Answers to written questions on notice

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1). If it is possible could you give a layman's description of the process for assessing the toxicity of a nanomaterial, including the general timeframe required for completing an assessment?

The aim of the regulatory toxicological assessment is to assess hazard potential – i.e. the nature of adverse health effects which could occur if sufficient exposure occurs, and some estimate of the degree of acute and chronic exposure (i.e. doses) required to produce those effects. These toxicological profiles are generally derived from a suite of studies in rats, mice and other animals, most often by oral exposure route, but where relevant, inhalational and/or dermal exposure studies may be included. It is then necessary to extrapolate the findings of these animal studies to assess their relevance to humans.

The processes for toxicological evaluation, and the legislated timeframes, differ according to the regulatory organization to which it is submitted. The TGA (therapeutic goods) and APVMA (AgVet chemicals) are **product**-based, rather than **substanc**e-based, regulatory systems. A new product containing nanomaterials would be evaluated for safety by the TGA or APVMA prior to market entry. The toxicological data requirements are set out more fully in relevant TGA and APVMA guideline documents. Data requirements for chemicals regulated by NICNAS tend to be more limited and are related to the basic properties of the chemical substance, and require less information addressing possible uses and exposures.

2). Are there standards in place for toxicity assessment that can be applied to nanomaterials? Is there a range of methodologies for tracking nanomaterials or nanoparticles when assessing their toxicity, if so does this pose any problems?

Nanomaterials may present different toxicological characteristics compared to larger-sized materials of the same composition, by virtue of their greater surface area, catalytic activity, and possibly because of their greater potential for bioavailability (access to sites within the body where larger particles may be excluded).

The most likely situation for novel toxic potential from nanoparticles to cause biological effects not seen in the bulk material, is if they are: *insoluble* nanoparticles

that *penetrate* biological membranes (imparting increased bioavailability compared to bulk material), and *persist* in the body (either through extensive tissue distribution and binding, or sequestration and slow remobilisation). Such a nanoparticle may be designated a "NP of concern" (NPOC) or "nanomaterial of concern" (NMOC).

The techniques for assessing this toxicological potential are essentially the same for any chemical substance, including nanoscale chemicals, but there may be a greater emphasis on understanding the ability of nanoparticles to alter the function of organs involved in removing particulates from the circulation, i.e. the liver and immune system (e.g. immune suppression or activation, and/or inflammatory responses).

Tracking the disposition of nanonaterials in the body can indeed be a difficult problem. Nanomaterials are usually not readily visualised except by sophisticated electron microscopic techniques, or unless they are labelled with a fluorescent marker, radioactivity, or with a stable (non-radioactive) isotope – however many of these methods will not show whether actual nanoparticles are still present in body tissues or fluids unless dual-labelling is also used (i.e. the nanoparticle has not dissolved if both labels are still present in very close proximity).

In the case of assessing the potential risk associated with exposure to a certain nanomaterial, it is important to evaluate its complete life-cycle (i.e. synthesis, manufacture, product formulation, transport, storage, use/abuse, disposal/removal/cleanup or recycle) to identify potential hotspots of nanoparticle release and exposure of humans and the environment. The potential routes of exposure can then be identified to help prioritise the exposure routes and organ/cell types to be examined by toxicity testing.

3). Submissions from the CSIRO and UTS both noted that some earlier toxicology studies on some nanomaterials were flawed due to the existence of contaminants and that there was a need for quality control on the sample being assessed. Is this an on-going problem?

It is very important that nanomaterials used in studies designed to understand their toxicology be adequately **characterised**. This would include description of their size, shape, surface area and impurity profile. It can be quite difficult to stabilise nanoparticles in a set nanosize range, because of their tendency to agglomerate in many types of experimental settings. To understand the mechanisms responsible for biological effects caused by exposure to nanomaterials, these nanomaterials should be accurately characterised during the toxicology study, in order to relate their actual physical and chemical state in the test system with the effects observed following exposure.

4). What was the identified need that led to the establishment of NanoSafe Australia? Can you describe the role and services that NanoSafe is currently providing?

With the increasing number of products coming onto the market that contain engineered nanomaterials, and several research groups across Australia developing new varieties of engineered nanomaterials, it became apparent that there was an urgent need for information about the potential adverse effects from exposure to certain nanomaterials, as well as advice about the safe handling of engineered nanomaterials. The NanoSafe Australia network was formed in December 2005 (initially named "NanoTox Australia", but renamed following meetings with industry representatives in early 2006). It is a group of Australian toxicologists and risk assessors, who have formed a research network to address the issues concerning the occupational and environmental health and safety of nanomaterials.

NanoSafe Australia aims to: (1) To support government, industry and non-government organisations (NGOs) in their efforts to understand the health and safety issues surrounding nanotechnology products and their manufacturing processes; and (2) To provide quality data for the appropriate risk assessments of nanomaterials. Consequently, NanoSafe Australia was commissioned by NanoVic in 2006 to provide a position paper on OHS best practice for the local nanotechnology industry and this is available on its webpages (http://www.rmit.edu.au/nanosafe) and a modified version was later published as: Harford A., Edwards J., Priestly B. and Wright P. (2007) "Current OHS best practices for the Australian Nanotechnology industry." Journal of Occupational Health and Safety - Australia and New Zealand, 23(4):315-331. ISSN 0815-6409. In the absence of toxicity data for most NPOCs, NanoSafe Australia recommends in its position paper that the precautionary approach be taken in workplaces handling engineered nanomaterials by using protocols based on "**A**s **L**ow **A**s **R**easonably **P**racticable (ALARP)" principle, while toxicology research develops data-sets for the identification and characterisation of the potential hazard and exposure associated with NPOCs.

NanoSafe Australia members (A/Prof. Paul Wright and Dr Neale Jackson of RMIT) also recently completed the first independent workplace audit of a major Australian nanotechnology facility, and are using this unique expertise for the Australian Safety and Compensation Council's project to evaluate the present evidence of the effectiveness of workplace controls for handling engineered nanomaterials, and also hopefully for conducting further nanotechnology workplace inspections nationwide. NanoSafe Australia members are also direct involved in important forums on the health and safety aspects of nanotechnology, including the Australian Research Council's Nanotechnology Network (ARCNN)

and the National Health and Medical Research Council's (NHMRC) Advisory Committee on Health & Nanotechnology (ACHN).

5). The submission from the NSW Government indicated that there was an opportunity and need to coordinate the toxicology research capability in NSW – possibly through the establishment of a network to create assessment capacity relevant to research and industry sectors in NSW. Do you see any pros or cons in the establishment of a number of State-based toxicology networks?

We favour support for individual scientists and groups working to exploit their expertise in specific areas (e.g. immunotoxicology, dermal penetration, occupational health & safety assessment). The work of these scientists would certainly be enabled by the establishment of suitable networks (e.g. NanoSafe Australia). However, we recognise that a case could be made to centralise the research effort through funding of a specialised institute. By comparison, funding an institute would require a substantially larger funding commitment, probably at a national level.

6). Currently where does the impetus and funding come from for toxicology research? Can you suggest anything that might make toxicology research attract more funding from the government or corporate sectors.

In Australia, funding for toxicology research is patchy and typically comes as a reaction to a problem that has already occurred, which needs an understanding of the toxic mechanisms involved to provide a suitable solution to the problem. In the case of nanotechnologies, we have the unique opportunity to be proactive and incorporate toxicity screening into the development of engineered nanomaterials before their application and release, as such information is very useful in re-engineering nanomaterials to reduce the potential risk associated with their use.

7). What factors need to be addressed in framing any risk communication strategy regarding nanotechnology? Do you think the results of any toxicology research should be made available to the public, and if so, in what form?

There is no doubt that a failure to engage effectively with the community could derail the development of nanotechnologies through misconceptions about the benefits and/or risks. The community backlash against genetically modifed (GM) technologies is often cited as a case study, and it is probably quite relevant to the risks of inhibiting nanotechnology development. Surveys already undertaken in Australia and overseas indicate that public awareness of nanotechnology is at a relatively low level, but concerns are being raised through campaigns demanding

that there be a moratorium on further development of nanotechnology until some of the safety and social issues have been better addressed.

We have attended at least one public forum organised by the Australian Office of Nanotechnology, which was designed to stimulate public debate. Our impression is that these meetings have been largely ineffective, due in part to the low turnout of the lay public, compared to a greater turnout of interested and involved scientists. As scientists with a commitment to improving community understanding of nanotechnology, we endorse the idea of better dissemination of information to the community.

When communicating information about a potential risk to the public from a particular nanomaterial and/or its application it should done be as part of a risk-benefit framework, so that the potential for significant benefits from this type of nanotechnology are not ignored. It is also important for the public to realise that there is not a single "nanotechnology" as such, but a very broad range of nanotechnologies with potential to provide targeted nanomedicines, cheap lighting and purified water, in addition to the many "green chemistry" applications of self-cleaning glass and clothing, etc (which reduce energy and water consumption and waste production). Therefore toxicology research findings about nanomaterials should be reported in context and compared to everyday common risks that people understand (e.g. smoking and car accidents).

8). A number of submissions have argued that any toxicology research should focus on nanomaterials that are being researched or developed for commercialisation. Do you agree that this would be the best approach?

To some extent it makes sense to focus on nanomaterials which are being developed for the Australian market. However, it is necessary for research activities to also encompass a broader range of nanomaterials to provide a better understanding of how variation in size, surface characteristics and/or surface coatings, can influence toxicological variability. A dual approach will assist scientists to predict the potential toxicity of the rapidly expanding nanomaterial classes and their hybrids, i.e. (i) developing toxicity screening systems to rapidly evaluate nanomaterials already being developed and produced; coupled with (ii) systematic research into understanding the relationship between the surface structure and biological activity of closely-related nanomaterials, to develop a predictive toxicology database of nanomaterials.

9). Coordination and the avoidance of duplication are important issues. Can you suggest how toxicology research should best be coordinated nationally?

It is important for Australian scientists to be aware of national and international directions in nanotechnology research, so that duplication of effort can be avoided to the maximum extent possible. NanoSafe Australia is well placed to assist in the co-ordination of nanotoxicology and nanosafety research, particularly with its direct input into the main national nanotechnology research network ARCNN, and NHMRC (through its ACHN).

10). What are the infrastructure requirements for toxicology research? Is the current infrastructure adequate?

Toxicology research requires high quality research infrastructure, including specialised cell culture, animal house, experimentation and analytical facilities. Nanotoxicology research requires the additional capacity of nanomaterials characterisation in the test systems. As indicated in the answer to Question 5, the creation of a specialised institute to conduct toxicology research for nanomaterials would require substantial funding commitment from both state and national levels.

11) Professor Priestly the Committee understands that you are involved with the National Health and Medical Research Council Advisory Committee on Nanotechnology and Health.
Can you briefly describe the objectives of this Committee?

The NHMRC Advisory committee on Health & Nanotechnology (ACHN) was established in part to provide advice to the NHMRC on research gaps to which it might direct funding. However, it also aims to provide expert advice on potential advances in medical treatment and diagnostics associated with biomedical applications of nanotechnology, and to foster the role of the NHMRC as a source of unbiased information on health and safety aspects of nanotechnology to the broader community. The ACHN is constituted to provide expert guidance in the areas of nanotoxicology, occupational health & safety, environmental effects, exposure assessment, and biomedical applications.

12). Some organisations, such as Friends of the Earth, have argued that if a size-based definition of a nanomaterial is used as the trigger for health and environment assessment then that definition should include nanoparticles up to 300 nanometres. What is your view on that position?

We do not hold a firm view of whether the definition should be expanded to include particles sized up to 300 nanometres. Any change in definition should be agreed at an international level, and we are aware that Australian government agencies are actively involved in such fora. What is more important is that

nanotechnology research should address, and encompass, an understanding of the criteria which determine how and when the toxicological properties of a nanomaterial undergo such a significant change that it should be treated as a truly novel material.

The concern about the specific particle size range of 300nm might be related to recent evidence suggesting that the penetrance of particulates through "high efficiency particulate air" (HEPA) filters is maximal at around 300nm (because both the smaller and larger particles are blocked or retained). However HEPA filters are still extremely effective as they are by definition at least 99.97% efficient in removing monodispersed particles with a diameter of 0.3 microns (i.e. 300nm) [U.S. Occupational Health & Safety Administration, OSHA].

Another nanoparticle size range of special interest relates to the potential elimination routes of very small particles from the body. Those nanoparticles that are not sequestered in the body's tissues or cells and have a diameter less than 5nm when present in the blood, rapidly pass from the blood volume and fluid surrounding the cells, through the kidney and into urine. But it must be noted that not all nanoparticles <5nm can be considered non-toxic, because uncoated "quantum dots" of this size are taken inside cells to release their toxic core components (e.g. cadmium and selenium). Such quantum dots can be coated with polymers to prevent uptake by cells and thereby reduce their toxicity, but this can result in extended periods in the blood circulation if the polymer coating also binds plasma proteins to make the actual diameter >5nm. These aspects highlight the importance of accurately characterising the behaviour of nanomaterials in biological systems.