Dr George Collins, Chief of Research, ANSTO (Submission 17)
Dr Miriam Goodwin, Senior Advisor, Research Management & Policy
4:30pm to 5:15pm

1) Your submission argues that NSW has the potential to be a leading location for research and development of nanotechnology using the state-of-the-art research facilities and capabilities that are located within the State. Can you advise what might need to be done to realise this potential, or conversely what barriers there are to NSW realising its potential?

NSW has foundations to grow in nanotechnology.

- We have one of the world's best research reactors in this city. That is an important part of being able to be a leading centre for nanotechnology because of the way in which neutron beams can be applied in nanotechnology.
- A number of companies have already been set up in the nanotechnology area.
- Universities in NSW are very active in this area.

Despite this, there is a perception that New South Wales is not as committed to nanotechnology as some other States have presented themselves as being. That is a barrier to realisation of full potential, but it could be addressed by increasing awareness and drawing together the various threads of activity and building on that.

For example, the Government could provide greater support to facilities in NSW that are available for nanotechnology R&D. There is potential across the State to try to bring researchers, industry and others together, and to give them a greater awareness of what is going on and what the potential applications are.

Regulation clarity is another barrier that a number of people have obviously raised in submissions to the Inquiry. There is a perception that there are potential risks in setting up in a business in nanotechnology because of concerns about regulation. So clarity about regulatory environment and requirements will be important.

2) Your submission notes that ANSTO is working with industry and universities in NSW on materials and process technology that incorporate nanomaterials and that you are developing novel materials with potential applications in solar cells, optics, optolectronics and as protective coatings for abrasion and corrosion resistance. Can you briefly describe these potential applications in terms of their potential benefits?

Over the past three years we have collaborated with an Australian-based manufacturer to develop a new process method (atomic layer deposition) for coating ophthalmic lenses. Our process technology allows thin films to be grown conformally on both surfaces of an ophthalmic lens, where present technology only coats one surface at a time.

In addition, we continue to research applications in this field with RMIT University – through sponsorship from the Australian Institute of Nuclear Science and Engineering (AINSE), in collaboration with A/Prof Dougal McCulloch – where we are modelling and developing graded optical coatings.

ANSTO is working with several partners to develop solar cells for market. Specifically we have an interaction with the Cooperative Research Centre for Polymers where we are engaged in developing particular polymer-ceramic materials and architectures in the nanoscale to produce light harvesting devices. These solar cells are lightweight, for markets in recharging consumer electronics. ANSTO is also working with an Australian start-up company to develop coatings to improve light harvesting.

In addition, ANSTO is working with a leading medical devices company to use nano-materials to enhance the performance of electroactive devices.

On the research front, we work closely with the University of New South Wales through Prof Robert Burford from the School of Chemical Sciences and Engineering, developing new materials for use as either barrier coatings for protection against moisture or as coatings for anti-fouling properties in membrane filters.

In addition, our group is working with Dr Dusan Losic from Flinders University in South Australia to modify pores in membranes to holes smaller than a human hair, which have the potential for use as gas and liquid separation.

3) With respect to Ceramisphere and Australian Membrane Technologies Pty Ltd, can you advise on the safety/toxicity of the nanomaterials involved and what assessment was or is being undertaken.

CeramiSphere

The safety and toxicity of CeramiSphere's nano-size product is under evaluation. Regarding the test we have conducted:

i) *In vitro* cytotoxicity on cells shows toxicity at dose 100 times larger than the administration dose. More importantly we observe no difference in dose response between 60 micron and 50 nm particles. I.e. size does not seem to matter *in-vitro*.

ii) The half a dozen different preclinical studies (*in-vivo* experiments) on rodents (rats and mice), via several routes of administration (intravenous, subcutaneous, oral) that we have performed so far have shown no adverse response and no premature death.

iii) Biodegradability of our particles has been studied in detail *in vitro* using a state-of-the-art methodology (the USP4 dissolution testing procedure). The particles have been shown to degrade rapidly, which is consistent with other reports of similar sol-gel silica.

iv) We are planning to conduct a full-blown toxicity study as part of our preclinical trials. This is estimated to cost $0.6 million, which is a lot of money for a small start-up. Of course, any help from the State Government would be welcome in funding this toxicity study.

From an occupational health and safety perspective, the ceramics industry has been handling fine (nano) powders for the last 100 years. In addition, most of the time the particles are handled in solution and amalgamated when dried. Although the prime reason for this is to avoid aggregation into micron-particles, this significantly minimises inhalation risk.

Australian Membrane Technologies

The potential for nano-toxicity and public health issues relating to the manufacture and applications of the nanoparticulate membrane bioreactor (NMB) technology has been considered extensively. The silica gel used in the manufacture of the NMB membranes starts as a nanoparticulate colloid that is gelled by adjusting the pH. Once gelled, the material is macroscopic, but does have a very high surface area, which makes it a very efficient dehydrating agent. The acute and chronic toxicity of exposure to silica gel has been studied extensively and the MSDS reports it as being moderately toxic with respect to body contact and chronic exposure. The inhalation of dust from the dried gel is the most serious risk, and long term exposure to high dust levels may cause pneumoconiosis, caused by particles less than 0.5 micrometers in diameter entering the remaining in the lungs. Skin contact causes dehydration and minor irritation.

Applications of the NMB are focused on sewage and wastewater treatment, but also include the culture of various microbes for biotechnological applications. In all of these applications the membranes remain moist, so no dust is generated. Silica gel is used in pharmaceuticals, abrasives (e.g. toothpaste), dietary supplements and as anti-caking agents, so the miniscule quantities of silica gel that may be ingested via the products of these applications is far smaller than is already approved by the US Food and Drug Administration and the Therapeutic Goods Administration here in Australia. Antibiotics and other pharmaceuticals biosynthesised in an NMB for intravenous, oral or topical applications (e.g. antibiotics) would be subjected to numerous downstream purification processes prior to use, so traces of silica gel would be removed to acceptable levels in this process.

As long as the OH&S regulations governing handling practices are adhered to in the manufacture of the NMB membranes, this technology poses no significant risks to the public or to those involved in its manufacture.

4) Your submission states that ANSTO has developed radiotracer technology for assessing the toxicity of a range of novel and commercially available nanoparticles. Can you provide the Committee with some detail on how and when this technology will be implemented and used.

Radiolabelling with the gamma-emitting radioisotope gallium-67 has been used extensively for *in-vivo* misdistribution studies of CeramiSphere's silica-based nanoparticles.

The major development activity in this area is a collaboration between ANSTO and the Australian Institute of Bioengineering and Nanotechnology at the University of Queensland. This work is in its early stages but currently involves two PhD students and has received support from the Australian Research Council's Centre of Excellence on Functional Nanomaterials and Queensland Smart State funding.

To date the team has successfully incorporated two gamma-emitting radioisotopes – cobalt-57 and gallium-67 – into nanoparticles without altering the physical properties of these layered double hydroxide (LDH) nanoparticles (average diameter of 65 nm). *In-vitro* studies have included quantitatively following the structural decomposition of the nanoparticles at a range of biologically relevant pH levels by monitoring the leaching of the radioisotope. *In-vitro* studies are continuing on biostability and interaction with biomolecules and will be followed by *in-vivo* studies of biodistribution, bioretention and bioaccumulation.

Due to the long half-life of the Co-57 (250 days) we will be able to monitor and model transport properties of these materials in various scenarios, such as transport via water and sediment and through ecosystems from low order species such as algae to higher order animals such as fish. We will design model system at ANSTO to mimic scenarios found in nature. The ability to model these systems will allow us to predict impact in our environment and assess levels of risk.

Ga-67 is a short-lived radioisotope (half-life of 3.5 days) commonly used in lymphoma imaging. Incorporation of Ga-67 into these nanoparticles will allow use of single photon emission computer tomography (SPECT) imaging to study their transport in animals and in humans, where relevant.

We have incorporated radiolabelling into the LDH structure, have commenced uptake in algae with CSIRO and had preliminary discussions regarding collaboration. We have also had preliminary discussions within ANSTO to look at imaging these materials in rats.

Polymeric materials have been radiolabelled with both Co-57 and Cu-64 (12.7 hour half-life), the latter for positron emission tomography (PET) imaging. The strategy used for radiolabelling is the Sarar platform technology, which is well developed for human use and can be applied to a range of polymeric systems, which means we can accurately compare the systems. Understanding the distribution of these materials in the body can also provide information on the therapeutic index of the material, which is particularly important for drug delivery applications. Hence the radiolabel is chosen depending on the type of study and to ensure the basic fundamental structure of the resultant material is conserved. We are also developing nanomaterials of different sizes to assess the effect of size on transport and bioaccumulation.

The Sarar technology cannot just be used to provide information on reengineering material but the same technology can be used to radiolabel particles with Cu-64 and conduct PET imaging.

This research program is part of a collaboration with Cambridge University and has a Commonwealth Government International Science Linkages grant. We presently have funds to support one postdoctoral fellow for one year.

<u>Eco-toxicology</u>

This research program is developing methodologies to screen a range of radiolabelled, commercially relevant and novel nanoparticles for their transport properties and

bioaccumulation from water and soil into organisms. A major challenge in aquatic biogeochemistry is determining a robust relationship that defines acceptable environmental concentrations that protect the environment and are realistic for regulators. That is, organisms will not assimilate (absorb) all nanoparticles that they are exposed to, and not all of the nanoparticles that are assimilated will induce toxicity.

Two other materials that we have earmarked for this program are hectorites and carbon nanotubes. We will incorporate the radiolabel Co-57 and Ga-67 into the structure of the hectorite but intend to use our hexaza cages for radiolabelling the carbon nanotubes. These hexaza cages can be radiolabelled easily with Cu-64 and Co-57 and therefore be used for risk assessment in animal models and humans as well as ecotoxicology studies.

5) Is there scope to expand ANSTO's current involvement in nanoparticle research for biosafety purposes?

Yes, if there is strong demand for ANSTO's unique capabilities in nuclear science and technology that can contribute towards this activity. However, a significant expansion of our involvement requires two preconditions:

1. Strong collaborations with other researchers
2. Additional staffing and funding from new sources or the redirection of resources from existing activities.

An alternate possibility is the transfer of the tools and techniques we develop to other researchers.

We have some funds from external sources but need further support to extend the program. We presently run these programs using PhD students and therefore require full-time postdoctoral fellows or relevant staff to focus on these matters. Preliminary discussions have begun with CSIRO (at Lucas Heights) and other ANSTO teams regarding potential links to develop robust and relevant assays to screen these types of materials.

For ANSTO one option is to use our research and safety capabilities to develop guidance on worker safety and health, similar to that established in the US National Institute for Occupational Safety and Health, NanoSafe in Europe and under the EU Framework Programs for research. This type of program could provide independent and relevant risk assessment to support the relevant regulatory bodies in place in Australia. This would avoid the establishment of a nanotoxicology regulatory body but would provide a 'hub' for investigations and answers to questions for regulatory bodies.

CSIRO (reported at ICONN 2008) is developing a nanotoxicology program for manufacturing but it is attempting to use enriched Zn-68 (18.8% naturally abundant). It proposes a manufacturing scenario at its research laboratories that could be used to determine where the Zn-68 went in the environment (using isotopic abundance ratios) and exposure to workers, but also wants to use this strategy for monitoring transport properties in humans. CSIRO has funds for one study but would look to industry to fund the rest of its program. There are presently no additional funds.

The sustainability of this type of experimental approach can be fundamentally challenged as it will require extensive funds to implement for every product and validation of techniques would have to be a large part of the research program (delaying the development pathway). Using radioisotopes we can make a Zn-62 isotope for imaging and Zn-65 for tracking long range studies. We can conduct these types of studies at much lower cost and undoubtedly with greater accuracy.

Any technology or methodology used for risk assessment for these materials needs to be easily implemented, low cost and offer fast throughput. ANSTO has the expertise and facilities to support a whole-of-life cycle of a material, that is, the assessment of structure (using neutrons, positrons and x-rays), function (using radiotracers), engineering of materials (using radiotracers) and risk assessment. If this approach were implemented in partnership with industry as part of the R&D development pathway of new materials, ANSTO could assist companies in getting to market earlier and also gain public acceptance for their technology and ours.

6) Your submission notes that ANSTO and its collaborators are developing a library of precisely engineered nanoparticles with different size and surface characteristics. Please tell us more about the importance of this work, including the basis on which items are included in the library. Is this library part of an international collaboration to develop an exhaustive library?

The researchers are planning to synthesise and characterise a tailored library of nanoparticles and have established strategies for investigating the in-depth biological interaction of three types of nanoparticles – carbon nanotubes, layered double hydroxide particles and hectorites. The objective is to undertake long-term tracking and understanding of specific biological interaction with biomolecules, cells whole organisms (such as algae) and animal models (fish, rats).

Radioisotope labelling offers a novel method of tracking these particles in biological systems and provides more accurate data with respect to their biological interactions.

Layered double hydroxide materials are already used in a range of consumer products. However, the use of these materials in food and health products is causing concern as there is no robust risk assessment. The risk assessment should be tailored to the applications. Last year they were incorporated into yoghurt without robust assessment and this created concerns in the European community.

The problems identified are ease of penetration of the particles throughout the body, the increase in reactivity (e.g. similar to found with catalysts) and increased absorption of all sorts of chemicals, because of the higher surface area. Qualities appropriately applied are beneficial in some applications but can be toxic in other scenarios. We need to manage the benefits and risks so that we can make better products.

We are also looking at the engineering of materials of different sizes but of similar composition as there is evidence that size of particles can have significant effect on chemical properties. Key in the development of nanotoxicology strategies is an understanding that chemical compositions, size and shape all have dramatic effects on the properties of these materials and therefore all need consideration in developing protocols to assess their impact in our environment.

While there is international interest in this work, at this stage the work is not part of any formal international collaboration.

7) The submission from the Australian Microscopy and Microanalysis Research Facility argues that Australia and NSW need future investment in next generation nanostructural analysis infrastructure such as transmission electron microscopy to enable tomorrow's science and technology. Do you agree with this view?

Access to state-of-the-art nanostructural analysis infrastructure is extremely important to enable tomorrow's science and technology. Recently Australia has seen a very significant investment in such infrastructure through establishment of OPAL and its world-class neutron scattering instrumentation, the Australian Synchrotron and other investment associated with the National Collaborative Research Infrastructure Strategy (NCRIS). NCRIS has contributed to the facilities at both OPAL and the Australian Synchrotron and has been the major impetus for the establishment of the AMMRF.

Continued investment will be necessary to ensure these facilities remain internationally competitive but this investment should be selective and driven by careful consideration of what tools can Australia most profitably use. There is no point in duplicating facilities that are available elsewhere if there is not strong existing or potential demand from within Australia and NSW.

TEM will make limited contribution to the issues of nanotoxicology. The information gained is only for part of a sample. It is also incredibly slow. Radiotracer techniques provide for opportunities to information on the bulk of material and the development of easy-to-use, fast throughout systems that are highly sensitive, accurate, flexible and independent of media, which cannot be challenged by any other technology available today.

8) Can you describe the process by which industry and universities may gain access to ANSTO facilities?

There are a number of ways by which industry and universities may gain access to ANSTO facilities:

i) If the work is entirely proprietary – i.e. all the results are to be the property of the external party – then access to facilities, services and contract research is provided at commercial rates.

ii) If ANSTO and the external party are to share the ownership of the results, (i.e. sponsored research) then the costs are also shared.

iii) For non-proprietary work – i.e. where the results are published in the scientific literature – access to the neutron scattering instruments at OPAL is provided free of charge through a merit review process involving international experts. For researchers from universities, travel and accommodation costs are covered by the Australian Institute of Nuclear Science and Engineering.

iv) AINSE also manages a merit review process to enable university researchers to access ANSTO's other facilities. In this case, AINSE pays a reduced rate for facility charges and covers travel and accommodation costs.

v) If the work is truly collaborative, with both ANSTO and the external party working towards shared objectives and covering their own costs, then ANSTO will usually provide its expertise and access to its facilities as part of its contribution to the collaboration. In some cases, the external party may contribute to the facility costs, particularly if there are large numbers of samples to be analysed.