RICE

CENTER FOR BIOLOGICAL AND
ENVIRONMENTAL NANOTECHNOLOGY

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NTP Nominations Faculty
National Toxicology Program/NIEHS
MD A3-01
P.O. Box 12233
Research Triangle Park, NC 27709

Dear Dr. Masten:

I am writing to nominate nanoscale materials as a class of substances for toxicological evaluation by the National Toxicology Program. Nanoscale materials are a broadly defined set of substances which all share the feature that at least one critical dimension is less than 100 nm. They can be made from nearly any substance; semiconductor nanocrystals, organic dendrimers, and carbon fullerenes and nanotubes are just a few of the many nanomaterial examples. These materials are central to a rich array of emerging nanotechnologies and scientists and lawmakers alike have invested substantial time and resources in the area over the past decade. In the last four years alone, the federal government has provided over one billion dollars in nanotechnology research funding. Already nanoscale pigments are used in consumer products such as sunscreens and cosmetics, and the market for these materials is estimated to grow to over eight billion dollars in the next decade. Clearly, nanomaterials and their associated nanotechnologies are here to stay.

The real value of nanomaterials lies in their unique chemical and physical properties, many of which are highly dependent on particle size and coating chemistry. For the purposes of this nomination, the term nanomaterials is restricted to mean nanoparticle systems with dimensions less than 20 nm. These solids have crystalline structures often similar to bulk materials, but because of their limited extent possess unique features. Semiconductor nanocrystals, for example, have strong and tunable emission throughout the visible and near-infrared; carbon nanotubes exhibit mechanical strength far greater than ordinary carbon fibers. Ceramic nanoparticles have highly active surfaces which can be used in catalytic and photocatalytic applications. These examples are only a few of the many ways that nanoparticle properties and surface areas are used in applications.

As this industry grows, it is essential that both private industry and public health and regulatory agencies begin to understand the toxicology of major nanomaterials classes. As nanotechnology develops, the general public will become exposed to increasingly higher amounts and more diverse forms of nanostructured materials. The health effects have not yet been considered, and only a handful of literature reports are available on the topic. It is important to complete the studies, now, before major applications become established. Only with such data can the public weigh the potential societal costs of these materials against their many benefits.

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Here at Rice, the Center for Biological and Environmental Nanotechnology which I direct has as its mission the characterization of the environmental impact of nanomaterials; our scope is limited, however, to only environmental engineering and consideration of the fate, transport, bioaccumulation and biodegradation of nanoparticles in aqueous systems. For our efforts to be informative, there must be ongoing work on nanoparticle toxicology and health effects. The NIEHS is the logical organization to engage in such research.

A number of potential health concerns were recently discussed at a symposium on the "Toxicology and Biological Interactions of Nanomaterials" at the 225th American Chemical Society National Meeting. This was the first symposium of its type and was coorganized by scientists from Rice University and the NIEHS. From the presentations at this symposium, it was clear that nanoparticles have the general ability to distribute throughout the body and be taken up by a variety of cell types in various tissues and organs. Certain materials collect in mitochondria, others in the nucleus, etc. The consequences of organ and cellular uptake are completely unknown, but research in ultrafine particles (which are also on the nanoscale) suggests the potential to influence tissue inflammatory and immunological processes, possibly leading to respiratory disease, cardiovascular disease, etc. It was also very clear that surface properties likely will determine both the degree of movement within the body, uptake by cells, and may greatly influence the ultimate toxic responses to these materials.

The characterization of the health impacts of nanomaterials presents many challenges to both nanotechonlogists and toxicologists. Most daunting is the extraordinary breadth of nanoscale materials: not only are there many different types, but they can be of many possible sizes and possess different surface coatings. Moreover, there isn't one 'most important' class of materials to focus, on now, nor is their likely to be in the near future. Like polymeric materials, nanomaterials are diverse and will be used in many forms and sizes. The strategy that we propose is to choose a limited class of materials which represent a cross-section of composition, size and properties, and use these as model systems to investigate fundamental questions concerning if and how nanomaterials can interact with the body.

We describe this 'model system' approach in the following. Rice University has the NSF sponsored Center for Biological and Environmental Nanotechnology of which I am the director. Toxicological studies are beyond the scope of our center, which focuses on developing new biomedical and environmental technologies based on nanomaterials. However, because of our focus, we have a number of chemists quite experienced with using inorganic nanomaterials in biological systems. These nanochemists can provide well characterized and controlled materials to toxicologists interested in uncovering general principles and mechanisms. The aim is to develop the baseline understanding of how nanomaterials may get into living organisms, how they are translocated, and predict some of their potential ultimate human health effects. This work will answer many complex questions concerning the protocols for nanotoxicology. (How are nanoparticles counted? How should they be measured?). Additionally, the results of the work will provide an important foundation for more specific characterization of the toxicology of particular materials in the future.

What follows is an outline of some specific health effects research initiatives that we believe should be addressed by the National Toxicology Program.

Nanotoxicology: Particle-cell interactions on the nanoscale

I. Consumers are getting increased exposures to nanoengineered particles

The objective of this work is to evaluate the toxicological effects of nanoengineered particulate matter. Nanotechnology is a new blend of science and engineering which seeks to create powerful new applications for materials that have been controlled on their nanometer scale. The growth of research in this area has been phenomenal; in addition to an exponential growth in the available literature on 'nanotechnology' there have been aggressive campaigns at universities everywhere to hire researchers in the area. There is no doubt that the center of gravity of knowledge in nanotechnology is in the universities, and that its study is transforming many branches of both science and engineering.

This intellectual development is being reinforced by substantial federal research dollars, and to a lesser extent the support of established industries. Over the past five years the federal government has invested well over one billion dollars in R&D nanotechnology across many agencies. Such direct support, primarily of physical sciences R&D, hasn't been seen since the heyday of the Manhattan project. Nearly all major industries are working hard to hire talent in this area- not so much because they clearly see a product from nanotechnology, but more because they have bought into the concept that it is the next big revolution in technology. Estimates for the size of the nanotechnology sector range from annual values of $1B in a few years to over $10B by 2010.

Its important to realize that one of the great marketing values of nanotechnology as a concept is that it is very broad. Nanotechnology can be low tech- for example, special nanoparticle additives in paint to provide a sparkly finish. It can also be high tech- such as wearable computers with nanoscale logic elements that can recognize faces for their wearers. It also has near-term markets, like in cosmetics that feel more silky because of small particle additives, and futuristic scenarios which envision systems of nanomaterials to store information and create energy. However, within this huge wealth of possible applications and timelines there is one common denominator: all nanotechnology has at its heart nanoengineered particles. Ultimately, nanotechnology will look a lot like a speciality chemical industry.

Right now, these nanoengineered particles are found in many places ranging from research labs to cosmetics. The number of research institutions preparing, processing and using nanomaterials has grown exponentially along with the funding in the area. More and more research workers are coming into contact with nanoscale metals, carbons and even polymers. There has been little to no studies of either the exposures of workers to these materials, or the consequences of accidental exposures. One OSHA study in carbon nanotube plant found few particles in the air, which is consistent with the fact that most nanomaterials are handled in liquids. Still, there are no guidelines for protection for researchers and no formal way to measure and minimize exposures.

Exposure to nanoengineered particles is not limited to workers; the use of nanoscale particles in cosmetics is a growing practice. Nanoparticles can make lotions deeper colors

(iron oxide pigments), and they can feel smoother when applied to the skin. In sunscreens ZnO has replaced other uv absorbing chromophores as the active ingredient, and antiperspirants are now clear because their active ingredient, alumina, is now nano and thus too small to scatter visible light. It is difficult to get details about the quantities and form of these particles in cosmetics; many industries in the area guard their formulations as trade secrets which makes it difficult to know how much, and of what size, nanoengineered materials may be in a product.

Nanotechnology isn't just a dream for the future. Nanoengineered particles are already in consumer products used daily by millions of consumers. Hundreds of research workers receive even more acute exposures, and handle these materials daily with no required protection or detection. These exposures will only increase substantially as nanotechnology reaches a wider variety of the society and its applications expand.

II. Nanoengineered particles are unlikely to be completely 'safe'

The unintended exposure of the population to increasing levels of nanomaterials would not be a concern if these materials were completely 'safe'. How safe are engineered nanomaterials? There is no peer-reviewed data on the topic. However, what is apparent is given the increasing exposures of consumers to these systems, the assumption that nanomaterials are 'safe as water' seems ill-advised. This problem can be analyzed from several perspectives to gauge quite generally the risk levels.

Because of the sheer diversity of nanomaterials, it is virtually certain that some examples will cause problems to our environment and human health. 'Nanoengineered particles' are a huge class of materials. They span sizes from 1 to 100 nm, with diverse compositions (ZnO to gold) and shapes. Moreover, the core inorganic species is only half or less of the major part of these materials- namely their surface. To consider only the core composition without concern for its surface chemistry and stabilization will surely lead to problems interpreting any data. There is not likely to be one simple answer when it comes to whether or not nanoparticles are 'safe'- it will depend on their size, composition and surface chemistry. This also points up the need to focus early on the general trends with size and surface so as to extrapolate possible issues in these diverse systems.

Nanomaterials are well known to have access to regions of the body not open to larger sized inorganic matter. They can cross the blood-brain barrier, for example, and leak out of capillaries. In some cases it appears that these materials take advantage of mechanisms for incorporation into cells that are quite distinct from those available to larger sized materials. These features are the basis for the great value of nanomaterials in many medical applications; however, it also indicates that these materials could avoid common clearance pathways for particulate matter. This observation complicates extrapolations of risks from one material to another and requires that a good understanding of biodistribution be developed.

There is also the issue of biological effect of nanomaterials. Already there is some indication that particles can cause unusual immune responses; larger particulate matter, for

example, known as 'wear debris' (from materials used in joint replacements) to bioengineers leads to auto-immune disorders over time. Nanomaterials are also much more soluble than larger particles- because of their high surface areas, they are potentially more subject to metabolic processes than are larger inorganic substances.

Finally, engineered nanoparticles present high surface areas in solution and can adsorb molecular contaminants. This coupled with their small size can provide such species access to areas of the body and cellular organelles not normally exposed. Facilitated transport of such impurities, exogenous or endogeneous in nature, could be an even larger problem in biological systems than the nanoparticles themselves.

Without hard data, one can only speculate what nanoparticles might do in the human body. Clearly, controlled engineered nanoparticles must be studied systematically to understand the general ways they can interact with cells and organisms.

III. Technical Approach

I. Objective: To characterize the basic interactions of nanomaterials with biological systems. Specifically we recommend studies of the following:

A) Evaluation of the size-dependent translocation of nanoparticles into organs using fluorescent quantum dots. Quantum dots are nanocrystalline fluorescent semiconductors (CdSe, GaAs ....) with highly controllable size (5-15 nm, sigma 5%); their emissive color has a strong dependence on nanocrystal size. We hypothesize that particles larger than 100 nm will exhibit very different organ distributions than those under 100 nm; below this limit distributions will depend only slightly on diameter. The role of surface chemistry can also be explored using particle coatings which make surfaces resistant to non-specific sorption of protein (e.g. PEG) as well as particle coatings that may promote protein sorption.

B) Characterize the inhalation toxicology of high aspect ratio materials, such as carbon nanotubes (single-walled or multi-walled), as a function of material purity and stabilization. The impurities in carbon nanotube samples may be highly inflammatory and possibly carcinogenic; however, once these impurities are removed the toxicity of this material may be limited. A critical issue is to minimize the amount of material required for this work, perhaps through recycling or careful experimental design, as carbon nanotubes are expensive and not commercially available.

C) Determine the immune response raised by nanoparticles from A and B with different surface coatings. We anticipate that the immune response of particles will be entirely dependent on the surface presented by the coating, rather than on the size and shape of the particles.

D) Study whether nanoparticle core composition influences the trends observed in A and C. If the surface coating is stable and non-fouling, we expect that the core composition to not play a role in governing particle toxicology.

E) Phototoxicology efforts. Many nanoparticles are highly opaque at visible and ultraviolet wavelengths. Their absorption of light often results in the production of active chemical species at their surfaces; in particular, ceramic particles such as titanium dioxide and zinc oxide are potent photocatalysts because of the generation of OH radicals through light absorption. The use of these particles in sunscreens and cosmetics suggests that the evaluation of their toxicity both when illuminated and in the dark would be highly relevant for the development of these and related consumer product applications.

A) Sample preparation capabilities of Rice University chemists

a. Semiconductor/Ceramic quantum dots.
   i. Particle preparation. CdSe and PbSe quantum dots can be prepared via standard methodologies in tri-octyl phosphine oxide. Such nanoparticles can be made in sizes ranging from 2 to 20 nm with size control better than 20% on the diameter. Typical yields are 1 gram per day.
   ii. Particle purification. Required particles can be purified with size selective precipitation; this will reduce yields, but narrow distributions to 5% or less. Byproducts from the synthesis can be removed with dialysis and centrifugation.
   iii. Stabilization in aqueous buffers. Particles will be coated with dedrimeric molecules which are reported to impart good stability in physiological buffers. Alternatively, materials may also be formed and coated with polyethylene glycol (PEG) possessing thiol substituents. Either way, particles can be prepared in the buffers of interest to toxicologists in typical concentrations of 10-100 mg/ml. Other surface coatings which impart water solubility (e.g. –NH2 or –COOH functionality) can also be prepared. In all cases non-specific sorption of proteins to surfaces can be quantified with analytical ultracentrifugation.
   iv. Particle characterization. Every sample will be evaluated using transmission electron microscopy and x-ray diffraction. If substantial particle samples are required, size-exclusion chromatography may be employed to save analysis time. Surface characterization will rely on NMR techniques. Cryo-electron microscopy and small-angle x-ray scattering methods may also be used to estimate the hydrodynamic radius of particles once they are coated.
   v. Particle fluorescence. Once coated, particle fluorescence will be evaluated in the visible and near-infrared. Each particle size will have a distinct emission profile. Thus, characterization of the emission of light will provide one method for quantitating particle amounts.
   vi. Particle properties and stability in physiological environments. An important issue is the extent to which particles remain encapsulated and stable in the body. We can evaluate particle stability over the span of days and weeks in appropriate tissue growth media and serum.
b. Single-walled Carbon Nanotubes (SWNT)

SWNT can be produced both by the HiPCo process as well as by laser vaporization methods. The material produced in these two methods differs in some important ways, notably catalyst concentration and mean SWNT length and diameter. Typical SWNT lengths range up to microns but purification methods can restrict particle populations to below 100 nm. Raw soot from the reactors can be purified by standard methods to remove amorphous carbon, multi-walled nanotubes and other impurities. Metal content in the purified powders is assessed via standard metals analysis methods.

iii. Particle solubilization in water and buffers will be accomplished using surfactants such as TWEEN. Near-infrared spectroscopy will be an important tool for evaluating the stability of these suspensions and the extent to which SWNT are isolated. The inclusion of model surfactants based on those present in the lungs may also be interesting for toxicology experiments.

Particle characterization directly in water environments will rely on cryo-TEM methodologies as well as near-infrared fluorescence and Raman spectroscopy.

B) Methods for detecting particles in tissue (for translocation studies)

i. Transmission electron microscopy. Tissue sections can be fixed and stained to visualize cellular structures. While isolated nanoparticles will not be visible, if aggregates form as they may be detectable. This method is the only one that could provide information about what organelles nanomaterials concentrate it.

ii. Atomic emission spectroscopy. Nanoparticles which have unique atomic composition, which is not present in large quantities in tissue backgrounds, would be amenable to quantitative analysis with AES. These would likely include cadmium, lead, zinc and titania. Additionally, isotopic substitutions- such as Fe-57 for the naturally occurring Fe-58- would also make this method feasible for systems like iron oxide. (Note: C-13 labeling of carbon nanostructures is a possibility, but the cost would be substantial). Nanomaterials would be extracted from tissue, and their total concentrations determined from the atomic concentrations in the tissue. This would provide the best quantitation of nanomaterial concentration in animals.

iii. Fluorescence microscopy. A unique feature of many nanomaterials is their strong emission at both visible and near-infrared wavelengths. While it is unlikely to be quantitative, fluorescence microscopy would allow for rapid screening of nanomaterial concentration and location in animal tissue.

Sincerely

Signature Redacted

Vicki Colvin
Director