulations, which are often implemented more rapidly and efficiently than most governmental regulations. A continued and consistent focus on "best practices for risk governance" should be a priority. As a stakeholder, industry requires the continued capability to ensure technology leadership, harmonized global standards for risk assessment that ensure workplace and consumer safety and health, and a validated scientific base for efficient, appropriate adoption of regulations by engaging with academic scientific teams, policymakers, and NGOs as credible dialogue partners.

4.8.5 NIOSH Guidelines for Occupational Safety, Including the Use of Monitoring Equipment to survey the Workplace

Contact person: Vincent Castranova, NIOSH

There has been a dramatic increase in production of ENMs—including CNTs—in recent years. Although aerosolization during handling of nanoparticles is feasible, data are lacking on the exposure levels in workplaces where nanoparticles are synthesized, packaged, used, or disposed. In addition, data concerning the effects of exposure to various types of nanoparticles are incomplete. The National Institute for Occupational Safety and Health (NIOSH) is conducting a multidisciplinary research program to (1) develop methods to monitor airborne levels of nanoparticles in the field; (2) determine airborne levels of nanoparticles in various workplaces and link peak exposures to certain work processes; (3) identify respiratory and systemic effects of pulmonary exposure of laboratory animals to various nanoparticles; (4) determine dose response, time course, mechanisms of action, and structure–function relationships; (5) develop models to relate responses in animal models to those in humans; (6) conduct risk assessment; and (7) evaluate the effectiveness of control technology and personal protective equipment. The NIOSH research plan is published in the Strategic Plan for NIOSH Nanotechnology Research and Guidance: Filling the Knowledge Gaps (NIOSH 2008). Progress reports are published by NIOSH on a regular basis (Progress towards Safe Nanotechnology in the Workplace; NIOSH 2007). As a result of available data, NIOSH (2009) has published Approaches to Safe Nanotechnology: Managing the Health and Safety Concerns Associated with Engineered Nanomaterial. This document suggests that in the absence of complete information, companies either manufacturing or working with nanomaterials should follow the precautionary principle and implement a risk management program in the workplace in order to minimize the risk of worker exposure to these materials. Critical elements of such a program include the following:

- Capability to anticipate new and emerging risks (hazard determination) and whether they are linked to changes in the manufacturing process, equipment, or introduction of new materials

- Installation and evaluation of engineering controls (e.g., exhaust ventilation and dust collection systems)
- Evaluation of the effectiveness of controls through monitoring of airborne nanoparticles in the workplace
- Education and training of workers in the proper handling of nanomaterials (e.g., in safe work practices)
- Selection and use of personal protective equipment (e.g., clothing, gloves, and respirators)

NIOSH has evaluated the air environment of several nanotechnology worksites using a sophisticated array of particle analysis instrumentation to determine particle size distribution, mass concentration, number concentration, mass median aerodynamic diameter, count median aerodynamic diameter, and particle surface area. Examples of the instrumentation are shown in Figure 4.8. Since this instrumentation is bulky and not commonly available to industrial hygienists, NIOSH has also developed the Nanoparticle Emission Assessment Technique that uses common, handheld, real-time monitors to evaluate workplace levels of airborne nanoparticles (Methner et al. 2010).

Figure 4.8. Example of field application of instruments needed for real-time measurement of number, mass, size distribution, and surface area of engineered nanomaterials (courtesy of A. Nel).

In addition, NIOSH is reviewing existing toxicology data and conducted risk assessment to recommend exposure limits to selected nanoparticles. A Current Intelligence Bulletin (2005) evaluated tumor induction data in rats after long-term inhalation of fine or nano-sizedTiO_2 and will recommend an exposure limit for the nanosized form that is an order of magnitude lower than for the fine form. This document is in the final stages of review before release. NIOSH is also drafting a Current Intelligence Bulletin (2010) that notes the congruence of data from the number of animal studies for granulomatous inflammation or fibrosis in response to SWCNTs and MWCNTs and will conduct risk assessments from these data to recommend an exposure limit. This development is illustrated in Figure 4.1.

4.9 INTERNATIONAL PERSPECTIVES FROM THE OVERSEAS WORKSHOPS

4.9.1 United States-European Union Workshop (Hamburg, Germany)

Panel members/discussants

Bengt Fadeel (co-chair), Karolinska Institutet, Stockholm, Sweden
André Nel (co-chair), University of California, Los Angeles (UCLA), United States
Peter Dobson, Oxford University, United Kingdom
Rob Aitken, Institute of Occupational Medicine, Edinburgh, United Kingdom
Kenneth Dawson, University College Dublin, Ireland
Wolfgang Kreyling, Helmholtz Centre, Munich, Germany
Lutz Mädler, University of Bremen, Germany
George Katalagarianakis, European Commission
Ilmari Pykkö, University of Tampere, Finland
Jean-Christophe Schrotter, Anjou Recherche, Water Research Center of Veolia Water, France

Overall, there has been a huge increase in activity in the nano-EHS field in the past decade, but emphasis has been on hazard assessment, and less progress has been made on exposure issues. Therefore, the available information is insufficient for predictions of *in vivo* effects or effects on human health. Moreover, the toxicological results generated to date do not allow for comprehensive conclusions on nanomaterial safety, due to conflicting data related to issues of physico-chemical characterization of materials but also due to the fact that the sheer numbers of different nanomaterials that are currently being produced and explored, with tunable compositions and structures, make it challenging to address EHS outcomes. More systematic research is thus needed. There is an awareness that nanomaterials need to be studied on a case-by-case basis in order to discern associations between specific material properties and hazardous effects. Hence, research on EHS issues pertaining to nanomaterials is an interdisciplinary exercise involving researchers in material sciences, biology, (eco)-toxicology, medicine, and so on. Moreover, paradigms have emerged to support our understanding of the interaction and/or interference of nanomaterials with biological systems.

The panel members agreed that there should be more focus on "nanosafety" instead of addressing only "nanotoxicology." In other words, safety assessment of engineered nanomaterials should not be a barrier to development but rather should enable the safe and sustainable development of nanotechnology. The concepts of "safety-by-design" (i.e., intelligent material design to mitigate adverse effects on human health and the environment) and proactive risk management of ENMs were also promoted by workshop participants. To this end, more systematic research is needed in the field of EHS, making use of high-throughput screening (HTS) and systems biology approaches. The implementation of such technologies could also aid in the reduction of the number of animal experiments by serving as a triage system for ENMs. The panel highlighted the need to focus on the following emerging topics:

- Development of new methods for detection and characterization of nanomaterials *in situ*, i.e., in living systems and relevant environmental matrices
- Standardization and validation of test methods for the assessment of hazards of nanoparticles as well as more complex nano-systems
- Understanding bio-nano interactions, including the behavior and fate of engineered nanomaterials *in vivo*, e.g., navigation of nanoparticles into the brain and other organs

- Long-term *in vivo* toxicity studies of selected nanomaterials, applying realistic doses, with assessment of genotoxicity end-points
- Monitoring of human as well as environmental/ecological exposure to nanoparticles to allow for risk assessment of these materials
- Development of HTS platforms and QSARs
- Systems biology approaches for profiling/fingerprinting of categories of ENMs
- Implementation of a "safety culture," i.e., a system of certified testing, labeling, etc., to manage the risk of nanomaterials

Overall, the emerging concept in the field of environmental, health, and safety (EHS) of nanomaterials assessment is "safety by design" as a result of the development of reliable and predictive test methods. Fostering international cooperation (as in the recent joint EU–US call on modeling) will be important, as will be sharing of research facilities and infrastructures (as in the European NanoSafety Cluster of FP6 and FP7 projects) (Riedicker and Katalagarianakis 2010). Moreover, interdisciplinary education of the next generation of nanosafety researchers is also needed.

4.9.2 United States-Japan-Korea-Taiwan Workshop (Tsukuba, Japan)

Panel members/discussants

Tatsujiro Suzuki (co-chair), University of Tokyo; Japan Atomic Energy Commission, Japan,
André Nel (co-chair), University of California (UCLA), United States
Masafumi Ata, National Institute of Advanced Industrial Science and Technology (AIST), Japan
Masashi Gamo, Research Center for Chemical Risk Management, AIST, Japan
Satoshi Ishihara, Japan Science and Technology Agency (JST), Japan
Chin-Chung Tsai, Chiao Tung University, Taiwan
Chung-Shi Yang, Center for Nanomedicine Research, National Health Research Institutes, Taiwan

The following is a summary of the key points discussed during the session.

Changes in the Vision over the Last Ten Years

- The need to address the potential risks by this new science is now widely acknowledged and has changed nanotechnology from a business and science dream to an inclusive societal feature.
- NGOs have advanced the codes of conduct, ethics, etc., of nanotechnology.
- Governments have begun to address regulatory issues, are viewing existing regulations, and looking at where there may be nanotechnology-specific issues.

Advances in Last Ten Years

- Many EHS studies have been conducted in all Asian countries, and some regulatory bodies have taken action, but no long-term policy or strategy has been established. Large companies are more active in addressing EHS issues than are small companies/startups.

Vision for the Next Ten Years

- In Asia, future funding trends for EHS research is uncertain. While steady funding in Taiwan is expected for next 5 years, it is not clear what will happen after the national program ends. Taiwan is spending about 10% of total R&D expenditure on EHS, while Japan is spending less than 2% on average; however, this expenditure fluctuates every year.

- While the United States is expecting that predictive toxicology for ENMs using computer and simulated modeling can play an important role, in Asia it is currently viewed as being of questionable value.
- All participants agreed that public involvement/outreach is critically needed in addressing potential EHS concerns.
- While there are new efforts on technology assessment in Japan, a new focus has emerged on the use of "distributed governance," which is premised on collective knowledge dissemination and not necessarily associated with government agencies. In Taiwan, the "nanoMark program" promotes best practices; this program has been successful in actively involving consumer groups.
- International collaboration in EHS research is considered a key factor. Japan and Korea are both involved in the Working Party on Manufactured Nanomaterials (WPMN) of the OECD, and Taiwan is interested in joining the WPMN sponsorship program. Japanese scientists also participate in the voluntary International Association of Nano Harmonization (IANH). The ISO Nanotechnologies Technical Committee (TC229) standards activities are also important.

Goals for 2020

- International harmonization is an important goal, for instance, in terms of development of standardized methods, risk evaluation, and risk assessment and management protocols. Korea has proposed in ISO/TC229 the use of nano-MSDSs, while the Taiwanese equivalent of the U.S. EPA uses a policy similar to TSCA in the United States.
- Classification of some ENMs as toxic substances should be considered an important goal.
- Institutionalization of technology assessment should be realized. It means that such activity needs to be an embedded function of societal efforts. The funding source should be stable and should be routinely carried out by an independent agency.
- Vision to develop tools and processes for public engagement is needed to assure responsible development of nanotechnologies. This vital effort should involve all stakeholders, such as the scientific community, public, government, industry, and media.
- International collaboration is important in sharing best practices for public engagement. The U.S. Centers for the Environmental Implications of Nanotechnology (CEIN) is an interesting model that could be followed in Asia.
- There is a strong need for information sharing, common databases, and for research that uses standard protocols to generate comparable data. Three Taiwan agencies are developing common databases. There is also a need to encourage industry to share data and information about the use of nanotechnology in its products. For example, there is a need to know what products have nanotechnology in them in order to assess exposure, hazard, and risks.

4.9.3 United States-Australia-China-India-Singapore Workshop (Singapore)

Panel members/discussants

Yuliang Zhao (co-chair), Chinese Academy of Sciences (CAS); CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, China
André Nel (co-chair), University of California, Los Angeles (UCLA), United States
Graeme Batley, Commonwealth Scientific and Industrial Research Organization, Australia
Graeme Hodge, Monash University, Australia
Joachim Loo, Nanyang Technological University, Singapore

Yiyan Yang, Institute of Bioengineering and Nanotechnology, Singapore
Yong Zhang, National University of Singapore

The following is a summary of the key points discussed during the session.

Changes in the Vision over the Last Ten Years

- The need to address the potential risks by this new science is now widely acknowledged and has changed nanotechnology from a business and science dream to an inclusive societal feature.
- NGOs have advanced the codes of conduct, ethics, etc. for nanotechnology.
- Governments have begun to address regulatory issues, are viewing existing regulations, and are looking at nanotechnology-specific issues.

Advances in Last Ten Years

- Over the last 10 years, more than 20 ENMs have been tested for potential toxicity. Although the initial toxicological data were inconsistent due to insufficient material characterization, current data reported in the literature are more consistent and reproducible due to more stringent characterization and harmonized test efforts
- The number of nanotechnology characterizations and definitions has narrowed, among which the recently published ISO definition of "nanotechnology" represents a significant advance.
- Rapid- as well as high-throughput screening techniques for assessment of potential toxicity of nanomaterials have been proposed, and implementation has begun.

Vision for the Next Ten Years

- Exciting advances in nanotechnology applications will occur, enabled by continuous incremental progress in nano-EHS issues.
- Since more is understood about the mechanisms and properties leading to nanomaterial hazard, safe implementation and design of nanomaterials have become possible.
- There is a need to establish nanotechnology-specific regulatory procedures for risk assessment of nanomaterials, including for governance.
- Guidelines must be developed for safe use of nanomaterials/nanotechnology in applications, and there should be continued development of self-regulation.

Goals for 2020

- **Goal 1:** Knowledge-generation about nanomaterial properties that could pose hazard at the biological level at a rate commensurate with the expansion of nanotechnology and new products.
  - Barrier: One-material-at-a-time analysis is impractical, given the large number of properties and the many new materials being produced.
  - Solution: Large-scale implementation of high-throughput screening techniques.
- **Goal 2:** To consider the safety of ENMs at the initial stages of their development and the development of the products incorporating them.
  - Barrier: Inability to predict whether ENMs with potentially hazardous properties may pose biological hazards.
  - Solution: Develop safe ENMs "by design" using principles similar to those of "green chemistry," e.g., coated materials to reduce/eliminate toxicity.

- **Goal 3:** The development of public confidence in/acceptance of nanotechnology as a result of all of the above.

Infrastructure Needs

- Funding for EHS research, integrated with applications development
- Nanomaterial reference libraries
- Databases of properties
- Instrumentation
- Environment detection poses a "grand challenge"

R&D Strategies

- Standardized assays and methodologies, validated and internationally accepted
- International cooperation, leveraging, e.g., OECD, WPMN, ISO, and others
- Industry participation (including funding support) and role in nano-EHS efforts

Emerging Topics and Priorities

- Occupational safety studies and defining of LOD and minimal exposure thresholds
- Mechanisms of nanotoxicity (important for predictable and designable nanotechnology), reliable ADME/Tox data (important for development of safety assessments)
- Modeling of risk assessment, fate and transport, and QSARs
- Nano-ESH methodology development
- Nano-informatics
- Addressing the current lack of knowledge concerning impacts on the environment, fate and transport, bioaccumulation, trophic transfer, etc.
- Knowledge translation: making "nano" accessible to the general public, increase the public's trust in science

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