

## Health impacts of nanotechnology 8 December 2003

### Summary of evidence presented to nanotechnology working group

**The views within this document are a summary of discussion and do not necessarily reflect the views of the Nanotechnology working group**

## Health & the environment workshop 8 December 2003

### Summary of evidence presented

## Afternoon session - health

### 1 Introduction

Professor Seaton introduced the afternoon session and outlined its aim, which was to discuss potential health impacts of nanotechnology.

### 2 Nanoparticle toxicology: presentation by Professor Ken Donaldson

Below is a summary of points made in the presentation. Please refer to the full presentation for further details.

In this work, a nanoparticle (NP) is classed in <100nm (<0.1micron) range, whereas the size of particles derived from the dusty trades such as mining usually examined toxicologically range from 100-10000nm (0.1-10 microns).

#### *a) Nanoparticles in the lungs<sup>1,2</sup>*

Work on lung inflammation caused by carbon black instilled into the lungs of rats was presented. Nanoparticle-sized carbon black caused significantly more inflammation than an equal weight of larger sized particles of the same material. Indices of inflammation increased in direct proportion to total particle surface area instilled, but an additional effect was found to be derived from the presence of transition metal ions on the surface of the particles. Similar results had been found with particles of other chemical constitution, leading to the conclusion that there may be a general inflammogenic effect of particles dependent on the surface area presenting to the lung but able to be enhanced by reactive chemicals on their surfaces. Thus given exposure to equal masses of nanoparticles and larger particles, greater toxicity would be expected from the former.

#### *b) Translocation of nanoparticles from lungs to brain<sup>3</sup>*

Prof Donaldson quoted (with permission) 'in press' research by Dr Gunter Oberdörster in Rochester NY in which rats had been exposed by inhalation exposure to nanoparticle C-13 for 6 hours. After an initial increase of C-13 to lung (to about 1.4 microgram C-13/organ), the amount in the lung was shown to decrease, whilst the amount in the olfactory bulb slowly increased (to approximately 0.4 micrograms C-13/organ). It appeared that under these experimental conditions NPs may gain access to the central nervous system via the olfactory nerve.

#### *c) Translocation of nanoparticles from the lungs to the blood<sup>4</sup>*

Work on translocation of radioactive NP carbon to the blood following inhalation was presented.

Technegas, <sup>99m</sup>Techetium-labelled ultrafine carbon, with dimensions of 5-30nm in cross-section and 3nm thick – was inhaled at an extremely low mass dose, and the subsequent radioactivity of the blood was measured. Under these experimental conditions, it had been shown that NPs depositing in the lung may pass into the bloodstream in very small amounts.

*d) Consequences of nanoparticle deposition in the lung on the blood<sup>5</sup>*

Work by Nemmar *et al*<sup>5</sup> had examined the effect of NP instillation on blood coagulability in hamsters. Pro-thrombotic effects such as the formation of clots were most prominent when the surface of the (lung-instilled) polystyrene beads was aminated, showing that this form of toxicity may be related to the surface reactivity of the particles.

*e) Cellular mechanism of nanoparticle effects: role of calcium*

Work by Stone *et al*<sup>6</sup> had shown that NPs of materials of otherwise low toxicity and low solubility may generate free radicals at their surface that were capable of driving cellular effects that lead to an alteration in calcium signalling, which can in turn lead to inflammation.

Other points

- If NPs were in the gut or delivered to various sites in the body for medical reasons, they may translocate to other sites.
- Experimentally, NPs in the lungs may cause increased blood coagulability either directly by translocating, or indirectly via release of cytokines, oxidants etc.

### **3 Discussion**

The presentation was followed by discussion of some of the issues raised, as well as other related points.

#### *Nanoparticles*

- These kinds of toxicity investigations are designed for investigating toxicological mechanisms and for comparing the effects of different particles. They necessarily require much higher doses often delivered in an unnatural manner. As a rough guide, it might require about 2 months to get the same dose (125 micrograms) in a normal environment as the doses used in the experiments outlined here.
- Much human epidemiology had shown unexpectedly consistent adverse health effects associated with very low concentrations of particulate exposure. The hypothesis therefore arose that the effect was due not to the mass but to the numbers of particles inhaled. This hypothesis has required testing experimentally and some of these results suggested that NPs behaved differently from larger particles in that they did not recognise cell membranes as a barrier.
- The surface reactivity of a particle, as well as its size, appears to play an important role in inflammogenic effects and so nanoparticle design may be critical to toxicology.
- The presence of transition metals on the surfaces of any particles can play an important role in toxicity; however, transition metals are not essential to the toxicity of all nanoparticles. Size alone appears to be a factor in some cases. An interesting issue for toxicological research would be the interactions of new nanoparticles such as buckyballs and metals such as iron (eg in the case of buckyballs filled with iron).
- Although the work presented involved introduction of ultrafine carbon black into the lung suspended in saline, nanoparticles introduced without saline would be expected to behave in the same way.
- Work did not address physical 'clearance' mechanisms for removing nanoparticles from lungs, but there is some evidence that nanoparticles may make this process slow down.
- Some experimental studies in susceptible humans are being done using aggregates of nanoparticles of about 1 micron in diameter. Detailed reports of this work are likely to be published in the near future.
- It was noted that nanoparticles have a tendency to aggregate.

#### *Nanotubes*

- The studies referred to in the presentation are on spherical particles – not platelets or tubes. The work of David Warheit<sup>7</sup> of the Haskell laboratories of Du Pont, USA on evaluating pulmonary toxicity of nanotubes in rats was raised, in which rats received approximately 1mg of nanotubes by lung instillation. It was reported that those highly dosed rats that died probably suffocated, and that the survivors showed scattered granulomatous reactions in their lungs. The authors found it difficult to explain this reaction, but participants pointed out that it was a common one to inhaled foreign particles and was probably enhanced by the instillation method producing a high dose of intra-airway fibres.
- The mechanism by which fibre-shaped particles such as nanotubes reach the gas-exchanging part of the lungs was discussed. A diameter of less than 3 micrometres was necessary and such fibres are likely to be retained if they are insoluble and greater than approximately 10 micrometres in length. If nanotubes clump together in the air like cotton wool (as happens in the lab), their ability to penetrate the deep lung would be reduced. However, individual long and durable nanotubes would have the potential to penetrate deeply into the lung, and could cause problems for the normal process of removal by macrophages. It was pointed out that it is now possible to manufacture nanotubes of a few nanometers diameter but up to about 1mm in length. It was thought that nanotubes are not likely to be soluble in the lungs, but a nanotube's solubility is affected by what it is attached to (eg in composites). As a general rule, the depth of penetration into the lungs of fibrous particles depends on their diameter and their toxicity to the lung depends on their length, durability and surface properties. Whether long, durable fibres cause lung damage rather than being cleared by the lungs depends on the dose of the fibres.
- In the few published papers on nanotube toxicology so far, doses have been very high and inevitably produced pathological reactions that would not be observed under everyday conditions. Much further work is required to assess the toxicity of nanotubes at plausible lung doses. In addition the possible exposures of individuals to these materials need to be assessed.

#### *General*

- Exposure to novel nanoparticles and nanotubes needs to be considered at three stages – production, use, and disposal. As they are in manufacture now, there is risk of exposure during the production of both nanotubes and nanoparticles. Their potential to interact with humans and the environment also depends on the use which materials are put to – for example, in paints which may be sprayed or used on surfaces subject to abrasion and thus may re-release particles/tubes.
- There are lessons to be learnt from epidemiological studies – for example, of the long- and short-term effects of air pollution on populations, on workers in the carbon black industry, and on welders – which include dose-response studies, and studies of pulmonary function. It was observed that ambient air pollution particles seem to have a greater overall effect on the heart than the lungs, but, despite exposures to very high numbers of particles, the health response is generally small.
- The possibility of using information from European Multicentre studies of air pollution to inform work here was discussed. It was commented that if the work had expressed doses as particle number or surface area this would be valuable, but unfortunately methods for doing this have only very recently been available.
- It was highlighted urban dwellers even in the low pollution conditions of today are constantly breathing in nanoparticles (both natural and man-made), at a rate of several million per breath, a concentration which the lung seems capable of dealing with without adverse effects in most individuals.

#### **4 Skin absorption of nanoparticles: presentation by Dr Sean Semple**

The following is a summary of points made in Dr Semple's presentation. Please refer to full presentation for further details:

- Exposure assessment for epidemiology and occupational hygiene has tended to focus mostly on the inhalation exposure route. However, in recent years there has been growing interest in the passage of chemicals through the unbroken skin, for example, due to occupational exposure in places where solvents, pesticides, or pharmaceuticals are manufactured or employed. Skin absorption also occurs in

non-occupational situations from the use of cosmetics and in the intentional application of topical creams and drug treatments.

- Methods of measuring dermal exposure and the associated difficulties were outlined.  
An explanation of skin structure was given. Materials deposited on the outside of the skin are generally of the micrometre size. If they are to pass through the skin they have to dissolve in the aqueous layer of sweat and sebum on the outer layer of skin (epidermis) and set up a concentration gradient between the epidermis and the dermis (a much thicker living layer of cells which includes blood vessels, nerves, hair follicles and sweat glands). This gradient produces a mass transfer that is dependent on the physical properties of the skin at that site and also the chemical properties of the substance. Diffusion across the complex membrane of the skin is regulated by Fick's Law, which states that the rate of diffusion across a barrier will be directly proportional to the concentration gradient.
- Chemical uptake through the skin is poorly understood and methods such as quantitative structure-activity relationships are being used to estimate permeation rates.
- The use of nanoparticles in sunscreens was outlined. This application is being driven by the advantage of having a 'cosmetically clear' material – compounds are mainly titanium dioxide (TiO<sub>2</sub>) and zinc oxide (ZnO). Sunscreen based on these materials is totally transparent due to the average particle size being less than 30nm.
- There is some debate as to the possibility that nanofine particles may be absorbed into the skin and increase the risk of formation of hydroxyl radicals which may then lead to oxidation and DNA damage. However, initial studies of absorption of these particles has proved inconclusive, some suggesting little penetration into the deep layers of epidermis and others using more complex skin flexing protocols showing absorption.
- Pharmaceutical companies are also now using ultrafine particles called nanosomes, solid lipid nanoparticles and Lipopearls to enhance the delivery of drug treatments and cosmetics to the skin. Work by Miyazaki<sup>8</sup> and colleagues has shown that nanocapsules loaded with indomethicin can be employed to improve transdermal delivery. Similarly a study by Haberland et al<sup>9</sup>, reports that lipophilic drugs such as steroids are absorbed up to four times faster when incorporated into solid lipid nanoparticles compared to standard emulsions. The absorption of these substances may be further increased, as these pharmaceuticals are likely to be applied to damaged/diseased skin which will have different absorptive properties to intact skin. Solid lipid nanoparticles can however also be employed to slow the rate of chemical release and skin uptake. Wissing and Muller<sup>10</sup> have recently demonstrated that an SLN formulation of sunscreen containing oxybenzone slowed the rate of release and hence penetration into human skin when compared with conventional emulsion sunscreen.
- There has been significant interest in the recent hypothesis that Chronic Beryllium Disease (CBD) may result from sensitisation caused by uptake of beryllium particles through the skin. Tinkle et al<sup>11</sup> have shown that 0.5-1.0 μm fluorospheres penetrate the epidermis reaching the dermis. This study is one of the few to employ a skin flexing protocol that is likely to be representative of real life conditions.

## 5 Discussion

The discussion covered some of the issues raised in the presentation, as well as other relevant ones.

### Sunscreen

#### *Titanium dioxide (TiO<sub>2</sub>)*

The issue of ultrafine TiO<sub>2</sub> and its ability to penetrate the skin was discussed. The European Commission's Scientific Committee on Cosmetics and Non-Food Products (SCCNFP) has stated in its Opinion on TiO<sub>2</sub> that TiO<sub>2</sub> is safe at any size, coated or uncoated, hydrophilic or hydrophobic<sup>12</sup>. It was stated that the SCCNFP had gathered evidence from industry on TiO<sub>2</sub> but had subsequently asked for additional tests from industry on nanosized TiO<sub>2</sub> before it could rule on its safety. The working group were informed that the dossier of work that informed the Opinion is confidential and the property of Physical Sunscreen Manufacturers Association

(PSMA)<sup>a</sup>. It was suggested that it would be useful if the working group could have access to this safety dossier to inform their work, as many of the toxicity studies will not have been published in peer-reviewed literature. The point was made that the work on which the SCCNFP had based its conclusions was done before 2000, and since then there had been developments in this area. The zinc oxide (ZnO) dossier is still being evaluated and the SCCNFP has not ruled on this yet.

The work of one attendee on TiO<sub>2</sub> was discussed. This had not shown that micronised TiO<sub>2</sub> could pass through the skin, but rather that, if it did, it had the potential to do damage to DNA. The potential damage varied by a factor of 100 depending on the grade of TiO<sub>2</sub>. It was also suspected that the least active TiO<sub>2</sub> was the coated variety. The use of these sunscreens had to be seen against the alternative, organic ones, some of which do penetrate the skin. Work by Lademann et al<sup>13</sup> has indicated that standard TiO<sub>2</sub> microparticles could not be detected in deeper layers of epidermis after application. This study also found that less than 1% of applied TiO<sub>2</sub> was found in the open part of the hair follicle where transdermal absorption may potentially be easier.

#### *Manufacture*

It was asked how these sunscreens were manufactured. One attendee responded that they are made either by precipitation or gas phase; nanoparticles are usually coated as this aids manufacture and dispersion. The nanoparticles used in sunscreen are typically between 20 – 100nm (under 20nm doesn't protect against UV).

#### *Testing*

The fact that sunscreen is tested as a cosmetic, not a pharmaceutical, was discussed. Toxicity tests for cosmetics are generally not as extensive as those for pharmaceuticals. Cosmetic products are also not tested on animals, so sunscreen toxicity is tested on a restructured epidermis, using an analysis of layers by electron microscopy, confocal Raman spectroscopy, and by chemical means. The safety assessment is equivalent to two weeks' use of sunscreen on a beach. However, the skin model has no blood supply and cannot flex or move and it was questioned how representative these tests could be.

It was asked whether there had been any toxicity tests on burnt or damaged skin, but it was thought that there had not. It was argued that sunscreen is not supposed to be applied to damaged skin (as it is a cosmetic), which led to discussion as to the extent to which sunscreen could be called a pharmaceutical. It was argued that if skin burns the advice is to get out of the sun rather than to apply sunscreen. However, there was another view that sunscreen fell on the border between cosmetic and pharmaceutical and it is likely that sunscreen would be used by some people on burnt or damaged skin.

The potential of nanoparticles to act as vehicles for transport of other chemicals was discussed. One attendee said that, as an example, there have been studies in the US which showed that uptake of pesticides was greater in individual exposed workers using sunscreen than in those not – but whether the transport was facilitated by nanoparticles or by another component of sunscreen was unknown.

#### ***Other uses of nanoparticles***

Nanoparticles of iron oxide have been used in lipstick, pharmaceuticals applied to skin, and nanocapsules for delivery of vitamins. According to one of the attendees, this latter application has shown nanoparticles only penetrate into the epidermis. TiO<sub>2</sub> is also a food additive (E171).

#### ***Regulation of nanoparticles***

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<sup>a</sup> The Executive summary of the Opinion is available on the SCCNFP website ([europa.eu.int/comm/health/ph\\_risk/committees/sccp](http://europa.eu.int/comm/health/ph_risk/committees/sccp)). The SCCNFP secretariat has provided the working group with a copy of the full Opinion but is not entitled to disseminate the contents of the safety dossier provided by the industry. They have stated that the reference list in the full Opinion gives all the studies enclosed in the safety dossier.

It was stated by one attendee that the existing methods of control depend on mass measurement, except in the case of fibres, which are counted. If particle number is the relevant measure of toxicity, however, mass measurements may not be sufficient, as they may not be accurately representing particle numbers. It is only recently (in the last 3-4 years) that a different toxic effect of nanoparticles has started to be considered based on surface area.

A number of problems were identified:

- There is a serious lack of toxicological information in this area – the knowledge base on lung and skin absorption of particles in the 0.2 – 1.0 micrometre range and their effects on cells and tissues is limited.
- The traditional particle count machinery has been impractical for widespread use in workplaces and laboratories. Nanotubes may be too small in transverse diameter to be detected by standard methods such as phase-contrast and scanning electron microscopy.
- If the particles aggregate, they may not be measured as nanoparticles, even though their surface area remains high and thus they may maintain the toxic properties of the individual particles.

If surface area proves to be the critical metric predicting toxicity, measurement of this could be an acceptable control measure, and work is starting in Health and Safety Executive after Christmas on this area.

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### Attendee list

Name	Affiliation
Ken Donaldson	Edinburgh University
Vicki Stone	Napier University
Vyvyan Howard	Liverpool University
Mike Brown	Boots plc
Anne Glover	University of Aberdeen
Maureen Meldrum	HSE
Bob Rajan	HSE
Christine Northage	HSE
Stuart Hawksworth	HSE
Chris Tuppen	BT
Sean Semple	University of Aberdeen
John Knowland	Oxford University
Graham Dransfield	Uniqema
Alistair McLeod	Imperial College London
Vic Hyde	The Cosmetics Toiletry and Perfumery Association
Francis Quinn	The Cosmetics Toiletry and Perfumery Association
Steve Downing	ICI
Ann Dowling	Nanotechnology working group
Roland Clift	Nanotechnology working group
Nicole Grobert	Nanotechnology working group
John Pethica	Nanotechnology working group
John Ryan	Nanotechnology working group
Anthony Seaton	Nanotechnology working group
Saul Tendler	Nanotechnology working group
Mark Welland	Nanotechnology working group
Roger Whatmore	Nanotechnology working group