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Fantastic voyage and opportunities of engineered nanomaterials: What are the potential risks of occupational exposures?

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> "Be precise. A lack of precision is dangerous when the margin of error is small."
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> Donald Rumsfeld (US Secretary of Defense)

In his legendary lecture at Caltech 50 years ago, Richard P. Feynman, introducing a new concept of nano-sized materials, noted: ". . . . a point that is most important is that it would have an enormous number of technical applications. What I want to talk about is the problem of manipulating and controlling things on a small scale." This prediction turned out to be a prophecy, and today we appreciate that quantum properties strongly affect physical and chemical properties of nanoscale objects, conferring electrical, optical, and magnetic features not present in materials at a larger scale. Based on this, devices enormously smaller than before have been created, which may have a potentially huge impact on engineering, chemistry, medicine, and computer technology. Not surprisingly, nanodevices have already found different applications as diagnostic and therapeutic tools in biomedicine and in numerous consumer products. The applications in biomedicine range from novel approaches to the design of artificial organs and tissues for replacement therapies to nanorobotic biosensors, diagnostic devices, and miniscule vehicles for targeted drug delivery.[1] Different types of nanoparticles are being considered, including carbon-based structures, such as carbon nanotubes, carbon nanocapsules, and fullerenes, or spherical lipid-based liposomes, which are already in use for numerous applications in drug delivery and in cosmetic products. Other types of nanomaterials envisioned for biomedical and other applications are based on metals and their oxides as well as ceramics. Nanotechnology industry is projected to exceed $1 trillion by 2015.[2]

The linear dimensions of nanomaterials are in the same range as the major cellular machineries and their components. Therefore, nano-sized materials are likely to interact and, more importantly, interfere with cellular organization and affect biological functions in ways that are unknown and cannot be deduced from previous experience with macro- or microsized objects. In addition, specific features of nanoelements may be realized through their unique electron donor/acceptor properties resulting in unexpected effects on redox balance and redox reactions in cells. This, again, emphasizes the poor predictive power of traditional toxicology as the basis for assessments potential damaging effects of newly created nanomaterials. These unusual and unpredictable properties of nanomaterials fostered the emergence of a new subdiscipline in the field of toxicology, nanotoxicology.[3] The latter can be defined as the field of science that investigates mechanisms and pathways through which nanoparticles or complex engineered nanostructures may interfere with the structural and functional organization of cellular and extracellular nano-sized machineries leading to cytotoxicity with adverse effects on human health and the environment.[4] This definition places emphasis on the specific responses that are directly related to the scaling and dimensions of nanomaterials. Recently, cases of such "molecular interferences" have been documented. For example, the narrow diameter (2–3 nm) and significant length (hundreds of nm) of single-walled carbon nanotubes (SWCNT) may facilitate their interaction with elongated biological structures of the mitotic apparatus. Indeed, in cultured human airway epithelial cells, SWCNT have been shown to cause fragmentation of centrosomes, multiple mitotic spindle poles, anaphase bridges, and aneuploid chromosomes. Confocal microscopy revealed an association of carbon nanotubes with cellular and mitotic tubulin as well as with chromatin.[5] It is noteworthy that studies of asbestos (crocidolite) fibers published almost 25 years ago demonstrated that Chinese hamster ovary cells, which contained fibers that were longer or equivalent to the diameter of the mitotic cell (20 μm), also showed different forms of mitotic abnormalities.[6]

Five organ systems—lung, skin, gastrointestinal tract, nasal olfactory structures, and eyes—are the major portals through which nanoparticles can enter the body as a result of inadvertent occupational or environmental exposures. Furthermore, nanoparticles that enter the body through these common routes could translocate into the circulation and travel to distant organs including the cardiovascular system and brain.[3] Of primary concern in the occupational setting are inhalation and skin exposure to nanoparticles. Identifying exposure scenarios that may occur during the production and use of nanomaterials is a central component of an overall risk management program.[7] However, the knowledge about workplace exposure to engineered nanoparticles is very limited at present. On

the other hand, until recently, no direct indications of the toxicity of nanoparticles to humans have been described.[2] However, Song et al[8] have reported on seven female workers suffering from occupational lung disease for which a potential link to nanoparticle exposure was suspected. Thus, seven women aged 18 to 47 years, exposed to nanoparticles consisting of polyacrylic ester in addition to an undefined mixture of other chemicals for 5 to 13 months, all with shortness of breath and pleural effusions, were admitted to hospital. In addition, all patients suffered from a rash with intense itching on their faces, hands, and forearms. A cursory survey of the workplace showed that the work was being conducted in a small room without adequate ventilation. Pathological examination of the patients' lung tissue revealed nonspecific pulmonary inflammation, pulmonary fibrosis, and foreign-body granulomas of pleura. Nanoparticles were detected in the cytoplasm of pulmonary epithelial and mesothelial cells as well as in the chest fluid of the patients, as evidenced by electron microscopy. Two of the women died from respiratory failure 18 to 21 months after onset of symptoms. The authors concluded that the study "may be the first study on the clinical toxicity in humans due to long-term exposure to nanoparticles." However, as noted in the introduction to this review, it is important to be precise. In fact, the aforementioned study has several major flaws including the fact that the title of the study seems to imply that exposure to "nanoparticles" (in general) is associated with severe lung disease.[8] However, such a causal relationship was not demonstrated by the authors. Moreover, while the clinical workup is comprehensive, the study suffers from a lack of exposure assessment in the workplace and also from a lack of characterization of the nanoparticles identified in the fluids and tissues of the patients. Therefore, the report by Song et al serves primarily as a tragic reminder of the importance of appropriate protective measures in the workplace. Well-established occupational hygiene measures could prevent the occurrence of such unfortunate outcomes in workers in industries in which nanomaterials are manufactured or applied, as in other industries. However, although this report does not conclusively illustrate adverse health effects of engineered nanoparticles, the gravity of the findings suggests that further investigation is needed. Several experts in the field have provided insightful comments and reflections on this study in a recent online survey posted on the SAFENANO web site (www.safenano.org). Hence, according to Prof. Anthony Seaton (University of Aberdeen), "similar episodes, almost always involving gases, have occurred in the past, but this one had unique features, notably the effect in causing effusion of fluid into the linings of the lung (the pleura) and heart (the pericardium), the finding of nanoparticles in the workplace and in the lungs and lung fluid of the workers, and the finding of a tissue reaction to particles in the lung lining." It is of interest to note that some of the clinicopathological findings in the report by Song et al are reminiscent of experimental findings detected after inhalation exposure of mice to carbonaceous nanoparticles.[9] Furthermore, Prof. Ken Donaldson (University of Edinburgh) remarks that "the damaging exposure was clearly a toxic cocktail of particles and chemicals and so is a highly unusual case that sheds little light on the hazards from the vast majority of nanoparticles used in workplaces, which do not have a reactive surface. It may yet turn out that the particles are a by-product of the chemical reaction and not the main cause of the injury." Despite the uncertainty surrounding the role of nanoparticles in this unfortunate incident, Dr Rob Aitken (Institute of Occupational Medicine, Edinburgh, and Director of SAFENANO) concludes that "it would certainly be ill advised to be too quick to dismiss the possibility."

Studies in animal models have described significant and at times high toxicity (compared to toxicity of microsized particles composed of identical or similar materials) of nanoparticles introduced in the body through different routes; however, the toxic mechanisms are based on typical pathways, commonly realized through a combination of inflammatory and oxidative stress responses. The time course and intensity of the body's reaction to nanoparticles may be unusual compared to microparticles. For example, we noted that inhalation of SWCNT caused pulmonary responses in mice characterized by an exceedingly high magnitude of oxidative stress and inflammation with very early onset of the fibrotic phase, but these responses are nonetheless associated with well described reactions and the accumulation of typical biomarkers in exposed animals.[9] Furthermore, the interplay between inflammation induced by nanoparticles and clearance of bacteria and resolution of infection is important to consider. This may be particularly relevant in the context of realistic exposures to nanoparticles occurring in conjunction with other pathogenic impacts such as microbial infections. For example, interactions between SWCNT administered through pharyngeal aspiration and bacterial pulmonary infection of C57BL/6 mice with *Listeria monocytogenes* (LM) showed that sequential exposure to SWCNT/LM amplified lung inflammation and collagen formation indicative of fibrosis.[10] Despite this robust inflammatory response, SWCNT preexposure significantly decreased the pulmonary clearance of bacteria in LM-exposed mice. Furthermore, decreased bacterial clearance in SWCNT-preexposed mice was associated with impairment of respiratory function. These data suggest that enhanced acute inflammation and pulmonary injury after SWCNT associated with delayed bacterial clearance may lead to increased susceptibility to lung infection in exposed populations.[10]

Nanomaterials are commonly designed to be mechanically strong and to display resistance to different chemical treatments. As a result, their biopersistance in tissues is expected to be very high. Nondegradable nanomaterials can accumulate in organs and inside cells where they can exert detrimental effects. For comparison, long-term accumulation of medicinal gold salts in the body has been linked to adverse effects in patients. SWCNT are known to be biopersistent and may remain inside macrophages in the spleen and liver for prolonged periods of time following parenteral administration.[11] Moreover, SWCNT have been observed in the lungs of exposed mice up to 1 year after pharyngeal administration (Shvedova et al, unpublished observation). On the other hand, biodegradation of nanomaterials could also yield adverse responses due to toxic degradation products. For instance, leaching of toxic core components such as cadmium from quantum dots with induction of oxidative stress has been suggested as a mechanism of in vivo toxicity of these nanomaterials. Controlled biodegradation of nanomaterials thus represents one of the important challenges not only in the field of nanotoxicology but also in nanomedicine, because the safe implementation of nanomaterials for biomedical purposes is contingent on the controlled degradation and/or clearance of the exogenous nanomaterials from the body. In a recent proof-of-concept study, Park et al[12] reported that multifunctional porous silicon nanoparticles self-destructed in a mouse model into renally cleared components—likely orthosilicic acid—in a matter of weeks, with no evidence of toxicity to animal tissues. Moreover, we have recently reported the enzymatic degradation of SWCNT via the plant-derived enzyme, horseradish peroxidase.[13] We also demonstrated biodegradation of carbon nanotubes by the human neutrophil enzyme, myeloperoxidase, thus pointing to possible strategies for the mitigation of unwanted inflammatory responses elicited by carbon

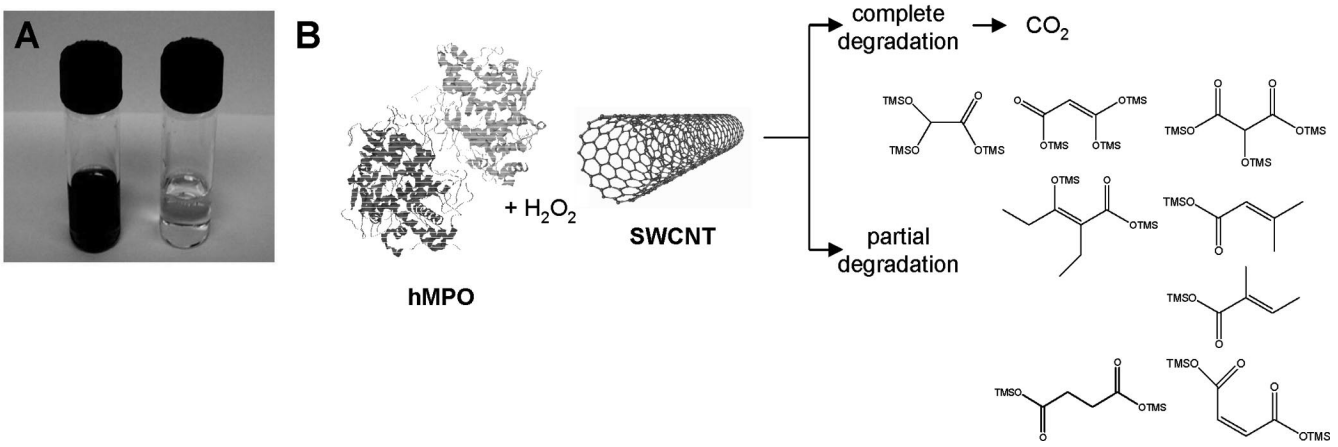

FIGURE 1. Biodegradation of carbon nanotubes. Recent studies have provided evidence of enzymatic degradation of SWCNT on incubation with horseradish peroxidase (HRP)[13] or human myeloperoxidase (hMPO) (Kagan et al, submitted for publication) and low amounts of H_2O_2. Detailed analysis of the biodegradation products showed that complete biodegradation produces CO_2. (A) Photograph showing enzymatic degradation of carboxylated SWCNT (left) and after 10 days of incubation with HRP and H_2O_2 (right). Reprinted with permission from American Chemical Society.[13] (B) Schematic of the biodegradation of SWCNT by neutrophil-derived hMPO. The chemical formulas depict short-chained tri-, di-, and mono- carboxylated alkanes and alkenes.

nanotubes in exposed individuals.[14] Although the intermediate products of these enzymatic reactions were aromatic and aliphatic hydroxylated and carboxylated species, the final product was CO_2 (Fig. 1). These results mark a promising technological possibility to develop "green" chemistry-based kits for biodegradation of inadvertently spilled carbon nanotubes by peroxidases in occupationally and environmentally relevant settings.

Regulation of the production and use of the increasing diversity of engineered nanomaterials is not only a huge and very difficult task to accomplish but also a very important one. Currently, attempts are initiated to regulate some classes of nanomaterials. For example, as a fibrous structure resembling asbestos in physical properties and health effects, SWCNT are currently under special attention regarding their possible carcinogenicity. The US Environmental Protection Agency generally considers SWCNT to be chemical substances distinct from graphite and other allotropes of carbon listed on the Toxic Substances Control Act Inventory. Many carbon nanotubes may therefore be considered as new chemicals under Toxic Substances Control Act. On June 24, 2009, EPA issued significant new use rules for single-walled and multiwalled carbon nanotubes for which EPA had received premanufacturing notices and negotiated so-called section 5(e) consent orders (see Ref. 15 and URLs contained therein). According to the Federal Register notice, the US Environmental Protection Agency negotiated the consent orders out of a concern that both the single-walled and multiwalled carbon nanotubes may cause "lung health effects" and health effects from skin exposure.

Insufficiency and ambiguity of the existing in vitro and particularly the in vivo toxicological data are hampering the risk assessment process required for scientifically sound regulatory and policy decision-making. Standardization of methods and reference materials are therefore a priority when conducting nanotoxicity testing. Furthermore, one should not confuse hazard data from toxicity studies with the concept of health risk: it must be remembered that exposure assessment is also an integral part of the equation.[16] More work is needed to develop appropriate methodologies with which to monitor nanoparticle exposure in the workplace and in the natural environment. Finally, one should take care not to generalize when reporting on potential adverse health effects of "nanoparticles," unless such generalizations are justified. The terms "nanoparticle" and "nanomaterial" are very broad (in principal, any material of any composition with one or more dimensions in the submicrometer range) and even a single class of nanomaterials, such as carbon nanotubes, can be subdivided into numerous categories with unique properties, based on physicochemical parameters such as size, charge, surface modification, etc. Material characterization is thus a critical component of understanding nanomaterial-related risk to human health.

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