Effects-Based Risk Assessment of Emerging and Existing Nanomaterials

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ABSTRACT

The phenomena of nanotechnology have been compared to the Industrial Revolution in its scope of potential applications, ability to transform current technologies and the number of scientific disciplines that is encompassed. However, the novel biological, physical, and chemical properties of nanoscale particles (NP) that are harnessed for new technologies may prove to be a Trojan horse. Although the majority of research is focused on product development the need for research to characterize toxicity of these NP is becoming increasingly evident. The lack of toxicity data is hampering the ability of government agencies to regulate the use of these particles effectively. This article discusses preliminary toxicity tests that were performed on selected ecological receptors from marine, freshwater, and terrestrial compartments. We also discuss the development of a risk assessment framework with a life-cycle focus in the context of managing environmental risks associated with nanotechnology.

Keywords: Nanotechnology, Nanotoxicology, Risk Assessment Framework

1 INTRODUCTION

Nanotechnology is a rapidly developing industry that has been compared to the industrial revolution. The harnessing of the novel biological, physical, and chemical characteristics of NP has been projected to realize many benefits that have previously been unattainable. However, along with these technological advances there also are responsibilities to understand and manage the hazards associated with exposures to NP.

NP are defined as a sub-classification of ultrafine particles with lengths in two or three dimensions greater than 0.001 micrometer (1 nanometer) and smaller than about 0.1 micrometer (100 nanometers) and which may or may not exhibit size related intensive properties [1]. Evidence is beginning to demonstrate that NP may prove to be somewhat of a Trojan horse; along with the tremendous potential to alter how we approach and view science and technology the unique physical and chemical characteristics of NP that can affect biological responses may pose human and ecological concerns distinct from their bulk counterparts.

NP can be made from a large number of compounds creating a complex group of materials to characterize and understand toxicity characteristics. There is a debate amongst the nanotechnology community of how to govern the associated risks of nanotechnology, whether hazard information can be applied to classes of nanoparticles or to individual NP and whether existing bulk hazard information is adequate. Currently there is not enough data to effectively inform the risk analysis and regulatory process.

This article discusses the preliminary findings of toxicity studies performed using 13 different commercially available NP on selected organisms from the marine water, fresh water, and terrestrial compartments. The development of a nanotechnology life cycle based risk assessment framework also will be discussed.

2 PUBLIC PERCEPTION OF RISK

The development of new technologies, including nanotechnology, is a process that involves input from a diverse group of stakeholders where the perception of risk weighs heavily in the successful progression of the technology (Figure 1). The evolution of any technology begins in the conceptualization realm with theoreticians who develop the initial idea or concept followed by scientists who move a theory from concept to the experimental stage. Once the technology has taken on a tangible form, entrepreneurs and sometimes venture capitalists begin to invest in the technology resulting in the emergence of consumer products. Ideally the development of new technologies progresses through this product development curve in a linear, albeit iterative, unhindered fashion. However, realistically other factors may potentially alter this path.

A major factor that may affect the realization of the full potential of nanotechnology is the publics' perception of the risks associated with nanotechnology with regard to socioeconomic and environmental health impacts [2,3]. Prior experience with the public backlash to genetically modified organisms (GMO; [4]) and the nuclear power industries provide the nanotechnology industry with precedence to consider these factors early in the evolution of development.

The occurrence of an event that causes morbidity or mortality due to use of or exposure to nanotechnology likely would trigger negative public perception and bring heightened attention from news media and activists groups.

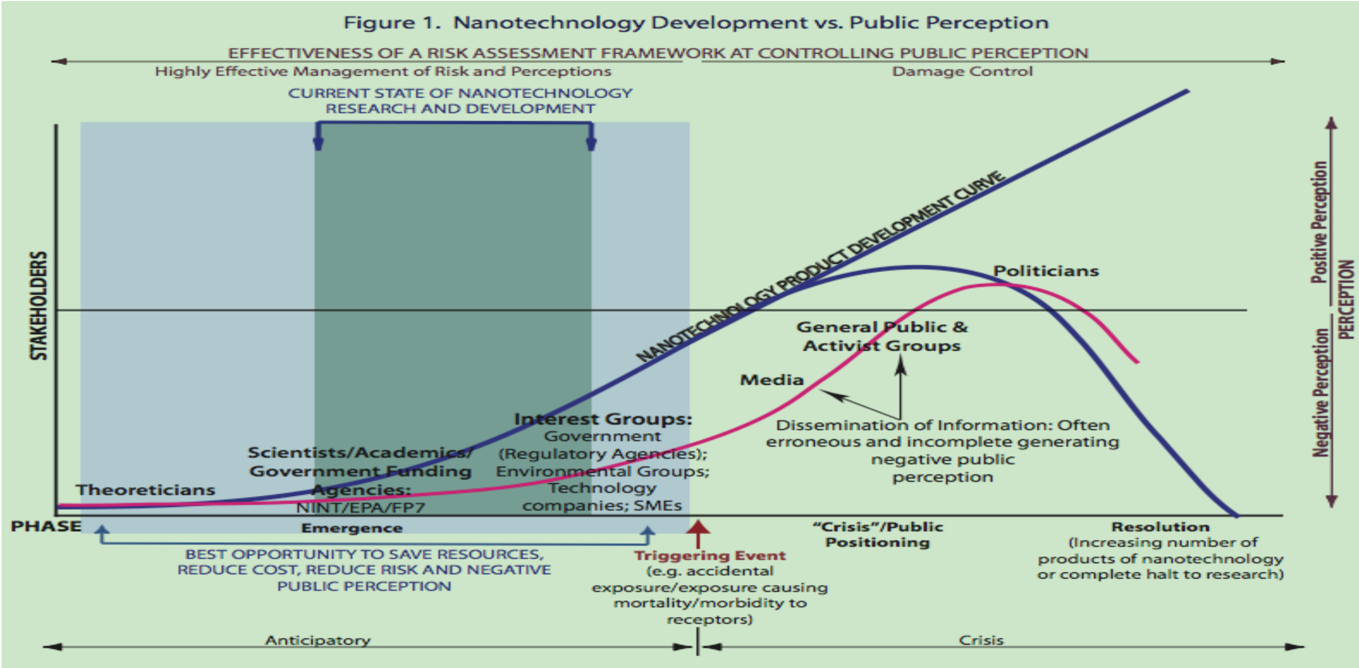

Figure 1. Nanotechnology Development vs. Public Perception

The question is whether nanotechnology will withstand consumer challenges or will it be constrained in a manner similar to the GMO industry. Much depends on the publics' confidence that appropriate measures to manage risks are being taken from the initial stages of product development through product disposal. Although consumer products that incorporate nanotechnology are rapidly emerging and already available [5] there is still opportunity to address environmental concerns and manage risks.

3 RISK ASSESSMENT FRAMEWORK

Public safety and environmental protection are important and shared obligations of proponents and regulators in any industry. The risk assessment framework provides one tool that can be used effectively to organize information that can be useful to foster informed dialogue and to assist in making critical decisions. There is growing recognition of the need for risk assessments of NP [6-9].

The risk framework can be applied at different stages of a product beginning with experimental work of formulation through disposal of all waste streams and products. The degree of sophistication used in assessing risks should increase along the product life-cycle. Characteristically, the assumptions used in assessing risk at the early stages are designed to be protective, that is to trip flags about possible problems so that more attention is focused on managing or mitigating such risks. As one progresses through the product lifecycle, more data become available and thus the assumptions used in the assessment become more realistic. At any level of analysis, absence of data typically triggers precaution. In the absense of solid defensible scientific information that addresses public concerns, the nanotechnology industry likely will face restrictive measures based on precautionary principles.

Clear communication on risk issues requires common understanding of terms. One important aspect is to distinguish between hazard and risk.

- Hazard – the inherent properties of a stressor (biological, chemical, or physical agent) that can have an adverse effect on a receptor (humans, other animals, plants, or microbes).
- Exposure – the magnitude, concentration, dose, or other measure of the degree of contact a receptor has with a hazard.
- Risk – the likelihood of an adverse effect occurring as a result of being exposed to a hazard.
- Risk Assessment – a process (usually within a formalized framework) that examines scenarios to evaluate the likelihood of an adverse event occurring. This is accomplished through estimation of the magnitude of exposure and relating effects that occur from such exposures to one or more hazards.

Studies of algae, invertebrates, plants, and fish show these organisms incorporate NP into their tissues and, at the concentrations/doses tested, exhibit some toxic responses. A key outstanding question is whether the laboratory test concentrations are realistic (i.e., will such concentrations occur in the environment? if so, under what circumstances?). At this stage, we do not know enough to judge whether adverse effects are likely to be manifested in occupational settings or the environment. Therefore, there is some urgency to adapt the risk framework to address the specific issues pertaining to the nanotechnology industry. Concurrently, the need is equally urgent for data on toxicity or hazard, fate and transport of particles across environmental media, and quantitation of exposure for various receptors (human and ecological).

4 TOXICITY TESTING

We have performed a series of laboratory toxicity tests to obtain preliminary indications of hazards associated with the various NP. In this section, we describe the types of NP tested, the test methods used to assess hazard, and the results of these studies.

4.1 Nanoscale Particles

Thirteen nanoscale particles (Table 1), purchased from Sigma-Aldrich (St. Louis, MO, USA) and Materials Technology Research (MTR, Cleveland, OH, USA), were used for toxicity testing. Nanoscale particle solutions (1000 mg/L) were made in freshwater (City of Calgary dechlorinated water) or in artificial marine water (30 000 ng/L, Instant Ocean Synthetic Seasalt®, Mentor, OH). For the sonicated samples each nanoparticle solution was sonicated immediately prior to testing (60 Sonic Dismembrator, Fisher Scientific, Nepean, ON, Canada) until the solution appeared non-particulate and as an even slurry. For the unsonicated samples, aliquots of solution were drawn from the top of the storage containers and used immediately for testing.

4.2 Experimental Testing

Test organisms were maintained in house according to our laboratory standard culturing conditions consistent with guidance from Environment Canada reference methods [10-12], the Alberta Energy Utilities Board Directive 50 Guidelines [13], USEPA standard methods [14], ASTM standards [14] and other standard methods [15]. Freshwater organisms: fathead minnows [14] (*Pimephales promelas*; Aquatox, Hot Springs, AK, USA), daphnids [11] (*Daphnia magna*; in house culture*)*, algae [12] (*Pseudokirchneriella subcapitata*; in house culture)], terrestrial organism: nematode [15] (*Panagrellus redivivus*; in house culture)] and marine organisms: sheepshead minnows [14] (*Cyprinodon variegatus*; Aquatox), *Vibrio fisheri* bacterium [13] (Microtox; Osprey Scientific, Edmonton, AB)] were tested following standard methods cited.

4.3 Statistical Analysis

Statistical analysis (student T-test) was performed on tests samples with differential survival using GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA).

5 RESULTS AND DISCUSSION

Many scientific, technological and health benefits may be realized with the evolution of nanotechnology. However, along with the beneficial aspects of nanotechnology is the added responsibility to understand and manage the hazards and risks associated with nanotechnology exposure.

This study was done to assess preliminary toxicities associated with a range of NP on a series of standard test species from the terrestrial, marine and freshwater ecological compartments. Unlike other studies where particles were suspended in organic solvents such as THF, we chose to use freshwater and marine water to mimic environmentally relevant conditions since some studies demonstrate that toxicity is due to solvent by-products [16,17].

In an attempt to establish homogeneous suspensions of the NP we examined the effects of sonicated and unsonicated samples. The exposure of NP to biological media (e.g. water) has a tendency to cause the particles to form larger fragments that may or may not be within the nanoscale range (1-100 nm) or have the same biological, physical or chemical properties of their native nanoscale counterparts. Sonication provides a physical force that decreases the size of these clumps.

In this study the exposure of marine and freshwater test organisms induced differential responses based on the nanoscale particle and aggregation state (Table 1). There is not a single particle that had completely benign or harmful effects, although copper, silver copper and silver were toxic to several of the organisms tested. Some NP had stronger toxicity effects, while others did not differ significantly from control tests.

In comparing the sonicated samples with the unsonicated samples sonication significantly ($p \leq 0.05$) increased toxicity induced by cerium oxide in sheepshead minnow, by diamond, titanium oxide and graphite in fathead minnows and by multiwalled nanotubes in daphnia. None of the other particles tested had statistically significant ($p > 0.05$) changes. Conversely, with silver, silver copper and copper treatments significant ($p \leq 0.05$) mortality was observed regardless of the aggregation state.

As previously mentioned, sonication decreases the size of the aggregated clumps into smaller particles. The smaller particles may be more toxic to the organisms tested due to altered surface characteristics or increased surface area and consequently higher degree of reactivity or a host of other factors that have not yet been characterized. A higher degree of reactivity may overwhelm the organisms' response against noxious stimuli or induced membrane damage [18,19].

Our laboratory study demonstrates that NPs suspended in environmentally relevant media have toxic effects within different media and trophic levels; the responses are highly particle, organism, and aggregation-state dependent. However, more sophisticated studies are required to characterize physical and chemical properties that may contribute to toxicity profiles of NP.

Although new products and novel uses for NP are constantly emerging on the consumer marketplace, the nanotechnology industry is still somewhat within the realm of scientists/academics and interest groups (Figure 1). So far, there has not been a major triggering event that has initiated a large negative public backlash, however, public action groups that allege governments are not doing enough to understand the risks associated with nanotechnology have begun to mobilize and are gaining momentum (ETC

Table 1. Toxicity Effects of Nanoscale Particles

COMPARTMENT	MARINE				TERRESTRIAL	FRESHWATER				
Organisms	**Sheepshead Minnow**		**Microtox**		**Nematode**	**Fathead Minnow**		**Daphnia**		**Algae**
Test Duration and Endpoints	96 Hours Mortality % Mortality		15 minutes Luminescence % Control		24 H Mortality % Mortality	96 H Mortality % Mortality		48 H Mortality % Mortality		72 H Growth Growth: + No Growth: -
Nanoscale Particle [1000 mg/L]	Unson	Son	Unson	Son	Son	Unson	Son	Unson	Son	Son
Diamond	0^a	0^a	103	7	1	7^a	$100*^b$	7^a	0^a	+
Cerium Oxide	0^a	$100*^b$	81	3	3	40^a	13^a	0^a	$47*^a$	-
Multiwalled Nanotubes	7^a	7^a	122	30	1	10^a	40^a	0^a	100^b	-
Titanium Oxide	0^a	0^a	105	5	6	13^a	$73*^b$	27^a	0^a	+
Aluminum Oxide	0^a	0^a	110	5	0	0^a	53^b	7^a	20^a	-
Silver	33*	N.D.	103	18	28	$100*^a$	$100*^a$	$100*^a$	$100*^a$	-
Silver Copper	$100*^a$	$100*^a$	57	1	21	$100*^a$	$100*^a$	$100*^a$	$100*^a$	-
Silicon Dioxide	0^a	0^a	112	28	3	7^a	27^a	67^a	$40*^a$	+
Barium Strontium Titanium Oxide	0^a	7^a	107	2	2	0^a	25^a	7^a	0^a	-
Carbon	0	N.D.	109	2	13	13^a	58^a	$100*^a$	$100*^a$	-
Copper	$100*^a$	$100*^a$	91	0	4	$100*^a$	$100*^a$	$100*^a$	$100*^a$	+
Graphite	0	N.D.	N.D.	2	2	13^a	$83*^b$	87^a	100^a	-
C60	0	N.D.	93	43	1	0^a	13^a	7^a	$27*^a$	-

*Significantly different than the control ($p \leq 0.05$); Means in the same row with different letters are significantly different

Group; Press Release). This speaks strongly to the need for scientists, industry, and regulators to address many of the public safety issues that have plagued the GMO, biotechnology, and nuclear power industries early on in the developmental process. Given the rapidity with which developments in the area of nanotechnology are occurring, it is imperative that relevant scientific evidence is available to both inform the regulatory process as well as guide practitioners so as to ensure safe and responsible use of nanomaterials [22].

6 CURRENT RESEARCH

We are currently evaluating the effect of NP exposure on root growth, shoot elongation, and nodule formation using alfalfa plants inoculated with rhizobium. We are also characterizing the NP before, during, and after testing using TEM.

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