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Creating a Safer and Healthier World by Advancing the Science and Increasing the Impact of Toxicology

SOT ISSUE STATEMENT

Nanoscience and Advanced Materials

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Thank you to SOT members Flemming Cassee, Alison Elder, Mary Gulumian, and Timothy Nurkiewicz for drafting this statement on behalf of SOT. This document is intended to be an overview of the issues and complexities associated with nanotoxicology. It is meant to provide a brief, up-to-date starting point for those scientists interested in this area of research.

Contents

Introduction

Nanoscience is the study and application of materials with dimensions between 1 and 100 nanometers. Nanoscience can be used across all other science fields, such as chemistry, biology, physics, materials science, and engineering. An engineered nanomaterial (ENM) is an anthropogenic material with at least one dimension that is less than 100 nanometers (nm)—throughout this document, we use ENM and nanomaterial synonymously. By this definition, fibrous or high-aspect ratio materials also can be considered as ENMs, provided that at least the diameter is less than 100 nm. The sometimes unique and desirable mechanical and optical properties of ENMs promoted their use in many fields, ranging from industrial to medical applications (Bonnard et al. 2019; Zhang et al. 2008). Advanced materials are materials that are specifically engineered to exhibit novel or enhanced properties that confer superior performance relative to conventional materials (Kennedy et al. 2019) and often incorporate ENMs. For example, they are used for groundwater remediation, drug delivery, antimicrobials, and biosensors; as cosmetic ingredients and imaging contrast agents; and in semiconductors and electronic parts (Arias et al. 2018; Jayapaul and Schröder 2019; McNamara and Tofail 2017; Patra et al. 2018; Rai, Yadav, and Gade 2009; Rai and Aswathanarayan 2011).

ENMs are highly diverse in physicochemical properties such as size, shape, and chemical composition, and advanced materials can be even more complex. Properties like morphology, surface area, biological and environmental persistence, surface redox activity, and the release of metal ion and organometallic moieties are associated with health-relevant adverse outcomes in hazard identification studies. For risk assessment, material properties also are relevant insomuch as they contribute to defining exposure, such as concentration (dose), intended use of the material, likelihood of release into the environment or workplace across the material's lifecycle, and stability after release (Arts et al. 2016; Wohlleben et al. 2019).

There are questions about the potential health and environmental risks associated with the use and manipulation of nanomaterials. Moreover, the success of this technology will require that the benefits outweigh the risks. This goal can be attained only by comprehensive toxicity testing of a growing number of nanomaterials. It is anticipated that alternative testing of toxicity with cell culture models that are validated with respect to *in vivo* responses will eventually significantly reduce animal testing as the standard for evaluating risk of nanomaterials (Braakhuis et al. 2021). A comprehensive risk evaluation process can be developed by assessing hazards and exposure, and this will ensure the safety and success of this new technology.

Unintentional exposure of human subjects to ENMs may result during synthesis or from the use of nanomaterial-containing products, while intentional exposure may result from the diagnostic or therapeutic application of such products. Concerns on the toxicity of ENMs used in these applications make it imperative to examine the adverse health effects, if any, that may result

from such exposures. Developing evidence suggests that short- and long-term ENM exposures can cause dose-dependent effects in portal-of-entry as well as distal tissues that may increase the risk for permanent damage and disease. There is, however, a range of toxic potencies among the ENMs.

This document highlights some of the unique issues related to the study of nanomaterials and the assessment of their risk potential.

Exposures Assessment

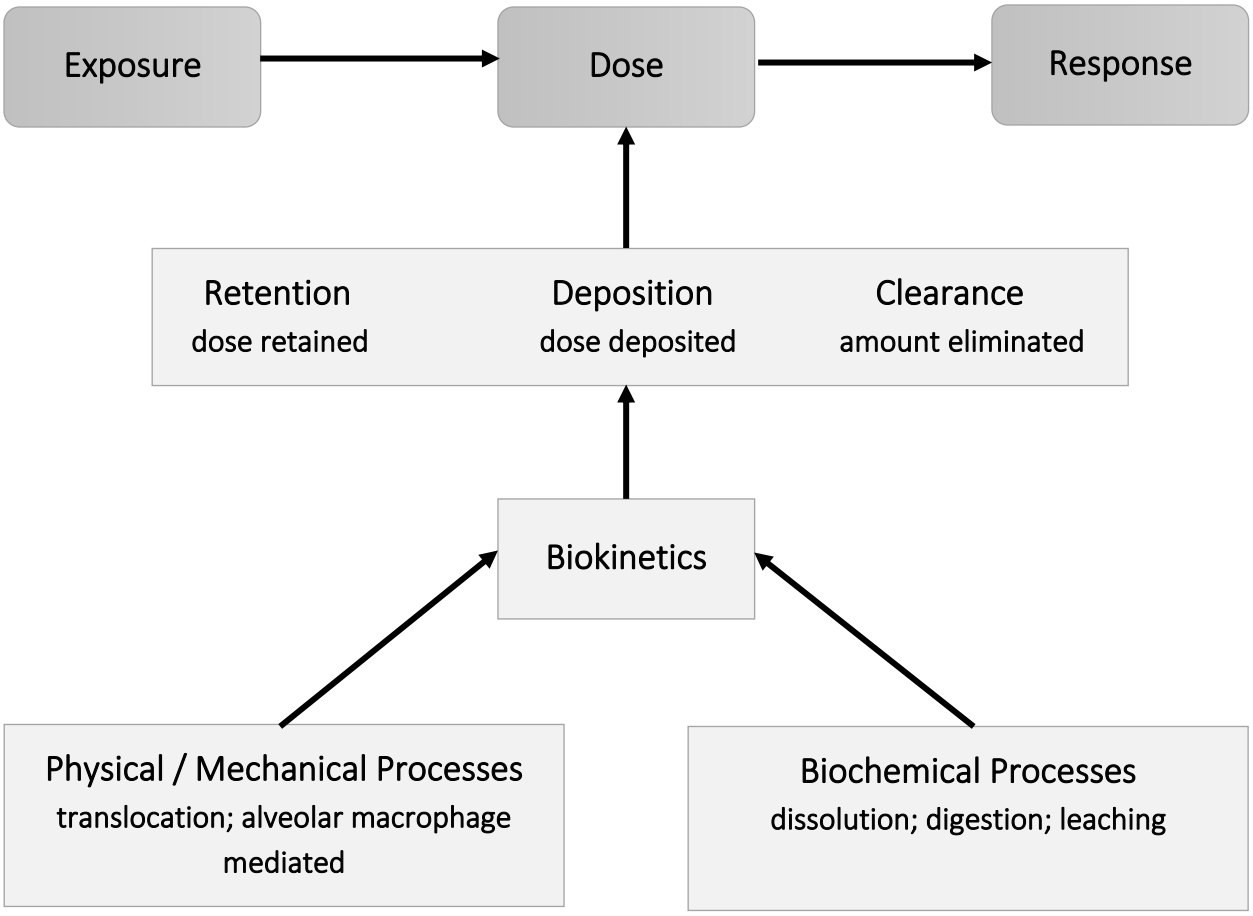
External Exposure

When describing exposure to nanoparticles, it is important to measure size distribution, number and mass concentrations, and surface area, as these are the parameters that are of relevance when determining delivered dose resulting from exposure to ENMs. Specialized instrumentation is used to collect continuous measurements. For example, a Scanning Mobility Particle Sizer (SMPS) is used to determine size distributions in air based on electrical mobility analysis. Upon collection, particles may be analyzed further using dynamic light scattering and/or transmission electron microscopy (TEM) for their size and scanning electron microscopy (SEM) for shape and surface morphology. Moreover, atomic force microscopy (AFM) also may be used for size and size distribution. It should be noted that these latter methods provide information about the physical dimensions and features of particles rather than their size in a medium like air or liquid which is what ultimately determines dose in the respiratory tract or cultured cells.

From Exposure to Dose

Inhalation exposure is arguably the most studied route of exposure receiving comments in the literature today. Once inhaled, ENMs have a high probability of deposition in the lungs (Kuempel et al. 2015). This deposition occurs primarily by diffusion throughout the entire respiratory tract and secondarily by temperature gradient effects in the first few airways of the lung during inhalation and exhalation. Fibers and platelets, like graphene, that are nano-sized in at least one dimension also are deposited in the lower respiratory tract, mainly by interception due to their small size and elongated shape. Once deposited, the chemical composition, including surface reactivity and dissolution rates, are the driving forces for toxicity.

Other routes of exposure also are reported, such as ingestion (Bergin and Witzmann 2013; Gaillet and Rouanet 2015), dermal (Crosera et al. 2009; Murray et al. 2013), and parenteral routes of exposure, including intraperitoneal and intravenous injection (Magaye et al. 2014; Fabian 2008). All routes of exposure are different from each other in terms of particle distribution and kinetics and often induce different toxicological effects (Ameh and Sayes 2019; Medina et al. 2007; Chen et al. 2009).



Adapted from Oberdörster 1991

Figure 1: Relationship of biokinetics to the exposure–dose–response paradigm.

Internal Exposure—Biokinetics

The internal exposure of ENMs is assessed by the amount present in specific target tissues or the whole body. Upon inhalation or injection, translocation to other organ systems via the blood stream can occur. Ingested particles also may gain entry to gut lymphatics and be translocated systemically. It is thought that ENMs do not undergo metabolism in the classic sense, but some types can be biotransformed via mechanical breakdown and/or dissolution, both of which may give an indication of their biodurability and retention in the body (Utembe et al. 2015). They also can be coated by serum or membrane proteins or other macromolecules, such as those found in the food matrix—known as the "corona" effect—which is one of the key factors determining the fate and outcomes of ENMs interacting with biological systems (Lundqvist et al. 2011).

During inhalation exposure, lung deposition is cumulative from the beginning to end of the exposure. Therefore, any extrapulmonary translocation that occurs would be progressive. Another consideration is that not all the surface area of a given alveolus or terminal bronchus is in immediate contact with a pulmonary capillary. Therefore, if inhaled ENMs are translocating from the alveoli, potential exists for entry into the systemic circulation via two routes: (1) across the capillary and into the blood and/or (2) into the interstitial space, ultimately returned to the right atrium via the interstitial fluid and lymphatics. In either regard, the initial delivery/dose to a target organ would be a function of the proportion of cardiac output directed to that organ, vascular resistance, and permeability within. For example, conditioning organs such as the liver and kidneys are prone to greater ENM exposure and accumulation as they receive a large portion of cardiac output and contain unique structures/physiology that favor ENM accumulation.

The intentional introduction of ENMs into the body via injection occurs in clinical or therapeutic applications (e.g., lipid nanoparticles, liposomes, emulsions). As with other nanotechnologies, the purpose is to improve aspects of the therapy (e.g., safety, compartment access, half-life). ENMs also are injected in animals for a variety of purposes. This may include demonstrations of biologic effects and/or toxicity independent of pulmonary involvement; drug development; tracers/labels; and antibody targeting. Injection produces higher, bolus ENM dose deliveries than inhalation exposures, but the biokinetics depend highly on the route into the body. Intravenous injections would follow a similar route in the cardiovascular system, as they would first return to the heart and then systemically be distributed. The biokinetics would be most rapid for this route. Intraperitoneally injected ENMs would require considerably more time to gain access to the systemic circulation via the interstitial fluid-lymphatic path.

Dose Metrics

Cell, tissue, and whole-body exposures to ENMs can be described by mass, number, or surface area of particles. It is, therefore, recommended to express concentrations in all three dose metrics, which may allow conversion from one dose to another when determining toxic effects. The implementation of dosimetry modeling also may assist in the determination of the delivered dose *in vitro* and *in vivo* (Cohen, DeLoid, and Demokritou 2015; Multiple-Path Particle Dosimetry (MPPD) Model). For mass deposition measurements specific to cell-based models, quartz crystal microbalance is a common analytical method used (Choi et al. 2012). For measuring concentration in the air for either *in vitro* or *in vivo* models, aerosol particle sizers (Sayes et al. 2010) and gravimetric determinations commonly are used.

Hazard Assessment

To be the most useful for both hazard identification and eventual risk assessment, studies on the effects of ENMs should be conducted over a range of concentrations/doses and should consider time—both duration of exposure and the period following exposure—as determinants of

response. Importantly, the range of doses should include a level (other than control) that does not induce the effects that higher concentrations/doses do (i.e., the no-observable-adverse-effects-level or the benchmark dose level). It should be noted that concentration and dose are not equivalent as a concentration is measured in the volume of air, food, fluid, etc., to which a cell, organ, or animal is exposed, and the dose is the amount of ENMs that actually comes in contact with the target.

Sample Preparation and Exposure Characterization

To assess the toxic potency of ENMs, materials have to be prepared in such a way that the biological test systems can be exposed in a meaningful way. Many *in vitro* test systems require that ENMs are dispersed in culture media that can affect the properties of the ENMs. For example, powder-based ENMs often are forming aggregates and, hence, possess a larger size than the primary material. Several methods have been published, and it should be mentioned that the way a sample is prepared for toxicological testing should be placed in the context of the objective of the study and the specific hypothesis or research question (e.g., DeLoid et al. 2017). Whatever the test system, a comprehensive description of the ENM characteristics in that system should be included to enhance replicability and better understanding of dose.

Standardized Testing (ISO and OECD)

The OECD Test Guidelines are accepted internationally as standard methods for safety testing and are indispensable for professionals working in industry, academia, and government on the testing and assessment of ENMs. One of the key features is the assurance of high-quality and reliable data and for countries and industry to fully benefit from the OECD agreement on Mutual Acceptance of Data (MAD) and avoid duplicative testing. In recent years, activities have launched to update various testing guidelines to accommodate testing of ENMs. For example, inhalation testing guidelines for sub-acute and sub-chronic testing have been updated with respect to the aerosol size range (no lower limit anymore) and information requirements when using poorly soluble, potentially more biopersistent particles. The OECD Working Party on Manufactured Nanomaterials (WPMN) continues to modify existing or develop new test guidelines for ENMs under the auspices of the OECD Working Group of the National Co-ordinators of the Test Guidelines Programme. The standard battery of genotoxicity tests may not be ideal—e.g., the use of bacteria, such as in the Ames test, is not recommended since "the gram-negative strains of bacteria used in the standard assays do not appear to have the capability for nanoparticle uptake, lacking mammalian mechanisms of endocytosis, pinocytosis, and phagocytosis" (Elespuru et al. 2018). The International Organization for Standardization (IOS) also develops standard operating procedures and standards to ensure the quality, safety, and efficiency of products, services, and systems.

Grouping Approaches

Due to the vast number of ENMs that already are developed, in production, or still in the design phase, it is almost impossible to test them all for their toxic potency in regulatory *in vitro* and *in vivo* toxicity testing. The alternative is to use as much information as possible derived from other materials with overlapping properties and dimensions to foster an efficient and affordable risk assessment. Both read-across and grouping approaches can be considered (Mech et al. 2019). Grouping of chemicals is well established and widely applied, and specific guidelines and informative documents are available from the European Chemicals Agency (e.g., ECHA 2013). As presented by Mech and co-authors (2019), most of the approaches, including those outlined by ECHA in the context of REACH, are not suitable with respect to nanomaterials. Recently, a framework for grouping and read-across of ENM-supporting innovation and risk assessment has been proposed in the context of the European project GRACIOUS (Stone et al. 2020). Based on the combination of available and newly generated information, a grouping hypothesis can be selected in risk assessment approaches.

Toxicity

The physicochemical diversity and complexity of ENMs suggest that many of the health effects that have been documented for larger-scale particles—and even some chemicals—would be expected with certain nanomaterials (Halappanavar et al. 2020). This is, indeed, the underlying premise of the aforementioned grouping schemes. Due to their surface reactivity, endpoints related to oxidative stress and inflammation often are evaluated, as well as fibroproliferative lesions and tumor formation after long-term exposures. The *in situ* release of metals, for example, could lead to interactions with endogenous proteins, leading to sensitization and the development of hypersensitivity reactions (Roach, Stefaniak, and Roberts 2019). The depletion of antioxidant defenses or other adaptive mechanisms could lead to heightened susceptibility to subsequent pathogen or chemical exposures.

Oxidative stress leading to inflammatory responses is a common biological response to ENMs and can lead to tissue damage, including permanent changes like fibrosis, lung cancer, and potentially mesothelioma related to biopersistent fibres (Stone et al. 2017).

Challenges in the Risk Assessment of Nanomaterials

Despite the great strides that have been made internationally in the development of standardized toxicity testing and exposure assessment for risk assessment methods for nanomaterials, there seems to be agreement that considerable work still remains to generate the required data for ensuring appropriate risk evaluation of nanomaterials. Knowledge gaps include the characterization of the wide range of nanomaterials being produced and information on their

complex behaviors in different media or the fact that the toxicity is specific to a tested nanomaterial and cannot be generalized or extrapolated, even within the same nanomaterial family. Also, there is a lack of chronic studies on the toxicity of different nanomaterials. Generalized exposure scenarios also have not been developed for risk assessment along the life cycle of a nanomaterial. Data on exposure of workers, consumers, and the public via the environment is available, but acquiring this data is challenging (Kuhlbusch, Wijnhoven, and Haase 2018). Not much is known about the levels of emissions from nanomaterial production facilities and the fates of these emissions in the environment, and therefore, they have been estimated using material flow models following the ENM lifecycle (Sun et al. 2016). Subsequently, without reliable data on effects and exposure, in-depth risk assessments of nanomaterials for developing risk-based management strategies or regulations are difficult to conduct. Despite these challenges, progress continues to be made by international agencies to contribute to the risk assessment of ENMs.

Future Directions

As presented by Stone and colleagues (2017), the toxicity and risk assessment of ENMs may have unique features, but there also are similarities with ultrafine particles that are omnipresent in the air we breathe. In essence, the hazard of nanomaterials can be assessed using commonly applied test methods and using mass as the unifying metric. However, as the number and diversity of novel nanomaterials and nano-enabled products increase, it will be impractical to test every new nanomaterial using *in vivo* models. Thus, approaches that are both validated with respect to *in vivo* responses to ENMs and accepted by the regulatory community are needed to assess and predict nanomaterial safety. It is clear that materials with exactly the same chemistry may have very different effects based on the physical aspects, including size, shape, and surface charge, which have to be taken into the equation when using read-across and grouping approaches. *In vitro* models have to include not only relevant biological endpoints that can be directly related to a human adverse outcome via adverse outcome pathways (Leist et al. 2017; Vinken et al. 2017), but also careful assessment of the biologically effective dose (DeLoid et al. 2017).

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