Toxicology

Nanotoxicology

K Donaldson, V Stone, C L Tran, W Kreyling, P J A Borm

A new frontier in particle toxicology relevant to both the workplace and general environment and to consumer safety

The revolution in nanotechnology is set to bring advantages in areas of our lives as diverse as engineering, information technology, and diagnostics. Part of this will necessitate large scale production of nanoparticles with new formulations and surface properties to meet novel demands. Our current knowledge of the toxicology of nanoparticles and nanotubes is poor but suggests that nanoparticles may be able to have adverse effects at their portal of entry, for example, the lungs, but that some nanoparticles may also escape the normal defences and translocate from their portal of entry to have diverse effects in other target organs. We suggest that a discipline of nanotoxicology be built up to address the new potential threats that widespread use of new nanoparticles could bring in support of the growth of a safe and sustainable nanotechnology industry.

THE NANOTECHNOLOGY REVOLUTION

There has been a great deal of interest in the scientific and general community in ''the nanotechnology revolution''. Nanotechnology can be defined as:

> ''… the manipulation, precision placement, measurement, modelling, or manufacture of sub-100 nanometer scale matter …''[1]

This manipulation of matter at the nanoscale will have diverse effects in manufacture, engineering, especially energy engineering, environmental technology, information technology, health and pharmaceuticals, etc. Currently there is production of a wide range of nanoparticles (NP) of different types and different properties which will be tested for their utility in various applications. It is envisaged that those found useful will be further developed into large scale manufacture. Any technology before introducing it to the marketplace and into the product chain needs careful evaluation with regard to its sustainability and risk perception. A number of studies have been conducted by US and EU nanotechnology expert groups as well as the German parliament.[2] Both European Union and European Science Foundation have also initiated activities to map the risks and opportunities from nanotechnology.[3] So far these studies illustrate the enormous opportunities of nanotechnology to give impetus to progress in both life sciences and information technology. However, there have already been a number of papers highlighting the potential environmental hazards arising from nanotechnology.[4–7] While some of the products that will contain nanoparticles are likely to have them fundamentally bound up in the structure, there is the potential for exposure to NP and nanomaterials throughout the product chain during manufacture, application, and waste management; subsequently there is a need for a toxicology that can assess the likely harm they may cause.

WHAT IS A NANOPARTICLE?

Particle toxicology is a mature science[8] which has addressed the mechanisms of lung injury caused by nanoparticles, and the term ultrafine particles has been in use for some time to denote nanoparticles.[9] There is no size cut-off below which particles suddenly become harmful, in the lungs at least. This is because harmful particles have their effects as a consequence of two factors that act together to determine their potential to cause harm: their large surface area, and the reactivity or intrinsic toxicity of the surface.[10 11] It is self evident that the smaller particles are, the more surface area they have per unit mass; therefore any intrinsic toxicity of the particle surface will be emphasised. As particles become generally smaller their likelihood of causing harm to the lung increases. NP are currently available in a variety of compositions that range from very simple—almost pure carbon or TiO_2—to very complex structures, where surface modifications are applied. Some of the most complex NP are likely to be produced for therapeutic purposes, with characteristics that are designed to give them properties of prolonging circulation in the blood, homing to specific organs or tissues, escape from phagocytosis, blood-brain barrier translocation, and sustained release of drugs.[12] Furthermore, because of their size and large surface area, NP binding to protein may result in a series of consequences not expected to occur when proteins bind to large particles. These could include:

- NP-protein complexes may be more mobile and, via protein metabolism, NPs may gain access to sites which large particles would not reach.
- Enhanced protein degradation at the large surface area of NPs may lead to functional changes of those proteins which would not occur at the relatively small surface area of large particles.[6 7]

DO NANOPARTICLES REPRESENT A TOXICOLOGICAL HAZARD?

Adverse effects of NP are likely to occur in very different scenarios. For NP made and handled in bulk there is potential for lung exposure. We are already exposed to large numbers of ambient NP in environmental air pollution[13] where the NP component has been the focus of much research as one of the likely drivers of adverse health effects.[14] For some NP, such as those in sunblock cream, dermal exposure is already occurring and the range of different NP in creams is likely to increase.[15 16] Nanoparticles in food are reported to cross into the gut lymphatics and redistribute to other organs more readily than larger particles.[17 18] A huge class of NP are designed to be introduced directly into the body for diagnostic and therapeutic reasons,[19] and for these there needs to be toxicology on the particles as well as for the drugs that they contain. Carbon nanotubes are long thin structures which can have diameters of a few nanometres, while the length can be up to many thousands of nanometres.[20] These could have very unusual toxicological properties, in that they share shape characteristics of both fibres and NPs; such limited toxicology as presently exists supports the contention that these may be harmful to the lungs.[21]

There is a considerable existing database in the lung particle toxicology literature that shows NP of various sorts to have extra toxicity,[22] by which we mean that the same material in the form of NP is more toxic than in the form of larger, still respirable, particles. Of special concern is the apparent ability of NP to redistribute from their site of deposition. Thus following inhalation exposure, NP have been reported to travel via the nasal nerves to the brain,[23 24] as has been described for polio virus,[25] and to gain access to the blood and other organs as reviewed by Kreyling and coworkers.[6]

Very small particles and structures could have a range of effects that are not seen with conventional particles For instance they may not be detected by the normal phagocytic defences, allowing them to gain access to the blood or the nervous system. Very small particles are smaller than some molecules and could act like haptens to modify protein structures, either altering their function or rendering them antigenic, raising the potential for autoimmune effects.

The same size of particle may be very different in its ability to translocate or have any of the effects mentioned above if its surface is altered chemically for special industrial or therapeutic application. A hallmark example in particle toxicology is quartz, which has been shown to have a variable hazard,[26] mediated by a very small level (<0.1%) of surface impurity.[27 28] Considering that surface modification is the fastest growing market for bulk NP application, the various effects of these treatments on the toxicology of NPs should be investigated.

NANOTOXICOLOGY

NP have greater potential to travel through the organism than other materials or larger particles. The various interactions of NP with fluids, cells, and tissues need to be considered, starting at the portal of entry and then via a range of possible pathways towards target organs. The potential for significant biological response at each of these sites requires investigation. In addition, at the site of final retention in the target organ(s), NP may trigger mediators which then may activate inflammatory or immunological responses. Importantly NP may also enter the blood or the central nervous system, where they have the potential to directly affect cardiac and cerebral functions.

We therefore propose that a new subcategory of toxicology—namely nanotoxicology—be defined to address gaps in knowledge and to specifically address the special problems likely to be caused by nanoparticles. Under the heading of nanotoxicology we consider that protocols should be developed for testing of all materials in the nanoscale, where there is the potential for substantial human exposure. Protocols already exist, for example, for the inhalation testing of particles in acute, subchronic, and chronic tests as specified by OPPTS in the USA and the OECD in Europe. These need to be modified to take account of the different potential toxicity of nanoparticles, with special reference to extra-pulmonary transport and burden of particles at sites distant to the portal of entry. Additionally, there is a strong need for a focused research programme directed towards fully understanding the relation of size and surface area on the deposition, translocation, and toxicity of small particles. In order to facilitate development of expertise in this multidisciplinary area, we recommend that test results should be exchanged via virtual networks that integrate toxicologists, material scientists, chemists, physicists, and medical doctors.

We believe that efforts to untangle science and science fiction regarding the risks from nanotechnology are needed and that a focus on the potential harmful effects of NP is both timely and necessary. The importance of nanotechnology to the economy and to our future wellbeing is beyond debate, but its potential adverse impacts need to be studied along the same lines. A discipline of nanotoxicology would make an important contribution to the development of a sustainable and safe nanotechnology.

Occup Environ Med 2004;**61**:727–728.
doi: 10.1136/oem.2004.013243

Authors' affiliations

K Donaldson, ELEGI Colt Laboratory, Wilkie Building, University of Edinburgh, Medical School, Teviot Place, Edinburgh EH9 8AG, UK
V Stone, School of Life Sciences, Napier University, 10 Colinton Rd, Edinburgh EH10 5DT, UK
C L Tran, Institute of Occupational Medicine, Research Park North, Edinburgh EH14 4AP, UK
W Kreyling, GSF National Research Center for Environment & Health, Institute for Inhalation Biology, Focus Network: Aerosols and Health, PO Box 1129, D-85758 Neuherberg/Munich, Germany
P J A Borm, Centre of Expertise in Life Sciences (CEL), Hogeschool Zuyd, PO Box 2606, 6401 DC Heerlen, Netherlands

Correspondence to: Professor K Donaldson, ELEGI Colt Laboratory, Wilkie Building, University of Edinburgh, Medical School, Teviot Place, Edinburgh EH9 8AG, UK; ken.donaldson@ed.ac.uk

REFERENCES

1 **Meyer M**, Persson O, Power Y, and the nanotechnology expert group. *Mapping excellence in nanotechnologies: preparatory study*. European Commission, 2001.
2 **Paschen H**, Coenen C, Fleischer T, *et al*. TA-Projekt Nanotechnologie, Buro fur technikfolgen beim Deutschen Bundestag. *Arbeitsbericht* 2003;**92**:445.
3 **European Science Foundation and Chair Prof Ruth Duncan**. Look-forward initiative on nanomedicine, 2003.
4 **Colvin VL**. The potential environmental impact of engineered nanomaterials. *Nat Biotechnol* 2003;**21**:1166–70.
5 **Brumfiel G**. Nanotechnology: a little knowledge. *Nature* 2003;**424**:246–8.
6 **Kreyling W**, Semmler M, Moller W. Dosimetry and toxicology of ultrafine particles. *J Aerosol Med*, In press..
7 **Borm PJA**, Kreyling W. Toxicological hazards of nanoparticles for drug delivery. *Journal of Nanoscience and Nanotechnology*, In press.
8 **Donaldson K**, Borm PJA. Particle paradigms. *Inhal Toxicol* 2000;**12**(suppl 3):1–6.
9 **Donaldson K**, Stone V, Clouter A, *et al*. Ultrafine particles. *Occup Environ Med* 2001;**58**:211–16.
10 **Tran CL**, Buchanan D, Cullen RT, *et al*. Inhalation of poorly soluble particles. II. Influence of particle surface area on inflammation and clearance. *Inhal Toxicol* 2000;**12**:1113–26.
11 **Donaldson K**, Tran CL. Inflammation caused by particles and fibres. *Inhal Toxicol* 2002;**14**:5–27.
12 **Oberdorster G**, Utell MJ. Ultrafine particles in the urban air: to the respiratory tract—and beyond? *Environ Health Perspect* 2002;**110**:A440–1.
13 **Maynard RL**, Howard B, eds. *Particulate matter: properties and effects upon health*. Oxford: Bios Scientific Publishers, 1999.
14 **Donaldson K**, MacNee W. Potential mechanisms of adverse pulmonary and cardiovascular effects of particulate air pollution (PM10). *Int J Hyg Environ Health* 2001;**203**:411–15.
15 **Tan MH**, Commens CA, Burnett L, *et al*. A pilot study on the percutaneous absorption of microfine titanium dioxide from sunscreens. *Australas J Dermatol* 1996;**37**:185–7.
16 **Pinnell SR**, Fairhurst D, Gillies R, *et al*. Microfine zinc oxide is a superior sunscreen ingredient to microfine titanium dioxide. *Dermatol Surg* 2000;**26**:309–14.
17 **Jani P**, Halbert GW, Langridge J, *et al*. Nanoparticle uptake by the rat gastrointestinal mucosa: quantitation and particle size dependency. *J Pharm Pharmacol* 1990;**42**:821–6.
18 **Hillery AM**, Jani PU, Florence AT. Comparative, quantitative study of lymphoid and non-lymphoid uptake of 60 nm polystyrene particles. *J Drug Target* 1994;**2**:151–6.
19 **Duncan R**. The dawning era of polymer therapeutics. *Nat Rev Drug Discov* 2003;**2**:347–60.
20 **Sun YP**, Fu K, Lin Y, *et al*. Functionalized carbon nanotubes: properties and applications. *Acc Chem Res* 2002;**35**:1096–104.
21 **Warheit DB**, Laurence BR, Reed KL, *et al*. Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats. *Toxicol Sci* 2004;**77**:117–25.
22 **Donaldson K**, Tran CL, MacNee W. Deposition and effects of fine and ultrafine particles in the respiratory tract. *The European Respiratory Monograph* 2002;**7**:77–92.
23 **Kreuter J**, Shamenkov D, Petrov V, *et al*. Apolipoprotein-mediated transport of nanoparticle-bound drugs across the blood-brain barrier. *J Drug Target* 2002;**10**:317–25.
24 **Oberdorster G**, Sharp Z, Elder AP, *et al*. Translocation of inhaled ultrafine particles to the brain. *Inhal Toxicol* 2004;**16**:437–45.
25 **Bodian D**, Howe HA. Experimental studies on intraneural spread of poliomyelitis virus. *Bulletin of the Johns Hopkins Hospital* 1941;**LXVIII**:248–67.
26 **Donaldson K**, Borm PJ. The quartz hazard: a variable entity. *Ann Occup Hyg* 1998;**42**:287–94.
27 **Schins RP**, Duffin R, Hohr D, *et al*. Surface modification of quartz inhibits toxicity, particle uptake, and oxidative DNA damage in human lung epithelial cells. *Chem Res Toxicol* 2002;**15**:1166–73.
28 **Knaapen AM**, Albrecht C, Becker A, *et al*. DNA damage in lung epithelial cells isolated from rats exposed to quartz: role of surface reactivity and neutrophilic inflammation. *Carcinogenesis* 2002;**23**:1111–20.