

# HEALTH PROTECTION AGENCY



## HPA BOARD MEETING

18 June 2008

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### **Nano-toxicology research at HPA Chilton Executive Sponsor: Roger Cox Presented by Gary Coleman**

#### **Introduction**

Nano-technology is likely to be one of the most important technological developments of the present century. Nano-technology involves the use of materials prepared so that the individual components, be they spheroids, fibres or tubes, are of very small size. The nanometer (nm) is used as the unit of measurement of such components:  $1 \text{ nm} = 1 \times 10^{-9} \text{ m}$  or  $1 \times 10^{-3} \mu\text{m}$ . It has been discovered that materials of such small dimensions have unusual and useful properties that are proving valuable in electronics, in opto-electronics, in imaging systems used for medical diagnostic work, in cosmetics, in making self-cleaning clothes and in food preparations. Such wide uses and unusual physico-chemical properties lead to questions about the safety of such materials and these have led to the rapidly developing field of nano-toxicology. New journals have been established to report the findings of toxicological studies and, already, monographs such as that edited by Monteiro-Riviere and Tran (2007), have appeared. Publications in the scientific, para-scientific and popular press are appearing at an extraordinary rate. Early fears of "grey goo" spreading widely have been allayed though a recent review used this unfortunate phrase in its title (Nohynek *et al*, 2007). Some of this activity has been driven by "hype" but real concerns exist and are being addressed in the UK and abroad. The Health Protection Agency has a responsibility to advise DH and others of the hazards and risks associated with exposure to nano-materials, especially via the environment. The Agency has responded to this challenge by establishing the National Nano-toxicology Inhalation Research Centre at HPA Chilton.

#### **The toxicological properties on Nano-materials**

Early studies of common materials presented in nano-form suggested that such formulations might have toxicological properties which, if not quite new, differed quantitatively from those of the same materials presented in larger, non-nano, form. Oberdorster *et al* (2005) have provided a comprehensive review of early studies. It now seems that nano-materials do not have entirely new toxicological properties: the key discoveries have been that nano-materials may reach parts of the body inaccessible to larger particles and that they tend to be more active than larger particles when activity is expressed on a "per unit mass" basis. Interestingly, the toxicological activity of nano-materials often seems to correspond to that of the same materials presented in non-nano form when the dose is expressed on a "per unit surface area" basis. This is, for example, the case for the common white pigment titanium dioxide (Oberdorster *et al*, 2005). This is very important in that it suggests that the inherent toxicological activity of the particle surface is not changed by distorting the surface to encompass a very small particle. Mechanisms thought to explain the toxicological activity of nano-particles are similar to those invoked to explain the activity of larger particles, for example free radical production, perhaps catalysed by metal species present at the surface of the particles seems to be important (Ayres *et al*, 2008).

Unfortunately the problem is not quite as simple as this. The effort needed to characterize adequately nanomaterials under investigation and to identify the most appropriate measurement to express dose should not be underestimated. Also as suggested above, nano-materials may be able to gain access to parts of the body which larger particles cannot reach and thus might produce novel effects. The novelty may not be due to novel intrinsic activity of the nano-materials but to the novelty of their distribution and thus of the sites at which activity is expressed. It has been shown, for example, that nano-materials can reach the brain by translocation along nerve fibres. Larger particles cannot do this though the polio virus (a 'natural' nano-particle) takes this route from skin abrasions to the spinal cord. The appearance of nano-particles in the brain can lead to inflammatory activity (Elder *et al*, 2006; Long *et al*, 2007; Kleinmann *et al*, 2008). Penetration to the blood stream via the lung has often been assumed though the evidence for such movement is contradictory (Nemmar *et al*, 2002; Mills *et al*, 2006; Weibert *et al*, 2006) and this possibility cannot be regarded as proven. But, the case for inhaled particles affecting the cardio-vascular system is, on the contrary, well established and is a topic of current research in the air pollution field (Delfino *et al*, 2005; Department of Health, 2006). Injection of carbon nano-tubes into the peritoneal cavity of rats has been shown to lead to the production of granulomata (Poland *et al*, 2008) and this has given rise to speculation that long, thin, insoluble carbon nano-tubes that are biopersistent may have toxicological properties similar to those of asbestos fibres.

### **National response to concerns**

Concerns relating to potential health effects led to calls for urgent assessment of the toxicological properties of nano-materials (Royal Society, Royal Academy of Engineers, 2004; Oberdorster *et al*, 2005). Funding for such research is being provided in the UK by the Department of the Environment, Food and Rural Affairs (defra), the Department of Health (DH) and the Medical Research Council (MRC). The Health Protection Agency (HPA) has responded to the challenge of nano-toxicology by developing the National Nano-toxicology Inhalation Research Centre (N-NIRC) at HPA Chilton. N-NIRC represents a collaboration between HPA, five leading UK universities and the MRC Toxicology Unit. Work at N-NIRC will focus on the kinetics of inhaled nano-particles and on the effects of nano-materials on the brain, the lung and cardiovascular systems. The initiative builds on expertise available within the Radiation Protection and Chemical Hazards and Poisons Divisions of HPA.

A major programme of international collaborative work on health and environmental safety of manufactured nanomaterials is being carried out as part of the OECD Chemicals Programme. ([www.oecd.org/sti/nano](http://www.oecd.org/sti/nano)). This work is being taken forward by the OECD Working Party on Manufactured Nanomaterials. An important aspect involves a sponsorship programme whereby countries agree to safety testing of a number of nanomaterials representative of groups currently in commercial use. Advice is being developed on appropriate test methods. The UK lead on this work is defra, with input from other agencies including the Health and Safety Executive (HSE), the Environment Agency (EA) and HPA

### **The National Nanotoxicology Inhalation Research Centre (N-NIRC)**

RPD Chilton has long experience of working with aerosols. It has been decided to capitalize on this expertise and to refurbish the RPD inhalation facility to allow a new series of studies of nano-materials. Money to support this activity has been provided from the HPA Development Fund. Contact with the leading German group, led by Dr Wolfgang Kreyling, has been established and staff from RPD have visited the GSF laboratory: state of the art equipment for generating aerosols of nano-materials and for measuring their concentration and specific surface area is currently being obtained. A series of experiments designed to examine the bio-kinetics of inhaled radioactive nano-particles will begin in 2009. The methods needed for such studies, including auto-radiography and tissue counting are available within PRD.

It was realized early on in developing N-NIRC that collaboration with external sources of expertise would be essential. A collaboration between five leading UK universities (Edinburgh, Birmingham, Imperial College, Kings, Cardiff), the MRC Toxicology Unit in Leicester and the HPA has thus been

established. A series of meetings have been held and three applications for external funding are in preparation. These are:

- Studies of the bio-kinetics and effects of inhaled carbon nano-tubes. Project being led by Professor Ken Donaldson (Edinburgh University)
- The importance of surface reactivity in controlling nano-particle toxicology. Project being led by Professor Frank Kelly (Kings College London)
- The neurotoxicological effects of nano-particles. Project being led by Professor Pierluigi Nicotera (Director MRC Toxicology Unit)
- These projects will lead to applications for funding to the MRC and EPSRC. If applications for funding are successful work on these projects will begin in late 2009/early 2010.

### **Project management**

The development of the NNIRC is being managed under the Prince2 project management system. Prince2 is the central government approved approach for large scale projects of this type. As required by Prince2 a Project Board has been constituted to oversee the project and a Project Manager and Project Team identified to plan and undertake the required tasks. The first official meeting of the Project Board was held on 23rd April 2008, however, meetings of those involved in the project commenced in an ad hoc manner in October 2007 and have been held regularly since. Project Team meetings are currently held weekly to monitor progress.

As CRCE expertise in Prince2 is limited an external consultant was employed in March 08 to provide assistance in the early project planning stages. This has proved very useful. The project has a number of phases. The project is currently in the 'Initiation' Phase. This is the detailed planning stage. The Initiation Phase will be completed by the end of June. At this point a detailed overall project plan for the set-up and management of the facility will have been completed with the associated Business Case. Some preparatory work has however been going on in parallel to this planning activity. The laboratory facility was previously used for the exposure of rodents to aerosols of plutonium and other alpha-emitting radionuclides. It is therefore necessary to clean-up the facility prior to development for its new use. Plans for the decommissioning and decontamination are well advanced and expected to commence this month.

The next stage is the detailed 'Specification' Phase. This involves defining all the equipment that needs to be purchased, designed and manufactured. This includes the aerosol production (ie spark generator and Ir-192 source) and measurement and characterisation equipment. Detailed plans for refitting the laboratory space will also be produced. To date draft technical diagrams indicating the configuration of the exposure equipment have been produced as have diagrams of laboratory lay-out. These were produced primarily to aid in the costing process for the Business Case but are a significant first step in the overall facility design process. These will be refined to ensure the facility meets all project objectives and all Health and Safety and Animal Welfare requirements.

The following Phase is 'Procurement'. To speed the lengthy EU defined tender process that is required for much of the equipment an EU tender call for the majority of the equipment has already been issued. This defines the equipment in general terms and thus does not constrain the design process. The final stage is the Build, Test Commission Phase.

Progress to date is consistent with plans to meet the HPA Business Plan Target of April 09 for undertaking experiments with rats using nose only exposure of Ir-192 aerosols.

## **Future requirements**

*Staff:* HPA has an important role to play in the nano-toxicology area and is well placed to become a Centre of Excellence for inhalation studies. To achieve this a long term commitment by the HPA Board to work in this area is sought. As the work expands, and as HPA develops in-house expertise in the nano-toxicology field, new staff will be needed. A senior aerosol physicist and a less senior worker in the same field will be recruited this year. In 2009 we should be looking towards recruiting a senior inhalation toxicologist to lead the biological aspects of the work. Technical support staff will also be needed.

*Funding:* Funds to maintain the in-house nano-toxicology programme will be needed. Successful grant applications will provide much of the funding needed for N-NIRC but core funding to maintain the facility and to push forward our own programmes will also be needed.

*Facilities:* The inhalation laboratory is being refurbished and equipped. A general purpose laboratory for use by visiting workers from the Collaboration will also be needed. Space for this has not yet been allocated. The UK lacks facilities for inhalation studies and it may be that a good deal of contract work could be taken on over the next five years. If this aspect develops a second inhalation facility will be needed.

*Conclusion:* N-NIRC is an exciting development for HPA. The Agency is uniquely well placed in the UK to make a major contribution in this area and to develop a Centre of Excellence. Support for the HPA Board for this activity is sought.

Prof. R L Maynard  
Dr R Fielder  
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