Clark, K.A.; White, R.H..; Silbergeld, R.K. **Predictive models for nanotoxicology: current challenges and future opportunities.** Regulatory Toxicology and Pharmacology, 59(3):361-363, 2011.

Postprint version	Final draft post-refereeing
Journal website	http://www.sciencedirect.com/science/journal/02732300
Pubmed link	http://www.ncbi.nlm.nih.gov/pubmed/21310205
DOI	10.1016/j.yrtph.2011.02.002

Title: Predictive models for nanotoxicology: current challenges and future opportunities

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Abstract

Characterizing the risks posed by nanomaterials is extraordinarily complex because these materials can have a wide range of sizes, shapes, chemical compositions and surface modifications, all of which may affect toxicity. There is an urgent need for a testing strategy that can rapidly and efficiently provide a screening approach for evaluating the potential hazard of nanomaterials and inform the prioritization of additional toxicological testing where necessary. Predictive toxicity models could form an integral component of such an approach by predicting which nanomaterials, as a result of their physico-chemical characteristics, have potentially hazardous properties. Strategies for directing research towards predictive models and the ancillary benefits of such research are presented here.

One of the greatest challenges for assessing the potential risks of manufactured nanomaterials (MNMs) is the lack of a rational evidence-based system for inferring the hazard of nanomaterials. This is particularly problematic in light of the increasing number of MNMs in commerce. The unique properties of MNMs, largely a function of their small size and associated high surface-area to volume ratio, can result in increased transport across membranes, binding to biological macromolecules, transport of molecules, biocidal properties, or may even result in biological properties that have not yet been predicted or recognized for nanomaterials. These properties, depending on the situation or purpose, may be beneficial, such as when optimized for drug delivery, but also may pose health and/or environmental risks as a result of unintentional exposures or environmental release.

Within a single class of MNMs (e.g., carbon-based nanomaterials, metal oxide nanoparticles), there can be a wide range of sizes and shapes, with different chemical composition and surface modifications, all of which may affect behavior and toxicity. When considering the variety of nanomaterials that are currently or will in the future be produced and used, it is clearly infeasible to evaluate hazard by testing all of these MNMs on a case-by-case basis, particularly with mammalian tests. Yet, for lack of a system for evaluating MNM hazard, there is little opportunity to compare the results of different studies or isolate factors that may translate into increased hazard and risks. The difficulties in assessing the risks of MNMs are well-recognized. Several articles have been published in recent years that outline options and challenges for applying traditional and alternative risk assessment strategies to MNM, both in terms of human and environmental risk (Grieger et al., 2010; Johnston et al., 2010; Linkov et al., 2007; Linkov et al., 2009; Morgan, 2005; Shatkin et al., 2010; Tervonen et al., 2009). Prominent among the alternative methods are the use of multi-criteria decision analysis (MCDA) and expert elictation, which incorporate expert judgment to a greater extent than traditional risk assessment methods. The over-arching theme of all these articles is the need for more data to conduct risk assessments that inform risk-based decision-making for nanomaterials.

A more intensive effort towards the development of predictive toxicity models is urgently needed. Predictive models have been used for decades to rapidly evaluate the potential hazard of chemicals for which there are inadequate or no data on potential hazard, as is the case for new chemicals evaluated under the US Toxic Substances Control Act. In addition, the use of these methods has been adopted under the High Production Volume Chemical Programs and the European REACH legislation (EPA, 1999; Hartung and Hoffmann, 2009; OECD, 2004). In parallel, the US EPA has recently released a report outlining the needs, in terms of environmental fate and transport of MNMs, for developing predictive models (Johnston et al., 2010). Efforts to address the human toxicity of nanomaterials should be directed towards developing models that predict associations between changes to MNM physico-chemical characteristics and hazardous properties. For example, small changes to size or surface functional groups may affect whether a MNM can cross physiological barriers, how it can interact with cellular structures, and ultimately, the types of responses it can initiate. If these relationships were understood, it would assist in developing safer MNMs, screening MNMs for regulatory purposes, and prioritizing MNMs for more comprehensive toxicological testing.

Several approaches to building predictive models in toxicology exist, ranging from relatively simple read-across methods to more complex computational models (e.g., QSARs). Regardless of model type and associated terminology, predictive models are developed by categorizing toxicological data by a concept of relational characteristics, with representative substances in a category used to infer toxicological information about other substances that can be assigned to the same category (OECD, 2007). The development of predictive models, particularly computational models, requires large amounts of high quality data. Data that are not sufficiently informative in the development of predictive models include data that are collected using diverse methods, that do not include a detailed description of the tested materials, measure different endpoints (or use different methods for quantifying endpoints), or are focused on one or a few MNMs. Several factors can hinder the development and predictive ability of models, thus presenting significant challenges to the development of predictive models for all

endpoints or classes of materials. These factors include limitations in the availability of the data needed to build models, in the understanding mechanism of action, and in the definition of the chemical space for which the model can be used (Cronin et al., 2003; Hartung and Hoffmann, 2009; Zvinavashe et al., 2008). However, the state-of-the-science for modeling of chemical-biological interactions is advancing, in part due to the continued development of high throughput testing and associated data analysis as well as advances in the understanding of the biological basis (e.g., modes of action) for chemical-induced health impacts (Rusyn and Daston, 2010).

Due to the differences between MNMs and the substances that are more traditionally the subjects of predictive modeling (i.e., molecules), the descriptors for building models for MNMs will necessarily be different and pose a significant challenge for toxicology (SCENIHR 2007). Puzyn, et al. (2009) discuss in detail the potential and challenges for developing nano-QSARs and provide examples of some initial QSARs developed for MNMs. The authors are optimistic that this methodology can successfully be applied to MNMs. Fourches, et al. (2010) have successfully developed two QNARs (quantitative nanostructure-activity relationships) using two large datasets from *in vitro* assays. Both models were statistically validated and externally predictive.

There are several aspects to a research strategy for developing predictive models for MNMs. First, there must be greater standardization at all stages of the toxicity studies, from starting materials to endpoint(s). Although standardization risks inhibiting flexibility and innovation in study design, for the purposes of developing models, data collected in the same manner are needed. Several organizations are currently evaluating testing protocols and developing guidelines and reference materials, including OECD, the International Organization for Standardization (ISO), and various other international projects. Material characterization continues to present a significant hurdle in terms of resources (e.g., costs and expertise) to many toxicologists: the continued development of well-characterized standardized reference materials for toxicity testing should assist in this area. Second, studies should be designed in ways that specifically and systematically evaluate the role of physico-chemical properties in MNM behavior. For example, a study

that tests the effect of a range of surface modifications on oxidative stress will be far more informative than one that measures oxidative stress following exposure to a single MNM. In general, systematic testing of variations on a MNM property (e.g., charge, size, surface properties, etc.) are needed to identify how physico-chemical properties influence biological activity. Finally, an open system for storing and sharing information is needed. Referring to the peer-reviewed literature for data is not useful because details are often missing, this system of sharing information is subject to publication bias (i.e., non-publishing of negative results) and many industry studies are not published. Successful data sharing systems must function in a way that users can enter data for sharing and access other data, all while maintaining confidentially of sensitive information to encourage broader participation. The DEREK system for predicting toxicity and the National Cancer Institute's caNanoLab provide examples of systems that could be adapted to a larger scale for sharing data (Gaheen et al., 2009; Marchant et al., 2008).

There are also secondary benefits from investing in the effort needed to develop predictive models, independent of models themselves. As noted above, only limited data are currently available for conducting hazard evaluations, and there is little agreement on the base set of toxicity assays and associated information needed to inform hazard assessment. A commitment to develop predictive models for nanomaterials will focus needed attention on improved nano-specific assays and developing open, curated databases of such knowledge. Alternative approaches for prioritizing risks in data poor areas, such as MCDA and expert elicitation, could also benefit from (and inform) the development of such information (Linkov et al., 2009; Tervonen et al., 2009). Additionally, the development of predictive models requires information that is focused primarily on interactions at the molecular and cellular level. It is generally recognized that high throughput/*in vitro* testing methods are needed to characterize the hazards of the large number of MNMs in production and use (Hartung, 2010). Such a research goal is well-aligned with recent European Union regulations seeking to minimize research in animals and efforts in the United States to better develop *in vitro* and *in silico* toxicology (NRC, 2007; Schoeters, 2010). As part of this effort, it is also critical to develop a basis for inferring relationships between *in vitro* and *in vivo* data for predicting human health

hazards (Warheit, 2010). Finally, as the field of nanotoxicology is relatively young, it is an ideal time to implement new and innovative strategies for testing. The successes and failures of these efforts can be used to inform toxicity l testing and associated risk assessment and risk management policy for other chemical classes or novel technological areas that have not been the target of increased research interest or coincided with efforts towards more advanced or alternative toxicity testing methods (Hartung, 2010).

The novel and advanced properties of MNMs calls for a novel and advanced approach to their testing. Several scientists have mentioned or urged the development of QSARs or other predictive models for MNMs (ICON, 2008; Meng et al., 2009). However, the concerted action to develop such an approach, both through funding mechanisms and the standardization of research methods (with predictive models in mind) is lacking. Now that there are initial efforts showing promise in the area of predictive models for MNMs, efforts must be made to increase the momentum in this area. As nanotechnology is redefining how we manufacture and use materials, it is only appropriate that health scientists meet this innovation with some innovation of their own.

The authors do not have any competing financial interests associated with this work.

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