

# Nanomaterial Categorization for Assessing Risk Potential To Facilitate Regulatory Decision-Making

Hilary Godwin,<sup>\*,†,‡,§,||</sup> Catherine Nameth,<sup>§</sup> David Avery,<sup>§</sup> Lynn L. Bergeson,<sup>⊥</sup> Daniel Bernard,<sup>#</sup> Elizabeth Beryt,<sup>§,¶</sup> William Boyes,<sup>□</sup> Scott Brown,<sup>■</sup> Amy J. Clippinger,<sup>○</sup> Yoram Cohen,<sup>§,||,●</sup> Maria Doa,<sup>△</sup> Christine Ogilvie Hendren,<sup>▲</sup> Patricia Holden,<sup>§,▽</sup> Keith Houck,<sup>□</sup> Agnes B. Kane,<sup>▼</sup> Frederick Klaessig,<sup>§,○</sup> Toivo Kodas,<sup>●</sup> Robert Landsiedel,<sup>††</sup> Iseult Lynch,<sup>‡‡</sup> Timothy Malloy,<sup>‡,§,§§</sup> Mary Beth Miller,<sup>⊥⊥</sup> Julie Muller,<sup>##</sup> Gunter Oberdorster,<sup>¶¶</sup> Elijah J. Petersen,<sup>□□</sup> Richard C. Pleus,<sup>■</sup> Philip Sayre,<sup>△,●●</sup> Vicki Stone,<sup>○○</sup> Kristie M. Sullivan,<sup>●●</sup> Jutta Tentschert,<sup>△△</sup> Philip Wallis,<sup>▲▲</sup> and Andre E. Nel<sup>\*,§,||,▽,▼,▼▼</sup>

<sup>†</sup>Department of Environmental Health Sciences, Fielding School of Public Health, <sup>‡</sup>Institute of the Environment and Sustainability, <sup>§</sup>University of California Center for Environmental Implications of Nanotechnology, and <sup>||</sup>California NanoSystems Institute, University of California, Los Angeles, California 90095, United States, <sup>⊥</sup>Bergeson & Campbell, P.C., Washington, D.C. 20037, United States, <sup>#</sup>CEA Nanosafety Platform, Grenoble, France, <sup>¶</sup>Luskin School of Public Affairs, University of California, Los Angeles, California 90095, United States, <sup>□</sup>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, United States, <sup>■</sup>DuPont Central Research & Development, DuPont Titanium Technologies, E.I. du Pont de Nemours and Company, Wilmington, Delaware 19803, United States, <sup>○</sup>PETA International Science Consortium Ltd., London, United Kingdom, <sup>●</sup>Department of Chemical and Biomolecular Engineering, University of California, Los Angeles, California 90095, United States, <sup>△</sup>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460, United States, <sup>▲</sup>Center for the Environmental Implications of Nanotechnology, Duke University, Durham, North Carolina 27708, United States, <sup>▽</sup>Bren School of Environmental Science and Management, University of California, Santa Barbara, California 93106, United States, <sup>▼</sup>Brown University, Providence, Rhode Island 02912, United States, <sup>○</sup>Pennsylvania Bio Nano Systems, Doylestown, Pennsylvania 18901, United States, <sup>●</sup>Cabot Corporation, Boston, Massachusetts 02210, United States, <sup>††</sup>Experimental Toxicology and Ecology, BASF SE, 67056 Ludwigshafen am Rhein, Germany, <sup>‡‡</sup>School of Geography, Earth & Environmental Science, University of Birmingham, Edgbaston B15 2TT, United Kingdom, <sup>§§</sup>University of California School of Law, Los Angeles, California 90095, United States, <sup>⊥⊥</sup>Applied NanoStructured Solutions, L.L.C., Lockheed Martin Company, Baltimore, Maryland 21220, United States, <sup>##</sup>Nanocyl, Sambreville, Belgium, <sup>¶¶</sup>University of Rochester, Rochester, New York 14627, United States, <sup>□□</sup>Material Measurement Laboratory, National Institute of Standards and Technology, Gaithersburg, Maryland 20899, United States, <sup>■</sup>Intertox, Inc., Seattle, Washington 98101, United States, <sup>○○</sup>School of Life Sciences, Heriot-Watt University, Edinburgh EH14 4AS, United Kingdom, <sup>●●</sup>Physicians Committee for Responsible Medicine, Washington, D.C. 20016, United States, <sup>△△</sup>Federal Institute for Risk Assessment, Berlin, Germany, <sup>▲▲</sup>SouthWest NanoTechnologies, Norman, Oklahoma 73071, United States, and <sup>▽▽</sup>Center for Nanobiology and Predictive Toxicology and <sup>▼▼</sup>Department of Medicine, Division of NanoMedicine, University of California, Los Angeles, California 90095, United States

## ABSTRACT

For nanotechnology to meet its potential as a game-changing and sustainable technology, it is important to ensure that the engineered nanomaterials and nanoenabled products that gain entry to the marketplace are safe and effective. Tools and methods are needed for regulatory purposes to allow rapid material categorization according to human health and environmental risk potential, so that materials of high concern can be targeted for additional scrutiny, while material categories that pose the least risk can receive expedited review. Using carbon nanotubes as an example, we discuss how data from alternative testing strategies can be used to facilitate engineered nanomaterial categorization according to risk potential and how such an approach could facilitate regulatory decision-making in the future.

Due to the tremendous potential for nanotechnology to revolutionize fields as diverse as electronics and medicine, the global community has invested considerable time and resources in the research and development of engineered nanomaterials (ENMs) and nanoenabled products. In the past decade, increasing emphasis has been placed on developing the science and tools needed to ensure that ENMs and nanoenabled

products are produced and used as safely as possible. In many countries, the time when new materials and/or products are first brought to market is a critical checkpoint for regulatory agencies to evaluate the potential risks of those materials and products and to put into place controls to ensure that the health and well-being of workers, the public, and the environment are well-protected. Although regulatory strategies for new chemicals and materials

\* Address correspondence to  
hgodwin@ucla.edu,  
anel@mednet.ucla.edu.

Received for review February 9, 2015

Published online March 20, 2015  
10.1021/acsnano.5b00941

© 2015 American Chemical Society

differ from country to country, almost all regulatory bodies grapple with the challenges associated with assessing the risks of ENMs in a timely fashion for decision-making. Categorization strategies are needed to enable regulators and industry either to predict ENM risk better or to allow prioritization of the testing (hazard, exposure, physicochemical) needed to estimate their potential risk while minimizing time-consuming and costly *in vivo* studies or traditional risk assessments.<sup>1</sup>

Developing scientifically based categorization strategies for regulatory purposes requires consideration of the needs, capacities, and input of regulators, affected businesses, and other stakeholders. Toward this end, the University of California Center for Environmental Implications of Nanotechnology (UC-CEIN) and the UCLA Center for Nanobiology and Predictive Toxicology convened a workshop for representatives from industry, government agencies, non-governmental organizations (NGOs), and academia at the Woodrow Wilson Center in Washington, DC, to discuss how categorization of ENMs coupled with alternative testing strategies (ATS) might be used to expedite hazard characterization and risk analysis, allowing for integrated environmental and occupational health and safety (EHS) decision-making for ENMs. Below, we present a synthesis (but not necessarily a consensus) of the perspectives of this diverse group of stakeholder representatives on this topic. To simplify the discussion, we have chosen to focus on carbon nanotubes (CNTs) as an illustrative example. We focus on CNTs both because of their importance in the world market and because grouping of CNTs currently poses a significant challenge to regulators. We discuss how CNTs are currently handled under the U.S. Environmental Protection Agency's (EPA) New Chemicals program and the challenges that have arisen when addressing these materials. Next, we discuss how new and emerging tools and approaches for

testing the safety of chemicals and materials (referred to herein as "alternative testing strategies" or ATS; see Figure 1) can facilitate grouping, ranking, and read-across for ENMs. In that section, we will introduce the concepts of adverse outcome pathways (AOPs) and structure–activity relationships that are based on ENM composition and physicochemical properties. Finally, we provide an example of how data from ATS could be incorporated into a revised decision analysis framework for ENMs. Although we have chosen to focus on CNTs and the EPA's New Chemicals program for the example discussed here, we feel that many of the principles and insights identified in this example are broadly applicable to other classes of ENMs and other regulatory paradigms. Thus, discussion of the CNT example will help to move the entire field forward.

#### **Handling of CNTs under the U.S. EPA's New Chemicals program: Challenges and Opportunities.**

In the United States, when companies are planning to manufacture a new chemical (e.g., a new nanomaterial), a premanufacturing notice (PMN) must be submitted to the U.S. EPA. After reviewing the PMN, the EPA can take a variety of actions, which often involves placing the chemical on the Toxic Substances Control Act (TSCA) Chemical Inventory without restriction or placing the chemical on the Inventory with restrictions (most commonly through a section 5(e) Consent Order and/or a Significant New Use Regulation or SNUR). Significant New Use Regulations are issued because submissions under the TSCA New Chemicals program are specific to the manufacturing, processing, and use that the submitter and downstream clients are undertaking; SNURs do not take into account different manufacturing, processing, and use scenarios.

The EPA's treatment of CNTs under its New Chemicals program is illustrative of the actions that are taken under this program (Figure 2). Under this program, the EPA uses 56 chemical categories to streamline

the review of new chemicals. The EPA has consistently considered CNT risks in the context of its "Respirable, Poorly Soluble Particulates" category. If a CNT is determined to fall into this category and sufficient risk exists as defined under section 5 of TSCA, a 90 day inhalation study is typically requested (once sufficient profits have accrued from commercialization of the subject CNT). The EPA has also promulgated SNURs for both generic single-walled CNTs and multiwalled carbon nanotubes (MWCNTs).<sup>2–10</sup> These SNURs require that, before certain new uses of CNTs (which are different from the uses provided in the initial submissions and with exposure potential) can begin, the company wishing to use the CNT in a new manner must provide notice to the EPA 90 days before commencing that new use. This regulation provides the EPA an opportunity to review the use and determine if it may present an unreasonable risk and take appropriate action. The review is similar to that conducted for the initial submission and builds upon the EPA's review of the initial submission (see Figure 2). For instance, if a 90 day inhalation study has not been submitted by the initial submitter, it may be required of the submitter of the notice of the new use. The primary challenge posed by the current approach is that it does not explicitly provide a methodology by which manufacturers or regulators can determine whether a "new" CNT is sufficiently similar to prior submissions such that the risk potential of the "new" CNT can be predicted based on data for existing CNTs. (This process is known as "read across"; see Figure 1.) Integration of methods for predicting the risk potential for CNTs based on data other than a 90 day inhalation study into the decision-tree approach would greatly facilitate improved decision making by both manufacturers and regulators.

Furthermore, the development of categories for nanomaterials would be helpful in the regulation of chemicals in countries other than

**Adverse outcome pathway (AOP)** refers to an analytical construct that describes a sequential chain of causally linked events at different levels of biological organization that lead to an adverse health or ecotoxicological effect. Adverse outcome pathways include effects at the macromolecular, cellular, organ, and organism levels [<http://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm>]

**Alternative test strategies (ATS)** in this context refers to alternatives to whole animal testing (particularly testing in mammals) or rapid testing approaches for nanomaterials that provide results that can be used to prioritize, and perhaps eventually replace, time-consuming whole animal studies. Alternative testing strategies include refinement, reduction and replacement of animal models when supplementing *in vitro* and *in silico* methods for generating safety data to be used for hazard and risk assessment and/or modeling.

**Categorization** refers to the grouping of chemicals. With traditional chemicals, a **chemical category** is a group of chemicals whose physicochemical and human health and/or ecotoxicological properties and/or environmental fate properties are likely to be similar or follow a regular pattern, usually as a result of structural similarity. Categorization strategies may include grouping, ranking, and read-across as examples of types of categorization. [<http://www.oecd.org/chemicalsafety/risk-assessment/groupingofchemicalschemicalcategoriesandread-across.htm>]. In the context of nanomaterials, additional factors could be considered such as grouping by mechanism of action at the nano/bio interface.

**Engineered Nanomaterial (ENM)** refers to an intentionally manufactured material, either in an unbound state or an aggregate, where one or more of the dimensions is in the size range of 1 – 100 nm.

**High-content screening (HCS)** usually refers to cell-based high-throughput screening that use microscopic or fluorescent images as assay readouts. Examples of HCS are quantitative measurements of multiple phenotypic changes in the same cell population.

**High-throughput screening (HTS)** refers to the use of automated tools to facilitate rapid execution of a large number and variety of biological assays (that may include 100s to 1000s of substances in each assay). The assays are for determining biological effects, and they may also be performed on cell-free biochemical assays, *in vitro* cell culture, or other conditions.

**Mechanistic toxicology** refers to a toxicological approach in which mechanistic screening (of pathways of toxicity or mechanisms of action) and establishing structure-activity relationships are used to estimate the likelihood and magnitude of adverse effects in animals and/or humans. One example is the use of carbon nanotube (CNT) libraries (in which a series of properties are accentuated) to show that the triggers of specific macrophage lysosomal and inflammasome injury responses can be used to compare and predict the major CNT properties that excite chronic lung inflammation and fibrosis.

**Quantitative structure-activity relationships (QSARs)** refers to the relationship between the chemical composition or the physicochemical properties of an ENM and a specific defined biological activity. This method could be refined to build quantitative mathematical relationships between the chemical or physicochemical structure and the biological activity of ENMs.

**Ranking** is used when ENMs are assigned a position on a scale, according to which ENMs may be classified based on their potential for exposure (e.g., high dustiness) and/or potential to cause harm due to physicochemical properties known to engage biological pathways and mechanisms of injury.

**Read-across** refers to the process where endpoint information for one chemical (the source chemical) is used to predict the same endpoint for another chemical (the target chemical), which is considered to be "similar" in some way (usually on the basis of similarities in physicochemical properties that are deemed to be indicative of risk, hazard, or exposure potential).

Figure 1. Key definitions.

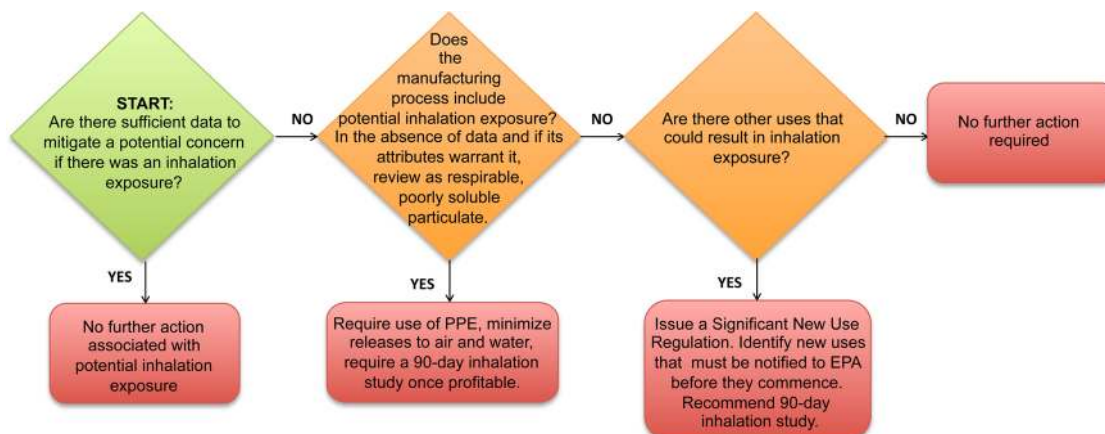


Figure 2. Decision-tree approach currently used by the EPA to characterize risk potential of carbon nanotubes (CNTs). The decision tree above provides an example of a regulatory program where CNTs are currently characterized by their risk and exposure potential under the Toxic Substances Control Act section 5 program. Currently, inhalation testing is requested most frequently as a result of concerns for worker exposure. Testing required as a result of risks to the general population or consumers, rarely if ever, has occurred to date for CNTs.



the United States. In Canada, for instance, under the New Substances program, information on particle size and distribution will be requested for those nanomaterials and classes of nanomaterials that are likely to behave differently than their non-nanoscale forms. Carbon nanotubes are one such class of nanomaterials.<sup>11</sup> Due to recent developments by the U.S.–Canada Regulatory Cooperation Council, the two countries will work to align their nanomaterials regulatory work better so as to have consistent approaches to risk assessment and the identification of categories of nanomaterials. In the European Union, under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) program, registrants can use read-across methods and categorization methods to fill data gaps for chemical safety assessment and for alternatives analysis.<sup>3,12</sup> Typically, these categories are based on structural similarities and physicochemical properties, as well as similarities in effects on human health and the environment. At present, the European Chemicals Agency (ECHA) has not issued formal guidance on categorization specific to nanomaterials, although ECHA and other regulators have developed reports and other materials addressing categorization of nanomaterials.<sup>13</sup>

**What Is Categorization and Why Is It Critical?** Preliminary grouping of ENMs could be helpful during the early stages of qualitative risk analysis either by manufacturers or regulators to identify “nanomaterials of concern”, which could then be targeted for more detailed testing, analysis, and verification. Several approaches involving different levels of stringency and completeness of data collection have been proposed<sup>1,14,15</sup> to group and to rank ENMs. These are briefly summarized in Table 1. Categorization methods should be premised on the best available evidence and take into consideration uncertainties concerning the hazard potential of specific ENMs at sites of potential

occupational or environmental exposures. The nature of the attributes used for categorization varies depending upon the type and purpose of the grouping as well as other factors. The relevant attributes may be human health outcomes (e.g., carcinogenicity), environmental endpoints (e.g., aquatic toxicity), physicochemical features of the substance, and/or the production volume of the material. Prudent risk management measures would include greater precaution in the handling and use of ENMs that lack sufficient data to estimate the health risks adequately.

The “holy grail” of this field is to be able to categorize the risk potential of ENMs based on their physicochemical properties because such an approach would allow manufacturers and regulators to make rapid decisions without requiring costly and time-consuming *in vivo* and/or *in vitro* data. A decision-tree approach for assessing nanomaterial health hazards has already been developed jointly by entities such as the U.S. EPA’s New Chemicals program, Health Canada, and Environment Canada under the Regulatory Cooperation Council.<sup>13</sup> However, practical implementation of such a decision tree for CNTs is not yet feasible. Carbon nanotubes have complex physical and chemical structures for which the contribution of individual properties to the causation of injury in relation to the complex, integrated structure has not yet been developed to the extent that risk can be predicted based on a selection of specific physicochemical properties alone. *In vitro* and *in vivo* toxicological testing has indicated that CNT wall number, synthesis method (which determines metal impurities and metallic or semiconductor status), length/aspect ratio, state of agglomeration, surface functionalization, hydrophobicity, suspension stability in aqueous environments, embeddedness in a matrix, and surface coating could all play roles in CNT toxicity. It is often impossible when looking at

the safety data sheet for CNTs to identify a single dominant feature that can be used to describe the potential hazard according to which the material can be classified. Moreover, how exactly these properties determine fate and transport, bioavailability, persistence, and triggering of biological injury responses is not well understood. As a result, some direct measures of biological activity and exposure potential are currently needed to categorize CNTs according to their risk potential. A possible exception is a new material that is very similar to some prior examples for which extensive hazard and exposure data are available. In most other cases, however, information about hazard and/or exposure potential is required at this stage for meaningful risk evaluation.

**Preliminary grouping of engineered nanomaterials could be helpful during the early stages of qualitative risk analysis either by manufacturers or regulators to identify “nanomaterials of concern”, which could then be targeted for more detailed testing, analysis, and verification.**

For decision makers to be able to identify whether a new material is similar to prior examples and to make informed decisions about the risk potential of ENMs, the broader community needs access to a shared data repository that integrates ENM physicochemical, hazard, and exposure data that have been curated for reliability and accuracy. In addition, we need more

**TABLE 1. Summary of Approaches To Grouping and Ranking Engineered Nanomaterials**

categorization according to physicochemical characteristics
<ul style="list-style-type: none"> <li>• Similarities in chemical composition (<i>e.g.</i>, carbon nanotubes, inorganic carbon, metal oxides, metalloid oxides, metals and metalloids, semiconductor quantum dots, organics, <i>etc.</i>)</li> <li>• Aggregation or agglomeration state (<i>e.g.</i>, readily dispersed particles that could be easily respired into the lung vs heavily agglomerated particles that are not respirable)</li> <li>• The media or matrix (<i>e.g.</i>, pure nanomaterials, liquid suspensions, and solid matrices; particles in diffuse coatings, durable coatings and composites, nanostructured products)</li> <li>• Classification according to physical state to improve safe handling and reduced occupational exposure (<i>e.g.</i>, bound or fixed nanostructures, liquid suspensions or liquid dispersions, dry dispersible nanomaterials, and nanoaerosols and gas phase synthesis)</li> <li>• Characteristics affecting particle toxicology (<i>e.g.</i>, size, shape, aspect ratio, surface area, surface reactivity or functionalization, surface coating, chemical composition and crystal structure, agglomeration and aggregation)</li> <li>• Categorization according to modes of action that predict biological risk (<i>e.g.</i>, higher solubility particles, poorly soluble/low toxicity particles, poorly soluble/high toxicity particles, fibrous particles)</li> <li>• Categorization according to the effects of the ENMs based on overall composition (<i>e.g.</i>, particles themselves, coatings, chemical effects such as ions or released molecules, molecules formed by the catalytic surface, molecules bound to the ENM)</li> </ul>
categorization according to exposure and use scenarios
<ul style="list-style-type: none"> <li>• Classification to predict likelihood of consumer exposure based on nanomaterial location in the product (<i>e.g.</i>, part of a bulk substance, such as nanoelectronics; surface location, such as on films; particles in liquid suspensions) or use of the product</li> <li>• Likelihood of inhalation based on dustiness or dispersibility</li> <li>• Exposure potential based on volume of production and life cycle analysis</li> <li>• Exposure based on environmental release from a manufacturing/processing site or a product matrix</li> <li>• Exposure of workers during production</li> </ul>
Categorization linking selected physicochemical properties to specific biological outcomes
<ul style="list-style-type: none"> <li>• Categorization based on the potential to induce acute oxidative stress and inflammation (including consideration of the dissolution, electronic properties, and surface catalytic activity of metal, metal oxide, semiconductor nanoparticles)</li> <li>• Categorization based on material aspect ratio and the capacity to induce lysosomal injury, inflammasome activation and initiation of chronic inflammatory responses and fibrosis (<i>e.g.</i>, carbon nanotubes (CNTs); metal and metal oxide nanowires, nanobelts, and nanorods; rare earth oxides)</li> <li>• Categorization based on surface functionalization of material groups that catalyze oxidative stress or lysosomal injury (<i>e.g.</i>, anionic, cationic, or neutral surface functionalization on CNTs; silica ring structure and surface silanol display)</li> </ul>

sophisticated tools for interrogating these data. Although resources such as NanoHUB and the Nanomaterial Registry provide a good start, comprehensive tool and data sets for providing these linkages and predicting behavior do not yet exist. We advise that, until such tools are developed, categorization approaches that consider the best available evidence are the only alternative for nano-EHS and regulatory decision-making by government agencies.

**How Alternative Testing Strategies Could Facilitate Categorization of CNTs.** While it is not currently possible to develop risk categories based on ENM physicochemical properties alone, the data obtained from ATS—including both *in vitro* assays as well as linked, predictive short-term animal studies—could provide data on which categorization can be based. Alternative testing strategy approaches include the use of mechanistic and predictive toxicological

assays to assign the materials to categories that are expected to have similar toxicological profiles among members in the group. Illustrative examples include the use of high-content screening (HCS) (Figure 1)<sup>16,17</sup> to develop hazard rankings for libraries of ENMs that have been assembled to represent systematic variation of physicochemical composition as well as accentuation of specific properties (*e.g.*, size, crystallinity, solubility, aspect ratio, electronic properties, surface functionalization, surface coating) within compositions such as CNTs, graphene, metals, metal oxides (including silica), and rare earth oxides. Where possible, this screening is premised on adverse outcome pathways (AOPs; *e.g.*, lysosome damage that triggers chronic inflammation and fibrosis; oxidative stress responses that trigger acute pulmonary inflammation) that also determine the pathophysiology of disease and are of potential use for

hazard ranking based on mode of action (MOA) and, in some instances, quantitative structure–activity relationships (QSARs). Different approaches for nanomaterial grouping that are based on ATS hazard assessment, exposure potential, risk assessments, and risk management have been published or are currently being developed.<sup>1,13–15</sup>

The assessment of mechanistic biological responses by high-content and rapid-throughput assays can speed up data generation to establish category-specific hazard profiling that can be used for initial regulatory decision analysis, including whether more costly and labor-intensive animal studies are recommended. Application of ATS data to ENM risk categorization also confers the advantage that these assays can be used to test pristine particles as well as nanoenabled products and environmentally aged particles. In addition, ATS approaches can be

Alternative testing strategy approaches include the use of mechanistic and predictive toxicological assays to assign the materials to categories that are expected to have similar toxicological profiles among members in the group.

used to facilitate the use of grouping according to exposure potential and product-use scenarios. Alternative testing strategy approaches that incorporate HTS/HCS assays are also potentially useful for rapid comparative analysis of material collections or ENM libraries to provide hazard ranking according to a tiered approach that may be used for limiting animal testing. Rapid-throughput screening can also be used to establish QSARs and to determine the hierarchical ranking of new materials that are assayed by HTS or HCS together with libraries of well-characterized materials. Thus, ATS could be used to establish low-risk categories that could receive rapid decisions, while materials of high-risk concern could receive more extensive evaluation. However, for such HTS/HCS assays to be broadly accepted in a regulatory context, they should also be evaluated for their relevance and reliability.<sup>18</sup>

To illustrate how ATS could facilitate grouping, ranking, and read-across of CNTs, a tiered approach to animal testing has been proposed to assess the risk of lung-based occupational exposures.<sup>19</sup> Under this paradigm, predictive toxicological paradigms based on AOPs could be used to prioritize materials within a category for further testing. The

results could then contribute to determining when a 90 day rodent inhalation study (currently considered by regulatory bodies as the most reliable indicator of adverse pulmonary effects in mammals and as a surrogate for worker adverse pulmonary effects) would be recommended. This approach enables stepwise investigation of large numbers and categories of materials, which can be compared, grouped, and prioritized for cellular, short-term *in vivo*, and, ultimately, long-term inhalation exposures if required to meet regulatory requirements. Key to the interpretation of these studies is the appropriate use of a wide dose range *in vitro*, to ensure that extrapolation (e.g., dose per unit surface area in the culture dish vs the lung) to the *in vivo* situation represents a dose–response relationship on the steep part of the dose–response curve, which can also be compared with occupational exposure measurements (available for CNTs). Used appropriately and in an iterative fashion to gain confidence during the introduction of new materials, the vision is to use the ATS-based tiered system to reduce animal testing. Moreover, the rapid knowledge gathering through ATS use can expedite new material testing as well as provide rankings for early decision analysis.

We have specifically chosen to focus on CNT risk categorization by using ATS that reflects injury to pulmonary cell types that can also occur in the intact lung during inhalation exposure in rodents and, by extrapolation, is possible for occupational exposures in humans. Based on predictive toxicological studies for lung injury, experimental data suggest that CNT properties such as hydrophobicity, state of dispersion, surface coating, length/aspect ratio, and surface charge play key roles in cellular uptake and bioavailability by lung cells. Other properties such as metal impurities, surface catalytic groups that can generate oxygen radicals, aspect

ratio, stability of the surface coating, and state of suspension in a low pH environment (e.g., lysosomes) are important for cellular injury (e.g., to the lysosome).<sup>19–23</sup> These findings stand in contrast with the popular impression that the fiber-like dimensions of rigid MWCNTs are the *defining* property of mesothelial and lung injury based on a frustrated phagocytosis paradigm. Although AOPs have not yet been developed for all pathways leading to pulmonary toxicity by CNTs (work is ongoing in this area), at least some AOPs are amenable to ATS. This strategy includes use of HTS/HCS approaches that assess profibrogenic effects at a cellular level as a result of lysosome injury, which predicts CNT fibrosis in the lung.<sup>20–23</sup> Based on these studies, it follows that ATS and grouping could be used to develop a tiered approach to assess CNT risk potential in the lung. Below, we discuss how such an approach potentially could be applied in a regulatory setting.

**A Proposed New Approach to Categorization of Engineered Nanomaterials for Regulatory Purposes.** Although no single categorization strategy is likely to work for all classes of ENMs in all regulatory situations, it may be possible to develop a general framework that can be adapted and customized for specific ENM compositions and specific regulatory contexts. For illustrative purposes, we will discuss a decision-tree approach for CNTs based on a predictive toxicological approach using ATS to prioritize material categories for further testing. We will also discuss potential barriers to incorporating ATS approaches into regulatory decision-making and how industry–academia–government–NGO cooperation could work to reduce these barriers.

A decision tree for assessing nanomaterial health hazards has already been developed jointly by the U.S. EPA's New Chemicals program, Health Canada, and Environment Canada under the Regulatory Cooperation Council (RCC).<sup>13</sup> However, the existing RCC decision

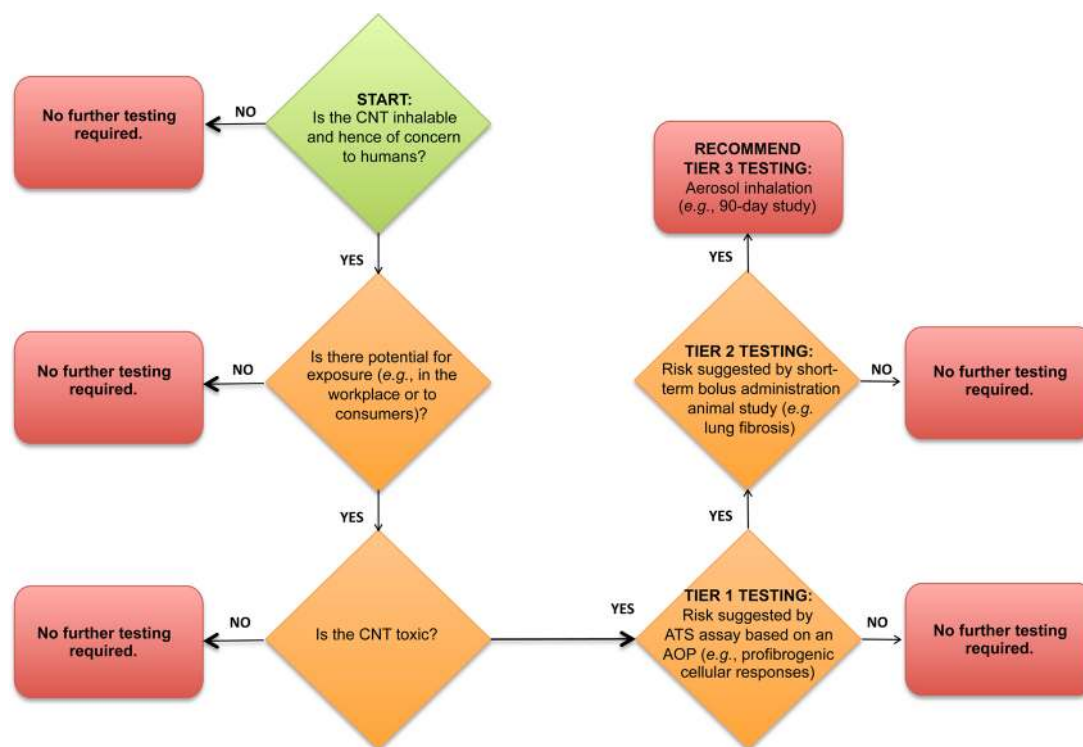


Figure 3. Proposal for how alternative testing strategy (ATS) data could be incorporated into a decision-tree approach for categorizing carbon nanotubes (CNTs) according to a tiered testing approach to rule out the need for 90 day inhalation studies when possible. We propose the use of ATS as a Tier 1 approach to determine whether short-term animal studies (Tier 2) are needed. A 90 day inhalation study would only be required if Tier 2 testing comes back positive or if validation studies suggest Tier 1 and/or Tests are not predictive for this class of materials. Prior to implementation, the tests used in Tier 1 and Tier 2 must be determined to be “fit for purpose” (*i.e.*, to yield results that are predictive of results obtained by Tier 3 tests and hence provide accurate predictions of risk potential for the class of materials being investigated).

Although no single categorization strategy is likely to work for all classes of engineered nanomaterials (ENMs) in all regulatory situations, it may be possible to develop a general framework that can be adapted and customized for specific ENM compositions and specific regulatory contexts.

tree relies on physicochemical properties alone to make decisions about

risk potential, which is not feasible as yet given gaps in our knowledge about the exact relationship of these properties to hazard and risk outcome. As a result, when regulators (*e.g.*, in the EPA New Chemicals program) determine that there is potential for inhalation exposure for a new CNT, they typically recommend that the manufacturer perform a 90 day inhalation study (see Figure 2). By contrast, the new decision tree proposed below explicitly articulates how ATS data potentially could be incorporated into decision points and minimize time-consuming and costly animal studies.

An example of such a decision tree for CNTs is depicted in Figure 3. This tree is structured around a tiered approach once a potential for inhalation exposure is identified. In the first tier, one or more *in vitro* assays that reliably recapitulate the potential to cause pulmonary inflammation could be used as an

initial hazard screen (Tier 1 Testing).<sup>20–23</sup> If (and only if) the results of the Tier 1 Testing suggest potential risk, then Tier 2 Testing is undertaken based on the ranking provided in Tier 1. Tier 2 Testing involves short-term bolus administration to the lung for determining injury potential (*e.g.*, lung fibrosis<sup>24</sup>). This testing also serves to confirm the predictive hazard potential based on the Tier 1 Test. If (and only if) the results of the Tier 2 Testing confirm and suggest *in vivo* risk potential, then performing Tier 3 Testing should be given consideration. Tier 3 Testing involves aerosol administration in rodents (*e.g.*, a 90 day study). This approach is preferable to asking for a 90 day inhalation study for every new material exposure and inhalation potential because 90 day inhalation studies are time-consuming and expensive: the EPA estimates that the cost is more than \$500,000 to perform such a



study on a single respirable, poorly soluble particulate material.<sup>25</sup>

The coordinated development of a decision-tree approach based on relevant and reliable ATS data would contribute to better-informed regulatory decisions about the potential risks of ENMs with data that could be collected in days to weeks and at relatively low cost. A decision-tree approach would also enable industry to anticipate the types of information that should be provided to the EPA for an expeditious EPA review. Similar approaches for assessing risk of ENMs postmanufacturing could be considered within the European regulatory system, REACH.<sup>26</sup>

Critically, for the type of decision-tree approach described above to be effective, both manufacturers and regulators will need to be in agreement that the assays used for Tier 1 and Tier 2 Testing accurately predict the risk potential for the specific class of materials being queried. Fortunately, significant progress has been made in the past decade in developing both *in vitro* assays and short-term *in vivo* assays to provide predictions that correlate well with data from conventional studies.<sup>20–24</sup> Nonetheless, employing ATS data in decision-making for regulatory purposes would be greatly facilitated by the establishment of government–academia–industry–NGO consortiums that could systematically investigate how results from different ATS approaches for a collection of well-characterized ENMs compare to those in historical *in vivo* outcome studies (e.g., from 90 day inhalation studies). Carbon nanotubes provide one possible example. The cross-comparison of ATS with standard assays would be an important step in a validation process for approaches such as those proposed here. Because the term “validation” has complex and variable meanings depending on the situation, for the purposes of this Nano Focus, we assume that the tools and methods employed would be sufficiently

validated to support the intended risk-assessment purpose. This concept is referred to as “fit for purpose” and recognizes that certain applications require different levels of stringency for validation than others.<sup>27</sup> Cross-comparison of results from tests in different tiers would also be greatly facilitated by the creation of a shared data repository similar to the one proposed earlier, which integrates ENM physicochemical, hazard, and exposure data that have been curated for reliability and accuracy together with effective tools for querying the data therein.

### CONCLUSIONS AND PROSPECTS

Categorization strategies are needed to allow regulators and industry to predict ENM risk and to prioritize the level of testing (hazard, exposure, physicochemical) needed to estimate potential risk while minimizing time-consuming and costly *in vivo* studies that characterize traditional risk assessment. To date, progress toward categorizing ENMs according to risk potential has been stymied by gaps in data and knowledge of how nanomaterial properties correlate with risk. Using input from a multistakeholder group of representatives from industry, government agencies, NGOs, and academia, we have synthesized the following insights that we feel provide constructive guidance on how to improve and to expedite categorization of ENMs according to risk potential:

- Physicochemical properties are not currently sufficient for ENM categorization for regulatory purposes.
- Categorization methods for regulatory purposes should include indicators of both hazard and exposure potential.
- Alternative testing strategies (ATS) may provide a useful means for expedited hazard screening for ENMs.
- Decision-tree approaches for categorizing CNTs according to their risk potential postmanufacturing could facilitate

decision-making in the EPA's New Chemicals program and in other frameworks.

- Targeted cross-comparison of ATS with standard assays may be needed for ATS to be incorporated as an accepted component of categorization strategies in some regulatory contexts.

Building upon these insights, we have developed an example of a tiered decision-tree approach for categorizing the risk of ENMs that incorporates data from ATS assays, which would minimize the need for costly and time-consuming long-term animal studies and greatly expedite effective decision-making in regulatory contexts. We have illustrated how this approach would work using available assays that link to adverse outcome pathways to assess the risk of CNTs in the context of the US EPA New Chemicals program. Many of the principles and insights identified through this example are broadly applicable to other classes of ENMs and other regulatory paradigms, including those for small molecular weight toxicants, where the use of ATS data for material characterization can also play a prominent role in guiding decisions by a broad range of stakeholders in different regulatory contexts.

**Disclosure:** This article has been subjected to review by the EPA and NIST and approved for publication. The views expressed are those of the authors and do not necessarily reflect EPA/NIST policy, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

**Conflict of Interest:** The authors declare the following competing financial interest(s): Several of the authors (S. Brown, T. Kodas, R. Landsiedel, M. B. Miller, J. Muller, and P. Wallis) are employed by companies which manufacture or utilize nano-objects to create materials or products. The authors alone are responsible for the content of the paper. The other authors declare no competing financial interest. ●● Formerly with U.S. EPA in Washington, D.C.

**Acknowledgment.** The UC Center for Environmental Implications of Nanotechnology is funded by a cooperative agreement from the National Science



Foundation and the Environmental Protection Agency (NSF-DBI-0830117 and NSF-DBI-1266377). The Center for NanoBiology and Predictive Toxicology is supported by the National Institute of Environmental Health Sciences (U19ES019528).

## REFERENCES AND NOTES

- Stone, V.; Pozzi-Mucelli, S.; Tran, L.; Aschberger, K.; Sabella, S.; Vogel, U.; Poland, C.; Balharry, D.; Fernandes, T.; Gottardo, S.; et al. ITS-NANO-Prioritising Nanosafety Research To Develop a Stakeholder Driven Intelligent Testing Strategy. *Part. Fibre Toxicol.* **2014**, *11*, 1–11.
- Multi-walled Carbon Nanotubes and Single-Walled Carbon Nanotubes; Significant New Use Rules, 75 FR 56880; Office of the Federal Register, National Archives and Records Administration, **2010**; Vol. 75, pp 56880–56889.
- ECHA Guidance on the Preparation of an Application for Authorisation. [http://echa.europa.eu/documents/10162/13637/authorisation\\_application\\_en.pdf](http://echa.europa.eu/documents/10162/13637/authorisation_application_en.pdf) (accessed January 25, 2015).
- Multi-walled Carbon Nanotubes; Significant New Use Rule, 76 FR 26186; Office of the Federal Register, National Archives and Records Administration, **2011**; Vol. 76, pp 26186–26192.
- Significant New Use Rules on Certain Chemical Substances, 78 FR 27048; Office of the Federal Register, National Archives and Records Administration, **2013**; Vol. 78, pp 27048–27057.
- Significant New Use Rules on Certain Chemical Substances, 78 FR 38210; Office of the Federal Register, National Archives and Records Administration, **2013**; Vol. 78, pp 38210–38223.
- Significant New Use Rules on Certain Chemical Substances, 78 FR 48051; Office of the Federal Register, National Archives and Records Administration, **2013**; Vol. 78, pp 48051–48068.
- Significant New Use Rules on Certain Chemical Substances, 79 FR 51899; Office of the Federal Register, National Archives and Records Administration, **2014**; Vol. 79, pp 51899–51913.
- Significant New Use Rules on Certain Chemical Substances, 79 FR 8273; Office of the Federal Register, National Archives and Records Administration, **2014**; Vol. 79, pp 8273–8293.
- Significant New Use Rules on Certain Chemical Substances, 79 FR 38464; Office of the Federal Register, National Archives and Records Administration, **2014**; Vol. 79, pp 38464–38475.
- Environment Canada, Advisory Note 2014-02 Program, N.S., **2014**.
- ECHA Guidance on Information Requirements and Chemical Safety Assessment. <http://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment> (accessed January 25, 2015).
- Arts, J. H. E.; Hadi, M.; Keene, A. M.; Kreiling, R.; Lyon, D.; Maier, M.; Michel, K.; Petry, T.; Sauer, U. G.; Warheit, D.; et al. A Critical Appraisal of Existing Concepts for the Grouping of Nanomaterials. *Regul. Toxicol. Pharmacol.* **2014**, *70*, 492–506.
- Hansen, S. F.; Larsen, B. H.; Olsen, S. I.; Baun, A. Categorization Framework To Aid Hazard Identification of Nanomaterials. *Nanotoxicology* **2007**, *1*, 243–250.
- Som, C.; Nowack, B.; Krug, H. F.; Wick, P. Toward the Development of Decision Supporting Tools That Can Be Used for Safe Production and Use of Nanomaterials. *Acc. Chem. Res.* **2013**, *46*, 863–872.
- Xia, T.; Malasarn, D.; Lin, S.; Ji, Z.; Zhang, H.; Miller, R. J.; Keller, A. A.; Nisbet, R. M.; Harthorn, B. H.; Godwin, H. A.; et al. Implementation of a Multidisciplinary Approach To Solve Complex Nano EHS Problems by the UC Center for the Environmental Implications of Nanotechnology. *Small* **2013**, *9*, 1428–1443.
- Nel, A.; Xia, T.; Meng, H.; Wang, X.; Lin, S.; Ji, Z.; Zhang, H. Nanomaterial Toxicity Testing in the 21st Century: Use of a Predictive Toxicological Approach and High-Throughput Screening. *Acc. Chem. Res.* **2012**, *46*, 607–621.
- OECD, Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment. Assessment, O. S. o. T. a., Ed. ENV/JM/MONO: Paris, France, **2005**.
- Nel, A. E.; Nasser, E.; Godwin, H.; Avery, D.; Bahadori, T.; Bergeson, L.; Beryt, E.; Bonner, J. C.; Boverhof, D.; Carter, J.; et al. A Multi-stakeholder Perspective on the Use of Alternative Test Strategies for Nanomaterial Safety Assessment. *ACS Nano* **2013**, *7*, 6422–6433.
- Wang, X.; Xia, T.; Duch, M. C.; Ji, Z.; Zhang, H.; Li, R.; Sun, B.; Lin, S.; Meng, H.; Liao, Y.-P.; et al. Pluronic F108 Coating Decreases the Lung Fibrosis Potential of Multiwall Carbon Nanotubes by Reducing Lysosomal Injury. *Nano Lett.* **2012**, *12*, 3050–3061.
- Wang, X.; Xia, T.; Ntim, S. A.; Ji, Z.; Lin, S.; Meng, H.; Chung, C.-H.; George, S.; Zhang, H.; Wang, M.; et al. Dispersal State of Multiwalled Carbon Nanotubes Elicits Profibrogenic Cellular Responses That Correlate with Fibrogenesis Biomarkers and Fibrosis in the Murine Lung. *ACS Nano* **2011**, *5*, 9772–9787.
- Li, R.; Wang, X.; Ji, Z.; Sun, B.; Zhang, H.; Chang, H. C.; Lin, S.; Meng, H.; Liao, Y.; Wang, M.; et al. The Surface Charge and Cellular Processing of Covalently Functionalized Multiwall Carbon Nanotubes Determine Pulmonary Toxicity. *ACS Nano* **2013**, *7*, 2352–68.
- Wang, X.; Duch, M. C.; Mansukhani, N.; Ji, Z.; Liao, Y.-P.; Wang, M.; Zhang, H.; Sun, B.; Chang, C. H.; Li, R.; et al. Use of a Pro-Fibrogenic Mechanisms-Based Predictive Toxicological Approach for Tiered Testing and Decision Analysis of Carbonaceous Nanomaterials. *ACS Nano* **2015**, *10*, 1021/nn507243w.
- Shvedova, A. A.; Kisin, E.; Murray, A. R.; Johnson, V. J.; Gorelik, O.; Arepalli, S.; Hubbs, A. F.; Mercer, R. R.; Keohavong, P.; Sussman, N. Inhalation vs. Aspiration of Single-Walled Carbon Nanotubes in C57BL/6 Mice: Inflammation, Fibrosis, Oxidative Stress, and Mutagenesis. *Am. J. Physiol. Lung Cell Mol. Physiol.* **2008**, *295*, L552–L565.
- Office of Pollution Prevention and Toxics, TSCA New Chemicals Program. Agency, Environmental Protection Agency, **2010**.
- European Union Environment Directorate-General and the European Union Enterprise and Industry Directorate-General, Fourth Meeting of the Competent Authorities for REACH and CLP (CARACAL). Brussels, Belgium, **2010**.
- Judson, R.; Kavlock, R.; Martin, M.; Reif, D.; Houck, K.; Knudsen, T.; Richard, A.; Tice, R. R.; Whelan, M.; Xia, M.; et al. Perspectives on Validation of High-Throughput Assays Supporting 21st Century Toxicity Testing. *ALTEX* **2013**, *30*, 51–66.