Article

Are Nanoparticles Safe?

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Introduction

Despite the wide application of nanomaterials there is a serious lack of information on their impact on human health and the environment. Engineered nanomaterials are currently found in diverse products: personal care items, sunscreens, abrasion-resistant materials, environmental catalysts, anti-fouling and anti-microbial coatings, in wood preservation, fuel cells, UV-attenuation, scratch resistant and charge dissipating coatings, and even food products.[1] Production ranges from the multi-tonnages of carbon black and fumed silica, for plastic fillers and car tyres, to microgram quantities of quantum dots as biological markers. Nanoparticles are small enough to penetrate small capillaries and pass through biological membranes such that nano-encapsulated therapeutic agents are being proposed or in clinical trials for a wide variety of treatments because of selective targeting and minimisation of side effects.[2]

But are nanoparticles safe to use? Their small size and unique properties may cause adverse effects not found in their larger analogues. Scientific evidence on possible effects on the human body and the environment is just beginning to emerge but there is minimal information on dominant exposure routes, exposure levels, and material toxicity. Consequently, advocates and opponents (who often erroneously equate asbestos to a nanoparticle) of nanotechnology have little information to support or refute their respective position. There is no doubt that toxological effects will vary with the structure, redox activity and preparative history of the nanoparticles in question. This, coupled with the diverse exposure routes, makes for a complex risk analysis.

Herein we look at the possible entry points into the human body and give an assessment of the known risk for some nanoparticles.

Portals for Nanoparticles

As already noted, the size of nanoparticles makes them highly mobile in both humans and the environment. Therefore, they can enter the body through several ports. Translocation can then occur via the blood stream leading to an accumulation in many tissues including the brain and testes.[3] It is still not known whether cells internalise nanoparticles, but at the cellular level nanoparticles can act as a gene vector.[4] Carbon black particles are thought to interfere with cell signalling,[5] an observation that has seen DNA used for the size separation of nanotubes (the DNA wraps itself around the nanotube if the tube diameter is right[6]) but this observation equally raises concerns over the effect of carbon nanotubes on the human body.

Skin

Human skin (ca. 1.5 m^2 in area in an adult human) normally functions as a strict barrier. Despite use in cosmetic and sunscreen products, there is only limited literature on, for example, the penetration of fine-size TiO_2, and none on nano-TiO_2. Nanoparticles may reach the epidermis, and occasionally the dermis, through mechanical agitation but penetration is limited by the hair follicle. There is no hard evidence to suggest they can enter the systemic circulation by this route.

Lung

Many nanomaterials and devices are formed from, or use, aerosols and colloidal suspensions so that exposure is most likely to happen through lung inhalation. While the airways are a relatively robust barrier, in the gas exchange area (the 300 x 10^6 alveoli) the barrier between the alveolar wall and the capillaries is very thin - merely 0.5 μ away from the blood flow. Spherical solid material can be inhaled when its aerodynamic diameter is <10 μm and so nanoparticles travel deeper into the lungs and will deposit in the alveoli via Brownian motion. If inhaled concentrations are low, then the retention time is about 70 days. Since the alveolar macrophages are the defence cells of the lung, nanoparticle accumulation could result in inflammation. Larger particles transmigrate from the alveolar regions to outside the lungs more rapidly, resulting in far greater particle clearance and less risk of inflammation.

Intestine

The intestinal tract is a more complex barrier, and while there are many similarities in the entry of nanomaterials here to that of the lungs, there are also important differences. Non-specific interaction often reduces the toxicity of ingested nanoparticles and consequently they may be less cytotoxic. The transit through the intestinal tract is relatively fast and, as nanomaterials do not remain long in the intestinal tract, their presence will not automatically induce an inflammatory response. In the intestinal tract, the ingested materials move from acidic (stomach) to basic conditions and this markedly changes solubility and the surface characteristics of the particle.

Translocation

Nanoparticles are most likely to enter the body via ingestion and inhalation. Enzymes and the physiological environment could change the properties of nanoparticles (particularly surface activity) and the question *What is the structure of* in vivo *nanoparticles?* has not been answered. This is a particular issue for redox-active metals because cationic nanoparticles would have an immediate toxic effect on the blood-brain barrier.

Once in the body there is no doubt that nanoparticles can translocate to organs and tissues and bioaccumulate. Systemic distribution to other organs, across the blood-brain barrier, and penetration of the blood–testis barrier has been demonstrated.[3] The passage of solid material from the pulmonary epithelium to the circulation system appears to be restricted to nanoparticles. Recent inhalation experiments with rats showed that nanoparticles (25 nm) had reached several organs after 24 h of exposure and (amazingly) the central nervous system. Transportation via the nerves was at a speed of 2.5 mm per hour! Nanoparticles that enter the liver have been found to induce local oxidative stress and, because of the production of radicals, modify the hepatocyte antioxidant systems, but there is no definitive evidence to implicate nanoparticles in liver damage in other than rats.

Translocation from the intestine to lymphatic tissue and capillaries undoubtedly is possible and immune responses may be triggered (such as implicated in Crohn's disease), but to date there are no data to suggest that humans may be affected by transport of the nanoparticle via this portal.

Specific Nanoparticles

Each type of nanoparticle will exhibit its own unique biological or ecological response that will also differ with shape and dosage. It is important to realise that a wide range of nanoparticles have been shown to create reactive oxygen species both *in vivo* and *in vitro* and hence have the potential to induce cell damage. We now provide specific data for nano-sized anatase (*p-TiO_2*), the archetypal industrial nanoparticle, and give an overview of the toxic response for some others.

Titanium dioxide

Fine-sized (<2.5 μm) TiO_2, consisting of agglomerates of needle-like particles ca. 20 nm x 100 nm in size (Fig. 1-upper), still is produced mainly by the classical batch sulfate or continuous chloride processes with an annual production of ca. 3.5 million tonne p.a. Nano-sized TiO_2 can be produced from the coarser material by aerosol or gel techniques but a variety of direct methods have been developed, especially starting from Ti(OPr-*i*)$_4$. These methods include mesoporous film formation from reverse micelles, supercritical fluid drying of gels, direct particle synthesis under supercritical conditions, and templated approaches.[7] The latter approach can also produce nanotubes (Fig. 1-lower). Gel methods are particularly important as they limit particle nucleation, growth and agglomeration[8] - a significant characteristic of nanoparticles. A recent survey of p-TiO_2 material sold in the US found that it had a size range of 20-50 nm. These are the materials now found in, *e.g.* sunscreens, cosmetics, bulk sprays, powders, coatings, scratch resistant sunglasses, stain repellent fabrics, and anti-graffiti coatings for walls.

TiO_2 is one of several dusts that are grouped into the category of poorly soluble particulates (PSP) by virtue of their low solubility in water and their toxicity. Pulmonary (lung) inflammation is a common response to the inhalation of PSP and has been closely associated with, *e.g.* fibrosis and cancer. In rats, it was found that p-TiO_2 cleared more slowly than fine-sized TiO_2 and translocated more efficiently to lymph nodes. Furthermore, the biological effects correlate better with surface area rather than mass.[9] One study suggested[10] that low exposures (10 mg/m^3) resulted in greater tumour incidence than high exposures (250 mg/m^3). Recent research using p-TiO_2 dots and rods indicated that the surface chemistry of p-TiO_2 may have a role.[11] With silica, cytotoxicity can be correlated with surface area which, in turn, influences the appearance of surface radicals and reactive oxygen species. The surface of TiO_2 is known to be activated to radical formation and analogous toxological responses to silica cannot be ruled out. Furthermore, size specific deposition of nanoparticles, when inhaled as single particles rather than aggregates, appear to contribute to their surface properties and free radical generation. Recent studies on a number of commercial formulations of pigment-grade TiO_2 particles indicate that different surface coatings and surface treatments can also influence the pulmonary toxicity.

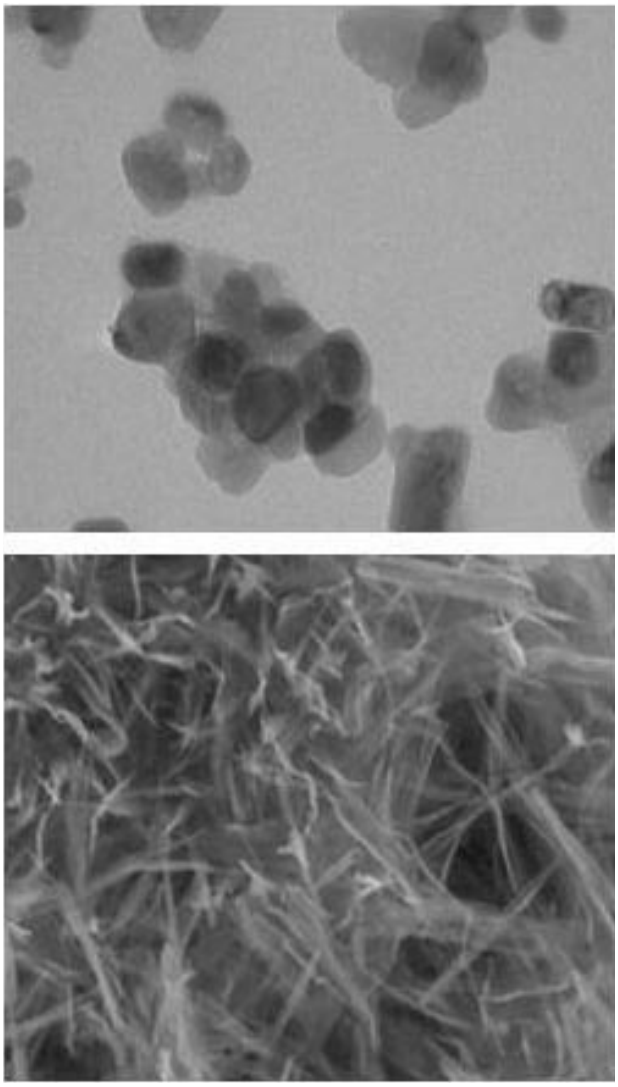

Fig. 1. (upper) Aggregated p-TiO_2; (lower) TiO_2 nanotubes (unpublished work, Otago.

Notwithstanding the experimental evidence for enhanced inflammatory response with p-TiO_2 in rats, we must be cautious in extrapolating this to human responses. Firstly,

nanoparticles have a tendency to clump together and may reach the body as aggregates rather than free entities; all laboratory studies have used artificial nanoparticle aerosols. Secondly, there is evidence to suggest that PSP-induced effects may be unique to the rat as they process inhaled particles in a very different manner to larger mammals and humans. Pulmonary TiO_2 overload leads to the development of pulmonary tumours only in rats[12] and they have a more severe and persistent pulmonary inflammatory response than either mice or hamsters to aerosol p-TiO_2. As yet there is no information on the effects on human health from p-TiO_2 inhalation. Studies involving coal miners exposed to coal-mine dust over a long period of time suggest that humans do not develop overload related tumours. Such evidence, combined with findings from the few studies conducted on particulate exposed primates, indicates that the lungs of larger mammals are less reactive to dust burden insults than rats.

Silver and other metal or metal oxide nanoparticles

Silver nanoparticles (Fig. 2), as antimicrobial agents, have been proposed as constituents of bone cement and other implantable devices.[13] There are clear toxological risks from such use as the nano-Ag could penetrate the dermis and then translocate. Both sperm-stem and liver cells have shown sensitivity to 15 nm Ag, in contrast to ultrafine $AgCO_3$ which had no effect. The cytotoxicity of Ag is related to oxidative stress. Nanoparticles of other metals, and many metal oxides are likely to generate reactive oxygen species but the limited evidence available suggests that nano-Ag is very toxic relative to most other metals and metal oxides. Studies using liver cells classed nano-Ag as highly toxic, nano-MoO_3 moderately toxic, and nano-Fe_3O_4, MnO_2, Al and W non-toxic at low dosage (10- 50 µg/ml); the toxicity increased at higher concentrations (>100 µg/ml).[14,15] Fortunately, some work suggests that metallic nanoparticles are less likely to translocate from the lung to extrapulmonary organs.[16]

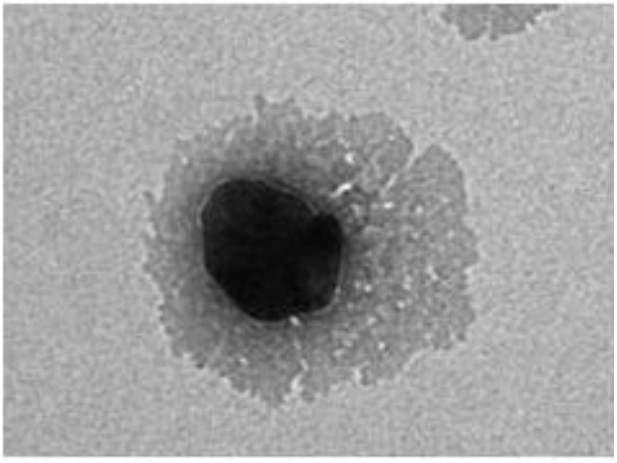

Fig. 2. Ag nanoparticles in chitosan matrix (unpublished work, Otago)

Quantum Dots

Quantum dots (QDs), such as CdSe, have been introduced as new fluorophores for use in bioimaging but to date there is little toxological information on them in the literature. While bulk CdSe is cytotoxic, it has been suggested that CdSe quantum dots are cytocompatible and safe for use in whole animal studies. This postulate is based in part on the use of protective coatings around the CdSe core of the quantum dot. Recent studies found that the cytotoxicity of CdSe QDs towards the liver correlated with the liberation of Cd^{2+} ions due to deterioration of the CdSe lattice.[15] These data suggest that while CdSe QDs may be nontoxic initially for *in vivo* use when appropriately coated, further work is needed on their long-term stability, both *in vivo* and when exposed to environmental conditions.

Nanocarbon

Humans have been exposed to carbon nanoparticles for millennia and carbon nanoforms have been a component of the natural atmosphere since combustion was discovered. As noted already, oxidative stress as a common mechanism for cell damage induced by nanoparticles and free radical cell damage has been demonstrated for C_{60} fullerenes and carbon nanotubes.[17]

Studies on single-walled carbon nanotubes have tended to use uncharacterised materials; it is unclear whether they are unaggregated, aggregated fibrils, nanoropes, carbon black, or mixtures. Therefore, results indicating that single-walled carbon nanotubes inhibit the proliferation of kidney cells in cell culture and cause lung inflammation must be treated with caution. Multi-walled carbon nanotubes (MWCNT) persist in the deep lung after inhalation and, once there, induce both inflammatory and fibrotic reactions in rats. They behave similarly to the notorious nanotube, *chrysotile asbestos*, suggesting that the health risks from exposure to carbon nanoforms may be severe, with an increased risk of carcinogenesis. Occupational exposure limits for the asbestos are ca. 10^6–10^7 fibres/m^3 over an 8 h period. Particle concentrations of nanocarbon aggregates from cooking are ~10^4–10^5/m^3, but as each aggregate may contain 10^3 MWCNT, the asbestos limit could be exceeded if around 10% of the MWCNT made their way to the lung during a 30 min. session at the stove or barbeque!

Charge properties, and the ability of carbon nanoparticles to affect the integrity of the blood-brain barrier as well exhibit chemical effects within the brain, have also been studied. It appears that neutral and low concentration anionic nanocarbon can serve as carrier molecules giving chemicals direct access to the brain; cationic nanoparticles have an immediate toxic effect at the blood-brain barrier.[18] Tests with uncoated, water soluble, colloidal C_{60} fullerenes have shown that redox-active, lipophilic carbon nanoparticles are capable of producing oxidative damage in the brains of aquatic species as well as humans.[19]

Conclusion

Whether or not the use of nanoparticles poses a significant health risk remains unknown. Careful scientific scrutiny of the sparse data, rather than journalistic hype, is required to give an answer to the question posed at the beginning of this article. There is no doubt that nanoparticles can enter the human body via the lungs and the intestines. Once in the body their translocation is a strong function of the surface characteristics of the particles. Nano-sized particles

are more likely to result in a higher lung burden, possibly amplifying any possible chronic effects. Recent studies on carbon nanotubes indicate that they can induce a rather general non-specific pulmonary response. But there is no universal nanoparticle and not all nanoparticles will be either benign or toxic. The presence of contaminants, such as metal catalysts in nanotubes, poses an added risk for evaluation. It is a challenge to devise high throughput and low cost toxological tests for nanoparticles without hindering the advancement of nanotechnology.

Our current understanding on their toxicity centres largely around a limited number of studies conducted (mainly) on laboratory animals, or cell cultures, where the response may not mirror that in a human, nor do they include any *in vivo* interactions.[20] Risk assessment must include the actual toxicity plus the exposure time, and the exposure component is largely unknown. There is also an assumption that because a fine-sized particle, *e.g.* TiO_2, has been approved for use and has no known carcinogenic properties, the nano-equivalent is also safe – a rather large assumption! Particle size is critical. Gold, usually considered inert, is a case in point. Nanogold particles are highly reactive and, for example, can move across the placenta from mother to fetus. The translocation of nanoparticles is certainly an issue the importance of which will only become evident over time.

Chemists in NZ should adopt a precautionary stance and assume that nanoparticles are a potential hazard until shown otherwise, eliminate their presence in the environment, and minimise their unintentional release. Many overseas universities, *e.g.* Cornell, and industrial laboratories have adopted this stance and developed protocols for safe working conditions.

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