

# Developing an Understanding of Nanotoxicology

Prof. Philip C. Burcham  
Pharmacology and Anaesthesiology Unit  
University of WA, Perth

## Introduction - the Nanotechnology Era

Although once the imaginative domain of science fiction movies such as *Fantastic Voyage* (1966), the increasing use of nanoobjects in many commercial settings confirms that the “Era of Nanotechnology” is upon us. As of early 2010, an estimated 1015 commercial products containing nanoengineered materials were in use in 24 nations around the world.<sup>i</sup> The diverse list of products which contain nanomaterials includes stain-, wrinkle- and odour-resistant clothing items; food enhancers and preservers; self-cleaning windows; high-performance sporting goods; automotive coatings; high density hard drives and transparent sunscreen lotions. Numerous medical and veterinary applications for nanotechnology are also under investigation, promising to provide new tools for medical imaging and diagnostics, drug delivery, tissue engineering, toxin detection as well as novel vectors for gene and vaccine delivery.<sup>ii</sup> Based on current rates of growth, some projections place the size of the global nanotechnology industry at \$2.6 trillion within 4 or 5 years (15% of global output).<sup>iii</sup>

While multiple chemistries are employed during nanomaterial fabrication, most existing approaches fall into three broad categories.<sup>iv</sup> First are the various polymeric species which are created by using simple monomeric building blocks (e.g. PEG, PLGA or HPMA) to assemble nanoscale-sized molecular structures (e.g. dendrimers). A second group comprises an assortment of inorganic materials and includes silver or gold nanoparticles, fullerenes, carbon nanotubes, quantum dots, silicates and metal oxides (e.g. titanium-, zinc- and iron-oxides). The final group includes a growing family of hybrid materials and includes PEGylated-quantum dots and DNA-gold hybrids.

Despite these diverse chemistries, nanoobjects, by definition, share the common feature of having at least one dimension within the 1 to 100 nm length range. Much of the hope – and perhaps hype – surrounding the economic prospects for nanotechnology stems from the expectation that materials in this size range will possess a range of novel capabilities. This is because nanosized objects inhabit an unexplored domain between the world of quantum mechanics that governs the behaviour of lone molecules and the world of classical physics which governs that of bulk materials. The unpredictable properties of matter in this uncharted territory will, it is hoped, display properties that can be harnessed to provide viable new technologies to rejuvenate flagging postindustrial economies.

## But Are They Safe? - the Need for Nanotoxicology

Application of the precautionary principle would dictate that we should expect that adverse or unwanted properties might just as likely emerge from nanosized materials as any vaunted beneficial powers. In the past 5 years, this expectation has spawned a new discipline – nanotoxicology, a branch of toxicology that concerns itself with characterising any adverse consequences resulting from interactions between nanomaterials and the similarly-sized molecular machines that underlie the stunning complexity of living cells.<sup>v</sup> While the toxicity of various naturally-occurring ultrafine particles has been appreciated for decades (e.g. asbestos, soot particulates, silicates, etc), until very recently, there has been no evidence for human toxicity involving synthetic or engineered nanoparticles. The publication in late 2009

of a controversial study attributing fatalities within a Beijing chemical plant to pulmonary inflammation caused by inhaled nanoparticles is the first report of this kind.<sup>vi</sup>

## Key Issues in Nanotoxicology

Although in its infancy and with new insights likely to emerge from ongoing nanotoxicological research, work to date has identified several issues of key significance to the toxicity of nanoparticles:

**A) Physicochemical Characterisation.** Many promising properties of nanomaterials are due to the high surface area of these species relative to conventional materials. Yet such properties also ensure nanoparticles are susceptible to clumping and agglomeration during evaluation of their toxic actions in model systems (e.g. in the gas phase during pulmonary inhalation studies or in culture media during studies in cultured cells). Nanomaterials are also prone to coating by proteins (e.g. by plasma proteins in vivo or animal serum proteins in culture media), a phenomenon that complicates study of their in vivo disposition and cellular effects. Nanoparticles are also prone to surface contamination by endotoxin, synthetic by-products and redox-active transition metals. On account of these properties, increasing stress is placed upon careful physicochemical characterisation of nanomaterials under conditions that are as close as possible to those used in toxicity bioassays.<sup>vii</sup>

**B) Dose Selection & Standardisation.** The toxicological literature contains a growing body of data documenting the effects of inorganic or polymeric nanoparticles on cellular growth or viability of cultured cells. Although such studies provide useful insights into the potential adverse cellular effects of nanoparticles, there is a growing concern that inadequate effort is being made to determine the human relevance of the doses used in many in vitro studies. A related awareness is that more careful attention to the quantities of nanoparticles that are translocated to secondary target tissues (e.g. heart, brain, liver) following pulmonary or dermal exposure to nanoparticles is needed to guide selection of doses used during mechanistic studies that employ secondary cell types in vitro.

A significant issue confronting efforts to characterise the dose-dependent toxicity of nanoparticles in animals concerns the metrics used to standardise doses. While metrics such as mg/kg or  $\mu\text{mol/kg}$  are common in conventional toxicology studies, a growing body of data suggests the total surface area of nanoparticles received is more closely related to the magnitude of any toxic response.<sup>viii</sup>

**C) Toxicokinetics (“ADME”).** In an analogous manner to the in vivo handling of conventional xenobiotics, several distinct phases can be distinguished during the disposition of nanoparticles within the body: absorption, distribution, metabolism and excretion. In general, the readiness with which nanoparticles undergo such processes varies according to their size, shape and surface properties, thereby necessitating careful study of each nanoparticle class on an individual basis.

Reflecting concerns over the potential exposure of workers to airborne nanomaterials during their production, transport, use and disposal, the lung has received the greatest attention during studies of the absorption of nanomaterials. While some nanoparticles are efficiently cleared from the lung, others appear to deposit in alveolar tissues for an extended period, undergoing only minimal distribution to

secondary tissues via the bloodstream and lymphatic system.

The possibility that nanoparticles might migrate from the upper respiratory tract (including the nasal cavity) to the CNS is receiving growing attention, although to date this phenomenon appears to have been mostly characterised using high doses of particles under acute, bolus exposure conditions. In general, metabolism is a minor contributor to the clearance of nanoparticles, although some polymeric forms may undergo metabolism following leaching of constituents into surrounding tissues. Depending on their size and charge, most elimination of nanoparticles likely occurs via the kidneys and bile, although clearance via phagocytic cells and the reticuloendothelial system is also significant for some classes of nanomaterials.

**D) Toxicodynamics.** As with most biological actions of nanoparticles, the type and severity of toxicity is strongly influenced by surface chemistry properties, a point that was underscored during early studies of the genotoxicity and cytotoxicity of quartz nanoparticles in which treatment with surface-modifying reagents attenuated the observed toxic effects.<sup>ix</sup>

Although multiple toxic outcomes can accompany nanoparticle intoxication in animals, most concern has focussed on two broad effects, namely the induction of oxidative stress and immunotoxicity. Within the lung at least, Single-Wall Carbon Nanotubes (SWCNT) appear the most active nanomaterials in inducing these effects.<sup>x</sup> Nevertheless, at high doses, care is needed to delineate between indirect induction of free radical production (via the oxidative burst elicited by phagocytic cells when engulfing nanoparticle deposits) and the inherent prooxidant properties due to the surface redox activity of these materials. Under the former scenario, induction of prolonged oxidative stress during “macrophage overload” can elicit fibrogenesis and tumourigenesis. Such considerations may cloud interpretation of lung pathology accompanying inhalation of high dose TiO<sub>2</sub> nanoparticles in rodents.<sup>xi</sup>

**E) Toxicity Prediction in the “Omics Era.”** The advent of global technologies providing a “bird’s eye view” of cellular changes in gene expression (“transcriptomics”) and metabolite profiles (“metabolomics”) in response to chemical exposure is revolutionising the practice of toxicology. As highlighted in the influential “Toxicity Testing in the 21st Century” report prepared by the National Research Council (USA) in 2007, increasing awareness of the “toxicity pathways” that are disrupted by particular types of toxicants (e.g. those inducing hepatotoxicity, nephrotoxicity or cardiotoxicity, etc) is likely to reduce the need to conduct large scale toxicity tests in rodents. Rather, it is hoped that high-throughput screening in human cells will suffice when characterising the toxicity of new and unknown chemical entities. With the dramatic increase in the diversity of nanoparticles in commercial development, nanotoxicology may be a special beneficiary of future advances in predictive toxicology that rely on changes in select “toxicity pathways” to detect rogue substances.<sup>xii</sup> Researchers from the National Toxicology Program (NTP) sketched the contours of future nanomaterial toxicity assessment in a recent publication.<sup>xiii</sup>

## Conclusion

The accelerating introduction of commercial products containing nanomaterials is challenging to a toxicological community unfamiliar with evaluating substances with such unusual properties. Vigilance is thus needed to ensure our ability to invent new nanoscale materials doesn't outstrip our capacity to assess their toxic potential. The increasing sophistication of toxicity screening - thanks to tools and insights borrowed from systems biology and bioinformatics - promises to facilitate this crucial task. Given the parlous state of toxicological research in Australia, urgent steps are needed to ensure our regulatory agencies are able to keep abreast of this exciting field of modern science.

---

<sup>i</sup> [www.nanotechproject.org](http://www.nanotechproject.org), maintained by Project on Emerging Technologies, Woodrow Wilson International Centre for Scholars, Washington, USA.

<sup>ii</sup> B Fadeel *et al* (2009) Nanomedicine: reshaping clinical practice, *J. Int. Med.*, 267: 2-8

<sup>iii</sup> See [www.nanotechproject.org](http://www.nanotechproject.org)

<sup>iv</sup> AJ Gormley and H Ghandehari, Evaluation of toxicity of nanostructures in biological systems, Chapter 7 in "Nanotoxicity – From In Vivo and In Vitro Models to Health Risks," Eds: SC Sahu and DA Casciano, 2009, Wiley.

<sup>v</sup> AA Shvedova *et al* (2010) Close encounters of the small kind: Adverse effects of man-made materials interfacing with the nano-cosmos of biological systems, *Annu. Rev. Pharmacol. Toxicol.* 50: 63-88.

<sup>vi</sup> Y Song *et al* (2009) Exposure to nanoparticles is related to pleural effusion, pulmonary fibrosis and granuloma, *Eur. Resp. J.*, 34: 559-567.

<sup>vii</sup> KW Powers *et al* (2009) Characterisation of nanomaterials for toxicological evaluation, Chapter 1 in "Nanotoxicity – From In Vivo and In Vitro Models to Health Risks," Eds: SC Sahu and DA Casciano, 2009, Wiley.

<sup>viii</sup> EK Rushton *et al* (2010) Concept of assessing nanoparticle health hazards considering nanoparticle dose-metric and chemical/biological response metrics. *J. Toxicol. Env. Hlth., Part A*: 73: 445-461.

<sup>ix</sup> RPF Schins *et al* (2002) Surface modification of quartz inhibits toxicity, particle uptake, and oxidative DNA damage in human lung epithelial cells, *Chem. Res. Toxicol.*, 15, 1166-1173.

<sup>x</sup> AA Shvedova and VW Kagan (2009) *J Internal Med*, 267: 106-118.

<sup>xi</sup> G Oberdorster (2009) Safety assessment for nanotechnology and nanomedicine: concepts of nanotoxicology, 267: 89-105.

<sup>xii</sup> For an example of how mRNA transcript profiling using microarrays is assisting characterisation of nanomaterial toxicity, see the recent paper by K Fujita *et al* (2009) Effects of ultrafine TiO<sub>2</sub> particles on gene expression profile in human keratinocytes without illumination: involvement of extracellular matrix and cell adhesion. *Toxicol. Letts.*, 191: 109-117. See also K Fujita *et al* (2009) Gene expression profiles in rat lung after inhalation exposure of C<sub>60</sub> fullerene particles. *Toxicology*, 258: 47-55.

<sup>xiii</sup> NJ Walker and JR Bucher (2009) A 21<sup>st</sup> Century paradigm for evaluating the health hazards of nanoscale materials? *Toxicol. Sci.*, 110, 251-254.