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Nanotechnologies for pesticides and veterinary medicines: regulatory considerations

Final report

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ACKNOWLEDGMENTS

This report, the first to deal with both agricultural and veterinary chemicals, was developed with the financial support of the Australian Government Department of Industry. The impetus behind it was to prepare a consolidated report which considered regulatory issues relating to nanopesticides and veterinary nanomedicines. It was prepared and written by specialists from Australia and overseas under the guidance and leadership of the APVMA's Chief Scientist Dr Phil Reeves.

Dr Reeves wrote Chapter 1, *Nanotechnology in Agriculture and Animal Husbandry—an Introduction*, with input from toxicology consultant Dr Gary Buffinton. Dr Les Davies (APVMA) reviewed the chapter. The APVMA's Dr Jan Klaver developed Chapter 2 on *Nanomaterials in Agricultural and Veterinary Chemicals: Legislative and Policy Considerations in the Australian Regulatory Framework*. Dr Reeves and Dr Davies reviewed it and contributed further material to augment the text.

Dr John Miles (retired from the NMIA) prepared Chapter 3 about *Nanomaterials in Agricultural and Veterinary Chemicals: Definitions, Metrology and Physicochemical Properties.* Dr Buffinton provided specific advice and added to the text. It was reviewed by Dr Reeves, Ms Nicola Hall (NICNAS), Dr Jan Hermann (NMIA) and Dr Davies. Dr Camille Roesch, an APVMA Visiting Pharmacist, prepared a first draft of Chapter 4, *Manufacture of Nanomaterials*, with significant input from Dr Reeves and Prof Terry Turney (Monash Uni). Dr Joseph Morrall (Dept of Agriculture) reviewed it.

An initial draft of Chapter 5, *Nanomaterials in Agricultural and Veterinary Chemicals: Human Health Risk Assessment Considerations*, was prepared by Dr Michelle Nic Raghnaill (APVMA Visiting Nanotechnology Consultant) with subsequent significant expansion from toxicology consultant Dr Nicholas Fletcher. Dr Reeves wrote the section on residues in food, which he based on extensive comments provided by Dr Adrienne Sips' group at the Netherlands National Institute for Public Health and the Environment (RIVM). The RIVM staff who made valuable contributions were Dr Gerrit Wolterink, Dr Trijntje van der Velde-Koerts, and Ms Sandra ten Voorde. The chapter was reviewed by Prof Brian Priestly, Head of the Australian Centre for Human Health Risk Assessment at Monash University (now retired).

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EXECUTIVE SUMMARY

Advances in nanoscale science, engineering and technology have paved the way for developing novel applications, devices and systems in agriculture and animal husbandry. Currently, the use of nanotechnology in these sectors is not widespread but is expected to change rapidly since more than 3000 patent applications have been lodged in the past decade for nanopesticides alone.

The interest in nanopesticides appears to focus predominantly on three formulation types: polymer-based nanoformulations, inorganic nanoparticles such as silica and titanium dioxide, and nanoemulsions. The benefits of these formulations compared to existing formulations include the release of active ingredients in a slow and targeted manner, protecting active ingredients against degradation and increasing the apparent solubility of active ingredients that are poorly water soluble. Other benefits such as a network of wireless sensors able to detect and locate pest-infested portions of a crop and communicate the information via satellite to a laptop computer, and nanoclay devices installed in drip irrigation lines that release agrochemicals on demand, are also envisioned. Deploying such technologies will reduce the environmental footprint and off-site impacts of chemicals through the use of smaller quantities and more targeted application.

Nanotechnology in animal husbandry is an important area of research and development, though to date, only one nanoproduct is registered for use in Australia. In particular, the use of veterinary drugs and vaccines is anticipated to increase in the short-term. The benefits of nanotechnology in drug delivery predominantly stem from improved stability and/or apparent solubility; an increased concentration of a drug at the intended site of action (increased efficacy); a decreased concentration of a drug in healthy non-target tissues (reduced systemic toxicity) and modified pharmacokinetics, including controlled release. Increased bioavailability as well as improvements in the ability to target and control drug delivery should improve safety-efficacy profiles. Advances in vaccine technology due to nanotechnology will include safer antigens consisting of synthetic peptides and recombinant proteins as well as novel nanoparticle-based adjuvants that can be highly tuned and engineered so vaccines may be administered less frequently. Nanotechnology-enabled products will increasingly find applications in food-producing animals, such as modifying animal feeds, maintaining herd health, improving fertility, promoting growth and preserving animal identity.

The unique physical and chemical characteristics of nanomaterials that offer so much promise to agriculture and animal health and livestock production may also pose risks to human health and the environment. The opportunities and potential risks associated with the use of nanomaterials in crop production and animal husbandry are discussed in Chapter 1.

The novel properties of manufactured nanomaterials are attributed to a combination of their small size, chemical composition, physicochemical properties and surface structure. As well as offering great benefits, these same properties may give rise to toxicity—the so-called ‘nanomaterials paradox’. This begs the question: ‘Are nanotechnology products safe?’ The OECD Working Party on Manufactured Nanomaterials has published a Series on the Safety of Manufactured Nanomaterials. These guidance documents will be amended and refined as necessary to reflect a rapidly growing knowledge base and are directly relevant to regulators and industry. In Australia, it is the role of the APVMA to ensure that the use of agvet nanoscale chemicals and chemical products do not harm human or animal health or the environment (see Chapter 2).

Information obtained from the characterisation of nanomaterials is a starting point for risk assessment. Characterising the relevant physical and chemical properties of nanomaterials may require access to specialised instrumentation that is not available in many test facilities. Also, nanoparticles may need to be characterised at various stages of their life cycle because their functionalities may change when exposed to different media. The characterisation of nanomaterials is addressed in 'Report of the OECD Expert Meeting on the Physical and Chemical Properties of Manufactured Nanomaterials and Test Guidelines', No. 41, and is covered in Chapter 3 of this report.

The manner in which nanomaterials are manufactured plays a significant role when considering risk. Because the relationship between manufacturing processes and risk is complex, it is not possible to make generalisations yet. What is of concern is the possibility that small changes to manufacturing processes may introduce unpredictable risks. Chapter 4 of the report discusses 'top-down' and 'bottom-up' nanofabrication as well as the numerous methods for manufacturing nanomaterials.

A key conclusion of the OECD Working Party on Manufactured Nanomaterials, as reported in 'Important issues on risk assessment of manufactured nanomaterials', No. 33, was that there is no significant evidence that the toxicological endpoints prescribed in the current Test Guidance document about 'normal-sized' materials are not adequate for nano-sized ones. However, some aspects of the risk assessment paradigm may need refining to reflect the increasing understanding of nanomaterial behaviour. A case in point is the self-assembly of certain nanomaterials into new structures in the body, which is not well captured within the current approaches to hazard assessment. The form of nanomaterials used in toxicology studies must be well-characterised. Issues such as physicochemical characterisation, preparation and characterisation of dosing suspensions and dose metrics are likely to need more detailed examination. Chapter 5 of the report reviews the potential risks to human health associated with agvet nanomaterials.

Chapter 6 discusses the regulatory considerations for nanoscale agvet nanomaterials in the environment. The adequacy of the current state of knowledge about the behaviour of nanomaterials in both terrestrial and aquatic environments is the basis for considering their potential environmental fate and effects. These can be very different compared with those of non-nanoscale chemicals. A whole life-cycle approach needs to be applied to the assessment of nanomaterials and should be considered during product development.

The report aims to inform and stimulate discussion about emerging nanotechnology and highlights the key regulatory considerations for agvet chemical nanomaterials based on the current state of knowledge. It systematically explores the opportunities and risks of these substances in Australian agriculture and animal husbandry and reviews the published work relevant to the registration of nanoscale agvet chemicals. It is not the report's purpose to provide formal guidelines since the field is advancing so rapidly they would likely be obsolete soon after their publication. Nor is the purpose of this report to describe a regulatory framework for agvet nanomaterials. The general consensus is that, for the foreseeable future, existing regulatory frameworks developed for macroscale chemicals will be used to regulate nanomaterials. Over time, however, the framework will evolve as new information highlighting limitations in the current risk assessment paradigm becomes available.

1 NANOTECHNOLOGY IN AGRICULTURE AND ANIMAL HUSBANDRY: AN INTRODUCTION

1.1 Background and historical context

A nanometre (nm) is one-billionth of a metre. To put nanoscale dimensions between approximately 1 and 100 nm into perspective, a sheet of paper is about 100,000 nm thick; a human hair is approximately 80,000 nm in diameter and most animal cells are 10,000 to 20,000 nm in diameter. The nanoscale dimension was described by Klaine et al (2012) in the following way: 'Imagine shrinking the moon to the size of a tennis ball. This is the same as shrinking the tennis ball to the size of a Buckminsterfullerene molecule' (Figure 1.1). This molecule made up of 60 carbon atoms is also known as a buckyball. It's spherical and has a diameter of about 1.5 nm.

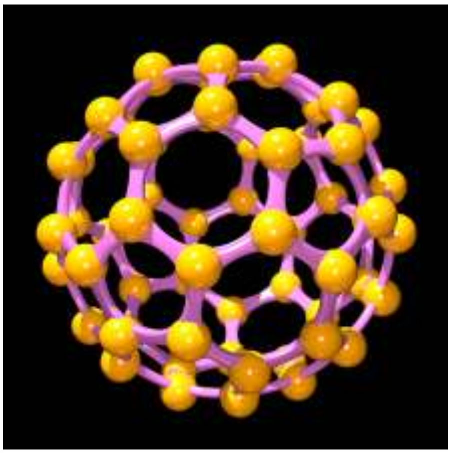

Figure 1.1: Model of a buckminsterfullerene (C_{60}). Image copyright Russell Kightley Media.

Nanotechnology promises benefits in a wide range of applications, from materials science to healthcare, food, cosmetics, chemicals (including industrial chemicals, pesticides and veterinary medicines), information and communication technology, transport and space, and energy generation and storage. The potential benefits to society include lighter and stronger materials, 'lab-on-a-chip' technology, environmental remediation technology, remote sensing and tracking devices related to food quality and spoilage, enhanced renewable energy from solar cells using silicon nanocrystals, increased computer speeds and self-cleaning surfaces.

But what is nanotechnology and how did it come about?

Nanotechnology is the application of nanoscience[1] to develop new materials and products, and involves manipulating matter at the nanometric scale (Health Canada, 2011). Although it has only recently attracted public attention, the field of nanotechnology has its roots in 20th century advances in materials science and high-resolution imaging and analytical techniques (Maynard et al, 2011). Indeed, it was a speech titled 'There's plenty

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[1] Nanoscience is the study of materials at dimensions between approximately 1nm and 100nm and the process for their manipulation.

of room at the bottom', delivered by Richard Feynman to a meeting of the American Physical Society at the California Institute of Technology way back in 1959, that is now credited with heralding the coming of nanotechnology.

Feynman's speech not only addressed manipulating and controlling matter on a smaller scale, he also anticipated many scientific and technical fields that are well established today. These include electron-beam and ion-beam fabrication, molecular-beam epitaxy, nano-imprint lithography, projection electron microscopy, atom-by-atom manipulation, quantum-effect electronics, spintronics, and microelectromechanical systems (Roukes, 2001).

Many significant events relating to the emergence of nanotechnology have occurred since Feynman's speech; key events are included in the following timeline.

1959	Richard Feynman's speech titled 'There's plenty of room at the bottom'
1974	Norio Taniguchi coined the term 'nanotechnology'
1981	Alexei Ekimov discovered nanocrystalline, semiconducting quantum dots in a glass matrix
	Gerd Binnig and Heinrich Rohrer invented the scanning tunneling microscope, allowing scientists to 'see' individual atoms for the first time
1985	Harold Kroto, Sean O'Brien, Robert Curl and Richard Smalley discovered Buckminsterfullerenes (C_{60})
	Louis Brus discovered colloidal semiconductor nanocrystals (quantum dots)
1986	Gerd Binnig, Calvin Quate, and Christoph Gerber invented the atomic force microscope
	Don Eigler and Erhard Schweizer manipulated 35 individual xenon atoms to spell out the IBM logo
	K. Eric Drexler published 'Engines of Creation: The Coming Era of Nanotechnology'
1991	SumioIijima is credited with discovering carbon nanotubes (CNT)
2003	Naomi Halas, Jennifer West, Rebekah Drezek and Renata Pasqualin developed gold nanoshells
2007	The journal *Nanotoxicology* was launched
2014	Lai-Sheng Wang and colleagues discovered borospherenes (B_{40})[2]

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[2] In early 2014, Wang and his colleagues reported clusters of 36 boron atoms forming one-atom-thick disks which they referred to as borophene. A short time later Wang and his research team reported clusters of 40 boron atoms forming a molecular cage. The new structure, referred to as borospherene, consists of 48 triangles, four seven-sided rings and two six-membered rings. Wang suggested the borospherene might have application in hydrogen storage.

The advent of nanotechnology has unleashed enormous prospects for the development of new products and applications for a wide range of industrial and consumer sectors.

The scope of the potential impacts of nanotechnology is highlighted by Richard Smalley[3] (2008), in a list of the Top Ten Problems Facing Humanity over the next 50 years:

1. Energy.
2. Water.
3. Food.
4. Environment.
5. Poverty.
6. Terrorism and war.
7. Disease.
8. Education.
9. Democracy.
10. Population.

The world's population in 2003 was 6.3 billion people and is predicted to increase to 8–10 billion people by 2050. This is an exponential increase that will result in a greater need for food, water, energy, healthcare and shelter in a world that is already struggling to meet these demands. Nanoscale science and nanotechnology have been demonstrated to have great potential in providing novel and improved solutions to many of these problems (Chen and Yada, 2011).

However, many of the same novel properties that give nanotechnologies the capacity to solve problems relating to the essential needs of humanity and the environment may also present novel and as yet unthought-of risks. Importantly, these potential risks may extend across the life-cycle of nanoproducts covering design and production, shipping, storage, use and recycling or disposal. All must be carefully considered and managed if society is to accept the new products and developments arising from the technology.

Nanotechnology is cutting-edge science offering considerable opportunities to develop innovative products and applications for numerous industrial and consumer sectors. It draws from a wide range of fields including physics, materials science, supra-molecular and polymer chemistry, interface and colloidal science, and from chemical, mechanical, biological, and electrical engineering. Cross-disciplinary research will be needed to overcome the major technical problems faced by researchers if they are to realise the paradigm-shifting advances they seek.

From a global industry perspective, one of the reasons for the excitement around nanotechnology and its alluring investment opportunities was the 2008 Lux Research forecast of a US$3.1 trillion market for nanotechnology-related industry by 2015. Due to the global economic slowdown and some concerns about the safety of

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[3] The late Professor Richard Smalley was awarded the Nobel Prize in Chemistry in 1996 for his role in discovering Buckminsterfullerene (C_{60}) in 1985. Upon his death in 2005, the US Senate passed a resolution to honour Smalley, crediting him as the 'Father of Nanotechnology'.

nanomaterials, this expected market growth never took place. The current assessment is that the nanotechnology industry will grow to $81 billion by 2015 (Nanomaterials and Markets, 2008–15).

1.2 Definitions and terminology

Many definitions of nanotechnology-related terms have been developed by expert bodies and regulatory agencies and are detailed in Chapter 3 of this report. Only a small sub-set of definitions derived from the International Organization for Standardization (ISO) (ISO, 2010) are presented in the box below.

The prefix ‘nano’ comes from the Greek word for ‘dwarf’ and nanoscience is the study of materials at dimensions between approximately 1 nm and 100 nm and the processes for their manipulation.

Nanotechnology is the application of scientific knowledge to manipulate and control matter in the nanoscale in order to make use of size and structure-dependent properties and phenomena, as distinct from those associated with individual atoms or molecules or with bulk or ‘normal-sized’ materials.

Nanoscale is the size range from approximately 1 nm to 100 nm.

Nanomaterial is material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale.

The APVMA’s working definition of a nanomaterial is ‘an intentionally produced, manufactured or engineered substance with unique properties that are directly caused by size features, with 10 per cent or more of the number-size distribution of these features lying in the range approximately 1–100 nm (the nanoscale)’. However, the APVMA acknowledges that human health and environmental issues may require a definition incorporating a different size range above 100 nm.

The manufacture of nanomaterials is detailed in Chapter 4 of this report and only three seminal definitions are presented here.

1. *Top-down* nanofabrication implies that structures are made increasingly small by progressively removing matter, usually by etching.
2. *Bottom-up* nanofabrication implies that atoms or molecules are distributed and rearranged to build new, functional nano-objects.

3. *Self-assembly* is the process in which a disordered system of molecules spontaneously forms an organised nano-structure or pattern that is at equilibrium or in a quasi-equilibrium state. The product formed has reduced free energy compared to the initial state of the disorganised molecules.

1.3 Properties and behaviours of nanomaterials

The physical, chemical, and biological properties of nanomaterials may differ in important ways from the properties of bulk materials and single atoms or molecules. Differences in magnetic properties, electrical conductivity and optical sensitivity attributed to quantum mechanics phenomena become prevalent at the nanoscale (Nel et al, 2006). For example, gold is very stable to oxidation as the bulk material but burns spontaneously at sizes below a few nanometres (Donaldson and Tran, 2002).

Batley and co-workers (2012) described seven main classes of manufactured nanomaterials: carbonaceous nanomaterials (eg carbon nanotubes), semiconductors (eg quantum dots), metal oxides (eg zinc oxide), nanopolymers (eg dendrimers), nanoclays, emulsions (eg acrylic latex) and metals (eg silver). They noted that these nanomaterials may exist in single, aggregated, or agglomerated forms and have various shapes, coatings and surface functionality.

It is important from a regulatory perspective to understand and consider the unique properties of nanomaterials and formulations (Eifler and Thaxton, 2011). For example, the similarity in size between natural biomolecules and manufactured nanomaterials raises concerns about nanomaterials interfering with biological processes, both on the cell membrane and within the cell. This point is illustrated by the diameters of a DNA double helix and a buckyball, which are approximately 2 nm and 1.5 nm respectively. The increased ability of nanosized particles to migrate into organisms and body tissues compared to non-nanoscale materials creates additional health concerns.

Table 1.1 illustrates the effect of particle size on particle number and the particle surface area/volume ratio for a given mass of a carbonaceous substance (Maynard et al, 2011).

In this conceptual model, sample A is comprised of micron-sized spherical particles (10 µm particle diameter) and sample B is comprised of nanoscale spherical particles (10 nm particle diameter). Sample A and sample B each contain 1 mg of particles. In this model, when particle diameter is decreased by three orders of magnitude, the number of particles increases by nine orders of magnitude, and the surface area/volume ratio of particles increases by two orders of magnitude.

Table 1.1: Effect of particle size on particle number and surface area/volume ratio[4]

Sample	Mass (mg)	Particle diameter (nm)	Number of particles	Particle surface area/volume ratio[5] (nm^{-1})
A	1	10^4	~ 10^{12}	0.006
B	1	10	~ 10^{21}	0.6

The relationships between particle diameter and particle number, and particle diameter and particle surface area/volume, have two important implications for nanomaterial behaviour. First, for a defined weight of nanoparticles, a larger number of smaller nanoparticles can increase the potential for disposition to more and different locations. Second, the surface area/volume ratio is higher for smaller particles and this is conducive to greater chemical reactivity since a greater proportion of atoms are located on the particle surface rather than in the inner bulk lattice.

The physicochemical properties of nanomaterials are considered in detail in Chapter 3 of this report.

1.4 Applications of nanomaterials

The applications of nanotechnology in healthcare and food science/technology and in the devices used in these sectors, as well as in the evolution of materials science, are all relevant to advancements in the agricultural and animal health sectors due to the cross-fertilisation between these sectors. The analysis that follows considers nanotechnology products and applications already on the market, or in the research and development pipeline. Figure 1.2 depicts the process from discovery of a nanomaterial to translation and marketing of a product.

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[4] Data in columns 2, 3, and 4 are from Maynard et al, 2011.

[5] Surface area and volume of spherical nanoparticles were estimated as $4\pi r^2$ and $4\pi r^3/3$ (where r = radius), respectively.

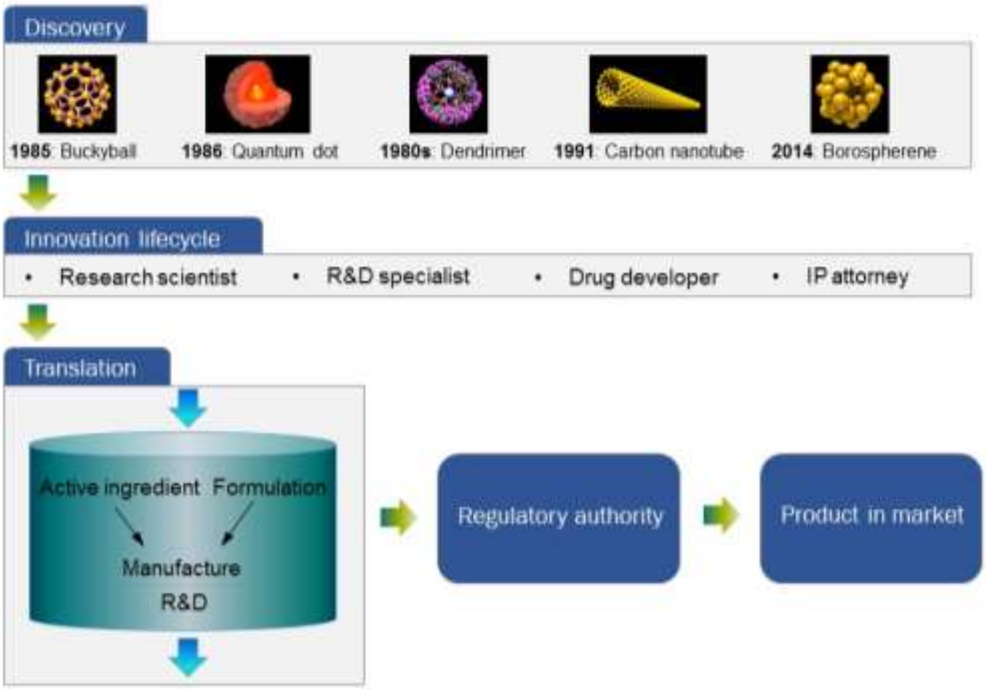

Figure 1.2: The pathway to market for a nanoproduct showing discovery of a nanomaterial, business considerations (eg intellectual property), R+D, manufacturing, regulatory approval and marketing.

Healthcare

In the healthcare sector, nanomedicines hold great promise to improve the prevention, detection, diagnosis and treatment of disease. Applying nanotechnology to drug reformulation has allowed some otherwise toxic drugs to be delivered more safely and effectively. These novel approaches are likely to help overcome disorders resistant to conventional therapy. Other novel nanomedicine applications display multiple functionalities such as diagnostics, targeted drug delivery, therapeutics and an ability to report back on the effectiveness of therapy.

A separate example involves magnetic nanoparticles, which are being investigated for a wide variety of biomedical applications, including improved contrast for magnetic resonance imaging (MRI), targeted drug delivery and hyperthermia treatment to destroy cancer cells. Cornell dots, which were first developed in 2005, received FDA approval in 2011 for human trials into improved cancer imaging and drug delivery. Cornell dots (also known as C-dots) comprise a silica shell less than 8 nm in diameter encapsulating near-infrared fluorescent dyes and a chemotherapeutic agent. The silica shell is coated with cancer-targeting molecules and with polyethylene glycol to increase residence time in the body. Researchers anticipate using C-dots to diagnose and treat cancer, in the staging of cancer disease, and assessing tumour burden via lymph node mapping (Benezra et al, 2011).

‘Nanosponges’ and ‘nanojuice’ have been reported in recent scientific literature. ‘Nanosponges’ are approximately 3000 times smaller than a red blood cell and comprise a biocompatible polymer core coated with segments of red blood cell membranes derived from the host (Hu et al, 2013). When injected into the bloodstream, the nanosponges attract pore-forming toxins produced by snakes, insects, bacteria etc which would otherwise perforate the outer membranes of erythrocytes causing cell lysis. In 2014, a new imaging technique involving nanoparticles suspended in liquid that patients drink (referred to as ‘nanojuice’) was reported (Zhang et al, 2014b). A laser light is used to activate naphthalcyanine dyes contained in the nanoparticles when the formulation reaches the small intestine, which is then imaged using photo-acoustic tomography. Human studies are now underway to determine whether this novel contrast agent is superior to those currently available for patients with celiac disease, Crohn’s disease or irritable bowel syndrome.

Many more novel materials with applications in nanomedicine are forthcoming. For example, applications of nanotechnology for regenerative medicine, such as repairing spinal cord injuries (Gelain et al, 2010) are expected. A separate example is a class of molecules called amphiphilic Janus dendrimers used to form evenly sized, stable vesicles. The unique onion-like structure of these evenly sized, stable vesicles may open the door to next generation nanomedicine through serial delivery of a drug from each of the 20 layers of the vesicle, or the release of a different drug from each layer of the vesicle (Zhang et al, 2014a).

Food

While there were no applications for manufactured nanomaterials in the food sector in Australia when this report was prepared, packaging and nanoencapsulation are reported to be the main applications received by agencies overseas (FAO/WHO, 2009). Examples of food packaging applications include plastic polymers with nanoclay to reduce oxygen permeability, nanosilver and nanozinc oxide for antimicrobial action, nanotitanium dioxide for UV protection, nanotitanium nitride for mechanical strength and as a processing aid and nanosilica for surface coating. While nanotechnology in food packaging has demonstrable benefits, there are also health concerns that nanomaterials might migrate from the packaging into food, and environmental concerns that when the packaging is disposed of, nanoparticles may enter landfills and cycle into other living organisms and even the food chain. While such impacts are unproven and uncertain, consumers expect to know what is in their food. Insurance professionals also require information on product labelling to evaluate underwriting risks.

Nanoencapsulation in the food sector, in the form of micelles, liposomes or biopolymer-based carrier systems, enables the development of delivery systems for additives and supplements in food and beverage products. Nano-encapsulated food additives include minerals, antimicrobials, vitamins and antioxidants. The most common objective of nanoencapsulation is to enhance the uptake and bioavailability of food additives; other benefits include improving taste, consistency, stability and texture (Chaudhry et al, 2008). One report describes the development of a colourless and tasteless beverage containing nanoencapsulated ingredients or additives that can be activated by a consumer at a particular microwave frequency. This activates selected nanocapsules, thereby releasing only the preferred flavour, colour or nutrients (Cientifica, 2006).

The food industry overseas is using nanocarrier technology. Examples include rendering water-soluble compounds like vitamin C to become fat dispersible, and rendering fat-dispersible compounds like vitamin A to make them water dispersible (FAO/WHO, 2009). New developments in nanotexturing achieve new taste sensations and improved textures in foods, and improved consistency and stability in food emulsions.

The industry is also developing nanosensors for food packaging to add an ‘intelligent’ function. These sensors are designed to ensure the integrity of foods packed under vacuum or an inert atmosphere by detecting leaks, indicating time-temperature variations such as occurs with freeze-thaw-refreezing, or revealing when food has spoiled. An example of the latter is a label for poultry meat based on a reaction between hydrogen sulphide and a nanolayer of silver (Smolander et al, 2004). The nanosilver layer is opaque light brown, but when meat starts to deteriorate silver sulphide is formed and the layer becomes transparent.

‘Smart’ labels that involve nanotechnology and have radio frequency identification displays (RFIDs) are being developed for foods with a limited shelf-life. The objective is rapid and accurate distribution of products. Self-healing nanomaterials that will repair small holes/tears in food packaging and respond to environmental conditions are also in the pipeline (Garland, 2004).

Further, an electronic ‘tongue’ for beer classification has been developed that uses an array of nanoscale sensors comprised of 21 ion-selective electrodes. These allow the device to distinguish between different varieties of beer with 82 per cent accuracy (Cetó et al, 2013).

Materials science

Several industry sectors are investigating the use of carbon-based nanomaterials as ultralight, high-strength composites and fibres. Carbon nanotubes have very high tensile strength. They are considered to be 100 times stronger than steel while being only one-sixth of its weight, making them potentially the strongest, smallest fibres known. Because of their strength, researchers are investigating the potential use of single-walled carbon nanotubes (SWCNTs) as reinforcing agents for intercalation matrices in polymer composites. Researchers are also developing smart nanomaterials with increasing functionalities, including responsiveness to external stress, electric and magnetic fields, temperature, moisture and pH.

Smart surface technology is another area that is advancing rapidly. It has potential applications in drug delivery systems, ‘lab-on-a-chip’ analytic systems, self-cleaning systems, liquid and chemical sensor systems and filtration systems. For example, nanostructured coatings for dirt-repellent surfaces have been reported with a cleaning action due to a ‘lotus effect’—the phenomenon that water beads and runs off the surface of lotus leaves due to nanoscale wax pyramids on the leaves’ surface.

1.5 Nanodevices and related technologies

Sensors

Nanotechnology is exerting a remarkable influence on the development of new sensing devices. Sensors are analytical instruments that generate quantifiable output signals, usually as a result of an analyte binding to a recognition element. In the case of biosensors, recognition elements include biological receptors such as antibodies, enzymes, aptamers[6] and peptides. The aim is to create nanodevices with new functions made possible due to the unique properties of nanomaterials, some of which can be precisely tuned. When coupled with materials capable of responding to external stress, electric and magnetic fields, temperature, moisture and pH, nanodevices can provide real-time, highly sensitive, analytical outputs. A recent development in the field of sensors is molecular imprinting, which is a powerful tool for generating tailor-made receptors for recognition elements in nanodevices. Molecularly-imprinted materials are easier to prepare than biogenic antibodies and equilibrate with analytes faster.

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[6] Aptamers are single-stranded DNA or RNA molecules that can bind to pre-selected targets including proteins and pesticides with high affinity and specificity. They are used as sensors and therapeutic tools and to regulate cellular processes, as well as to guide drugs to their specific cellular targets.

‘Lab-on-a-chip’ devices

A laboratory-on-a-chip integrates laboratory functions on a chip that is only millimetres or centimetres in size. The technology promises faster reaction times, reduced use of materials and high product yields. It has the potential to improve and reduce the cost of healthcare. ‘Lab-on-a-chip’ devices are already used in a wide range of applications, including fast and accurate hand-held sensors for environmental monitoring, medical diagnosis and process control in manufacturing. ‘Lab-on-a-chip’ devices equipped with integrated electronic sensors will allow scientists and healthcare professionals to make better-informed analyses (Grissom et al, 2014).

1.6 What nanotechnology could mean for agricultural and veterinary chemicals

Advances in nanoscale science, engineering and technology have paved the way for novel applications in agriculture and animal husbandry. While the main advantages that nanotechnologies offer over existing technologies arise from the improved or novel functionalities of nanomaterials, not all nanomaterials have relevance to the agricultural and animal husbandry sectors. To date, relatively few applications have been commercialised in these sectors globally and only one product has been registered in Australia. However, this situation is expected to change rapidly as more nanoproducts move through the R&D phase.

A diverse array of potential nanotechnology-derived applications for the agricultural and animal husbandry sectors has been reported (Scott and Chen, 2002; Chen and Yada, 2011; Underwood and van Eps, 2012). Existing applications, and those expected in the immediate future, include nanoformulations that promise enhanced efficacy, better product stability and smaller environmental footprints; ‘smart field systems’ able to detect pests as well as adverse conditions in field crops and apply pesticides, water and fertilizers to crops only as needed; ‘smart herd systems’ that detect and treat subclinical illness in a single infected animal in a herd; nanoscale identity preservation for the continuous tracking and recording of the history of agricultural commodities and ‘smart fabrics’ able to monitor the vital signs of the wearer.

In the agrochemicals sector, nanotechnology will offer significant advances, such as pesticides delivered to plants by novel routes. An increasingly important consideration when formulating nanopesticides is reducing potential harm to the environment. As well as reducing pesticide use and off-site impacts through more targeted pesticide application, ‘greener’ nanopesticides are being developed to achieve environmental sustainability benefits. Potential candidates include naturally-occurring active ingredients, such as pheromones and essential oils and safer adjuvants such as biodegradable polymers.

The commercial application of nanotechnology-enabled products in the animal health sector is in its infancy, but anticipated applications for companion animals will include diagnostics, targeted drug delivery and effective therapy associated with minimal adverse side effects. Such applications are not dissimilar to those used in human nanomedicine. Consequently, the research underpinning certain human nanomedicines will likely be used to develop veterinary nanomedicines for companion animals. By comparison, nanotechnology-derived products for food-producing animals are expected to focus on modifying animal feeds, maintaining herd health, improving fertility, promoting growth and preserving animal identity.

1.6.1 Nanotechnology and agricultural chemicals

Australia has a modern agricultural system and its farmers are among the most efficient in the world. Even so, innovation through R&D will be required to increase current food production levels while improving the sustainability of production. Nanotechnology has the potential to increase the amount of food produced and sustain the systems that produce it.

More than 3000 patent applications for nanopesticides have been lodged in the past decade (Kah et al, 2012). Therefore, despite few nanopesticides being marketed so far, considerable activity is occurring. A more recent literature review of the different types of nanopesticides identified polymer-based nanoformulations, inorganic nanoparticles (eg silica and titanium dioxide) and nanoemulsions as the formulation types most reported (Kah and Hofmann, 2014). The authors noted that polymer-based nanoformulations have greater efficacy compared to conventional formulations and have many applications.

Nanotechnology may modify the behaviour of agrochemicals by one or more of the following mechanisms:

- increasing the apparent solubility of poorly soluble active ingredients
- releasing active ingredients in a slow/targeted manner
- protecting the active ingredient against premature degradation.

The mechanisms by which nanoformulations increase efficacy have not been fully characterised, although the behavioural effects noted above may contribute to the observed increase. For example, increasing the apparent solubility of poorly water-soluble active ingredients results in improved tank mixing, cuticle penetration and uptake. The drivers for developing slow-release formulations include improved operator safety and reduced application rates due to less pesticide losses from degradation, leaching and/or volatilisation. Slow/targeted release formulations are particularly important for active ingredients that degrade rapidly.

Polymer-based nanoformulations

It is anticipated that a range of polymers will be used in agrochemical formulations in the short term. The main types of polymer-based nanoformulations are polymeric nanospheres (Figure 1.3) and polymeric capsules. These allow the rate of release of active ingredients to be adjusted by changing the proportions and molecular weights of the polymers used. For example, depending on the polymer matrix used, the release half-life of carbofuran in water ranged from 7.5 to 55 days. Other possible indications of polymer-based formulations include drift control agents, foam control agents[7], and improved safety in case of accidental ingestion (reduced absorption from the gastrointestinal tract).

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[7] Foam control agents provide antifoaming and defoaming properties. They include technologies based on surfactants, silicone oils, mineral oils and waxes.

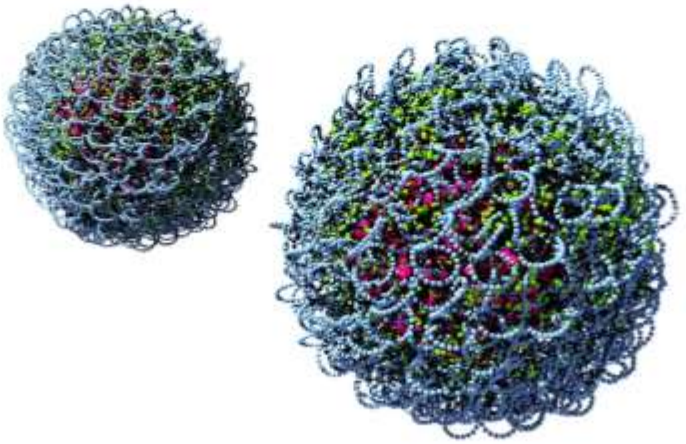

Figure 1.3: Polymer-based nanospheres (polymers shown in blue; active ingredient shown in magenta). Image copyright Russell Kightley Media.

Inorganic nanoparticles

The majority of studies investigating inorganic nanoparticles have considered silica, titanium dioxide, silver and copper. Data generated by Yuvakkumar et al (2011) in laboratory and field studies showed that silica nanoparticles increased seed germination and water use efficiency. However, other workers reported that comparable application rates of silica nanoparticles and diatomaceous formulations were required to achieve similar effectiveness (Debnath et al, 2011). Meanwhile, the evidence base showing the potential beneficial effects of titanium dioxide in agriculture, when applied alone, is growing. Owolade and Ogunleti (2008) and Moaveni et al (2011) reported increased yields from cowpeas and barley respectively, while Zheng et al (2005) reported improved spinach seedling growth. Titanium dioxide nanoparticles have also been shown to reduce the incidence of some diseases in the field (Owolade and Ogunleti, 2008). The presumed active mechanisms of titanium dioxide nanoparticles include protection against disease and increased photosynthesis. Nanoparticles of silver and copper were trialled in laboratory, glasshouse and field studies and shown to curtail the growth of fungal and bacterial plant pathogens (Rai and Ingle, 2012; Lamsal et al, 2011).

Porous hollow silica nanoparticles

Porous hollow silica nanoparticles (Figure 1.4) are promising agents in applications requiring sustained pesticide release, especially for photosensitive active ingredients. They have a shell thickness of approximately 15 nm, a pore diameter of four to five nanometres, and facilitate a high pesticide loading. The UV-shielding properties of porous hollow silica nanoparticles have been demonstrated to significantly improve the photostability of avermectin[8] entrapped in the hollow core of the nanoparticle carrier. Moreover, the entrapped avermectin demonstrated sustained-release behaviour (Li et al, 2007). Controlled delivery from porous hollow silica nanoparticles has also been reported for the water-soluble pesticide validamycin (Liu et al, 2006).

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[8] The avermectin investigated in this study was abamectin. The avermectins are a series of 16-membered macrocyclic lactone derivatives generated as fermentation products of the soil actinomycete, Streptomycetes avermitilis. The avermectins display potent anthelmintic and insecticidal properties.

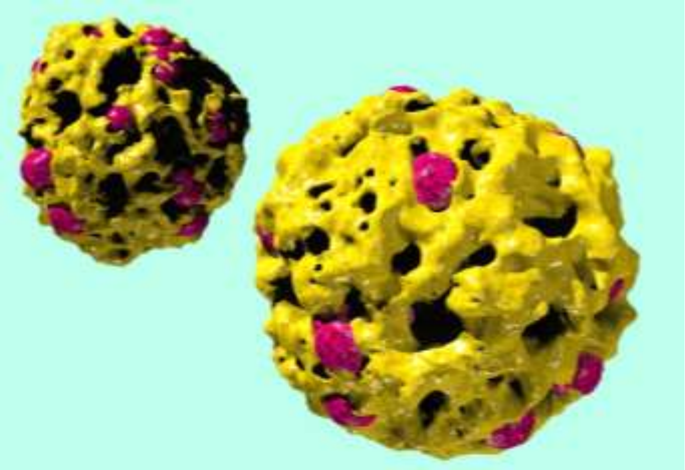


Figure 1.4: Porous hollow silica nanoparticles (active ingredient shown in magenta; porous hollow silica shown in yellow). Image copyright Russell Kightley Media.

Nanoemulsions

The range of drop sizes typically quoted for nanoemulsions is 20–200 nm (Kah et al, 2012). Nanoemulsions are mixtures of two immiscible liquids that contain lower concentrations of surfactants compared to microemulsions (typically 5–10 per cent surfactant compared to 20 per cent in microemulsions). Their major use in the agrochemical sector is to increase the apparent solubility of poorly soluble active ingredients while limiting the concentration of surfactants present in the formulation. They have achieved efficacy similar to or slightly greater than that of current formulations. The greater efficacy is thought to result from a slower release of labile active ingredients from the protective environment of the nanoemulsion (Kah and Hofmann, 2014).

Solid lipid nanoparticles

Solid lipid nanoparticles have been investigated for controlling the release of pesticides (Frederiksen et al, 2003) and protecting pesticides from photodegradation (Nguyen, 2012a, b).

Nanodispersions

Nanodispersions (also called nanosuspensions) result from the dispersion of nano-crystals (crystalline or amorphous particles consisting of 100 per cent active ingredient) in liquid media (Figure 1.5). The aim is to maximise the surface area (relative to volume) of the active ingredient to increase the dissolution of poorly water-soluble compounds. Nanodispersions have relatively low production costs and reduced impact on the environment.

Figure 1.5: Nanodispersions with nano-crystals shown in magenta. Image copyright Russell Kightley Media.

Nanogels

Nanogels are composed of a cross-linked polymer network or hydrogel. Those proposed for use in agriculture tend to be insoluble in water and therefore less prone to swelling or shrinking with changes in humidity. They also demonstrate good pesticide loading and release profiles.

Electrospun nanofibres

Electrospun nanofibres are being investigated for plant protection purposes. These fibres are obtained by using an electrical charge to draw the fibres from a liquid. Xiang et al (2013) incorporated in the fibres a non-ionic hydrophobic dye with a molecular weight and partition coefficient that mimics a systemic agrochemical. They showed that the dye’s release profile was superior to those of spheres and capsules.

Nanoclays

Nanoclays are thin sheets of silicate materials in the order of 1 nm thick and 70–150 nm wide. They are derived from montmorillonite clays commonly found in volcanic ash and their size is reduced and surface modified to form nanoclays that are biocompatible and have low toxicity. A promising group of these inorganic materials are the layered double hydroxides, or so-called anionic clays. They are layered solids consisting of cationic layers and exchangeable interlayer anions. They have already been used as hosts for the controlled release of plant growth regulator α-naphthalene acetate and for the controlled release of the herbicide 2,4-dichlorophenoxyacetate (Bin Hussein et al, 2005). Other potential uses include the slow/targeted delivery of pesticides, plant nutrients and fertilisers.

Carbon nanotubes

Carbon nanotubes are an allotrope of carbon that take the form of cylindrical carbon atoms (Figure 1.6). Carbon nanotubes are reported to have demonstrably positive effects on plant growth. Khodakovskaya and co-workers (2009) reported carbon nanotubes penetrating tomato seeds and increasing their germination and growth rates. In laboratory studies, carbon nanotubes were found to improve shoot and root growth in chickpeas (Tripathi et al, 2011).

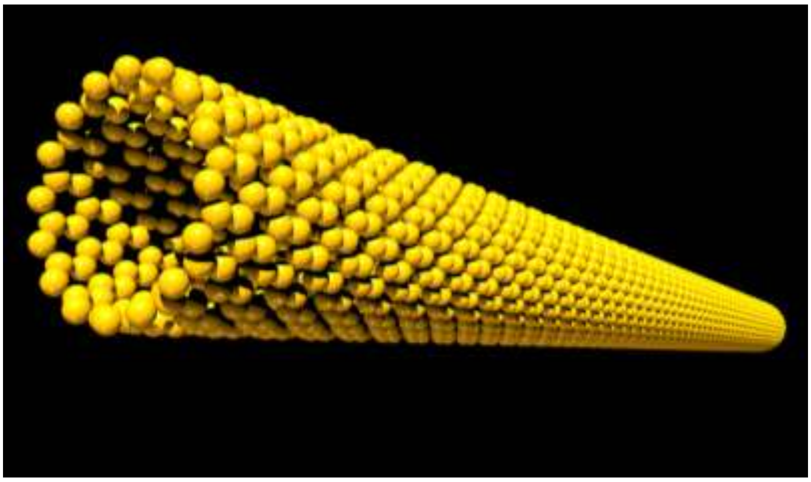


Figure 1.6: Carbon nanotube. Image copyright Russell Kightley Media.

Biosensors

Nanotechnology also has potential applications in agricultural biosensors. These nanodevices are likely to be used increasingly to detect environmental contaminants, including pesticides. Other potential uses for biosensors are to detect diseases and/or pests, including in imported agricultural produce, and testing food safety at the farm gate.

Wireless sensor networks

Wireless sensor networks are collections of very tiny, ultra-low-power sensor nodes, capable of sensing and communicating within a few tens of metres to fulfil complex, large-scale monitoring tasks. They have a wide variety of potential applications, including in precision agriculture. Researchers believe wireless sensors will be able to detect and locate portions of a crop that are pest-infested and communicate the information via satellite to a laptop computer (Figure 1.7). Another benefit of this technology will be targeted application of smaller quantities of pesticides, thereby reducing their environmental footprint.

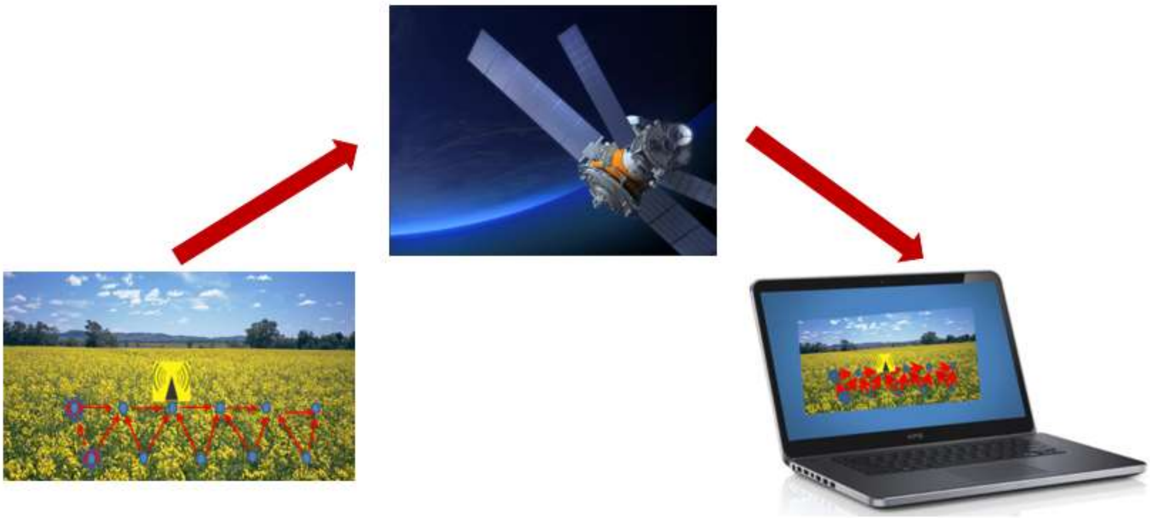


Figure 1.7: A wireless sensor network used in precision agriculture to detect diseases or pests, or the need for irrigation. Source: Khot et al, 2012.

Environmental Remediation

Nanotechnology can also be used to remediate agricultural land. For example, methods involving nanotechnology are being used to reverse the effects of pesticides in soil and groundwater (Baruah and Dutta, 2009). Similar technologies are available to treat waste water streams to remove pesticide contaminants (Mueller and Nowack, 2010).

1.6.2 Nanotechnology and veterinary chemicals

Nanotechnology has the potential to revolutionise animal health. Many applications for companion animals and food-producing animals have been reported, some of which are current while others are in the R&D phase. Nanosensor devices such as a 'lab-on-a-chip' for in vitro applications are also envisioned. The discussion that follows focuses on the benefits of nanotechnology-derived products in the animal health sector.

An area expected to increase in activity in the near term is the delivery of veterinary drugs and vaccines. The benefits of nanotechnology in drug delivery are predominantly the result of improved stability and/or apparent solubility; an increased concentration of drug at the site of action (increased efficacy); a decreased concentration of drug in healthy non-target tissues (reduced systemic toxicity) and modified pharmacokinetics, including controlled release. Increased bioavailability as well as improvements in the targeted and controlled delivery of existing drugs and their application through nanotechnology should make administering them easier while also improving their safety-efficacy profiles. The number of drugs available to a veterinarian may also be extended. Drugs that previously were not available due to their pharmaceutical behaviour (eg poor solubility), pharmacokinetics (eg too rapid elimination), pharmacodynamics (eg adverse side effects), or therapeutic response (eg lack of efficacy for a specific condition) may soon be used safely. Nanotechnology offers opportunities to address many of these shortcomings and overcome some clinical problems resistant to conventional therapy. Advancements in vaccine technology include safer antigens consisting of synthetic peptides and recombinant proteins (Nordly et al, 2009); novel, nanoparticle-based adjuvants that are highly tunable and can be engineered so that vaccines may be administered less frequently and via a convenient administration route (Scheerlinck et al,

2006) and, in humans, administration methods that allow patients to safely treat themselves (eg the NanoPatch® vaccine; Corrie et al, 2012) may become available for pet owners to use on their companion animals.

An important advance is in ‘smart’ drug delivery which allows specific sites to be targeted and drug release to be controlled. The strategy generally involves attaching targeting ligands such as monoclonal antibodies to the surface of nanoparticles, which are then transported in the systemic circulation to the target tissue. With tumours, infections and inflammation, the situation is different due to the vasculature being permeable to nanoparticles, allowing for their extravasation and accumulation in the target tissue. This phenomenon is known as the ‘enhanced permeability and retention’ (EPR) effect, and it facilitates both active and passive targeting of nanoparticles to specific sites. Passive targeting involves the movement of small particles through leaky vasculature to the target tissue. Active targeting relies either on targeting ligands attached to the surface of the nanoparticles, or on applying an alternating magnetic field to direct magnetic nanoparticles to the desired site of action. Schiffelers et al (2001) report targeting intracellular parasitic, fungal and viral infections in cells of the mononuclear phagocyte system with uncoated nanoparticles, which showed rapid cellular uptake. This novel approach is counter to the one generally practised whereby nanoparticles are coated with hydrophilic substances such as polyethylene glycol to reduce opsonisation and prolong circulation time.

A variety of nanoformulations for animal drug delivery are in use or are proposed for the foreseeable future. The following is a brief account of these different nanoformulation types.

Drug nanocrystals

When the bioavailability of poorly water-soluble drugs is limited by their rate of dissolution, nanosizing can markedly improve bioavailability. The observed improvement is attributed to the surface area (relative to volume) of a drug nanocrystal being orders of magnitude greater than that of its conventional counterpart. Another advantage is less variability in bioavailability for the fed and fasted state. Drug nanocrystals may be produced using either top-down technology (ie subjecting micronized particles to milling or grinding) or bottom-up technology (ie the nanoprecipitation of molecules).

Liposomes

Liposomes are self-assembled vesicles comprised of a central aqueous cavity surrounded by a lipid membrane(s) or lamella(e) (Figure 1.8). Hydrophilic and hydrophobic drugs in the core or lamella of liposomes, respectively, are protected from degradation during the absorptive and distributive phases following oral administration. This protection is lost if a drug is prematurely released into the gastrointestinal tract. The circulation time of liposomes is prolonged by coating them with polyethylene glycol. On contact with biological cells, liposomes tend to unravel and merge with the membrane of the cell, releasing their payload of drugs or other agents. Liposomes encapsulating imaging contrast agents have also been used for in vivo diagnostics. However, such use has been restricted following concerns over toxicity and safety relating to complement-mediated hypersensitivity reactions. The latter occur in 5–45 per cent of human patients during liposome administration (Szebeni et al, 2007).

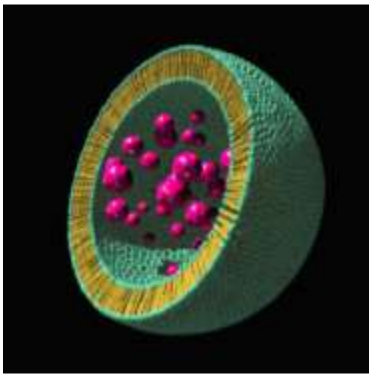


Figure 1.8: Liposome in cross section (active ingredient is shown in magenta). Image copyright Russell Kightley Media.

Polymer-drug conjugates

Drugs conjugated with polymers demonstrate slower degradation than drugs alone and the degradation time varies with different polymers. Polymers synthesised for this purpose are generally biodegradable. Slower degradation of polymer-drug conjugates results in a prolonged circulation time which offers two benefits. First, such conjugates may be administered less frequently to maintain effective blood levels. Second, a prolonged circulation time allows for greater extravasation of a drug by the enhanced permeability and retention (EPR) effect, resulting in higher drug concentrations at the site of action. Polymer-DNA conjugates or polyplexes, which are similar in concept to polymer-drug conjugates, are used in gene therapy.

Dendrimers

Dendrimers are highly branched polymers consisting of an initiator core, interior layers composed of repeating units and terminal moieties that can be functionalised to modify the solubility, miscibility, and reactivity of the resulting macromolecule. The synthetic process controls the size and structure of dendrimers as well as their biocompatibility and biodegradability. High loadings of a drug can be incorporated in the dendrimer core, or attached to the terminal moieties on the dendrimer surface.

Polymeric micelles

Polymeric micelles comprise a core protected by a hydrophilic outer shell formed by amphiphilic block copolymers. The advantages of polymeric micelles for drug delivery include solubilisation of poorly soluble molecules and sustained drug release attributed to the drug’s encapsulation protecting it from degradation and metabolism. Polymeric micelles can also enhance the delivery of drugs to desired biological sites, thereby improving therapeutic efficacy and reducing unwanted side effects. An example is micelles containing attached sugar-group ligands that specifically target glycol-receptors in cellular plasma membranes.

Solid lipid nanoparticles

Solid lipid nanoparticles demonstrate excellent physical stability and protect an incorporated drug from chemical degradation. A disadvantage is that they have low drug loading capacity. It is likely that solid lipid nanoparticles will be developed that are suitable for delivery by most routes of administration.

Polymeric nanoparticles

The two main forms of polymeric nanoparticles are polymeric nanocapsules and polymeric nanospheres. From a drug delivery perspective, polymeric nanocapsules demonstrate a high drug loading capacity. They also allow for increased drug bioavailability and controlled drug release compared with the conventional drug counterpart. Other applications of polymeric nanocapsules include the detection (including imaging), diagnosis and treatment of disease. Unlike the situation with polymeric nanocapsules, the drug in polymeric nanospheres is physically and uniformly dispersed in a dense polymeric matrix.

Magnetic nanoparticles

Drug-coated magnetic nanoparticles (Figure 1.9), generally larger than 50 nm in size, are used in drug delivery. After drug administration, a magnetic field is used to direct the magnetic nanoparticles to the desired site of action and keep them there. Smaller magnetic nanoparticles, often approximately 5 nm in diameter, are used to generate therapeutic hyperthermia, which is created by applying an external alternating magnetic field to the tissue in which the magnetic nanoparticles have accumulated. This causes localised cellular necrosis in, for example, a targeted cancer.

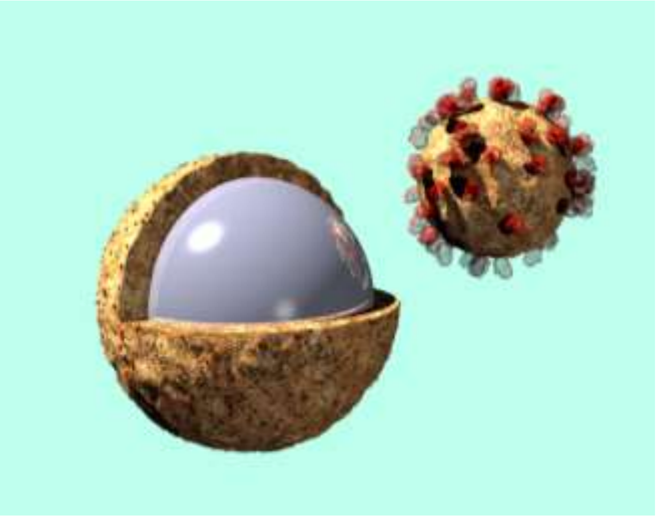


Figure 1.9: Two magnetic nanoparticle coated with drug (brown), one with attached antibodies (red). Image copyright Russell Kightley Media.

Nanoclays

Minerals that exist in nature, and volcanic ash in particular, are processed to form nanoclays. The layered double hydroxides (LDHs), which comprise two layers of positive charge balanced by intercalated hydrated anions, are one category of nanoclays. An example of an LDH is nanobiohybrids, which are nanoclay hosts with various negatively charged biomolecules intercalated between the layers. Replacing the hydrated anions with DNA using ion exchange results in a nanobiohybrid with applications for gene therapy (Figure 1.10). Following delivery to a biological system, nanobiohybrids are phagocytosed and the biological material released from the inorganic host either by dissolution of LDHs in the acidic environment of lysosomes, or through reverse ion-exchange within the cellular fluids.

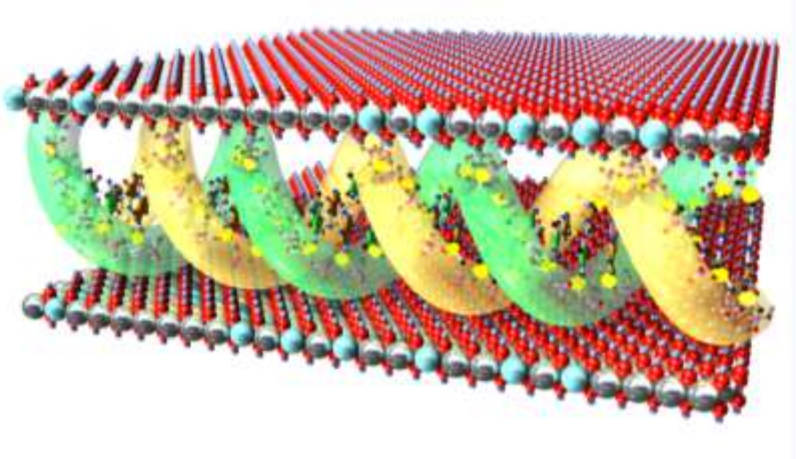


Figure 1.10: Nanobiohydrid comprising layered double hydroxide and intercalated DNA.
Image copyright Russell Kightley Media.

Gold nanoshells

An advantage of gold nanomaterial is its biocompatibility. Gold nanoshells (Figure 1.11) are used to diagnose and treat diseases such as cancer. In this application, gold nanoparticles are coated with surface moieties specific for the tissue being targeted (eg monoclonal antibodies) and a hydrophilic substance such as polyethylene glycol to prolong circulation time. The gold nanoparticles are irradiated with near infrared to visualise and destroy gold-targeted cancer cells.

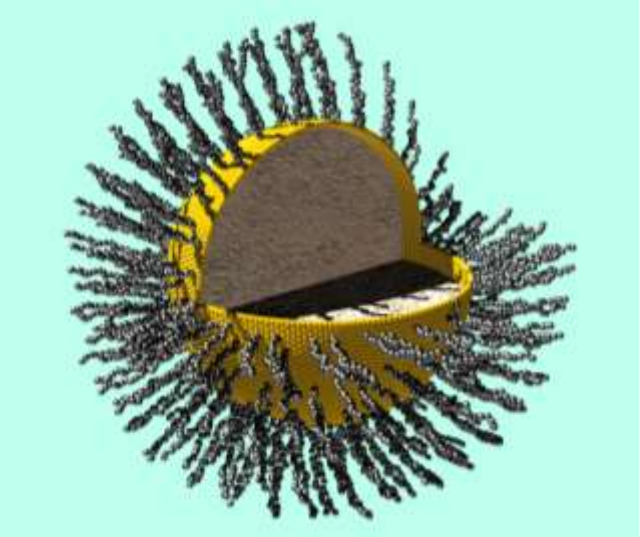


Figure 1.11: Gold nanoshell coated with surface moieties (typically drug or monoclonal antibodies).
Image copyright Russell Kightley Media.

Carbon nanotubes

These nanomaterials have a high optical absorbance at infrared frequencies and may in future have similar applications to gold nanoshells. Carbon nanotubes are also being investigated as nanovector systems. However, contrasting these potential benefits are concerns also under investigation regarding bioaccumulation and possible long-term toxicity.

Quantum dots

Quantum dots (Figure 1.12) are colloidal semiconductor nanocrystals with unique optical properties. Their in vitro toxicity is related to the composition of the core (typically cadmium selenium) and can generally be overcome by coating the core with other metals, such as zinc sulfide, or adding a protective hydrophilic coating like polyethylene glycol. Quantum dots demonstrate high level fluorescence, long-term stability, simultaneous detection of multiple signals, and tunable emission spectra. They hold promise as a multifunctional therapeutic for lymph node mapping, identifying molecular targets, photodynamic therapy, drug delivery, and surgical oncology. Further research is necessary to evaluate the long-term stability of quantum dots in vivo.

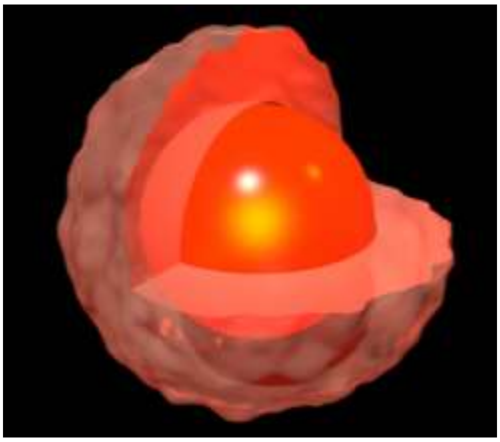
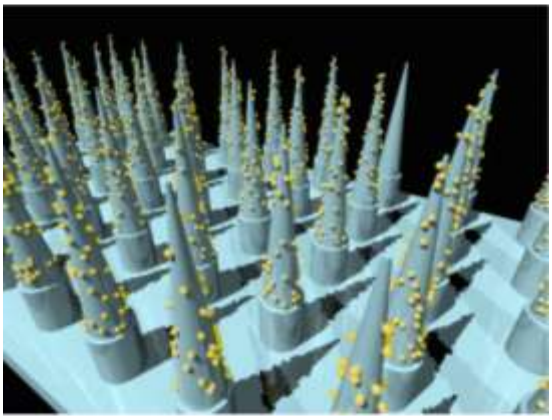


Figure 1.12: Quantum dot sectioned to show the outer shell and the inner core.
Image copyright Russell Kightley Media.

Vaccines and vaccine adjuvants

By 2008, nanoparticle vaccine delivery systems for more than 40 animal diseases had been reported as either successfully developed or under development (Scheerlinck and Greenwood, 2008). A shift from antigens comprising inactivated microorganisms to safer synthetic peptides and recombinant proteins has also been reported (Nordly et al, 2009). This was partially attributed to earlier research into nanoparticle adjuvants including emulsions, liposomes, nanobeads, immune stimulating complexes (ISCOMs) and inorganic particles for improving immunogenicity (Scheerlinck et al, 2006). Nanoparticle adjuvants can be engineered to be highly tunable to elicit prolonged immunogenic responses and to allow for convenient administration.

Nanotechnology-enabled vaccines have been noted to decrease unwanted inflammatory responses at injection sites in food-producing animals. This is thought to be caused by nanosized adjuvants that mimic the size of viruses being well tolerated by cells. Advancements are also being reported in vaccine delivery. The NanoPatch® (Figure 1.12) is an example of a nanotechnology-enabled device that delivers vaccines dermally to humans (Fernando et al, 2012), though the concept applies equally to animals. Studies in mice have found that a comparable immunogenic response is elicited by one one-hundredth of the dose delivered conventionally by a needle and syringe, a finding consistent with skin having more immune cells than muscle. The NanoPatch® promises significant benefits, particularly in developing countries where access to healthcare professionals and facilities is limited (Chen et al, 2011).



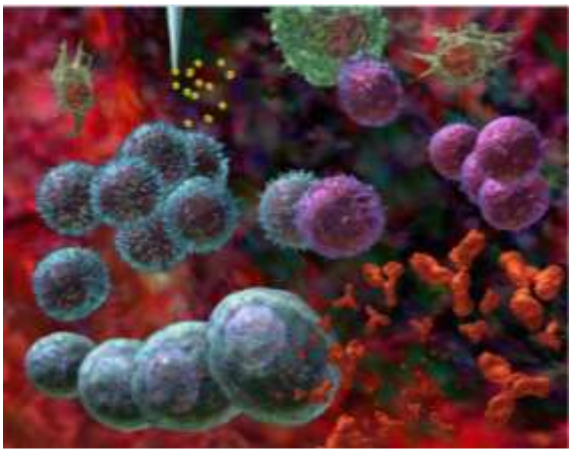


Figure 1.13: The NanoPatch® vaccine. Panel A: Schematic of a NanoPatch® showing projections (pale blue) coated with vaccine (yellow). Panel B: The elicited immune response to the vaccine (yellow) showing antigen presenting cells (Dendritic cells) (pale green cells with red nuclei and irregular cell membranes in the background), T helper cells (pink), B cells (blue with red nuclei), plasma cells (large spherical cells in foreground), and antibodies (red, Y-shaped bodies in foreground).

Nanotechnology applications in food-producing animals

Nanotechnology-enabled products will increasingly find other applications in food-producing animals. For example, animal feeds may incorporate nutritional supplements in nanoparticular form to increase the bioavailability of a mineral or vitamin. In this respect, fat-soluble vitamin E is more stable in the aqueous environment of the gastrointestinal tract of animals when encapsulated in liposomes as a nanodispersion. The bioavailability of vitamin E in this formulation is increased compared to the conventional counterpart. Nanotechnology-enabled feed additives to protect animals against mycotoxins or to remove food-borne pathogens in the gastrointestinal tracts of livestock have also been reported. An example of the latter are nanoparticles that adhere to E. coli; the nanoparticles used consist of a polystyrene base, a polyethylene glycol (PEG) linker, and a mannose-targeting biomolecule. Other opportunities that nanotechnology offers for food-producing animals relate to growth promotion, fertility and breeding, and animal health. All of these applications may involve implantable self-regulating drug delivery systems. One report envisages a system for the detection, diagnosis and therapy of sub-clinical bacterial infections in food-producing animals (Scott and Chen, 2002). Major benefits of using smaller quantities of antibiotics are reduced pressure for the emergence of antibiotic resistance and markedly reduced residues of antibiotics in food commodities.

In vitro nanosensor devices

In vitro nanosensor devices will play an increasingly important role in the animal health sector. A ‘lab-on-a-chip’ is an example of an in vitro nanosensor device. A ‘lab-on-a-chip’ integrates laboratory functions and promises faster reaction times (allowing for point-of-care diagnostics), reduced material use and a high product yield. The very latest advances in plasmonics[9], nanofabrication, microfluidics[10] and surface chemistry underpin the capabilities of nanosensor devices. As a result, novel diagnostic assays are available that link functionalised nanoparticles to biological molecules such as antibodies, peptides, proteins and nucleic acids (Driskell et al, 2005; Luchini et al, 2010). A ‘lab-on-a-chip’ able to detect protein cancer markers in blood is a case in point. A drop of blood injected into the chip circulates through the micro-channels and any cancer markers present will stick to gold nanoparticles located on the microchannels, setting off changes in ‘plasmonic resonance’ that are monitored by the device. In the veterinary field, a multitude of nanoparticle-based detection systems have been successfully validated to detect viral, parasitic and bacterial pathogens (Kumanan et al, 2009; Yuan et al, 2009).

1.7 Potential risks posed by nanomaterials

While nanotechnologies offer a multitude of opportunities for innovation in agriculture and animal husbandry, a balanced view is critically important because the unique physical and chemical characteristics of nanomaterials that offer potential benefits may also pose risks to human health and the environment (SCENIHR, 2007a). Eifler and Thaxton (2011) noted that effective regulation of nanotechnology-enabled products requires an understanding and consideration of their unique properties. A better understanding of the link between nanomaterial properties and biological behaviour in humans, animals and the environment will increase the robustness and relevance of

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[9] Plasmonics is the study of the interaction between an electromagnetic field and free electrons in a metal.

[10] Microfluidics is the science of designing, manufacturing, and formulating devices and processes that deal with volumes of fluid on the order of nanolitres or picolitres.

risk assessments. Detailed accounts of the regulatory considerations for assessing the risks of nanomaterials are contained in subsequent chapters.

The discussion that follows addresses some of the potential risks posed by nanomaterials and issues raised in public comments in response to the release of a draft version of this report.

Manufactured nanomaterials are found in many consumer products on the market today, including sunscreens, cosmetics and clothing (Oomen et al, 2011). However, the safety of nanomaterials is an emotive and controversial matter. The public want to know if these products are safe; some people contend that the risks posed by nanomaterials are unknown and there have been calls nationally for mandatory labelling and a register of nanomaterials as a means of providing society with its right to know. Both mandatory product labelling and the development of a register of nanomaterials have been considered, and dismissed, by a previous Australian Government.

The safety of nanomaterials often features in the popular press. The three cases that follow are typical of those reported.

This first case involves safety concerns relating to nanoparticle-based sunscreens. Media reports assert that nanoparticles incorporated in some UV filters penetrate the skin, enter the bloodstream and possibly cause adverse effects. Scientists contend there is no definitive evidence for nanoparticles in sunscreens penetrating the skin (TGA, 2013). Rather, the nanoparticle ‘marker ions’ detected in the blood and urine of human trial subjects who have applied sunscreens containing nanoparticles result from the dissolution and ionization of nanoparticles on the skin—so it’s the ions and not nanoparticles per se that have been absorbed percutaneously (Gulson et al, 2010). Dermal absorption studies including the findings of a review of nanoparticle-based sunscreens are discussed in Chapter 5 of this report.

Nanoscale silver (nanosilver) has been the subject of numerous media reports. Most products containing nanosilver are used for their antibacterial properties; in the home to keep surfaces clean or to reduce odours, as a coating in medicinal applications such as artificial joints and pacemakers and in ceramic filters for water purification. The principal concern about nanosilver is environmental risk, which is addressed in Chapter 6. However, it is also feared that the widespread use of nanosilver in consumer products could select for bacterial resistance that undermines its use in hospitals. Nanosilver highlights the importance of life-cycle considerations, including production, transport, storage, use and disposal or recycling, when regulating nanomaterials.

A third case focuses on media reports about research published by Poland et al (2008) that injecting carbon nanotubes (CNTs) into the abdominal cavities of mice resulted in asbestos-like lesions. The study demonstrates that CNTs conform to a structure-activity relationship based on aspect ratio[11], to which asbestos and other macro- and micron-sized pathogenic fibres conform. While the findings confirm that fibres with high aspect ratios can result in long-term harm, this behaviour is not restricted to nanofibres but applies to all fibres, regardless of size.

Size matters!

Size affects the potential risks posed by nanomaterials in at least three ways. First, materials at the nanoscale dimension are of similar size to natural biomolecules. For example, the diameter of a DNA double helix is about

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[11] Aspect ratio describes the primary dimension over the secondary dimension(s).

2 nm while the diameter of buckyballs is about 1.5 nm. The similarity in size translates into an increased potential for manufactured materials to interfere with biological processes. This could include the behaviour of cell membranes, biochemical pathways in cells, or the functionality of DNA/RNA processes. Second, the surface areas (relative to volume) of nanomaterials are orders of magnitude larger than those of non-nanomaterials, therefore, the properties of the surface molecules and functional groups are more likely to dominate overall biological interactions (Klaine et al, 2012). It follows that surface chemistry is an important determinant of nanomaterial hazard. Third, nanoparticles may translocate to tissues in the human body or the environment in a manner differently from their conventional chemical counterparts. All three of these size-related nanomaterial behaviours may increase or decrease potential risks to human health and the environment, and are addressed in registration applications and assessed by Government regulators.

An accurate characterisation of manufactured nanomaterials is required in order to fully understand both the benefits and the potential toxicity of nanoparticles in biological systems (Royal Society and Royal Academy of Engineering, 2004). When a full characterisation is not possible and it becomes necessary to prioritise the parameters for characterisation, Oberdörster et al (2005a) propose that the following criteria be considered:

- the context within which a material is being evaluated
- the importance of measuring a specific parameter within that context
- the feasibility of measuring the parameter within a specific context.

In 2014, the OECD Working Party on Manufactured Nanomaterials published ‘Report of the OECD Expert Meeting on the physical chemical properties of manufactured nanomaterials and test guidelines’, No. 41 (OECD, 2014). The report provides guidance for assessing the aggregation and agglomeration of nanomaterials, and determining the size, surface area, porosity and surface reactivity of nanoparticles. A detailed account of the findings of the report is presented in Chapter 3 of this report.

Testing the toxicity of nanomaterials also requires awareness of special considerations which are covered in the OECD Working Party on Manufactured Nanomaterials Report, No. 33, titled ‘Important issues on risk assessment of manufactured nanomaterials’ (OECD, 2012a). Traditional test methods need to be applicable to the nanomaterial under consideration. For example, whether a material is soluble, insoluble, or partially soluble may affect the suitability of a traditional toxicity test. If a traditional toxicity testing method cannot be satisfactorily modified to be fit for purpose, new methods may be needed. In addition, caution is needed if extrapolating the results of in vitro studies to an in vivo assessment since a direct translation seldom applies. It is also important to keep in mind that in vitro tests are most useful in providing information on mechanistic processes and in clarifying mechanisms and modes of action suggested by studies in whole animals.

Pharmacokinetic studies, that is the absorption, distribution, metabolism and excretion (ADME) of test substances in test animals, are used to determine the systemic exposure of organs and tissues. Where the unique function of the nanomaterial is to act as a ‘Trojan horse’ for localised dosing in a target tissue, the methodology would also act as a carrier of any contaminants present in the active constituent preparation (Shipley et al, 2009) and would need to be considered in the overall risk assessment.

The impact of nanomaterials on the environment has been the subject of intense and ongoing research.
The impact is more pronounced with non-biodegradable nanoparticles that persist and accumulate in the environment. The current knowledge base highlights the need to track the full life-cycle of manufactured

nanomaterials and adopt ‘safe by design’ concepts for reducing, or even preventing altogether, the detrimental impact of nanomaterials on the environment. Two examples of ‘safe by design’ concepts applied to nanoparticles are those designed to dissolve very slowly, and those coated with inert compounds. The OECD Working Party on Manufactured Nanomaterials, Report No. 33, discusses in detail the environmental risk assessment framework as well as elements that need to be considered when assessing ecological risks (OECD, 2012a).

Nanotechnology-enabled products used in food-producing animals and crops need to be evaluated for their potential to leave residues in food. A recent expert meeting noted that the current risk assessment approaches used by FAO/WHO and Codex for residues in food are suitable for manufactured nanomaterials, but any additional safety concerns arising from the characteristic properties of nanomaterials would need to be addressed (FAO/WHO, 2009). It is also noted in reference to Codex standards that ‘neither the specifications nor the ADIs for food additives that have been evaluated in other forms are intended to apply to nanoparticulate materials’ (WHO, 2007).

1.8 Regulation of nanomaterials in Australia

A question asked increasingly by consumers and scientists alike is ‘Are nanotechnology products safe?’ A related question is ‘What is known about the fate of nanotechnology products in the body and in the environment?’ While it is generally accepted that many areas of nanotechnology do not present new hazards, there remain information gaps in our understanding of nanotechnology products that only research can fill.

In Australia, the APVMA is responsible for regulating agvet chemicals and formulated chemical products, including those based on nanotechnology, up to the point of retail sale. Protecting human health and the environment from any harmful effects of these substances is a legislated responsibility of the APVMA. However, regulating an emerging technology such as nanotechnology is not a unique problem. The challenge of dealing with the regulatory requirements of nanomaterials has been minimal because, at the time this report was prepared, the APVMA had received only one application to register a nanotechnology-enabled product. For the foreseeable future, the existing regulatory framework developed for non-nanoscale agvet chemicals, in conjunction with a case-by-case approach, will be used to regulate nanomaterials. Over time the framework will evolve as new information highlighting limitations in the current risk assessment paradigm becomes available. The development of a rational regulatory framework for nanopesticides and veterinary nanomedicines will be guided by a better understanding of the biological behaviour of nanomaterials in humans and in the environment.

1.9 Summary of public comment on the draft report

The draft report was released for public comment in October 2014 and was subsequently amended in appropriate sections to accommodate a number of the comments and opinions submitted. The following summary represents a condensed version of further comments which did not lead to report amendments. To a large extent, these issues have been flagged in the report as requiring further consideration. Indeed it is exactly these concerns that have led the APVMA to suggest a case-by-case approach to the risk assessment of nanomaterials. The concerns included suggestions that the risks associated with the use of nanomaterials in agricultural and veterinary chemical products have not been considered in sufficient detail and that the precautionary principle has not been followed. They also suggested the report did not consider that the use of nanomaterials in agricultural production might help perpetuate the belief that the agricultural industry is resource, energy and chemically intensive. A more general comment was that definitions of nanomaterials require further development and research.

There was a concern that the current regulatory framework, presented in detail in Chapter 2, is inadequate to capture information about whether (or not) new product applications (including variations/reformulations) contain nanomaterials. It was considered that it could be possible, or become normal practice, for the regulator to become less likely to subject new applications containing nanomaterials to appropriate scrutiny. As noted above, the APVMA has a legislated responsibility when approving agvet chemicals to protect human health and the environment.

Comments on the definitions of nanomaterials were general in nature and consistent with issues already raised in Chapter 3 and elsewhere in this report as well as in, for example, OECD (2014). Further commentary presented broad information on nanomaterials which are either in current human medicines or in development (and are likely to be used in humans) where the upper dimension ranged from 100–400 nm. These upper dimension values for human medicines were presented as evidence that the dimensions of nanoscale veterinary and pesticide products are greater than the nominal definition range of 1–100 nm. This report recognises that some non-nanoscale human medicines are also suitable as veterinary therapeutics and likely to be registered as such.

Other comments raised issues with aspects of Chapter 2, which describes the legislative and policy considerations in the Australian Regulatory Framework and consideration of the level of risk. One view on established toxicological studies, for which an accepted premise is the relationship between dose and toxicity, was that this relationship may not be valid for nanomaterials due to differing physicochemical properties and affinities to host macromolecules, implying that the predictive value of established test guideline studies would be decreased. Such interactions are recognised as a possibility in the literature and in the note from the OECD on toxicity testing of nanomaterials (OECD, 2012a). The comment suggested these effects were unknown or yet to be characterised. However, these considerations, such as unique changes in physicochemical properties, dermal transfer, binding to host macromolecules and possible synergistic effects, are key components necessary for interpreting results from standard pharmacodynamic, pharmacokinetic, toxicokinetic, and general toxicity studies. The standard evaluation practice for regulatory quality studies minimises the likelihood of a unique hazard remaining undetected. Indeed, as discussed in Chapter 5, the oral toxicity of some nanoscale metals has been found to be markedly lower than the non-nanoscale metal ion preparation, suggesting that established test guideline protocols are likely to be suitable for characterising the toxicity profile of many nanomaterials.

Further opinions received on the effectiveness of the Australian Regulatory Framework considered that the current approach of using the existing framework for non-nanomaterials to regulate nanomaterials is inappropriate, even if it is just while the existing framework evolves to incorporate nanomaterials. To support these comments, previous submissions to the APVMA on the ineffectiveness of the APVMA Chemical Review process for non-nanomaterial agricultural chemicals were cited; these submissions suggested that the process put the community at increased risk of exposure to hazardous chemicals. This, and the recent decision by the Australian Government not to pursue a re-registration scheme for active constituents and associated products that were grandfathered into the National Registration Scheme in 1996, were considered to be sufficient ‘evidence’ that the Australian Regulatory Framework could not be relied upon to protect the public from agricultural and veterinary nanomaterials.
The APVMA considers these comments present an incomplete view of the way in which the existing Chemical Review program operates.

Some observers expressed concern that the possible environmental impacts of nanomaterials are yet to be defined. They claimed that soil contamination by nano metal oxides reduced plant/crop growth, and that appropriate disposal requirements of such materials have been inadequate. They suggested these areas need further research. These concerns are discussed in Chapter 6.

Other comments on the report called for a mandatory register of nanomaterials and the labelling of nanomaterials with nano-specific regulation, although it was conceded that such a proposal was likely to be the responsibility of the *National Industrial Chemicals Notification and Assessment Scheme* (NICNAS) and not the APVMA.
The concern was that APVMA legislation and/or current application requirements, evaluation practices and post approval/registration activities were not adequate to deal with the regulation of nanomaterials. One purpose of labelling chemical products is to manage the hazards to human health, and environmental safety. A risk of adopting a mandatory register and specific labelling of products which contain nanomaterials, is that the listing and labelling may signal to less informed users or consumers that *all* products containing a nanomaterial are hazardous, no matter how minimal the overall content of nanomaterial is and no matter what the actual hazards and relative risks of the product might be.

1.10 Conclusion

It is critical that strategies are available to minimise the potential risks of nanomaterials such that the social, environmental, and economic benefits of nanotechnology are fully realised. The APVMA has a key role in ensuring that nanotechnology-enabled agvet chemicals and chemical products are introduced to Australian agriculture and animal husbandry in a safe and responsible manner.

The objective of this report is to highlight the regulatory issues that need to be considered when bringing agvet chemical products of nanotechnology into the Australian market. Chapters of the report address relevant aspects of nanotechnology including definitions, metrology, physicochemical properties, manufacture, and the potential impacts on human health and the environment. Every attempt has been made to ensure the information on this rapidly-evolving field was current at the time of writing. The report represents a first attempt to offer a blueprint on the regulatory considerations applicable to nanotechnology in Australian agriculture and animal husbandry.

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2 NANOMATERIALS IN AGRICULTURAL AND VETERINARY CHEMICALS: LEGISLATIVE AND POLICY CONSIDERATIONS IN THE AUSTRALIAN REGULATORY FRAMEWORK

Abstract

The novel nature of nanotechnology has prompted national governments around the globe, including Australia, to develop appropriate regulatory frameworks to deal with a range of new legislative and policy considerations. Working with existing nanomaterials and engineering new ones has the potential to create new human health, environmental safety and trade issues that may require the development of new or revised approaches to govern their safe use. As Australia's national pesticides and veterinary medicines regulator, the APVMA is at the forefront of regulatory development for nanomaterials as 'new' substances in agriculture and animal husbandry. This requires a thorough understanding of nanomaterial properties, behaviours and their potential applications, in order to develop appropriate risk assessment methodologies and controls based on sound science.

2.1 Legislative and policy considerations

2.1.1 Global context

Worldwide regulatory approaches to nanotechnology vary but are becoming increasingly consistent. In 2007 when Ludlow et al reviewed nanotechnology and applicable Australian regulatory frameworks for industrial, human therapeutic and agvet chemicals, international regulators were also just commencing their own considerations.

Since then there have been a number of significant steps taken overseas. Charrière and Dunning (2014) provide a detailed timeline and overview of nanotechnology policy and regulation in Canada, Australia, the European Union, the United Kingdom and the United States.

These reviews have identified challenges common across international regulators that include terminology, definitions, measurement calibration, standardised measurement and analyticals, certified reference materials, testing methods and standards (Purushotham, 2014).

2.1.2 Australian context

The APVMA has specific regulatory oversight of nanomaterials if they constitute, or are intended for use in, agricultural or veterinary (agvet) chemical products. For these materials, the APVMA is involved at several stages of the 'nanofamily lifecycle' identified by Ludlow et al (2007). These stages are importation, manufacture and product supply.

Importing the active constituents (that are neither approved nor exempt) for a proposed or existing chemical product requires the APVMA's consent. Likewise, importing a chemical product that is not registered or exempt requires consent.

The APVMA administers a Manufacturing Licensing Scheme where use of Good Manufacturing Practice (GMP) ensures that manufacturing processes are consistent, are adequately supervised, and have effective quality control. Australian manufacturers of veterinary chemical products must be licensed by the APVMA through the Manufacturing Licensing Scheme. Licenses are issued only to manufacturing facilities within Australia that have demonstrated compliance with the Manufacturing Principles and the Australian Code of Good Manufacturing Practice for Veterinary Chemical Products. Facilities undergo audits to ensure they comply with these requirements before being issued with a license and audits continue at regular intervals while the license is in force. The APVMA also operates an Overseas GMP Scheme, which requires that the manufacturing quality of imported products meets GMP standards equal to those applied to Australian manufacturers.

The *Agricultural and Veterinary Chemicals Code Act 1994 (AgVet Code)* (Commonwealth of Australia, 1994) prohibits the supply of agvet chemical products or active constituents unless the substances have been authorised by the APVMA via registration, approval, permit or some other form of exemption. There are some exceptions to this requirement provided in the *Agricultural and Veterinary Chemicals Code Regulations 1995* (Commonwealth of Australia, 1995). For example, Regulation 40 allows imports of quantities of active constituents and products without consent if they meet certain quantity requirements, and are for the purpose of research.

The base regulatory triggers relate primarily to the function and intended purpose of a chemical substance and are informed by understanding its chemical composition and any risks arising from its proposed use.

The schedule to the *Agricultural and Veterinary Chemicals Code Act 1994*, defines agricultural chemical products as follows:

> ‘ … a substance or mixture of substances that is represented, imported, manufactured, supplied or used as a means of directly or indirectly:
>
> a) destroying, stupefying, repelling, inhibiting the feeding of, or preventing infestation by or attacks of, any pest in relation to a plant, a place or a thing; or
>
> b) destroying a plant; or
>
> c) modifying the physiology of a plant or pest so as to alter its natural development, productivity, quality or reproductive capacity; or
>
> d) modifying an effect of another agricultural chemical product; or
>
> e) attracting a pest for the purpose of destroying it’.
> (Agvet Code s.4)

The schedule to the *Agricultural and Veterinary Chemicals Code Act, 1994*, defines veterinary chemical products as:

> ‘ … a substance or mixture of substances that is represented as being suitable for, or is manufactured, supplied or used for, administration or application to an animal by any means, or consumption by an animal, as a way of directly or indirectly:
>
> a) preventing, diagnosing, curing or alleviating a disease or condition in the animal or an infestation of the animal by a pest; or
>
> b) curing or alleviating an injury suffered by the animal; or

c) modifying the physiology of the animal:

d) so as to alter its natural development, productivity, quality or reproductive capacity; or

e) so as to make it more manageable; or

f) modifying the effect of another veterinary chemical product'.
(Agvet Code s.5)

Additional provisions and regulations further refine these definitions by specifying the inclusion and exclusion of certain substances in certain circumstances (*Schedules 3 and 3AA to the Agricultural and Veterinary Chemicals Code Regulations 1995*).

Amendments to agvet legislation took effect on 1 July 2014. The AgVet Code restates provisions clarifying that the health and safety of human beings, animals and the environment are the priority of the regulatory system. Among other things, the amendments emphasise the need to align regulatory effort with risk. New provisions are drafted to emphasise that the AgVet Code must be implemented using science-based risk analysis processes (Commonwealth of Australia, 2013).

The legislation now includes references to safety criteria, trade criteria and efficacy criteria and outlines the circumstances in which these criteria are relevant to the APVMA's consideration of product registration or active approval, or the granting of an APVMA permit.

The safety criteria reflect the pre-2014 consideration of safety. However, there is now scope for the APVMA to determine when the criteria become relevant, based on risk. The APVMA must have regard to safety criteria before deciding whether to approve a new active constituent. Similarly, before registering a chemical product, the APVMA must have regard to the safety criteria and/or an established standard for the product and, in certain circumstances, the APVMA must also have regard to trade and efficacy criteria. The APVMA's satisfaction with regard to safety must endure, and the APVMA may reconsider registered products or approved active constituents to determine whether they continue to meet the safety criteria.

Additional guidance is provided in legislative instruments and guidelines prepared under Section 6A of the AgVet Code (APVMA, 2014a).

2.1.3 Safety criteria

The 5A definition of 'meets the safety criteria' in the Code Act is:

For the purposes of being satisfied whether an active constituent meets the safety criteria, the APVMA:

a) must have regard to the following:

  i. the toxicity of the constituent and its residues, including metabolites and degradation products, in relation to relevant organisms and ecosystems, including human beings;

  ii. the method by which the constituent is, or is proposed to be, manufactured;

  iii. the extent to which the constituent will contain impurities;

    iv. whether an analysis of the chemical composition of the constituent has been carried out and, if so, the results of the analysis;
    v. any conditions to which its approval is, or would be, subject;
    vi. any relevant particulars that are, or would be, entered in the Register for the product;
    via. whether the product conforms, or would conform, to any standard made for the product under section 6E to the extent that the standard relates to matters covered by subsection (1);
    vii. any matters prescribed by the regulations; and

b) may have regard to such other matters as it thinks relevant.

For the purposes of being satisfied as to whether a chemical product meets the safety criteria, the APVMA:

a) must have regard to the following:
    i. the toxicity of the product and its residues, including metabolites and degradation products, in relation to relevant organisms and ecosystems, including human beings;
    ii. the relevant poison classification of the product under the law in force in this jurisdiction;
    iii. how the product is formulated;
    iv. the composition and form of the constituents of the product;
    v. any conditions to which its registration is, or would be, subject;
    vi. any relevant particulars that are, or would be, entered in the Register for the product;
    via. whether the product conforms, or would conform, to any standard made for the product under section 6E to the extent that the standard relates to matters covered by subsection (1);
    vii. any matters prescribed by the regulations; and

b) may have regard to one or more of the following:
    i. the acceptable daily intake of each constituent contained in the product;
    ii. any dietary exposure assessment prepared under subsection 82(4) of the *Food Standards Australia New Zealand Act 1991* as a result of any proposed variation notified under subsection 82(3) of that Act in relation to the product, and any comments on the assessment given to the APVMA under subsection 82(4) of that Act;
    iii. whether any trials or laboratory experiments have been carried out to determine the residues of the product and, if so, the results of those trials or experiments and whether those results show that the residues of the product will not be greater than limits that the APVMA has approved or approves;
    iv. the stability of the product;
    v. the specifications for containers for the product;
    vi. such other matters as it thinks relevant.

2.1.4 Trade criteria

The 5C Definition of ‘meets the trade criteria’ in the Code Act is:

1. A chemical product meets the trade criteria if use of the product, in accordance with instructions approved, or to be approved, by the APVMA or contained in an established standard, does not, or would not, unduly prejudice trade or commerce between Australia and places outside Australia.
2. For the purposes of being satisfied as to whether a chemical product meets the trade criteria, the APVMA must have regard to the following:
    - (a) any conditions to which its registration is, or would be, subject;
    - (b) any relevant particulars that are, or would be, entered in the Register for the product;
    - (ba) whether the product conforms, or would conform, to any standard made for the product under section 6E to the extent that the standard relates to matters covered by subsection (1);
    - (c) any matters prescribed by the regulations.
3. For the purposes of the operation of this Code in relation to a particular chemical product, the APVMA is required to have regard to the matters set out in subsections (1) and (2) only:
    - (a) to the extent prescribed by the regulations; or
    - (b) if there are no such regulations—to the extent that the APVMA thinks the matters are relevant.

2.1.5 Efficacy criteria

The 5B Definition of ‘meets the efficacy criteria’ in the Code Act is:

1. A chemical product meets the efficacy criteria if use of the product, in accordance with instructions approved, or to be approved, by the APVMA for the product or contained in an established standard, is, or would be, effective according to criteria determined by the APVMA by legislative instrument.
2. For the purposes of being satisfied as to whether a chemical product meets the efficacy criteria, the APVMA must have regard to the following:
    - (a) whether any trials or laboratory experiments have been carried out to determine the efficacy of the product and, if so, the results of those trials or experiments;
    - (b) any conditions to which its registration is, or would be, subject;
    - (c) any relevant particulars that are, or would be, entered in the Register for the product;
    - (ca) whether the product conforms, or would conform, to any standard made for the product under section 6E to the extent that the standard relates to matters covered by subsection (1);
    - (d) any matters prescribed by the regulations.

3. For the purposes of the operation of this Code in relation to a particular chemical product, the APVMA is required to have regard to the matters set out in subsections (1) and (2) only:

   (a) to the extent prescribed by the regulations; or

   (b) if there are no such regulations—to the extent that the APVMA thinks the matters are relevant.

2.1.6 Standards for chemical products and actives

Section 87 of the *Agricultural and Veterinary Chemicals Code Act 1994* requires chemical products to comply with the standard prescribed for the product (if any). (For the purposes of s.87 of the Act, Regulation 42 of the *Agricultural and Veterinary Chemicals* Code *Regulations 1995* prescribes all chemical products.)

Where a standard applies to a constituent of a chemical product, the constituent must comply with that standard. Standards that may be applied are in the form of a hierarchy. Standards developed by the APVMA take precedence, followed by (for veterinary substances) standards specified in the *British Pharmocopoeia*, the *British Pharmacopoeia (Veterinary)*, the *European Pharmacopoeia* or the *United States Pharmacopoeia*, followed by standards specified in the *FAO and WHO Specifications for Pesticides*. If no standard is applicable then the standard is as set out in the table at Regulation 42(4) of the *Agricultural and Veterinary Chemicals Code Act Regulations 1995*.

The APVMA has not yet needed to develop standards for nanomaterials as no applications have been made for it to approve nanomaterial-active constituents. Additional guidance is provided in APVMA guidelines prepared under Section 6A of the Agvet Code (APVMA, 2014a).

In 2007 ‘A review of possible impacts of nanotechnology on Australia’s regulatory framework’ (Ludlow et al, 2007) identified various areas of potential concern regarding the Australian regulatory oversight of nanomaterials. These areas remain relevant to the future APVMA approach to regulating chemical products based on nanotechnology and are issues also identified by international regulators.

The key issues identified in the 2007 review by Ludlow and co-workers can be grouped into three main topics: whether nanoform materials should be considered new substances; metrology and definitions, including threshold measures for triggering regulatory action; and risk assessment methodologies. These are discussed below.

2.1.7 Nanomaterials as new substances

A key issue is whether materials in nanoform should be considered ‘new’ substances. Many international regulators have grappled with this issue, for instance, in 2014 the US decided that nanomaterials would be regulated as ‘new’ substances.

The APVMA is not limited in this regard; irrespective of ‘nanoform’, the APVMA legislative framework allows it to consider products (mixtures of chemical substances) if they are represented or intended for use as agricultural or veterinary chemical products that would require approval or registration. Regulatory guidelines prepared by the APVMA include the requirement to indicate whether any constituent used in a product has nanoscale properties (APVMA, 2014b). Similarly the Regulatory Guidelines for chemistry and manufacture include detailed advice about the information required in cases involving nanoscale materials (APVMA, 2014c).

2.1.8 Metrology, definitions and thresholds

Threshold weights or quantities of nanomaterials which trigger regulatory oversight vary internationally. The EU trigger quantity is one tonne of materials. While risks are mitigated via mandatory reporting schemes, this figure was under review when the APVMA was preparing this report.

APVMA regulatory triggers do not rely on threshold weights or volumes of materials, though there are exemptions in APVMA regulations for quantities of materials used in research.

Relevant to these issues of amount, specific exemptions for research and development uses that currently apply to conventional materials may assume greater significance for potentially hazardous nanomaterials and their products.

Authorisations via an APVMA permit require similar consideration of safety criteria as for products and actives. Applications for permits require a declaration regarding nanomaterial content.

A related factor is consideration of the threshold definitional values for nanomaterials. For instance, what are the threshold values for considering an active or product as a nanomaterial? While the APVMA currently requires a declaration from an applicant for active approval or product registration if it contains nanomaterials, this declaration relies on the definition, and also on an applicant reasonably understanding the presence or absence of nanomaterials. The APVMA has prepared regulatory content including the definition of nanomaterials (APVMA, n.d.).

APVMA Regulatory guidelines state that, where size distribution shows that, by number of particles, 10 per cent or more of a substance is at the nanoscale, the substance will be considered a nanomaterial for risk assessment purposes (APVMA, 2014b). This is consistent with the European Food Safety Agency guidelines regarding safety and uncertainty (EurActiv 2014). The APVMA's partner regulator for Industrial Chemicals (NICNAS) adopts a similar approach (see Chapter 3).

2.1.9 Other issues relating to the regulation of nanomaterials

A less-defined series of issues identified in the 2007 study by Ludlow and co-workers relate to the understanding of risks associated with nanoform materials. Without a clear understanding of the particular risks (or absence thereof), regulatory processes triggered by threshold risks may not be invoked where effects of engineered nanomaterials on human health are currently unknown. There is scope also for risk to accrue based on a variety of factors including the inherent toxicology of the parent material when rendered in nanoform and risks inherently introduced via the manufacturing process.

Irrespective of the presence of nanomaterials, the APVMA needs to be satisfied with the safety criteria (see Section 2.1.3 above) before approving an active constituent or registering a chemical product. Later incorporation of nanoscale-registered constituents into already registered products would render the product non-compliant with particulars recorded at the time of registration.

The APVMA currently requires applications for permits, product registration or active constituent approval to include a declaration about whether the proposed active constituent or product includes nanoparticles. This is in effect a notification scheme for new products or actives, but will not capture research activities covered by general APVMA permits (eg PER7250) or where the weights of materials concerned are excluded by Regulation.

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3 NANOMATERIALS IN AGRICULTURAL AND VETERINARY CHEMICALS: DEFINITIONS, METROLOGY AND PHYSICOCHEMICAL PROPERTIES

Abstract

Before it is possible to regulate nanomaterials they need to be defined and characterised according to their physical and chemical properties. This is necessary because the behaviour of some materials at normal scale is quite different at the nanoscale. However, size does not imply specific risks or hazard properties per se. This behaviour makes finding a universally appropriate definition for some nanomaterials difficult. Some countries and international organisations have offered their own definitions but there is no universally agreed definition. New instruments and techniques to measure the characteristics of these materials, such as their size and shape, chemistry, mass, charge and crystallinity, have been required to facilitate regulation. Measurements of size and the number size distribution are considered the most universally applicable and suitable measurands for nanoparticles. Techniques used include electron microscopy, atomic force microscopy, dynamic light scattering, laser diffraction, particle tracking analysis, aerosol characterisation and surface area measurement.

3.1 Introduction

The regulation of nanomaterials requires a definition, validated methods and instrumentation to detect, characterise and analyse nanomaterials.

This chapter begins by considering the definition of a nanomaterial. It is followed by a review of the physicochemical parameters of nanomaterials, a pre-requisite for risk assessment and a brief description of the instruments and techniques used to measure these parameters. A detailed account of the instruments and techniques used in nanometrology is presented in Appendix 1 of this report.

3.2 Defining nanomaterials

The term ‘nanomaterial’ informally refers to materials with external dimensions or an internal structure in the nanometre length range, a nanometre being one billionth of a metre. These materials typically exhibit different or additional properties and behaviour compared to the same material without nanoscale features.

Government agencies, industry, the scientific community, standards organisations, non-government organisations and others have expended much effort in recent years in attempting to define a nanomaterial. Different stakeholders have ‘varying interpretations of what a nanomaterial is, and confusion over the underlying science and its implications to risk’ (Maynard, 2011). A wide range of ensuing definitions have common elements. The search for relatively consistent and agreed definitions required to deal with the regulatory issues pertaining to nanomaterials has become a priority.

Regulatory definitions are used to delineate those substances that are to be captured within regulatory frameworks. Risk assessments for regulatory purposes determine the hazard and exposure of humans and the environment to these substances and, if possible, identify measures to manage any potential risks. The protection of human health and the environment are the primary objectives of risk assessment. Regulatory definitions are also important in enforcement activities. Recommended general reading on regulating nanotechnology includes Hodge et al. (2007, 2010, 2013).

The point at which nanomaterials change their behaviour from conventional to unconventional behaviour depends on the particular material and, not uncommonly, the context in which the issue is being considered. The boundary between nanoscale and non-nanoscale material behaviour is often indistinct and may depend on many parameters, including size, particle shape, porosity, surface area and chemistry. This has led some to suggest that a ‘one size fits all’ general definition of an engineered nanomaterial will inevitably fail to capture what is important for addressing risk (Maynard, 2011). Regulators consider all of these factors when assessing the potential risks posed by nanomaterials.

A detailed analysis of 27 existing definitions of nanomaterials from four different sources, namely academic institutions and scientific advisors, regulators, non-government organisations and four international organisations (Saner and Stoklosa, 2013) shows that the most common elements of these definitions are size, structure and properties/novel phenomena.

Note that the word ‘size’ is used commonly in the literature with units of nanometres, when what is meant is ‘length’. ‘Size’ strictly refers to the dimensions, proportions, amount, or extent of something and can include mass, volume, area and number, whereas ‘length’ should be used when referring to a distance. To avoid confusion, this chapter will continue to use the word ‘size’.

The following are reviews of some of the more significant published definitions of the term ‘nanomaterial’ and related terms. A more detailed review may be found in Lövestam et al (2010).

3.2.1 International Organization for Standardization (ISO)

ISO is the world's largest developer of standards. It is a non-governmental network of the national standards bodies of 157 countries, supported by the Central Secretariat based in Geneva, Switzerland. The principal deliverables of ISO are international documentary standards embodying the essential principles of global openness and transparency, consensus and technical coherence. ISO standards are developed by experts nominated by the national member bodies contributing to the work of the particular committee responsible for the subject matter under consideration.

ISO Technical Committee TC229 (TC229) was formed in 2005 and is the main ISO technical committee responsible for international standardisation work related to nanotechnologies (International Organization for Standardization ISO/TC 229 Nanotechnologies, 2005). It was created to complement and coordinate the nano-relevant standardisation work already undertaken by other ISO technical committees. TC229 has formal liaisons with several other major players in the nanotechnology standardisation arena, including (1) the Organisation for Economic Cooperation and Development (OECD), which has devoted two working parties to the topic (Working Party on Manufactured Nanomaterials, WPMN, and Working Party on Nanotechnology, WPN), (2) the International Bureau of Weights and Measures (BIPM), (3) the European Commission's Joint Research Centre (JRC), a research-based policy support organisation and (4) the Versailles project on Advanced Materials and Standards (VAMAS), which is active in pre-normative research.

Initially, three working groups were established by TC229, namely WG1: Terminology and Nomenclature, convened by Canada, WG2: Measurement and Characterisation, convened by Japan and WG3: Health, Safety, and Environmental Aspects of Nanotechnologies, convened by the USA.

The work of WG1 continues to be a critical foundation and priority for ISO TC229, as the development of standards for measurement, characterisation and health and safety cannot be completed until consensus on terminology, a controlled vocabulary and nomenclature is reached. It follows that regulations, legal contracts and health and safety guidelines cannot be written until there is agreement on terminology.

Core Terms (TS 80004-1)

Important definitions in TS 80004-1 are:

- **Nanoscale**

  Size range from approximately 1 nanometre (nm) to 100 nm.

  Note 1 – Properties that are not extrapolations from a larger size will typically, but not exclusively, be exhibited in this size range. For such properties the size limits are considered approximate.

  Note 2 – The lower limit in this definition (approximately 1 nm) is introduced to avoid single and small groups of atoms from being designated as nano-objects or elements of nanostructures, which might be implied by the absence of a lower limit.

- **Nanotechnology**

  Application of scientific knowledge to manipulate and control matter in the nanoscale in order to make use of size and structure-dependent properties and phenomena, as distinct from those associated with individual atoms or molecules, or with bulk materials.

- **Nanomaterial**

  Material with any external dimension in the nanoscale, or having internal structure or surface structure in the nanoscale.

  Note 1 – This generic term is inclusive of nano-object and nanostructured material.

  Note 2 – See also engineered nanomaterial, manufactured nanomaterial and incidental nanomaterial.

- **Nano-object**

  Material with one, two or three external dimensions in the nanoscale.

  Note – Generic term for all discrete nanoscale objects.

- **Nanostructure**

  Composition of interrelated constituent parts, in which one or more of those parts is a nanoscale region.

  Note – A region is defined by a boundary representing a discontinuity in properties.

- **Nanostructured material**

  Material having internal nanostructure or surface nanostructure.

  Note – This definition does not exclude the possibility for a nano-object to have internal structure or surface structure. If external dimension(s) are in the nanoscale, the term nano-object is recommended.

- **Engineered nanomaterial**

  Nanomaterial designed for a specific purpose or function.

- **Manufactured nanomaterial**

  Nanomaterial intentionally produced for commercial purposes to have specific properties or specific composition.

A review of TS 80004-1 is due for completion in 2015.

Nano-objects—nanoparticle, nanofibre, nanoplate (TS 27687)

TS 27687 is also being reviewed and when completed, it will be renamed ISO/TS 80004-2: Vocabulary for nano-objects, nanoparticle, nanoplate, and nanofibre.

Important definitions are:

- **Nanoparticle**

  Nano-object with all three external dimensions at the nanoscale.

  Note – If the lengths of the longest and the shortest axes of the nano-object differ significantly, the terms nanorod or nanoplate should be considered. 'Significantly' is considered to mean more than three.

  Section 4 of TS 27687 is concerned with assemblies of particles and defines agglomerates and aggregates as:

- **Agglomerate**

  Collection of loosely bound particles or aggregates, or mixtures of the two, where the resulting external surface area is similar to the sum of the surface areas of the individual components.

  Note 1 – The forces holding an agglomerate together are weak forces, for example van der Waals forces, as well as simple physical entanglement.

  Note 2 – Agglomerates are also termed secondary particles.

- **Aggregate**

  Particle comprising strongly bonded or fused particles where the resulting external surface area may be significantly smaller than the sum of calculated surface areas of the individual components.

  Note 1 – The forces holding an aggregate together are strong forces, for example covalent bonds, or those resulting from sintering or complex physical entanglement.

Note 2 – Aggregates are also termed secondary particles and the original source particles are termed primary particles.

The ISO definition of a nanomaterial is very broad. It includes all nano-objects (nanoparticles, nanofibres and nanoplates) and nano-structured materials (including aggregates and agglomerates). The central use of the term ‘nanoscale’ means that a nanomaterial is essentially categorised according to the size of its constituent parts. It does not require a nanomaterial to display unique or specific properties or have a specific risk.

The 1–100 nm range specified in the ISO definition of nanoscale is commonly used as a threshold in the field of nanotechnology. However, there is no scientific evidence to support this. Indeed, it is now apparent that, in many cases in medicine, cosmetics and food, the unique properties and phenomena associated with size and shapes in engineered materials extend above and below the nanoscale. Also, single thresholds do not take into account the fact that the constituents of most nanomaterials have a size distribution. According to the European Commission Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), when only a part of the nanomaterial has a size within the size range of the definition, it should be clear whether, and when, such a material would be considered a nanomaterial (SCENIHR, 2010).

A number of additional ISO Standards have been developed that are of relevance in analysing, determining and defining particle size from a nanotechnology perspective (ISO 14887:2000; ISO 13318-1:2001; ISO 13318-2:2007; ISO 14488:2007; ISO 9272-6:2008; ISO 22412:2008) (ISO, 2001a, 2001b, 2007a, 2007b, 2008a, 2008b).

3.2.2 Organisation for Economic Co-operation and Development (OECD)

The OECD established a Working Party on Manufactured Nanomaterials (WPMN) in 2006 and adopted the draft TC229 definition of nanomaterial as a working definition for the term ‘manufactured nanomaterial'. This was later modified to:

> Manufactured nanomaterials: Nanomaterials intentionally produced to have specific properties or specific composition, a size range typically between 1 nm and 100 nm and material which is either a nano-object (ie that is confined in one, two, or three dimensions at the nanoscale) or is nanostructured (ie having an internal or surface structure at the nanoscale) (OECD Working Party on Manufactured Nanomaterials, 2008).

This definition adds the deliberate intention to produce unique physicochemical properties to the size-based ISO definition. The ISO and OECD definitions specify an approximate size range for nanomaterials; however they do not address the issue of size distributions and are difficult to apply in a regulatory context.

3.2.4 European Union (EU) Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR)

In a 2007 report SCENIHR (SCENIHR, 2007b) concluded that the term nanomaterial is a categorisation of a material due to its size, and defined a nanomaterial as:

> Any form of a material that is composed of discrete functional parts, many of which have one or more dimensions of the order of 100 nm or less.

SCENIHR also stressed that size distribution should be taken into account when defining a nanomaterial. This accounts for the materials in which only a part has a size within the size range of the definition. It was suggested that this could be achieved by considering the percentage of the number size distribution that is above or below the threshold. For example, a material could be considered a nanomaterial when more than 0.15 per cent of the material, as indicated by the number concentration, has a size below the designated upper size limit.

In 2010 SCENIHR published a report on the scientific basis for the definition of nanomaterial. It concluded that:

'Whereas physical and chemical properties of materials may change with size, there is no scientific justification for a single upper and lower size limit associated with these changes that can be applied to adequately define all nanomaterials.

There is no scientific evidence for a single methodology (or group of tests) that can be applied to all nanomaterials.

Size is universally applicable to define all nanomaterials and it is the most suitable measure. Moreover, an understanding of the size distribution of a nanomaterial is essential and the number size distribution is the most relevant consideration.

In order to define an enforceable definition of "nanomaterial" for regulatory use it is proposed to set an upper limit for nanomaterial size and to add to the proposed limit additional guidance (requirements) specific for the intended regulation. Crucial in the guidance that needs to be provided is the extended description of relevant criteria to characterise the nanoscale. Merely defining single upper and lower cut-off limits is not sufficient in view of the size distributions occurring in manufactured nanomaterials.

Alternatively, a tiered approach may be required depending on the amount of information known for any specifically manufactured nanomaterial and its proposed use.

The scientific opinion recognises however that specific circumstances regarding risk assessment for regulatory purposes for certain areas and applications may require the adaptation of any overarching definition.

It should be stressed that "nanomaterial" is a categorization of a material by the size of its constituent parts. It neither implies a specific risk, nor does it necessarily mean that this material actually has new hazard properties compared to its constituent parts or larger sized counterparts.'

The SCENIHR report was the first to highlight the need to consider a size distribution as well as a size range in defining a nanomaterial. The report also emphasised the lack of a scientific basis for a simple size range such as

1–100 nm, implying that the use of such a size range in a nanomaterial definition would be a policy decision rather than a scientific one.

3.2.5 Joint Research Center (JRC) of the EU

The JRC's reference report (Lövestam et al. 2010) did not include a specific definition for a nanomaterial. But it did recommend that a definition for regulatory purposes should use size as the defining property, should include size distribution considerations and, significantly, only take into account particulate materials.

The justification for restricting a definition for regulatory purposes to materials which are in a particulate form at the nanoscale, and which are mobile in their immediate environments, is that it is only these materials that raise human health and environmental safety concerns.

The JRC 2010 report specifically targeted a nanomaterial definition for regulatory purposes. It therefore focused on identifying materials that may pose risks to health, safety or the environment. This leads to the restriction of a definition to particulate materials, a significant reduction in generality.

3.2.6 European Commission (EC) and European Union (EU)

The EC has definitions of nanomaterial in several regulations, including one on cosmetics (European Commission, 2009):

> Nanomaterial means an insoluble or biopersistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm.

and one on food (SCENIHR, 2010):

> Engineered nanomaterial means any intentionally produced material that has one or more dimensions of the order of 100 nm or less or that is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic of the nanoscale.

Another definition of a nanomaterial published by the EC (European Commission, 2011) is based on the JRC report (Lövestam et al, 2010), the SCENIHR opinion (SCENIHR, 2010) and the definition of nanomaterial developed by the ISO.

Their recommended definition is:

> Nanomaterial means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 per cent or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm to 100 nm.
>
> In specific cases, and where warranted by concerns for the environment, health, safety or competitiveness, the number size distribution threshold of 50 per cent may be replaced by a threshold between 1 and 50 per cent.

The definitions of particle, agglomerate and aggregate are essentially the same as the ISO definitions for these terms.

This definition includes a statement that by derogation[12], fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials.
Also, compliance with this definition may be determined on the basis of the specific surface area by volume, namely a material should be considered as falling under the definition if the specific surface area by volume of the material is greater than 60 m^2/cm^3. This recommendation has been incorporated into a European Union (EU) regulation for biocidal products (EU, 2012).

The EU recommendation:

- includes incidental and natural materials as well as engineered materials
- is restricted to nanoparticles
- includes aggregates and agglomerates of nanoparticles
- focuses on the size of the nanoparticles
- includes a specific size distribution requirement, and
- allows the number distribution threshold to be varied depending on environmental, health and safety concerns.

The EU definition does not use the ISO defined term ‘nano-object’, choosing instead the term ‘nanoparticle’.

The EU recommendation has been controversial and has prompted global debate (Foss Hansen et al, 2013; Bleeker et al, 2013). Member states, EU agencies, and economic operators within the EU are invited to use the definition, but very few regulations have been introduced that use it. It is not harmonised with other jurisdictions, including the USA.

Indeed, some have argued that the EU definition uses criteria that are not supported by current data on nanomaterial risk and that perhaps nanomaterials should not be explicitly defined at all (Maynard, 2011).
The alternative view is that a definition is required for labelling purposes, and would assist industry and regulators in identifying when specific safety assessments might be necessary (Stamm, 2011).

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[12] Derogation is the partial revocation of a law, as opposed to abrogation or the total abolition of a law.

The EU recommendation is currently undergoing review by the JRC. Two of the three reports in the Review Series have been published and can be accessed at: bookshop.europa.eu/en/towards-a-review-of-the-ec-recommendation-for-a-definition-of-the-term-nanomaterial--pbLBNA26567/ and http://bookshop.europa.eu/en/towards-a-review-of-the-ec-recommendation-for-a-definition-of-the-term-nanomaterial--pbLANA26744/ respectively.

3.2.7 North America

The **United States National Nanotechnology Initiative (NNI)** describes nanotechnology as 'the understanding and control of matter at dimensions between approximately 1 and 100 nanometres, where unique phenomena enable novel applications' (National Nanotechnology Initiative, 2009).

Adding intentional manufacture to this leads to the NNI definition of an engineered nanomaterial as:

> A material that has been purposely synthesized or manufactured to have at least one external dimension of approximately 1 to 100 nanometres—at the nanoscale—and that exhibits unique properties determined by this size (National Nanotechnology Initiative, 2011).

The **United States Food and Drug Administration (FDA)** states that while there is no formal agency definition, it does offer 'guidance' on its 'current thinking'. When considering whether an FDA-regulated product contains nanomaterials, or otherwise involves the application of nanotechnology, the FDA will ask:

> Whether an engineered material or end product has at least one dimension in the nanoscale range (approximately 1 nm to 100 nm), or whether an engineered material or end product exhibits properties or phenomena, including physical or chemical properties or biological effects, that are attributable to its dimension(s), even if these dimensions fall outside the nanoscale range, up to one micrometre (1000 nm) (U.S. Department of Health and Human Services Food and Drug Administration Center for Food Safety and Applied Nutrition 2012).

In June 2014 the FDA updated its 'guidance for industry', saying it would only consider products to be 'engineered' to have certain dimensions or exhibit certain properties if they have been subject to 'deliberate and purposeful manipulation' by nanotechnology. It said the incidental presence of particles in the nanoscale range in 'conventionally-manufactured' products did not fall under the guidance if they had not been deliberately manipulated to be that size. Similarly 'familiar' biological and chemical nanoscale substances such as microorganisms and proteins are not covered by the guidance.

The FDA added that while it has no opinion on whether nanotechnology is 'inherently safe or harmful,' its use 'may result in product attributes that differ from those of conventionally-manufactured products and thus may merit particular examination'.

The **United States Environmental Protection Agency (EPA)** also has no formal agency definition. However, it has outlined key criteria across several documents including:

- particle size between 1 and 100 nm in at least one dimension
- the material exhibits unique properties compared to larger sized particles
- the material is engineered at the nanoscale
- inclusion of aggregates and agglomerates, and
- a distribution of particles with greater than 10 per cent by weight less than 100 nm.

The Office of Pesticide Programs (OPP), part of the EPA, has a working definition of a nanoscale material (U.S. Federal Register, 2011), namely:

An ingredient that contains particles that have been intentionally produced to have at least one dimension that measures between approximately 1 and 100 nanometres.

Health Canada published a definition of nanomaterials in a policy statement that applies to all substances that it regulates including consumer products, industrial substances, food, therapeutic and agvet products (Health Canada, 2011) namely:

Any manufactured substance or product and any component material, ingredient, device, or structure is considered to be nanomaterial if it is at or within the nanoscale in at least one external dimension, or has internal or surface structure at the nanoscale, or if it is smaller or larger than the nanoscale in all dimensions and exhibits one or more nanoscale properties/phenomena.

For the purposes of this definition:

- ‘nanoscale’ means 1 to 100 nm, inclusive
- ‘nanoscale properties/phenomena’ means properties which are attributable to size and their effects; these properties are distinguishable from the chemical or physical properties of individual atoms, individual molecules and bulk material
- ‘manufactured’ includes engineering processes and the control of matter.

Health Canada is the only North American organisation to formally define a nanomaterial.
As noted above, US organisations and agencies typically do not formally define nanomaterials, instead giving a list of key criteria that a nanomaterial must satisfy. However it is noted that the Canada-US Cooperative Council Nanotechnology Initiative is developing common criteria for identifying characteristics of nanomaterials of concern, or of no concern—see actionplan.gc.ca/en/page/rcc-ccr/nanotechnology-work-plan.

3.2.8 Australian agencies

National Industrial Chemicals Notification and Assessment Scheme (NICNAS)

NICNAS is the only Australian Government agency that has developed a working definition for regulatory purposes (NICNAS, 2010, 2013). This working definition is broadly consistent with other available international definitions.

The NICNAS working definition is:

> …industrial materials intentionally produced, manufactured or engineered to have unique properties or specific composition at the nanoscale, that is a size range typically between 1 nm and 100 nm, and is either a nano-object (ie that is confined in one, two, or three dimensions at the nanoscale) or is nanostructured (ie having an internal or surface structure at the nanoscale).
>
> Notes to the working definition:
>
> - intentionally produced, manufactured or engineered materials are distinct from accidentally produced materials
> - 'unique properties' refers to chemical and/or physical properties that are different because of a material's nanoscale features when compared with the same material without nanoscale features, and result in unique phenomena (eg increased strength, chemical reactivity or conductivity) that enable novel applications
> - aggregates and agglomerates are considered to be nanostructured substances
> - where a material includes 10 per cent or more number of particles that meet the above definition (size, unique properties, intentionally produced) NICNAS will consider this to be a nanomaterial.

The NICNAS definition emphasises intention and engineering. It is not restricted to primary nanoparticles but specifically includes aggregates and agglomerates.

NICNAS has also published detailed guidance in relation to nanomaterials, accessible at nicnas.gov.au/regulation-and-compliance/nicnas-handbook/handbook-appendixes/guidance-and-requirements-for-notification-of-new-chemicals-that-are-industrial-nanomaterials/specified-conditions-for-requesting-additional-data-requirements and nicnas.gov.au/regulation-and-compliance/nicnas-handbook/handbook-appendixes/guidance-and-requirements-for-notification-of-new-chemicals-that-are-industrial-nanomaterials/guidance-on-providing-additional-data-requirements. In addition, NICNAS has made a number of administrative changes relating to the regulation of nanomaterials which are described at nicnas.gov.au/communications/issues/nanomaterials-nanotechnology/nicnas-regulatory-activities-in-nanomaterials.

Australian Pesticides and Veterinary Medicines Authority (APVMA)

The APVMA is currently developing a working definition for a nanomaterial based closely on the NICNAS definition. The review of current definitions of a nanomaterial given above would indicate that such a working definition should consider the following;

A nanomaterial should be an intentionally produced, manufactured or engineered substance with unique properties that are directly caused by size features with 10 per cent or more of the number size distribution of these features lying in the range approximately 1–100 nm (the nanoscale). There should be recognition that biological and EHS issues may require a different size range above 100 nm.

3.3 The metrology of nanomaterials

3.3.1 The parameters used to characterise nanomaterials

The appropriate characterisation of manufactured nanomaterials is critical for many fields, including research, manufacturing, and regulation (food, cosmetics, human and animal medicines, and pesticides). There have been numerous studies on which physicochemical parameters should be used to characterise nanomaterials generally (eg Stone et al, 2010; Stintz et al, 2010), but it is now well established that a single list of parameters, covering all industry sectors, is not possible.

However, there is some consensus on the parameters needed for risk assessment and hence regulation (OECD, 2009a). Lists of such parameters, developed in recent years, include those from the organisations listed below.

In May 2008, WG3 of TC229 produced a focused list titled 'Physico-Chemical Characteristics of Engineered Nano-Objects for Toxicological Assessment' namely:

- agglomeration state/aggregation
- composition (eg chemical composition and structure)
- concentration
- hydrophobicity
- manufacturing process
- oxidizing properties
- particle size/size distribution
- purity
- shape
- solubility
- stability
- surface area
- surface chemistry
- zeta potential.

A SCENIHR report concentrating on the risk assessment of products of nanotechnologies (SCENIHR, 2009) concluded that the main physicochemical parameters of interest with respect to nanoparticle safety are:

Physical properties

- size, shape, specific surface area, aspect ratio
- agglomeration/aggregation state
- size distribution
- surface morphology/topography
- structure, including crystallinity and defect structure
- solubility.

Chemical properties

- structural formula/molecular structure
- composition of nanomaterial (including degree of purity, known impurities or additives)
- phase identity
- surface chemistry (composition, charge, tension, reactive sites, physical structure, photocatalytic properties, zeta potential)
- hydrophilicity/lipophilicity.

The OECD published a list focused on the safety aspects of nanomaterials. It concluded that the majority of the end-points and the test guidelines regarding physicochemical, environmental fate, ecotoxicological and toxicological properties found in existing OECD test guidelines were relevant and applicable to nanomaterials (OECD, 2009a, 2010a, 2010b).

In 2010, the OECD WPMN listed 17 physicochemical properties for characterising nanomaterials (OECD, 2010a) namely:

- shape
- agglomeration/aggregation
- water solubility/dispersability
- crystalline phase
- dustiness
- crystallite size
- representative electron microscopy (TEM) picture(s)
- particle size distribution—dry and in relevant media
- specific surface area
- zeta potential (surface charge)
- surface chemistry (where appropriate)
- photocatalytic activity

- pour density
- porosity
- octanol-water partition coefficient, where relevant
- redox potential
- radical formation potential.

A report from a workshop on nanoparticle metrology (Stintz et al, 2010) summarised the properties of interest as follows:

Morphology

- characteristic length and areas in 2D-projection
- parameters describing aggregates/agglomerates
- shape parameters from morphology data, eg sphericity, aspect ratio, fractal dimension.

Size-related properties based on hydrodynamics and/or interaction with external fields

- diffusion coefficient, hydrodynamic diameter (of translation)
- settling velocity, Stoke's diameter
- aerodynamic diameter
- acoustophoretic mobility
- (partial) scattering or extinction cross section.

Surface area of the dispersed phase

- via adsorption of gases
- via small angle X-ray scattering
- via titration experiments with surfactants, polyelectrolytes etc.

Chemical composition and phase

- crystallinity (amorphous fraction vs crystalline fraction)
- phase fractions of different crystallographic phases.

Concentration of particles

- mass, surface, number concentration
- total or fractional concentration.

Interfacial properties

- surface charge
- zeta potential

- surface conductivity
- pristine point of zero charge and iso-electric point (for different charge determining ions).

Interaction with continuum/suspendants

- solubility and dissolution kinetics
- ROS (radical oxidising species) potential
- wettability.

It is important to note that for all of these ‘lists’, typically only a few of the parameters will need to be measured for a given application. It is also apparent that size and number size distribution are the two parameters universally applicable in characterising nanomaterials (Linsinger et al, 2012). The other parameters most commonly used are state of agglomeration and aggregation, shape, surface area, surface and bulk chemistry and zeta potential.

The ‘Report of the OECD Expert Meeting on the Physical Chemical Properties of Manufactured Nanomaterials and Test Guidelines’ in the Series on the Safety of Manufactured Nanomaterials, No. 41, was published in 2014 for the analysis of the physicochemical properties of manufactured nanomaterials and consideration of endpoints for regulatory purposes (OECD, 2014). Understanding these primary properties and the possible exposure pathways of nanomaterials are the starting points for relevant risk assessment and risk management strategies for regulating nanomaterials.

The workshop also considered how existing OECD Test Guidelines apply to the physicochemical properties of manufactured nanomaterials and whether there is a need to update or develop new OECD Test Guidelines and/or OECD Guidance Documents for safety and regulatory decision-making. These regulatory challenges were considered in terms of:

- state of dispersion, aggregation and agglomeration, solubility, zeta potential and octanol-water partition coefficient
- size (and size distribution)
- surface area and porosity
- surface reactivity.

Each of these parameters is discussed below.

Dispersion/agglomeration/aggregation

The workshop agreed not to discuss the measurement of the strength of agglomerates and aggregates. With respect to the latter, it recognised that the term ‘agglomerate’ is used to describe weakly bonded particles, and ‘aggregate’ to describe strongly bonded particles, with the understanding that the boundary between them depends on the circumstances. The workshop concluded that there is a need for a new Test Guideline on dispersion/agglomeration/aggregation, which can refer to existing ISO standards.

Water solubility

The workshop emphasised the importance of the difference between solubility and dispersibility. For example, if after dissolution the nano-specific effects of nanomaterials were no longer present, it concluded the material should be assessed as any other soluble chemical.

Zeta potential/surface charge

Zeta potential is a key factor governing whether nanoparticles agglomerate, aggregate, settle, flocculate, or remain in suspension. Measuring the zeta potential to determine the iso-electric point is considered especially useful and relevant for the fate and exposure of nanomaterials. The workshop concluded that the zeta potential of a nanomaterial is a relevant property and there is a need for a new Test Guideline (which can refer to existing ISO standards). The Test Guideline would specify the intended use of the measurements and the relevant dispersion protocols for the different end-points when assessing zeta potential.

Octanol-water partition coefficient

The distribution and fate of nanomaterials to fatty or aqueous media are significant.
However, the workshop considered that the distribution of nanomaterials is not governed by the fundamental mechanisms for which the octanol-water partition coefficient test was developed. It concluded that the octanol-water partition coefficient should not be assessed for nanomaterials. However, other kinetic and surface chemical effects would need to be discussed at future meetings.

Size and size distribution

Particle size has a direct impact on the reliability of nanoparticle-based products and provides essential underpinning metrology for toxicological studies of nanoparticles. Particle sizing methods are based on a range of different physical principles for determining particle size and particle size distribution. The report provides guidance for preferred nanoparticle size, and nanoparticle size distribution measurements of spheroidal, non-spheroidal and environmental, health and safety samples.

Surface area and porosity

Surface area is considered an important parameter for predicting exposure and may be a predictor of toxicity when extrapolating within the same type of nanomaterial. The workshop identified SAXS (small angle X-ray scattering) as a possible technique for surface area determinations (in liquids), though there are no methods currently available that can be adapted as technical standards for liquids so this represents a priority development. This endpoint is important for regulatory bodies because most industrial nanomaterials are synthesised and used from liquid phases and fate and toxicity testing are conducted in liquid media. Porosity may be important for nanostructured materials. However, current methods for measuring porosity are currently not specific for nanomaterials, though may be applicable or adaptable.

The workshop agreed that further clarification is needed on the relevance of surface area in regulatory assessments. It concluded there is sufficient information to proceed with a technical standard for a Brunauer Emmett Teller (BET) method for dry powdered metal oxide nanomaterials, though BET methods for other classes of nanomaterials still require further research (Brunauer et al, 1938).

Surface reactivity

Surface charge is very important in predicting qualitatively potential toxicity and the media used can have a pronounced impact on cytotoxicity and other fate and effect endpoints. However, the need for quantitative information on surface reactivity is not required for regulatory purposes yet. Both qualitative and quantitative methods are available for characterising surface chemistry but other methods may also need to be developed to determine functional groups, orientation on the surface, coverage on the surface, information on the core, covalent versus non-covalent interactions and packing density.

Conclusions

The workshop concluded there is a need to document the techniques and methods currently available for measuring the surface chemistry of nanomaterials and for defining any caveats relating to specific techniques and methods, eg, a method for determining a particular endpoint for certain classes of nanomaterials which may not be generally applicable to other nanomaterials. The workshop also considered that access to specified instrumentation and/or establishing specified techniques should not represent an unreasonable regulatory constraint when formulating test guidelines for nanomaterials.

Meanwhile, before addressing the specific measurement issues involving nanomaterials, general matters concerning metrology and nanometrology need to be addressed.

3.3.2 Metrology—the science of measurement

Metrology, the science of measurement, is a well-developed scientific discipline with a long history. Metrology in the nanoscale is known as nanometrology. For further reading see Miles (2007, 2010) and Jamting and Miles (2013).

The current international measurement system began in 1875 when 17 nations signed the Metre Convention, recognising the need for measurements to be uniform internationally. A further 37 member states are now signatories, with Australia signing in 1947. The Metre Convention established the structure and processes for worldwide uniformity in measurement, firstly through a harmonised set of units of measurement, the International System of Units (SI), and secondly through a recognised method of establishing measurement standards that realise these units. The international structures established under the Metre Convention cover scientific and industrial measurements and are described by the International Bureau of Weights and Measures (BIPM), located in Sèvres, France. They are overseen by the peak international expert metrology body, the International Committee for Weights and Measures (CIPM). Australia's National Measurement Institute (NMIA) is Australia's official representative to the Metre Convention's activities.

The SI system is a set of agreed definitions duplicated in many countries. BIPM's mission is worldwide consistency of measurements traceable to the SI. Traceability (see below) relates a measurement result, or the value of a standard, to references at higher levels ending at a national primary standard. In doing so it uses a chain of comparisons, all having stated uncertainties. International traceability allows nationally realised standards to be linked and known in terms of the SI units.

The BIPM cooperates with appropriate national authorities, normally the relevant National Metrology Institute (NMI). All Member States of the Metre Convention support a NMI that has, in general, the role of maintaining national measurement standards, ensuring their suitability for national needs, and transferring measurement traceability, metrological expertise and knowledge to national users through high level calibration services, advice and other assistance.

Some of the key terms and concepts used in the field of metrology (Joint Committee for Guides in Metrology, 2008) include:

Measurand – the quantity intended to be measured. This needs to be clearly defined and understood.
For example, the measurand for the size of a complex-shaped nanoparticle may involve lengths in three dimensions, the aspect ratio, the temperature and the measuring technique used. The correct and full description of the measurand is a prerequisite for a successful measurement.

Reference – a measurement unit, a measurement procedure, a reference material, or a combination of them all. For example, the length of a given rod may be 5.34 m, a product of a number and a measurement unit, namely the metre.

Calibration – an operation that, under specified conditions:

- in a first step establishes a relation between the values of the quantity to be measured with measurement uncertainties provided by measurement standards, as well as corresponding indications with associated measurement uncertainties, and
- in a second step uses this information to establish a relation for obtaining a measurement result from an indication. In other words, the operation that relates a measurement standard to the reading of an instrument.

Measurement uncertainty – non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used. The measurement uncertainty is an estimate of the range of values within which the true value lies. It is a fundamental parameter, as important as the measurement result itself. For example, the diameter of a nanoparticle may be written as 10 nm ± 2 nm, where ± 2 nm is the measurement uncertainty.

Estimating the measurement uncertainty involves considering all known sources of uncertainty in the measurement process, and has to be done in accordance with the ISO Guide to the Uncertainty of Measurement (International Organization for Standardization and International Electrotechnical Commission). Typically, the measurement uncertainty is reported as the standard uncertainty multiplied by a coverage factor $k = 2$, which for a normal distribution corresponds to a coverage probability of approximately 95 per cent, ie the correct value of the measurand is within the range (measured value ± expanded uncertainty) at a confidence level of about 95 per cent.

Metrological Traceability – property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty.
The traceability of measurements typically relates measurements to the SI, maintained by NMIs, which regularly perform international key and supplemental comparisons to ensure that their national references agree with those of other countries. Establishing metrological traceability is crucial if measurements are to be compared and accepted internationally. It allows meaningful comparison of measurement results, made at different times and different locations.

High quality measurements that may be relied upon for legal and regulatory purposes require clearly specified measurands and measurements that are traceable and made with calibrated measuring instruments by skilled observers in a suitable measuring environment. The measurement uncertainty must be properly determined and appropriate for the needs of the measurement.

3.3.3 Nanometrology

Significant efforts are ongoing nationally and internationally to achieve a harmonised and valid nanometrological measurement system. Programs are now in place in many countries, including Australia, to develop capability for performing high quality nanometrological measurements (NMIA, n.d.).

Traceability for length measurements in the nanoscale is typically achieved at the NMI level by transferring the realisation of the primary standard for the metre down to measurements at the nanometre level. This is normally done using primary length standards to calibrate high magnification microscopes, such as an electron microscope (EM) or an atomic force microscope (AFM), fitted with optical interferometers on the translation axes. These microscopes are then used to calibrate the grids, gratings and line scales that are used to calibrate secondary AFMs or EMs. These secondary instruments are in turn used to calibrate reference standards or materials for the calibration of instruments in testing and industrial laboratories.

More generally, properly certified reference materials are being developed internationally and nationally (Roebben et al, 2011, 2013) as they are crucial for instrument calibration. Instrument manufacturers often provide reference materials to monitor the performance of their instruments, but these can lack traceability.

High quality nanometrology requires laboratories with independently proven competence, normally achieved by third party accreditation (NATA in Australia: nata.asn.au/) using laboratory audits and interlaboratory comparisons to support method validation.

A significant complexity of physicochemical characterisation of nanomaterials is that the properties may depend both on the employed methodology as well as the properties of the medium supporting the sample. The requirement that measurements be performed in various media adds significant complexity to the design of instruments, the handling of reference materials and the test methods.

It cannot be stressed too strongly that the results for the measurement of a given physicochemical parameter of a nanoparticle will differ depending on the instrument and technique used and the supporting medium. It is equally important to realise that characterising a given nanomaterial demands the use of more than one measurement method.

a) *Instruments and techniques used in nanometrology*

Nanometrology uses a very large range of instruments, techniques and physical principles. Because the trend in regulating nanomaterials is to focus on nanoparticles, the rest of this report will likewise focus on the characterisation and nanometrology of nanoparticles.

Particle characterisation techniques may be classified into three different classes. Firstly, there are ensemble techniques that average over a large number of particles and measure an average for the system as a whole. These techniques provide good statistical representation of the particle system but are often unable to resolve contributions from individual particles or from small parts of a broad particle size distribution.

Secondly, there are single particle analysis techniques that measure the properties of individual particles and can resolve particle size distributions in great detail but are limited by small sample sizes. Although it is possible to increase the number of particles that are measured, the time and expense involved is often prohibitive.

Thirdly, there are separation techniques. These are based on a separation step before applying detection and measuring techniques. Fractionation allows the sample to be separated into smaller volume fractions which can be detected with either an ensemble technique, now capable of detecting contributions to the measurement from each fraction, or further analysed using singe particle analysis techniques.

Particle characterisation techniques may also be classified according to the parameter of interest, and then technique, as follows:

Size and shape

Microscopy, including scanning electron microscopy (SEM), transmission electron microscopy (TEM), scanning probe microscopy (SPM), atomic force microscopy (AFM), scanning tunneling microscopy (STM), near-field scanning optical microscopy (NSOM), fluorescence microscopy (FM) and confocal optical microscopy (COM).

Scattering techniques, including dynamic light scattering (DLS), small angle x-ray scattering (SAXS), small angle neutron scattering (SANS) and particle tracking analysis (PTA).

Aerosol characterisation, including condensation particle counting (CPC), differential electrical mobility classification (DEMC) and a differential mobility analysing system (DMAS).

Separation techniques, including field flow fractionation (FFF), differential centrifugal sedimentation (DCS) and size exclusion chromatography (SEC).

Surface area measurement, including the Brunauer-Emmett-Teller (BET) method.

Chemistry

Surface and bulk chemical analysis, including fluorescence spectroscopy, UV–Vis spectroscopy, fluorescence correlation spectroscopy (FCS), Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, electron energy loss spectroscopy (EELS), auger electron spectroscopy (AES), secondary ion mass spectrometry (SIMS), x-ray photoelectron spectroscopy (XPS), inductively coupled plasma mass spectrometry (ICP-MS), nuclear magnetic resonance spectroscopy (NMR) and energy-dispersive x-ray spectroscopy (EDS).

Charge in suspensions

Electrophoresis mobility (from which zeta potential is calculated).

Mass

Quartz crystal microbalance (QCM), differential scanning calorimetry (DSC).

Crystallinity

X-ray diffraction, electron backscatter diffraction (EBSD)

b) *Sampling and dispersion*

Prior to measuring any parameter related to a nanoparticle, it is critical to first prepare a representative sample (Jamting and Miles, 2013). Some of the issues with sampling are:

- ensuring that any sub-sample is representative of the whole sample
- minimising sampling errors
- ensuring that the main sample is well mixed before sampling
- choosing appropriate sampling techniques.

If the nanomaterial is already a dilute liquid suspension, the sampling process is straightforward but care needs to be taken to ensure that the main sample is well dispersed before measurement. If the nanomaterial is in the form of a dry powder, dispersion of the sample must be concerned with:

- choice of a suitable suspendant
- choice of surfactant (if any)
- method of dispersing the powder into the suspendant
- wettability of the dry particles by the suspendant
- suspension stability.

Sample dispersion can also be a challenge, particularly for dry powders. Nanoparticles have a strong tendency to aggregate when dried and some form of energy, such as ultrasonication or vortexing, is often required to break up the aggregates in the suspension. Care has to be taken when using ultrasonication, as high power levels can damage the particles themselves.

There are two ISO standards that deal with the issues related to sampling and dispersion: (ISO, 2007b; ISO, 2001a). These standards, along with other publications (Allen, 1997; Jillavenkatesa et al, 2001; Merkus, 2009) provide guidelines for sampling and dispersion of particles.

The remainder of this report now focuses not only on the characterisation and nanometrology of nanoparticles, but more specifically on measurements of size and shape. The nanometrology of the other parameters listed above (chemistry, charge, mass and crystallinity) will not be considered for reasons of economy.

c) *Reference materials*

The dependence on the instrument for a measurement result, as well as technique and supporting media in nanometrology, means that reference materials (RMs) are very important. They allow the instrument performance to be checked and verified under conditions very similar to the actual measurement conditions. Most of the particle characterization documentary standards referenced in this report recommend regular verification procedures be established.

Reference materials (Joint Committee for Guides in Metrology, 2008) must be sufficiently homogeneous and stable regarding their specified properties, which have been established as fit for intended use in measuring or examining nominal properties. The German Federal Institute for Materials Research and Testing (BAM) has developed a database in collaboration with TC229, which lists RMs with properties at the nanoscale (BAM, 2008).

A Certified Reference Material (CRM) is a reference material accompanied by documentation issued by an authoritative body. It provides one or more specified property values with associated uncertainties and traceabilities using valid procedures (Joint Committee for Guides in Metrology, 2008). Metrological traceability of a technique or measurement may be established using CRMs.

Certified reference materials are one of the most important tools for ensuring appropriate quality and reliability of measurements. Private companies do not typically provide the more complex reference materials for calibration and quality control, such as nanomaterial CRMs, in sufficient variety, quantity and quality. Government intervention is therefore necessary to remove this obstacle to the free movement of goods and innovation. The JRC's institute for Reference Materials and Measurements (JRC-IRMM) develops and markets CRMs for standardization and metrology in nanotechnology (irmm.jrc.ec.europa.eu/).

d) Size and shape techniques and instruments

Measurements of size and the number size distribution are considered the most universally applicable and suitable measurands for nanoparticles (SCENIHR, 2009).

The adequate description of three-dimensional objects, such as nanoparticles, poses a challenge. If the particles are spherical, their size could be described by a single diameter but for non-spherical particles, other descriptors have to be used. Standard terminology and methodology have been developed specifically for this purpose (ISO, 2008a).

A common method is to use the equivalent diameter, namely the diameter of a sphere that produces a response by a given particle-sizing method, which is equivalent to the response produced by the particle being measured. For example:

- volume diameter, x_v, the diameter of a sphere having the same volume as the particle (measurand in, for example, laser diffraction measurements)
- surface volume diameter, x_{sv}, the diameter of a sphere having the same surface to volume ratio as the particle (measurand in, for example, gas adsorption measurements)
- Stokes' diameter, x_{Stk}, the free-falling diameter of a particle in the laminar flow region (measurand in, for example, disk centrifugation measurements)
- projected area diameter, x_a, the diameter of a circle having the same area as the projected area of the particle resting in a stable position (measurand in, for example, microscopy-based image analysis measurements)
- projected area diameter, x_p, the diameter of a circle having the same area as the projected area of the particle resting in random orientation (measurand in, for example, dynamic image analysis measurements)
- perimeter diameter, x_c, the diameter of a circle having the same perimeter as the projected outline of the particle
- Feret's diameter, x_F, the mean value of the distance of parallel tangents of the projected outline of the particle position (measurand in, for example, microscopy-based image analysis measurements)

- Martin's diameter, x_M, the mean chord length of the projected outline of the particle (measurand in, for example, microscopy-based image analysis measurements)
- hydrodynamic diameter, x_h, the diameter of a sphere which has the same drag coefficient as the particle position (measurand in, for example, DLS and PTA measurements)
- radius-of-gyration, xGyr, a measure of the distribution of mass about a chosen axis, given as the square root of the moment of inertia about that axis divided by the mass (measurand in, for example, static light scattering, small angle neutron scattering and small angle x-ray scattering).

As discussed above, particle characterisation techniques comprise three classes: ensemble techniques, single particle analysis techniques and separation techniques. These are discussed further in Appendix 1.

3.4 Conclusion

The proposed APVMA working definition of a nanomaterial must be suitable for both nanopesticides and veterinary nanomedicines. Currently, there is no universal consensus regarding the definition of either a nanopesticide or a veterinary nanomedicine. Several workers (Kah et al, 2013; Kah and Hofmann, 2014; Kookana et al, 2014) have highlighted a number of issues that need to be considered when developing a definition of nanopesticides. For example, the nanomaterial found in a nanopesticide may be either the active ingredient or a non-active 'carrier'; the size may exceed the traditionally accepted upper limit (100 nm) of the nanoscale dimension; and the durability of a nanopesticide varies markedly such that the retention of the nano characteristic may be either transient or persistent. The SCENIHR (2010) report cited earlier notes that 'additional guidance (requirements) specific for the intended regulation' may be necessary. It is proposed that the APVMA working definition of a nanomaterial should have an upper limit to the nanoscale dimension of 100 nm; however, a size range exceeding 100 nm may be required to address human health and environmental safety concerns. The need will be determined on a case-by-case basis. The definition applies to nanopesticides and veterinary nanomedicines for the purposes of both risk assessment and enforceability by the APVMA.

There are challenges associated with nanometrology, particularly in characterising nanoparticle-based nanomaterials. The measurement of parameters such as size and size distributions lead to unique problems, including different measurement methods not providing comparable results. In addition, the analysis of nanomaterials in complex media is especially challenging due to a lack of validated and cost-effective measuring methods.

Using several different techniques that complement each other as well as providing some redundancy is a more suitable approach to better understand nanoparticle systems. Samples of nanoparticles may have very uniform distributions of shapes and sizes, but often they are more complex. The challenge is then to characterise an ensemble of particles by a small number of descriptors such as, for example, size. Also, it is often necessary to use separation techniques. These present the particle ensemble to the measurement technique in such a way that sub-populations can be measured separately.

For most of the techniques presented in this report there are established protocols, such as ISO standards, that can be used to calibrate instruments and verify measurement techniques. These documentary standards also provide a greater insight into the limitations of the applied method. Using RMs and CRMs in combination with test protocols ensures that the instruments are functioning correctly and giving accurate, reliable results.

Detailed information on specific techniques and instruments used to measure the size and shape of nanoparticles is presented in Appendix 1 of this report.

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4 MANUFACTURE OF NANOMATERIALS

Abstract

This chapter describes a variety of manufacturing processes specific to nanomaterials. From a risk perspective, it is important to appreciate that subtle changes in the method of preparation can lead to significant alterations in the physicochemical properties and morphologies of the resulting nanoparticles. Using Green Chemistry and Green Engineering manufacturing methodologies in the production of nano-based agvet chemicals will help reduce their environmental footprint, create less waste, involve safe solvents and renewable raw materials and lead to materials which are safe by design, innocuously breaking down to fewer pollutants. Continuing collaborative interactions with other regulators and drawing on the knowledge of nanotechnology experts will be important in assessing the anticipated risks associated with nano-based manufacturing processes.

4.1 Introduction

In much the same way as manufacturing processes influence the quality of non-nanoscale active constituents, manufacturing processes for nanomaterials can play a significant role in determining how hazardous the manufactured material might be. Manufacturing techniques can dictate particle qualities, including impurity, and are significant for assessing occupational exposure during manufacture and subsequent stages of product life cycle. However, the relationships between manufacturing processes and hazard are not straightforward and it is not possible to make generalisations. What regulators need to be aware of is the possibility that small changes to manufacturing processes may introduce unpredictable hazards.

Regulations authorising the marketing of nanopesticides and veterinary nanomedicines are product-based rather than manufacture or process-based. However, regulators must also consider the handling of these materials as they move through their ‘life-cycle’ from being raw materials to become products that are retailed, used and discarded.

Nanoscale materials may offer advantages over conventional manufactured products and accord with the principles of ‘Green Chemistry’ and ‘Green Engineering’. Explanatory information about these sets of green principles is presented on the US Environmental Protection Agency (EPA) websites at *Green Chemistry and Nanotechnology* and at *Green Engineering for a Sustainable Environment a*s well as in a presentation on nanochemicals by Naidu (2009). The Twelve Principles of Green Chemistry (Anastas and Warner, n.d.) are a guiding framework for the design of new chemical products and processes, applying to all aspects of the process life-cycle from the raw materials used to the efficiency and safety of the transformation, the toxicity and biodegradability of products and reagents used.

The twelve principles are: waste prevention, synthetic methods that consume all starting materials, less hazardous syntheses, chemicals that are safe by design, use of safer solvents and excipients, renewable raw materials, reduction of synthetic by-products, catalytic reagents in favour of stoichiometry, designed to innocuously decay, manufacturing interventions to prevent pollution, and choice of the safest chemical forms to prevent accidents (Anastas and Eghbali, 2010).

A similar set of twelve principles apply to Green Engineering (Anastas and Zimmerman, 2003).The authors note that ‘Green engineering focuses on how to achieve sustainability through science and technology. *The 12 principles of green engineering* (see box) provide a framework for scientists and engineers to follow when designing new materials, products, processes, and systems that are benign to human health and the environment. A design based on the 12 principles moves beyond baseline engineering quality and safety specifications to consider environmental, economic, and social factors’.

The 12 principles of green engineering

Principle 1:	Designers need to strive to ensure that all material and energy inputs and outputs are as inherently nonhazardous as possible.
Principle 2:	It is better to prevent waste than to treat or clean up waste after it is formed.
Principle 3:	Separation and purification operations should be designed to minimise energy consumption and materials use.
Principle 4:	Products, processes, and systems should be designed to maximise mass, energy, space and time efficiency.
Principle 5:	Products, processes and systems should be ‘output pulled’ rather than ‘input pushed’ through the use of energy and materials.
Principle 6:	Embedded entropy and complexity must be viewed as an investment when making design choices on recycle, reuse or beneficial disposition.
Principle 7:	Targeted durability, not immortality, should be a design goal.
Principle 8:	Design for unnecessary capacity or capability (eg ‘one size fits all’) solutions should be considered a design flaw.
Principle 9:	Material diversity in multicomponent products should be minimised to promote disassembly and value retention.
Principle 10:	Design of products, processes and systems must include integration and interconnectivity with available energy and material flows.
Principle 11:	Products, processes and systems should be designed for performance in a commercial ‘afterlife’.
Principle 12:	Material and energy inputs should be renewable rather than depleting.

Source: Anastas PT, Zimmerman JB (2003).

Aside from the need to consider manufacture of the nanoparticles, introducing nanomaterials into conventional manufacturing also needs consideration. Nanomaterials can offer a range of ‘green’ advantages over conventional manufacturing, including waste and by-product reduction, elimination of impurities and more efficient use of chemical resources. They can also introduce novel hazards such as increased physicochemical reactivity and other new risks, particularly around occupational exposure during manufacturing processes and subsequent use.

Various statutory criteria must be satisfied before APVMA will approve active constituents or register products. Manufacturing aspects of nanomaterials are a relevant consideration and thus the APVMA's Manufacturing Licencing Scheme (MLS) applies to conventional and nanoscale veterinary medicines alike. As a result, nanomaterials' manufacturing processes will be assessed and may become a relevant particular requirement for enduring approval of the active constituents.

4.2 Manufacturing risks

Approaches to nanomaterial regulation internationally have not yet resulted in any standardised risk-based approaches around manufacture. Many of the issues arising during manufacture are a result of the different materials needed to facilitate the fabrication process. As the US FDA (US FDA, 2014) notes: 'Changes in the manufacturing process, including use of different solvents, time/temperature conditions and changes to the starting chemicals (eg alternative starting materials, different purity levels or different concentrations of the chemicals used in the process) may change the types and/or quantities of impurities in the final product. Additional agents, such as dispersing agents and surface modifiers, are often used in the manufacture of nanomaterials. These additional agents and impurities should be considered in the safety substantiation for nanomaterials.'

4.3 Methods for manufacturing nanomaterials

Whitesides and Love (2007) have described how nanofabrication methods can be divided into two categories: top-down methods, which carve out or add aggregates of molecules to a surface, and bottom-up methods, which assemble atoms or molecules into nanostructures. There are many specific manufacturing pathways for engineered nanomaterials within these two broad categories.

The bottom-up method starts with atoms or molecules and builds up to nanostructures. This method is used to fabricate both small nanostructures with dimensions between two and 10 nanometres, eg quantum dots and carbon nanotubes and hierarchical structures containing nanoscale dimensions, eg zeolites[13] and metal-oxide frameworks. The bottom-up approach is the way by which all living biological systems are created and many significant mineral deposits are formed.

There are also hybrid processes incorporating both top-down and bottom-up elements.

4.3.1 Top-down nanofabrication

The 'top-down' approach to nanofabrication involves creating 'nanostructures' from a large parent entity via precision engineering and the cutting, etching and grinding of a starting material. The techniques used are derived mainly from those used to fabricate microstructures in the semiconductor industry and involve three important steps:

1. The deposition of thin films/coatings on a substrate.
2. Obtaining the desired shapes via photolithography.
3. Pattern transfer using either a lift-off process or selective etching of the films.

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[13] Zeolites are microporous, aluminosilicate minerals commonly used in commercial absorbents and catalysts.

The four most common approaches to top-down nanofabrication are:

1. Mechanical: cutting, rolling, beating, machining, compaction, milling, atomisation, pearl/ball milling and high pressure homogenisation.
2. Thermal fabrication: annealing, chill-block melt spinning, electrohydrodynamic atomisation, liquid dynamic compaction, gas atomisation, template synthesis and evaporation, sublimination, thermolysis, combustion and carbonisation of copolymers.
3. High-energy and particle fabrication: arc discharge, laser ablation, sputtering[14], solar energy vaporisation, ion milling, electron beam evaporation, reactive ion etching, pyrolysis, combustion and high-energy sonication.
4. Chemical fabrication: chemical etching, chemical mechanical polishing, electropolishing, anodising and combustion.

Advances in top-down nanofabrication techniques yield almost atomic-scale precision and will most likely remain the method of choice for building complex devices for some time to come. Lithography (also known as photoengraving) represents one of the most prominent techniques for top-down nanofabrication. In its simplest form, lithography is a planographic printing process, whereby an image or pattern is transferred to a substrate. A diverse array of lithographic methodologies is available and of these, photolithography is the most widely used, particularly for the mass production of integrated circuits. Top-down nanofabrication via attrition or milling, which yields nanoparticles of variable size (10–1000 nm) and shape/geometry, is used in the pharmaceutical industry to enhance the dissolution of poorly water-soluble compounds and to increase bioavailability of oral dosage forms. The technique is expected to have similar applications in veterinary medicine.

4.3.2 Bottom-up nanofabrication

Bottom-up manufacturing involves atomically precise engineering-chemical synthesis. The five most common approaches are:

1. Gas-phase fabrication: chemical vapour deposition, atomic layer deposition, combustion, thermolysis, metal oxide organometallic vapour phase epitaxy, molecular beam epitaxy, ion implantation, gas phase condensation and solid template synthesis.
2. Emulsification: diffusion and supercritical fluid precipitation.
3. Liquid-phase fabrication: molecular self-assembly, supramolecular chemistry, precipitation, nucleation and sol-gel processes, oxidation or reduction of metal compounds, nanocrystal growth, electrodeposition, electroplating, electroless deposition, anodising, electrolysis in molten salt solutions, solid template synthesis, liquid template synthesis and supercritical fluid expansion.
4. Solid-lithographic fabrication: nanolithography, dip-pen methods, nanosphere template methods, nanopore template methods, block copolymer lithography, local oxidation nanolithography and scanning tunneling or atomic force-microscope writing.

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[14] Sputtering involves the physical vaporisation of atoms from a surface by momentum transfer from bombarding energetic atomic-sized particles.

5. Biological and inorganic fabrication: protein synthesis, nucleic acid synthesis, membrane synthesis, inorganic biological structures and crystal formation methods. Many of the biological fabrication methods are special cases of liquid-phase fabrication with highly specific structure-directing templates.

4.3.3 Hybrid and other processes

Hybrid processes incorporate both top-down and bottom-up nanofabrication methodologies.

Self-assembly involves atoms or molecules arranging themselves into ordered nanoscale structures via physical or chemical interactions. This method of manufacture is used for producing ‘smart’ objects, crystals, films and tubes.

Positional assembly involves the deliberate manipulation of molecules and is reportedly used in the manufacture of modular composite nanosystems (Wong, 2013) and printed electronics (Fachot, 2013).

4.4 Possible production methods for agvet nanomaterials

Although ‘top-down’ or ‘bottom-up’ are convenient process descriptors, within these broad generalisations there are many specific manufacturing pathways for engineered nanomaterials. For the purpose of this chapter, production methods have been allocated to one of seven generic categories:

1. nano/micro- emulsion methods.
2. synthesis by precipitation or chemical reaction.
3. vapour phase synthesis.
4. sol-gel processing.
5. hydrothermal/solvothermal processing.
6. directed assembly methods, eg dendrimers.
7. size reduction methods, eg grinding.

It should be noted that few agvet nanomaterials have been manufactured to date. It is for this reason that human drugs/drug delivery systems and not agvet chemical examples are cited in some of the discussion that follows.

4.4.1 Nano/micro-emulsion methods

Nano/micro-emulsion methods for synthesising nanoparticles involve two steps: (1) generating nanoparticles in a two (or more) phase system; and (2) nanoparticular stabilisation to inhibit bulk phase separation. The latter commonly involves polymerisation, electrostatic charge or the addition of surfactants. Oil-in-water-in-oil (o/w/o) systems and water-in-oil-in-water (w/o/w) systems are variations of the general theme for nano/micro-emulsion methods for nanoparticle synthesis.

In 2007 Letchford and Burt published a review of the formation of amphiphilic block copolymer nanoparticles for use in human drug delivery. The structures generated are micelles, nanospheres, nanocapsules and polymersomes, all of which display distinct structural and physicochemical properties. The advantages offered by these nanoparticulates as drug delivery systems include prolonged circulation, passive targeting and enhanced solubilisation of hydrophobic drugs. Information from a review relating to the generation and stabilisation of four polymeric nanoparticles is presented in Table 4.1.

Table 4.1: Information on the generation of amphiphilic block copolymer nanoparticulate structures (sourced from Letchford and Burt, 2007).

Nanoparticle	Factors to consider	Method of dispersion to obtain the nanoparticles	Means of stabilising the nanoparticles to inhibit bulk phase separation
Micelles	For relatively water soluble copolymers	Direct dissolution[a]	Polymerisation
		Film casting[b]	Polymerisation
	For copolymers that are not readily water soluble	Dialysis[c]	Polymerisation
		Oil-in-water emulsion[d]	Polymerisation
Nanospheres	If polymerisation is required	Emulsification	Polymerisation
	If polymerisation is not required	Solvent displacement (nanoprecipitation)[e]	Polymerisation
		Emulsification/solvent evaporation	Polymerisation
		Emulsification/solvent diffusion	Polymerisation
		Salting out	Polymerisation
Nanocapsules		Interfacial deposition of preformed polymers[f]	Polymerisation
		Modified solvent displacement	Polymerisation
Polymersome		Film hydration[g]	Polymerisation

[a] the copolymer is added to the aqueous media at a concentration above the critical micelle concentration and the drug is allowed to partition into the core of the micelles.

[b] the copolymer and drug are dissolved in a volatile solvent which is then evaporated; warm buffer or water is added to the resultant film with agitation to dissolve the polymer film.

[c] the copolymer and drug are solubilised in a water-miscible organic solvent and micelles are formed by adding water.

[d] a solution of copolymer drug and a volatile non-water-miscible organic solvent is added to aqueous media, with or without a surfactant, that is being rapidly stirred; the solvent is then evaporated.

[e] the polymer is dissolved in an organic, water-miscible solvent which is then added to the aqueous phase in the presence or absence of surfactant; the organic phase diffuses out leading to precipitation of the polymer and formation of nanoparticles.

[f] to a solution of drug in a water-miscible organic solvent, an oil which is miscible with the solvent but immiscible with the mixture is added; upon moderate agitation, the solvent diffuses into the aqueous phase and the polymer aggregates around the oil droplets.

[g] the copolymer is dissolved in a volatile organic solvent such as chloroform; the solvent is evaporated under a stream of nitrogen gas to leave a thin film of polymer which is rehydrated with the aqueous phase with vigorous agitation to yield submicron vesicles.

The following discussion, which cites a paper by Cushing et al (2004), describes the preparation of inorganic nanoparticles using liquid-phase synthesis, a procedure first reported in the early 1980s. Because microemulsions are thermodynamically stable, they are used extensively as micro and nano-reactors. Reverse micelles consist of oil, water, surfactant and co-surfactant; the core and outer shell are composed of water and oil respectively. The small size of reverse micelles subjects them to continuous Brownian motion causing them to collide frequently. Approximately one in 1000 collisions result in short-lived dimers, formed by the expulsion of some surfactant molecules into the bulk oil phase. Two reverse micelles will exchange the contents of their aqueous cores during the estimated 100 nanosecond lifetime of the dimer. This interaction of reverse micelles makes them suitable as nano-reactors. Importantly, the size of the water pool in the cores of reverse micelles can be controlled and the nanoparticulate products of the reactions formed inside the micellar cores have nearly uniform size and shape.

Microemulsion synthetic methods have been reported for metals and metal oxides (by reduction); metal oxides (by co-precipitation of one or more metal ions) and core-shell and onion-structured nanoparticles. Because many metallic nanoparticles, particularly iron, are susceptible to rapid oxidation, gold coatings of these nanoparticles are of particular interest. In addition to addressing oxidation, gold coatings allow the surfaces of metallic nanoparticles to be subsequently functionalised with various organic species by taking advantage of the very strong gold-sulphur bonds. The technique for applying gold coatings on metal nanoparticles is relatively straightforward in reverse micelle synthesis.

4.4.2 Synthesis by precipitation or chemical reaction

Precipitation (or co-precipitation when the process involves the precipitation of multiple species simultaneously) exhibits the following characteristics. The products of precipitation are generally sparingly soluble species formed under conditions of high supersaturation, which usually results from a chemical reaction. Nucleation will be a key step in the precipitation process and results in a large number of small particles being formed. Finally, processes secondary to the precipitation process, such as Ostwald[15] ripening and aggregation, will dramatically affect the size, morphology and properties of the products.

Although precipitation can be induced by numerous methods, precipitation induced by chemical reactions is the most common method for the synthesis of nanoparticles. When the process contains only one or two elements, precipitation reactions are relatively straightforward. In more complicated ternary and quaternary systems, the process becomes more complex as multiple species must be precipitated simultaneously, ie co-precipitation. Co-precipitation reactions involve the simultaneous occurrence of nucleation, growth, Ostwald ripening (also known as coarsening), and/or agglomeration. The processes of nucleation and growth govern the particle size and morphology of products in precipitation reactions. The key to any precipitation process is the degree of supersaturation. When precipitation begins, numerous small crystallites initially form (nucleation) but they tend to quickly aggregate together to form larger, more thermodynamically stable particles (growth). The growth process of the precipitated particles can be either diffusion-limited or reaction-limited; however, the overwhelming majority of precipitation reactions are diffusion-limited. As such, concentration gradients and temperature become the predominant factors determining growth rate as new material is supplied to the particle surface via long-distance mass transfer. Ostwald ripening is the phenomenon by which smaller particles are essentially consumed by larger particles during the growth process.

To produce nanoparticles, the nucleation process must be relatively fast while the growth process remains relatively slow. The formation of particles with a narrow size distribution further requires that the nuclei of all species present form simultaneously and without subsequent nucleation of smaller particles.

The agglomeration of small particles precipitated from solutions is practically inevitable in the absence of a stabiliser. Therefore the presence of a stabiliser at termination of the growth process is particularly important. There are generally two approaches to nanoparticle stabilisation:

1. Steric repulsion between particles caused by surfactants, polymers or other organic species bound to the nanoparticle’s surface (generically referred to as capping ligands or capping agents).
2. Electrostatic (van der Waals) repulsions resulting from the chemisorption of charged species (usually, though not necessarily, H^+ or OH^-) at the surfaces.

Of these two approaches, steric stabilisation is somewhat more common, probably due to issues concerning the chemical stability of the nanoparticles at very high or very low pH values.

Commercial products, which can extend into the <100 nm size scale, are the various yellow-brown ‘Bayferox’ iron oxide pigments, created as by-products of the reduction of nitrobenzene to aniline by metallic iron. These iron oxide pigments are produced in many thousands of tonnes annually and are found in various common household products, ranging from cosmetics to fence-stains.

Green chemistry approaches to production of nanoparticles are also being developed. They rely on using plant products or microbial metabolites as reagents. Therefore, the biosynthesis of a wide range of metal-containing nanoparticles has been developed, using various bacteria, actinomycetes, fungi, yeasts and viruses (Narayanan

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[15] Ostwald ripening is an observed phenomenon in solid solutions or liquid sols that describes the change of an inhomogeneous structure over time ie small crystals or sol particles dissolve, and redeposit onto larger crystals or sol particles.

and Sakthivel, 2010). The biodiversity of microbes, and in particular various extremophiles, offer considerable potential for tailored design and production of nanomaterials. This field is in its infancy, with improvements in microbial cultivation methods and the extraction techniques still needed in order to overcome problems in producing and isolating monodisperse nanomaterials at a sufficiently rapid rate. Natural products, eg plant extracts, tea, coffee, banana, wine, sugar and polyphenols, can also be used as reductants to produce nanoparticles, or as capping agents to stabilise existing nanomaterials (Kharissova et al, 2013). The application of such green processing methods to organic nanomaterials of agvet interest has yet to be explored.

In recent years, quantum dots have stimulated interest in chalcogenide[16] nanoparticles and their syntheses. Precipitates consisting of agglomerated crystallites with widely disparate sizes and shapes occur instantaneously when metal and chalcogenide ions react. Studies by Peng and co-workers (1998) showed two distinct processes occur during precipitation that affect size and size distributions of nanocrystals; these are a focusing phase and a defocusing phase. The precipitation process can also be controlled when the reactions are performed in heterogeneous media such as reverse micelles. More recently, the temporal separation of nucleation and crystal growth has resulted in the formation of nanoparticles with a narrow size distribution. These are important advances since the physical and optical properties of quantum dots are particle size-dependent and therefore tunable.

Topotactic growth[17] of quantum dots and other nanoparticles during liquid-phase synthesis is important in making hybrid structures. Seed crystals are first precipitated and subsequently grown by adding a different reactant. Thus by appropriate choice of reactants, it is possible to create shell-core structures. For example, it is possible to create gold-silver mixed structures, where either gold or silver may form the core and the other metal is the outer shell. These have been designated as Au@Ag or Ag@Au core-shell nanoparticles (Tsuji et al, 2012). Formation of such controlled nanoscale hybrids is important. For example, the photoluminescence (a visual functional property of quantum dots) can be enhanced by using different capping agents and depositing a shell of zinc sulphide around a cadmium selenide core (a 'CdSe@ZnS' nanoparticle).

4.4.3 Vapour phase synthesis

The history of vapour phase condensation (also known as gas phase condensation) for the production of metal nanopowders can be traced to a patent filed by Bottoms in 1930. Briefly, a metal is introduced onto a heated element, which results in rapid melting and the generation of a pre-determined vapour pressure. A gas introduced into the chamber under high pressure moves the evaporated metal away from the heated element, thereby cooling it. The liquid particles of metal formed have nanoscale dimensions and at this stage the particle specifications are determined by the controlled environment. Because the coalescence and agglomeration of particles is a challenge associated with condensation growth, coatings are applied to the particles after they have cooled and solidified.

Spray pyrolysis is another method involving the vapour-phase synthesis of nanomaterials. The method involves an aerosol process that atomises a solution and by a series of steps (evaporation → precipitation → drying → decomposition → sintering[18]) produces solid particles (Iskandar, 2009). Because precipitation only occurs in that

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[16] A chalcogenide is a chemical compound consisting of at least one chalcogen anion and at least one more electropositive element. Although all group 16 elements of the periodic table are defined as chalcogens, the term chalcogenide is more commonly reserved for sulphides, selenides, and tellurides, rather than oxides. Many metal ores exist as chalcogenides.

[17] Topotactic growth describes growth, within a crystal lattice, that involves the displacement or exchange of atoms.

[18] Sintering is the process of compacting and forming a solid mass of material by heat and/or pressure without melting it to the point of liquefaction.

part of the droplet where the solute concentration is higher than the equilibrium saturation, precipitation can be controlled. Porous silica nanoparticles synthesised by spray pyrolysis were investigated for reducing the photodegradation of pesticides (see chapter 1)

Chemical vapour deposition (CVD), which is a method of vapour phase fabrication, involves a chemical reaction in a vapour or gas phase followed by deposition of the reaction product on a heated surface. For the chemical reaction to proceed, activation energy is required and this is provided by one of several different methods. Thermal CVD activates the reaction at a temperature in excess of 900ºC; plasma CVD activates the reaction by plasma at temperatures between 300ºC and 700ºC; laser CVD utilises laser thermal energy to heat an absorbing substrate that results in pyrolysis and photo laser CVD induces the chemical reaction with UV radiation. CVD finds many uses including in the manufacture of semiconductors. However, CVD technology is unlikely to have applications in the manufacture of nanopesticides or veterinary nanomedicines. A variation of CVD, where the vapour of a metal or metal compound is reacted with oxygen in the gas phase at low pressure to produce a condensate of metal oxide nanoparticles, is a commercially established process for a wide variety of nanomaterials. Indeed, such a process is akin to the “chloride route” for producing millions of tonnes of TiO_2 pigment used in paints (the particle size of the TiO_2 produced is ~250 nm, which exceeds the upper limit of 100 nm for the nanoscale).

4.4.4 Sol-gel processing

Sol-gel fabrication is a liquid-phase fabrication method which involves conversion of monomers into a colloidal solution (or sol) that acts as a precursor for an integrated network (or gel) of either discrete particles or network polymers.

Common to most sol-gel processes is a series of distinct steps:

1. Formation of a stable sol solution.
2. Gelation via a polycondensation or polyesterification reaction.
3. Aging of the gel (or syneresis) into a solid mass, accompanied by contraction of the gel network and expulsion of solvent from the gel pores.
4. Drying of the gel to remove liquid phases, which can lead to fundamental changes in the structure of the gel.
5. Dehydration at temperatures as high as 800ºC, during which surface-bound M-OH groups (M in the chemical formula represents a metal) are removed, thereby stabilising the gel against rehydration.
6. Densification and decomposition of the gels at high temperatures (T>800ºC), which collapses the pores of the gel network and volatilises remaining organic contaminants.

Sol-gel methods are commonly used to synthesise oxides and in this context, sol-gel processing refers to the hydrolysis and condensation of alkoxide-based precursors such as tetraethyl orthosilicate. However, the nature of the specific reactions involved in hydrolysis and condensation differs substantially between the various types of precursors.

A modified sol-gel process has been developed for metals that are not suitable for traditional sol-gel type reactions due to their unfavourable hydrolysis equilibrium (Pechini, 1967). An advantage of this method is the lack of a requirement for the metal used to form suitable hydroxo complexes. The primary disadvantage of the method lies in the lack of control over particle size, shape and morphology.

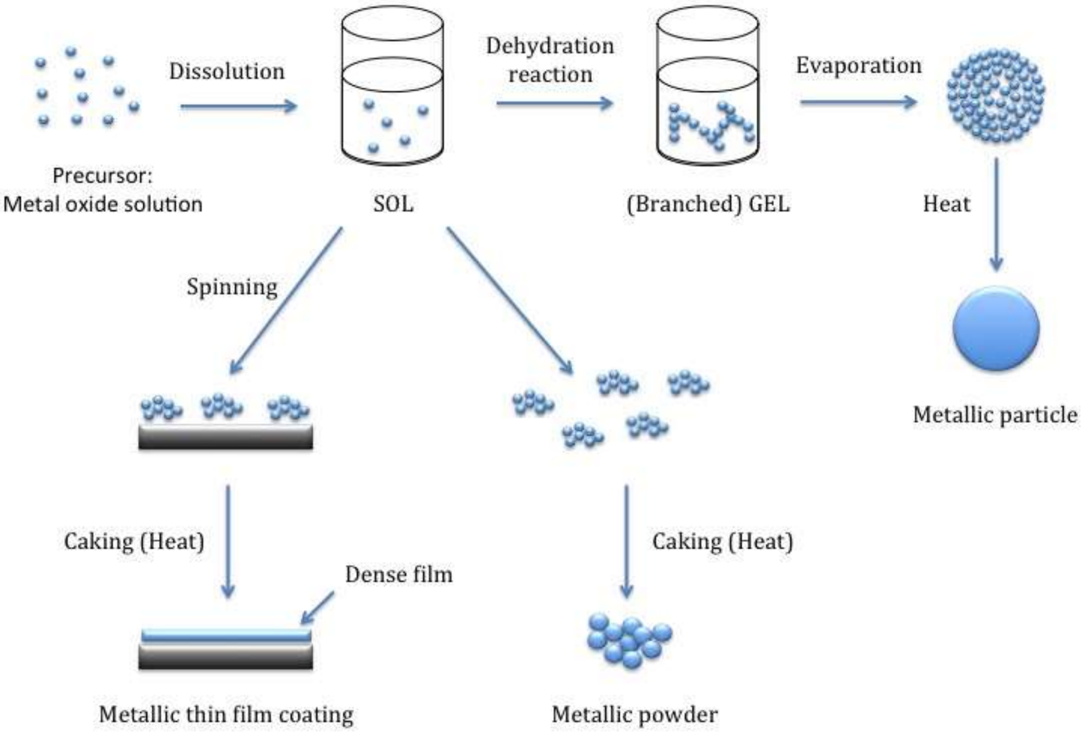

Figure 4.1 The sol-gel process of manufacture (depicted is the fabrication of a metallic thin film coating, metallic powder and metallic particles)

4.4.5 Hydrothermal/solvothermal processing

Hydrothermal synthesis is generally defined as crystal synthesis or crystal growth under high temperature and high pressure water conditions from substances which are insoluble at ordinary temperature and pressure (<100ºC, < 1 atm) (Hayashi and Hakuta, 2010). The term *solvothermal* (rather than *hydrothermal*) is used to describe any chemical reaction in the presence of a non-aqueous solvent. Another term, *supercritical fluid technology* is used when the reactions are performed at temperatures above the supercritical point of the solvent.

Hydrothermal methods allow the synthesis of crystals of substances that are unstable near their melting points. These methods involve closed-system physical and chemical processes flowing in aqueous solutions at temperatures above 100ºC and pressures above 1 atm. Autoclaves are commonly used for this purpose. Under these conditions, substances such as some oxides, silicates and sulphides, that are practically insoluble under normal conditions, can be diluted in water and aqueous solutions. Zeolites, used for water purification and petroleum refining, are produced in many thousands of tonnes by hydrothermal methods.

Hydrothermal technology is used in processing nanomaterials such as metals, metal oxides, and semiconductors, for a wide variety of technological applications. Processing metal oxides under hydrothermal conditions constitutes an important aspect of hydrothermal processing because of its advantages in the preparation of highly monodispersed nanoparticles with a control over size and morphology. The hydrothermal technique offers a unique method for coating various compounds on metals, polymers and ceramics as well as for fabricating powders or bulk ceramic bodies. It is also ideal for processing designer particulates. The term ‘designer particulates’ refers to particles with high purity, high crystallinity, high quality, are monodispersed and with controlled physical and chemical characteristics. The hydrothermal technique is considered the most suitable technique to prepare the materials for advanced drug delivery systems and hyperthermia. These applications require control of the size and shape of the synthesized physicochemical characteristics. Importantly, the hydrothermal process is compatible with green and sustainable chemistry.

Supercritical methods have not yet found extensive application in nanomaterial production. Supercritical water is a favourable particle formation medium; its critical temperature and pressure are 374^{o}C and 22.1 MPa, respectively. Supercritical water (and supercritical fluids) allow the reaction rate and equilibrium to be varied by shifting the dielectric constant and solvent density with respect to temperature and pressure. The dielectric constant and solubility of many compounds change significantly under supercritical conditions. A case in point is the dielectric constant of water which is 78 at room temperature and below 10 under supercritical conditions. Similarly supercritical carbon dioxide (T_c = 31^{o}C, T_p = 7.4 MPa) is used in the pharmaceutical industry to synthesise drugs with controlled particle size. Various supercritical fluid precipitation techniques have been developed, including antisolvent precipitation methods and rapid expansion from supercritical solutions (Rowe and Johnston, 2012).

4.4.6 Directed assembly methods, eg dendrimers

Dendrimers are produced by several methods, two of which are described below.

a) *Divergent-growth method*

This method is depicted in Figure 4.2. The initiator core (shown in black) is reacted with a reagent comprised of a reactive branch unit (shown in grey) with protective groups (shown in blue) to form a first generation dendrimer (also known as a 1G dendrimer). The production of higher generation dendrimers (ie 2G dendrimers and 3G dendrimers) requires the protective groups to be removed and the ‘growth’ reaction repeated until a dendrimer of the required size is formed.

A disadvantage of the divergent-growth method is the number of steps involved.

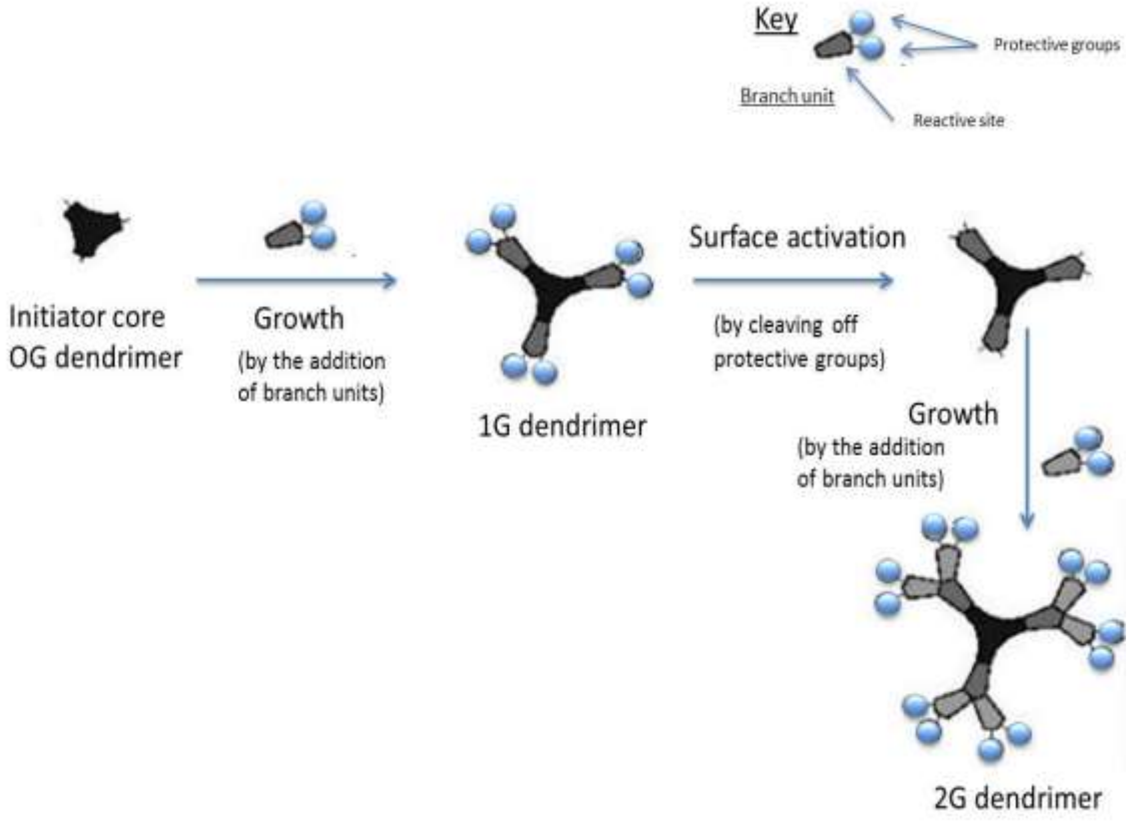

Figure 4.2 The divergent-growth method of dendrimer synthesis.

b) Convergent-growth method

The convergent-growth method for producing dendrimers was developed in response to divergent synthesis being considered too slow. Convergent growth begins at what will be the surface of the dendrimer, and works inwards by gradually linking surface units together. Figure 4.3 depicts a surface unit comprised of a reactive branch unit and surface groups, and a growth unit comprised of a focal point and a reactive branch unit.

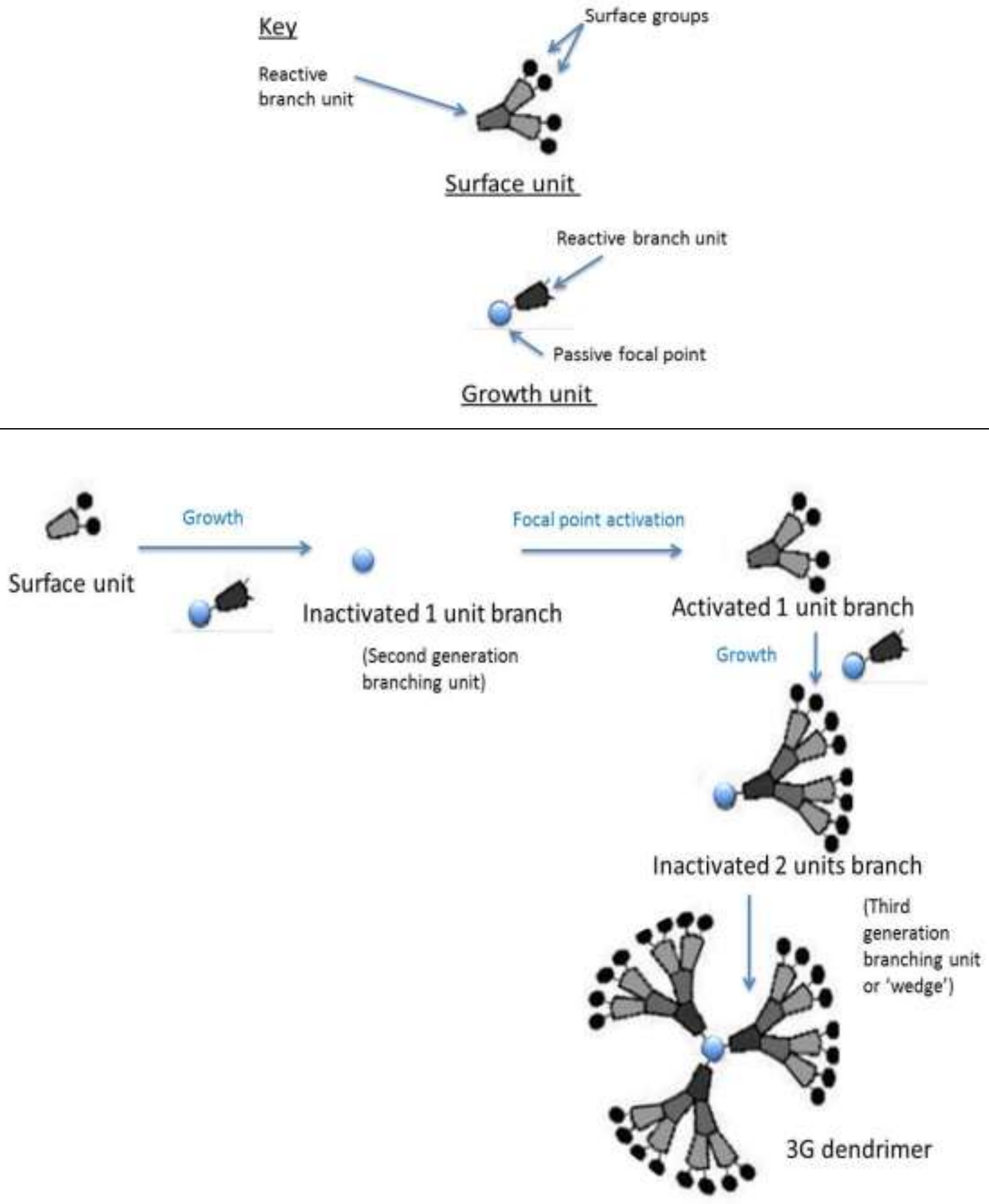

Figure 4.3 The convergent-growth method of dendrimer synthesis.

4.4.7 Size reduction methods, eg grinding

Particle size reduction techniques are widely practised in the agvet sector for the production of fine powders or dispersions, using a wide variety of mill design, eg pin mills, jet mills and ball mills. However, it becomes increasingly difficult to transfer mechanical energy to the material undergoing size reduction when the particle size drops into the sub-micron region (Baláž, 2008). High energy attrition (also called 'stirred media'; Figure 4.4) mills have been specifically designed and are now commercially available to grind materials to nano-dimensions (examples of production mills are accessible online eg buhlergroup.com/global/en/products/bead-mill-micromedia.htm#.VSmp9JO2XyA; www.netzsch-grinding.com/en/products-solutions/wet-grinding/zeta-rs-nano-mill.html#!tabs/technique; www.hockmeyer.com/products/wet-grinding/particle-reduction-mill-detail.html). These agitator bead mills have various designs including annular gap, basket and rotating peg geometries. Although not currently widely used to produce nanosized agvet products, these mills are capable of ready scale-up to enable processing of tonnage quantities and have the potential to produce slurries directly stabilised with various dispersants and excipients (see next section) for end-use application.

Figure 4.4 Laboratory 'stirred media' mill (with permission T.W. Turney)

4.5 Excipients used in agvet nanoformulations

Excipients are incorporated in agvet formulations for many reasons, including:

- to aid in the manufacture of product
- to enhance product performance
- to improve product stability.

Examples of excipients and their function include:

- solubility enhancing agents
- plant penetration enhancing agents
- buffering agents to control pH
- surfactants to inhibit protein adsorption to interfaces
- preservatives to prevent microbial growth
- carbohydrates as bulking agents to facilitate lyophilisation
- polymers to increase solution viscosity
- salts or sugars to stabilise proteins and obtain physiological tonicity and osmolality.

Excellent reviews on excipients in non-nanoformulations and the function of these excipients are available in the scientific literature (Chi, 2012).

All considerations that are relevant to the excipients in conventional agvet chemical formulations apply equally to excipients in nanoformulations. However, the stability of nanoformulations generally requires additional consideration. This reflects the fact that nanoformulations harness the unique properties of nanomaterials, making it critical for particle size to be maintained throughout the shelf life of the product. This is a major challenge for formulation chemists and is usually addressed by using stabilising excipients to prevent agglomeration and/or aggregation of nanoparticles. The discussion that follows focuses principally, though not exclusively, on excipients with a primary function of stabilizing agvet nanoformulations.

Stabilisers (for keeping nanoparticles in suspension)

Many agvet chemical nanoproducts are formulated as suspensions and with these formulations stabilisation is required to prevent agglomeration and/or aggregation of nanoparticles from occurring. Agglomeration/aggregation involves inter-particle forces such as van der Waals and hydrophobic attractive forces, electrostatic and steric repulsive forces. When the net inter-particle force is attractive, agglomeration/aggregation is very likely to occur. By contrast, when the net inter-particle is repulsive, agglomeration/aggregation is unlikely to occur.

Van der Waals forces and electrostatic forces are the best understood of the attractive and repulsive forces, respectively. Van der Waals attractive forces between two atoms or molecules are relatively short range, extending over only a few-tenths of a nanometre. In a particle, however, there is an additive effect on the forces between the 10^6–10^{10} atoms of a particle that can generate a long-range attraction between particles of up to 5–10 nm. It is noteworthy that van der Waals attractive forces are stronger in inorganic nanoparticles than in organic nanoparticles.

Electrostatic stabilisation is commonly used to generate repulsive forces in aqueous media. Because electrostatic stabilisation is generally ineffective in organic media, steric stabilisation is the preferred approach. Steric stabilisation, which generally involves coating particles with a soluble polymer, causes steric repulsion to occur when the coated particles approach each other to a separation distance less than twice the thickness of the polymer layer.

Stabilisers (for maintaining the native conformation of proteins)

Stabilisers act by a number of mechanisms to maintain the native conformation of proteins present in formulations. In nearly all cases, thermodynamically unfavourable excipient-active principle interactions (Chi, 2012) exclude excipients from the protein surface.

Examples of stabilisers include sugars, polyols, amino acids, amines and salting-out salts. Of these, the most frequently used stabilisers in nanoformulations are sugars such as sucrose and trehalose. Polyols, which are carbohydrates excluding sugars, are also used as stabilisers and, in this regard, larger polyols provide better stabilisation than do smaller polyols. Several amino acids including histidine, arginine, glycine, methionine, proline and lysine, as well as glutamic acid-arginine mixtures, are used to stabilise solutions of nanoparticles. A variety of mechanisms account for the improved stability observed with amino acids, one of which involves amino acids allowing for better molecular conformation through linking the amino acids to the nanoparticles (Chi, 2012). The stabilisation of nanoformulations caused by amines and salting-out salts is attributed to electrostatic repulsive forces.

Surfactants

Toxicity is an important consideration when selecting surfactants to control and stabilise the size and shape of nanomaterials. It may lead to alternatives to surfactants being considered for controlling and stabilising nanoparticle size and shape during synthesis and when new functionalities are being incorporated on the particle surface (Van Arnum, 2008; Ray et al, 2009). Non-ionic surfactants are generally non-toxic and are widely used to stabilize nanosuspensions/nanodispersions; to suppress aggregation of nanoparticles; and to control the refolding of protein. Examples of non-ionic surfactants include Polysorbate 80 (Tween 80) and Polysorbate 20 (Tween 20). They have been widely incorporated in marketed protein pharmaceuticals at concentrations in the 0.0003–0.3 per cent range (Chi, 2012).

A separate consideration is the extent to which a surfactant can cover the surface of a nanomaterial without adversely affecting the nanomaterial’s desired effect. A case in point is the use of surfactants such as Bry 35, Triton X-10, Pluronic F12 and sodium dodecyl sulphate (SDS) during nanomaterial synthesis when creating a particular surface property. In order to achieve the desired effect, the surface must not be completely covered by the surface modifier and it may be necessary to monitor the progress of reactions using techniques such as the measurement of zeta potential and hydrophobic interaction chromatography (HIC) analysis.

Polymers

As mentioned above, coating nanoparticles with soluble polymers is a technique used to generate steric repulsive forces—a process known as steric stabilisation. With this approach, it is important to ensure that the extent of polymeric coating does not adversely affect the nanomaterial’s desired effect. Applying this procedure to metallic nanoparticles avoids the aggregation of nanoparticles, and reduces or eliminates the formation of an oxide layer on the surface of the nanoparticles. Examples of the hydrophilic polymers used include chitosan, carbopol, dextran, hydroxyl ethyl starch (HETA), gelatin and PEG-4000 (PEGs with higher molecular weights have been found to be more effective than those with smaller molecular weights). As well as soluble polymers generating steric repulsive forces, the non-polar moieties on certain polymers, such as PEGs and pluronics[19], can decrease water surface tension, which suppresses surface adsorption-induced aggregation.

Where agricultural chemicals are being applied via droplet spraying, small amounts of polymer are often added to control viscosity of the droplet. Such additions can have a substantial effect on the efficiency of delivery apart from just controlling droplet size. Thus, by adding very small amounts of a water-soluble polymer to a dispersion, it is possible to inhibit droplet rebound on a hydrophobic surface and markedly improve deposition without significantly altering the viscosity of the solution (Bergeron et al, 2000).

Coupling agents

Coupling agents are mainly used to modify the surface characteristics of nanoparticles; however, they are also used in the synthesis of nanomaterials. An example of a coupling agent being used for surface modification involves porous hollow silica nanoparticles prone to agglomeration due to the presence of a terminal NH_2 group on the silane structure. Agglomeration in turn leads to an increased rate of hydrolysis which induces particle growth. This can be overcome using the coupling agent, 3-aminopropyltrimethoxysilane (APTMS), in a specific co-condensation process (Kobler and Bein, 2008).

Preservatives

Preservatives are included in formulations to prevent microbial growth, particularly for multi-dose formulations of veterinary medicines. Examples of preservatives include benzyl alcohol, phenol, m-cresol and antioxidants such as sulphites and ascorbic acid. In some situations, preservatives will cause the aggregation of nanoparticles, and this is especially prevalent in those nanoformulations containing proteins.

Chelators

Chelators or sequestrating agents are organic complexing agents that inactivate metallic ions (Knepper, 2003). They reduce the aggregation of gold and other metallic/magnetic nanoparticles. Examples of chelating agents are phosphonates, ethylenediamine-N,N'-disuccinic acid (EDDS) and citric acid.

Thickeners and emulsifiers

These classes of excipients are used to:

- avoid phase separation of emulsions and suspensions
- improve the stability of solutions of nanoparticles
- dissolve or disperse lipophilic drugs
- increase the bioavailability of drugs administered orally.

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[19] Pluronics comprise a diverse group of compounds. An example is pluronic block copolymers (‘poloxamers’) consisting of hydrophilic poly(ethylene oxide) and hydrophobic poly(propylene oxide) blocks arranged in A-B-A tri-block structures. The key attribute for the biological activity of pluronics is their ability to incorporate into cellular membranes and translocation into cells where they affect various cellular functions.

Examples of thickeners and emulsifiers include alginates, saccharose (unstable in acidic solutions), cellulose and Polysorbate 80.

Significant advances in the development of nanotechnology-enabling excipients with the capacity to contribute unique functionality to medical drug products have been reported (Bansal, 2014). Examples include increased bioavailability of poorly soluble active ingredients and the targeting of drugs to specific tissues. While desirable, testing excipient functionality in a formulation or manufacturing process is not always possible. Recently, the European Pharmacopoeia introduced the concept of ‘Functionality Related Characteristics’ (FRC) in the non-mandatory section of the excipient monograph. An FRC is a controllable characteristic of an excipient that is shown to impact its functionality (Bansal, 2014). Since functionality can only be tested in the context of a particular formulation and its manufacturing process, customised FRCs for excipients may be required.

Examples of nanoscale excipients used in human medical products (Bansal, 2014) that, in future, are likely to be used in veterinary medicinal products, are listed below:

Polymeric nanoparticles

- sodium alginate
- gelatine
- polylactic acid
- polyglycolic acid
- dextran.

Solid lipid nanoparticles

- tricaprin
- tristearin
- glycerylmonostearate
- cetylpalmitate
- palmitic acid.

Emulsions

- soybean lecithin
- egg lecithin
- polaxamer 188
- polysorbate 80
- tyloxapol.

Vesicle-based systems

- phosphatidylcholine
- distearoyl

- dipalmitoyl phosphatidic acid
- cholesterol.

Stabilisers

- polyvinylalcohol
- polyvinylpyrrolidone (PVP)
- hydroxypropyl methyl cellulose (HPMC).

Finally, a major challenge in developing bio-functionalised nanoparticles is the limited stability of immobilised proteins during production and storage and, most importantly, the loss of biological function during sterilisation. A report by Tscheliessnig and co-workers (2012) described the development and characterisation of a nano-coating formulation capable of maintaining the functionality of proteins on biological-device combination products, including during terminal sterilisation. This research may offer a solution to these challenges.

In conclusion, various methods have been used to synthesis nanomaterials that involve two main approaches: top-down (or physical) and bottom-up (or chemical). Hybrid processes incorporate both of these methods. In this chapter, these broad categories have been assigned to one of seven generic categories:

- nano /micro-emulsion methods
- synthesis by precipitation or chemical reaction
- vapour phase synthesis
- sol-gel processing
- hydrothermal/solvothermal processing
- directed assembly methods
- size-reduction methods.

To date, the number of nanoscale agvet chemicals manufactured using these methods is very small, though this situation is likely to change in the immediate future. It is critically important that the synthesised nanomaterials consistently demonstrate the desired physiochemical characteristics. In order to assess the anticipated risks associated with nano-based manufacturing processes, the APVMA will continue collaborative interactions with other regulators and draw on the knowledge of nanotechnology experts.

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5 NANOMATERIALS IN AGRICULTURAL AND VETERINARY CHEMICALS: HUMAN HEALTH RISK ASSESSMENT CONSIDERATIONS

Abstract

Nanotechnology promises significant benefits for mankind, but gaps in contemporary knowledge about how nano products behave require new thinking in relation to their potential for harm and how the novel risks they pose should be assessed. International and national agencies, such as the APVMA, are adapting conventional risk assessment methodologies and creating new ones as they investigate the possibility that some engineered nanoparticles may pose a threat to human health. Scientists believe that existing risk assessment procedures are adequate for evaluating the toxicokinetics and toxicodynamics of nanoparticles at present. However, they understand that the rapid expansion of the nano industry is leading to the creation of new nanomaterials with more complex behaviour and thus some modifications to toxicity testing endpoints may be required. Current risk assessment protocols will also need to be refined and expanded as our knowledge of nanomaterials increases.

5.1 Introduction

Nanotechnology provides the opportunity to manufacture a diverse group of materials with properties that offer potential benefits for use in agricultural and veterinary chemical products. However, there has been some concern about the potential risks that these materials may pose to human health and the environment. One of the key concerns is that nanoparticles may cause harm in a manner that is not assessable or predictable, based on current approaches to risk assessment.

This overview of the available literature on the toxicokinetics and toxicology of nanoparticles will consider the suitability of the current risk assessment paradigm and toxicity testing methodologies. The risk assessment model depicted in Figure 5.1 is an adaptation of the classical human health risk assessment framework. By identifying the main stages of the risk assessment paradigm, it highlights the regulatory considerations applicable to the potential human health risks associated with the use of nanotechnology-enabled products in agriculture and animal husbandry. This chapter will provide general guidance about assessing human health risks associated with using nanomaterials in agricultural and veterinary chemicals; the intended audience includes those involved in their regulation and their manufacture.

It is generally agreed that increased surface area, altered surface chemistry, and improved dissolution of nanoparticles potentially create a toxicity profile that deviates from conventional materials of the same composition. Therefore, regulators may need to give greater consideration to nanoparticle ingredients that remain in particulate form in the final product. This may include, for example, more or different testing to characterise the physicochemical properties of the test material, as well as the material’s properties in dosing suspensions.

Conversely, there is less likelihood of novel toxicities due to nanoparticles if they are soluble in the product formulation. Similarly, there is little likelihood of nanoparticle-related toxicity if they rapidly undergo dissolution or biodegradation in, for example, water, lipid, food or feed, or biological fluids to form soluble non-nanoform degradation products. The toxicity of these materials would then be due to the constituent ions or monomers or metabolites, ie novel toxicities would not be anticipated since the solubilisation removes the particulate nature of the material. These materials can be assessed using conventional risk assessment processes and methodologies.

In conducting this review of human health, available documents related to nanoparticle risk assessments produced by other national and international agencies and bodies such as the Therapeutic Goods Administration (TGA), the National Industrial Chemicals Notification and Assessment Scheme (NICNAS), Safe Work Australia, United States Food and Drug Administration (US FDA), European Medicines Agency (EMA, formerly the European Medicines Evaluation Agency [EMEA]), Food and Agriculture Organization of the United States (FAO), World Health Organization of the United Nations (WHO) and European Food Safety Authority (EFSA) have been considered. The recent work of the OECD Working Party on Manufactured Nanomaterials (WPMN), which has conducted one of the most comprehensive nanomaterial research programs into the health and safety of nanoparticles, has also been considered.

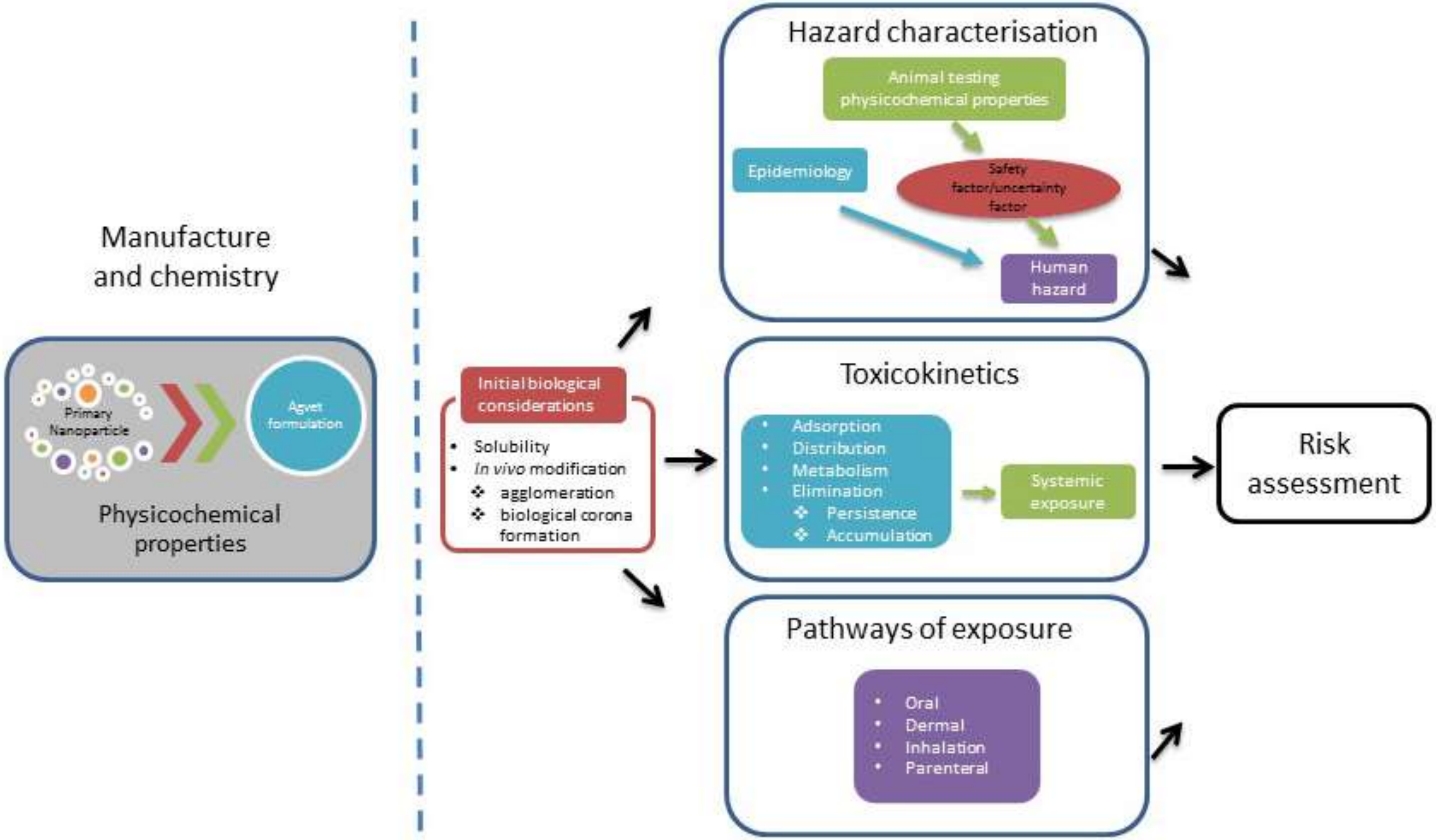

Figure 5.1: Graphic depicting, from a risk assessment perspective, the linkages between hazard characterisation, toxicokinetics, and pathways of exposure.

5.2 Applicability of the current risk assessment framework

The current risk assessment framework for human health includes four main steps: hazard identification, hazard characterisation, exposure assessment and risk characterisation. A number of international agencies and bodies have evaluated the applicability of this framework and found it to be generally appropriate to address risks posed by nanomaterials, although some modifications in methods are anticipated (COT 2005, 2007; EFSA, 2009, 2011; FAO/WHO, 2009; US FDA, 2007; OECD, 2013a, 2013b; SCENIHR, 2005, 2007a).

The current risk assessment framework has been applied successfully to a number of nanoscale materials in the food sector including cyclodextrins, silicon dioxide, large structured molecules and polymers (FAO/WHO, 2009). The United States (US) and European Union (EU) have similarly approved medical products composed of a range of nanoscale materials using the framework. Relevant examples include liposomes (Ambisome®, Doxil®, Visudyne®), nano-emulsions (Diazemuls®, Diprivan®, Intralipid®), micelles (Taxol®, Konakion MM®, valium MM®), polymer protein conjugates (PegIntron®, Somavert®) and polymeric substances (Copaxon®) (EMEA, 2006; FAO/WHO, 2009).

5.3 Adequacy of existing test guidelines

The OECD WPMN reviewed the OECD Test Guidelines for the Safety Testing of Chemicals to determine their applicability to manufactured nanomaterials (OECD, 2009a). It found they are generally applicable for investigating the health effects of manufactured nanomaterials. However, more consideration should be given to characterisation of the physicochemical properties of the materials. In some cases, test guidelines should also be modified, particularly in relation to toxicokinetics and inhalational toxicity testing. Those recommendations are addressed in subsequent sections (refer to Sections 5.6.6 and 5.7.9, respectively).

The WPMN concluded that the toxicological endpoints prescribed in the current test guidelines are applicable to the testing of nanoparticles (OECD, 2009a). However, the OECD notes that the ‘frameworks and other management systems may need to be adapted to take into account the specific properties of manufactured nanomaterials’ (OECD, 2013a, 2013b).

Toxicological endpoints generally apply to the whole of an organism or tissue investigated, eg histopathology, and therefore should equally apply to soluble chemicals and nanoparticles. However, the WPMN recognised that future research may identify modes of action unique to nanoparticles; thus some modifications to toxicity testing endpoints may be necessary.

5.4 Physicochemical characteristics

The reader is referred to Chapter 3 for a detailed discussion on the physiochemical characterisation of nanomaterials.

Adequate particle characterisation is a necessary element in assessing the potential toxicity of nanoparticles to biological systems. It is important to ensure that the material used in toxicology studies has properties within the range of the material for which approval is sought. The physicochemical properties of the naked particle and the nanoparticle in the final product, as well as any changes that may occur through the product’s life-cycle, should be addressed.

Particle characteristics that may trigger the need for toxicological testing include: particle size, size distribution, aggregation, agglomeration state, shape, chemical composition, surface area, surface chemistry, dissociation constant, crystal structure, surface charge, zeta potential, Hamaker constant[20], interfacial tension and porosity (OECD, 2010a, 2012b). The relevant particle characteristics are determined on a case-by-case basis, depending on the nature, functionalities, and intended uses of the material. From a manufacturing perspective, it is critically important that the nanomaterials being synthesised consistently demonstrate the desired physiochemical characteristics.

5.4.1 Sample preparation for safety testing

Due to the wide variety of nanomaterials, it is difficult to develop advice on sample preparation that is applicable to all nanomaterials. Nonetheless, sample preparation is a critically important consideration for toxicity studies to minimise uncertainty and bias, and to yield meaningful and reproducible results. For instance, the choice of stirring versus sonication during sample preparation can result in markedly different results. This is the context in which the OECD (2012b) developed guidance on sample preparation for the safety testing of nanomaterials; this guidance document applied to water insoluble manufactured nanomaterials but not to soluble nanomaterials, which are considered unlikely to need different sample preparation compared to conventional chemicals.

The behaviour of the nanoparticles in dosing suspensions is also critical to interpreting toxicity studies. Nanoparticles can agglomerate or aggregate to form larger structures when dispersed in air, food/feed, liquid vehicles and biological media. Batch variations and ageing effects may also be more significant for nanoparticles than small molecules. Other factors, including nanoparticles adhering to the walls of vessels, may result in significant overestimation of exposures.

5.5 Dose metrics

Dosimetry refers to estimating or measuring the amount (in terms of mass, number, surface area, volume etc) of a particle at a specific biological target site at a particular point in time (OECD, 2012b). Any of the possible dose metrics may be sufficient to describe the dose-response for a given particle type and size.

The relationship between toxicity and various dose metrics is a subject of continuing discussion in the scientific community (Oberdörster et al, 2005b; Warheit et al, 2005; 2006; Donaldson and Poland, 2013; Pauluhn, 2009). Despite reviews of this issue it is not yet possible to recommend one dose metric over another to fit all cases (Seaton et al, 2010; Maynard et al, 2006).

Doses are generally expressed as mass per kg body weight or mg/m^3. However, other interrelated dose metrics, including particle number or surface area, may also need to be considered when describing nanoparticle dose-response relationships. On that basis, it is desirable to characterise the properties of the nanoparticle sufficiently (as above) to provide information so the mass dose can be converted to other metrics, if relevant. This may be useful when comparing the toxicity of a nanoscale particulate material with a conventional (non-nano) material of the same composition.

Scientific justification for the selection of the most appropriate dose metric should be provided.

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[20] The Hamaker constant provides a means of determining the Van der Waals particle-particle interaction while ignoring the influence that the intervening medium between the particles has on this interaction.

5.6 Toxicokinetics

A sound knowledge of the toxicokinetics[21] of nanoparticles is critical to understanding potential novel toxicities. It is an area generally recognised as under-researched for both nanoparticles and conventional particles, and presents challenges not typically encountered for soluble chemicals.

A summary of the available literature on the toxicokinetics of nanoscale and microscale particles following inhalational, oral and dermal exposure is presented below. In general, the available data indicate that the respiratory tract, gastrointestinal tract and skin represent substantial barriers to the absorption of nano and microscale particulates.

5.6.1 Inhalation

A number of comprehensive reviews are available on the fate of insoluble particles in the respiratory tract (eg Geiser and Kreyling, 2010; Hagens et al, 2007; Hoet et al, 2004; Johnston et al, 2013; Landsiedel et al, 2012; Oberdörster et al, 2005a; Pauluhn, 2009; Rogueda and Traini, 2007).

The relative disposition of inhaled particles in the nasopharyngeal, tracheobronchial and alveolar regions of the respiratory tract depends on particle size, species and the structure of the respiratory tract. Moreover, the relationship between size and disposition is complex and may be non-monotonic, depending on the region of disposition; Oberdörster et al (2005a) describes the predicted fractional deposition of inhaled particles in the nasopharyngeal, tracheobronchial, and alveolar region in the human respiratory tract following nose breathing.

The International Commission on Radiological Protection (ICRP) model (1994) predicts that approximately 90 per cent of inhaled 1 nm particles are deposited in the nasopharyngeal region, 10 per cent are deposited in the tracheobronchial region, while deposition in the alveolar region is negligible. Conversely, 5 nm particles are distributed relatively evenly in the three sections, while approximately 50 per cent of 20 nm particles may be deposited in the alveolar region (Oberdörster et al, 2005b).

Following deposition in the respiratory tract, deposited particles are cleared either by chemical dissolution, or a physical translocation of particles (Oberdörster et al, 2005b). Particles which readily undergo dissolution can dissolve in lung fluid and act locally, or be absorbed systemically (Rogueda and Traini, 2007). Relatively insoluble nanoparticles are removed mainly by luminal macrophages and neutrophils, which internalise particles and degrade them, or carry them to the mucociliary escalator (Landsiedel et al, 2012; Rogueda and Traini, 2007).

The mucociliary escalator is an efficient transport system which pushes the mucus and trapped solid materials towards the mouth. The process of phagocytosis takes place within 6–12 hours, but the subsequent clearance is much slower. A retention half-time of solid particles in the alveolar region based on this clearance mechanism has been estimated to be approximately 50–70 days in rats in non-overloading conditions, and up to 700 days in humans (Rogueda and Traini, 2007; Oberdörster et al, 2005b; Pauluhn, 2009).

Several studies in rodents have demonstrated that microscale and nanoscale particles can translocate from the lung to the pulmonary interstitium and local lymph nodes. This is not a nano-specific effect and has been observed

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[21] Toxicokinetics is the study of the absorption, distribution, metabolism and excretion of potentially toxic substances from the body. It essentially has the same meaning as pharmacokinetics but does not relate to pharmaceutical substances.

for larger particles and fibres, such as asbestos (reviewed in Donaldson and Poland, 2013). While the translocation of particles to local lymph nodes and the pleural cavity is well accepted, it has been a subject of debate as to whether insoluble nanoscale nanoparticles deposited in the lung are able to translocate to any significant extent to other secondary organs.

Some recent studies have attempted to measure particulate translocation from the lungs to secondary organs:

- Kreyling et al (2009) conducted inhalation studies in rats to estimate the amount of iridium (Ir) and carbon (C) nanoparticles translocated from lungs to the blood and secondary organs. Nanoparticles composed of chain aggregates (and agglomerates) with primary particle sizes of 2–4 nm (Ir) and 5–10 nm (C) were labelled with ^{192}Ir. Rats were exposed via inhalation for 1 h and radioactivity was measured in the lungs, blood, liver, spleens, kidney, heart, brain and carcass. Virtually all radioactivity was recovered in the lungs at 24 h. Recovery of radioactivity in the liver, spleen, kidneys, heart, brain and bone was low (<0.05 expressed as a fraction of radioactivity recovered in tissues and excreta). In another study, Kreyling et al (2010) found that approximately 2 per cent of 20 nm TiO_2 nanoparticles deposited in the lungs of rats were translocated to secondary organs.
- Klein et al (2012) reported the results of organ disposition of a number of different nanoparticles administered by inhalation in a series of short-term inhalation toxicity studies. Concentrations of TiO2, cerium oxide (CeO_2), Al-doped CeO_2, polymer-coated synthetic amorphous silica and barium sulfate ($BaSO_4$) were measured in the lung, mediastinal lymph node, liver, kidney, spleen, brain (olfactory bulb) and blood. The authors observed that, in general, test materials were not measured in tissues other than the lymph nodes at concentrations above the detection limit (0.5 µg per tissue). Polymer-coated synthetic amorphous silica was the exception and was detected in the spleen but the measured concentration was not reported.
- Semmler-Behnke et al (2008) reported that 24 h after intra-tracheal instillation of 18 nm gold nanoparticles, approximately 99.8 per cent of the administered dose was retained within the lungs. Conversely, for 1.4 nm gold nanoparticles approximately 91.5 per cent of the dose was retained in the lungs and 8.5 per cent of the dose was recovered in secondary organs (lung, liver, blood, spleen, skin and carcass).
- Nemmar et al (2002) demonstrated an apparent rapid absorption of inhaled 99mTechnetium-labelled carbon nanoparticles from the lungs of healthy humans. However, these findings could not be replicated in similar studies using the same label (Brown et al, 2002; Hagens et al, 2007; Wiebert et al, 2006; Mills et al, 2006). Similarly, in a more recent study, Moller et al (2008) found that most inhaled ultrafine carbon particles are retained in the lung periphery and in the conducting airways, without substantial systemic translocation or accumulation in the liver after 48 hours. As such, it can be reasoned that the studies of Nemmar et al (2002) reported the absorption of 99mTechnetium species rather than the 99mTechnetium-radiolabelled nanoparticles (reviewed in Hagens et al, 2007).

Together, the available data suggest that systemic translocation of nanoparticles following deposition in the lung is generally very low at non-inflammatory exposure levels.

5.6.2 Oral exposure studies

Several comprehensive reviews are available on particulate absorption from the gastrointestinal tract (GIT), mainly focused on the potential use of nano and microparticles for oral delivery of drugs and vaccines (des Rieux et al, 2006; Florence and Hussain, 2001; Hussain et al, 2001; O'Hagan, 1996; Shakweh et al, 2004). Other investigators have focused more generally on the potential human health hazards that may be associated with absorption of insoluble nanoparticles from the gastrointestinal tract (Hagens et al, 2007; Hoet et al, 2004; Landsiedel et al, 2012; Oberdörster et al, 2005b).

Conceptually, in order to be absorbed intact from the GIT, a particulate administered in the diet (nanoscale or microscale) must first resist dissolution and degradation in the stomach and intestine and be able to pass to the apical surface of the epithelial cells lining the mucosa. Dissolution can be defined as a dynamic process by which a particle, which has some solubility in the local environment, goes into a solution phase to form a homogenous mixture. The rate of dissolution is influenced by size, solute concentration, surface area, surface morphology, surface energy, dissolution layer properties and aggregation (reviewed in Borm et al, 2006).

The gastrointestinal epithelium is permeable to substances of low molecular weight, including monomers of nutrients such as amino acids, fatty acids and saccharides, but relatively impermeable to macromolecules and particles. This low permeability means that most insoluble material has poor oral bioavailability and passes through the gastrointestinal tract to be eliminated from the body unchanged. Nevertheless, evidence accumulated over the past 40 years appears to indicate that some, albeit generally low, absorption from the gastrointestinal tract does occur (des Rieux et al, 2006; Florence and Hussain, 2001; Hussain et al, 2001; O'Hagan, 1996; Shakweh et al, 2004).

The mechanism of absorption is generally considered to involve M-cells, which are associated with submucosal lymphoid follicles of Peyer’s patches (PP) and other sites of gut-associated lymphoid tissue (GALT) (reviewed in Gebert et al, 1996). M-cells, named for their micro-fold and membranous appearance, are specialised epithelial cells which sample gut antigens. There is also some evidence that small amounts of nanoscale materials may be absorbed through normal columnar epithelial cells. Some relevant examples using different nanoscale particulates and experimental protocols are described below.

In a 2012 study, radiolabelled gold nanoparticles of different sizes (1.4–200 nm) with negative surface charge, and 2.8 nm nanoparticles with opposite surface charges, were administered by intra-oesophageal instillation to rats (Schleh et al, 2012). Radioactivity was measured in selected organs and excreta for 24 h by gamma-spectroscopy. Approximately 99.6 per cent or greater of administered radioactivity was associated with the gastrointestinal tract or faeces. The maximum absorption (0.37 per cent of administered radioactivity) was observed for negatively charged 2.8 nm gold nanoparticles. This is the only identified study in which the administered dose was completely recovered. However, the results of earlier more qualitative studies described below, were generally consistent in observing a low level of gastrointestinal absorption.

Mice received 20 ppm of 4, 10, 28 or 58 nm gold particles in drinking water for seven days ad libitum. The mice were killed and tissues including blood, brain, lung, heart, kidney, spleen, liver, small intestine and stomach samples were collected. Elemental gold was found above background levels in all nine tissues sampled for 4, 10 and 28 nm particles. Gold was not found in most tissues for 58 nm particles. Concentrations of gold in tissues ranged from approximately 5 ng/g in the brain to about 75 ng/g in the kidney for 4 nm particles. The authors reported that gold particle uptake occurred through single enterocytes that had died, and were in the process of being excluded from the villi. Uptake of gold particles was not seen through or between normal enterocytes, nor was it seen to occur through M-cells in the PP region of the ileum (Hillyer and Albrecht, 2001).

Titanium dioxide (TiO_2) particles (rutile; 500 nm) were administered by gavage as a 12.5 mg/kg bw suspension to rats, daily for 10 days. Urine and faeces were collected daily. Animals were killed 15 h after the final dose and the stomach, intestine, colon, peritoneal tissue, liver, spleen, kidney and heart were removed. Analysis was by light microscopy, scanning electron microscopy (SEM) and inductively coupled plasma-atomic emission spectroscopy (ICP-AES). Microscopic examination found TiO_2 particles in the PP, connective tissues of the mesentery network and the mesenteric lymph node. Particles were also found in the Kupffer cells of the liver, but not in the kidney, heart or in lung macrophages. Using ICP-AES, the authors estimated that total systemic uptake of particles was approximately 6.5 per cent of administered dose taking into account values in blood (0.02 per cent), PP and mesentery network and nodes (2.9 per cent), liver (2.4 per cent), lungs (1.2 per cent), spleen (0.02 per cent) and heart (0.04 per cent) (Jani et al, 1994).

Polystyrene spheres (50, 100, 300, 500, 1000 and 3000 nm) with covalently-linked fluorescein were administered by oral gavage to rats at a dose of 1.25 mg/kg bw for 10 days. Stomach, small intestine, colon, liver, spleen, heart, blood, kidney and lungs were analysed for the presence of polystyrene by gel permeation chromatography. Uptake of polystyrene into the PP was observed for all particle sizes. Distribution to liver, spleen, blood and bone marrow showed size dependence, and was estimated to be up to approximately 6 per cent of administered dose for 50 nm particles; 1 per cent for 1000 nm and 0 per cent for 3000 nm particles (Jani et al, 1990).

In a longer term study, Joel et al (1978) investigated the uptake of carbon particles (estimated to be of diameter 20–50 nm) from the intestinal tract of mice following gavage for 2–8 days, or administration in the drinking water (1.5 per cent carbon suspension) for up to 12 months. Following gavage, carbon particles were identified in the PP but not in other tissues, including the mesenteric lymph nodes. After exposure in drinking water for two months, carbon particles were observed in the PP and the mesenteric lymph nodes, but not in the liver, spleen or lungs. Particles remained in PP and macrophages in the mesenteric lymph node for four months after exposure had ceased. Similar findings were observed after six months but there was some equivocal evidence of carbon particles in the liver, and macrophages containing carbon particles were frequently seen in the lung. There were no remarkable differences at 12 months.

Limited observations supporting intestinal absorption of non-biodegradable particulate material are also available for humans. Powell et al (1996) reported the presence of granular pigment in GALT from surgically resected intestinal tissue. Pigmented macrophages containing numerous phagolysosomes, rich in submicron-sized particles, were characterised by morphological assessment, X-ray microanalysis and image electron energy loss spectroscopy to be: (1) spheres of TiO_2 (anatase 100–200 nm); (2) aluminosilicates characteristic of the natural clay mineral kaolinite; and (3) mixed environmental silicates without aluminium (100–700 nm in length). Böckmann et al (2000) also reported that TiO_2 nanoparticles (160 and 380 nm) administered to human volunteers resulted in generally increased blood titanium levels for up to 24 h, which appears to provide some qualitative evidence that non-biodegradable particles can be absorbed across the intestinal mucosa and reach the systemic circulation in humans.

5.6.3 Dermal absorption studies

The potential for nanoscale materials to be absorbed across the skin has received considerable attention due to their potential for use in sunscreens, cosmetics and therapeutics (Desai et al, 2010; Hagens et al, 2007; Landsiedel et al, 2012; Nohynek et al, 2007; Prow et al, 2011; Stern and McNeil 2008; TGA, 2013). In general, the consensus is that healthy skin represents a substantial barrier so the penetration of most environmental nanoparticles, including viruses, dusts, allergens and other materials is negligible. On that basis, it has been argued that, if nanoparticles are considered to resemble macromolecules of high molecular weight, significant absorption from healthy skin is considered unlikely (Maynard et al, 2011).

Zinc oxide (ZnO) and TiO_2 particles into animal and human skin appear to be the most studied of all nanoparticles in experimental studies of penetration due to their common use in sunscreens. The weight-of-evidence suggests that nanoparticles applied to the skin only penetrate into hair follicle openings and skin furrows, and that little material penetrates below the surface of the stratum corneum (Nohynek et al, 2007). This conclusion is supported by the European Commission’s Scientific Committee on Consumer Safety (SCCS, 2012) which concluded there was no evidence that ZnO nanoparticles are absorbed through the skin.

Similarly, a recent review of the scientific literature by the Australian TGA found that the current weight of evidence suggests TiO_2 and ZnO nanoparticles do not reach viable skin cells or the systemic circulation, and that a potential for harm has not been demonstrated in short-term studies (TGA, 2013). This review considered the results of an Australian study which investigated the potential for systemic exposure of humans to nano-ZnO in a sunscreen formulation over a period of five days (Gulson et al, 2010). It concluded that while the results indicated increased levels of ^{68}Zn (based on changes in $^{68}Zn/^{64}Zn$ ratio) from ^{68}ZnO particles, the analytical method could not differentiate between nano-particulate or ionic zinc.

There is some evidence suggesting that certain nanoparticles may penetrate into or through skin under particular conditions. For example, Ryman-Rasmussen et al (2006) found that quantum dots (QD; particle size 4.6 or 12 nm) with neutral or cationic coatings may penetrate into the epidermis or dermis of intact porcine skin at alkaline pH, whereas QDs with anionic coating penetrated to a small extent into the epidermis after 24 h of exposure.
This finding was further investigated in a study in which QDs with three surface modifications, polyethylene glycol (PEG), PEG-amine (PEG-NH2) and PEG-carboxyl (PEG-COOH), were evaluated for human skin penetration from aqueous solutions at pH 7.0, 8.3 (PEG, PEG-NH_2) and 9.0 (PEG-COOH). There was some penetration into intact viable epidermis of skin for the PEG-QD at pH 8.3 but not at pH 7.0, and no penetration into living skin was observed for any other QDs at the pHs used. However, all QDs penetrated through the viable epidermis and into the upper dermis within 24 h following tape-stripping of the stratum corneum (Prow et al, 2012).

Overall, while there is no evidence of significant penetration by nanoparticles in sunscreen through healthy human skin, there is some evidence that under certain conditions, including alkaline pH and compromised skin conditions, some limited penetration of certain nanoparticles through the stratum corneum to the viable dermis is possible. However, the toxicological significance of this finding is unclear, given the limited amounts of dermal penetration likely to be encountered under normal conditions.

5.6.4 Parenteral administration

Typically, naked (uncoated) nanoscale particles are removed from the circulation in a matter of minutes and sequestered into organs of the mononuclear phagocyte system (MPS) (Owens and Peppas, 2006).
Most nanoscale and microscale particles, upon contact with biological matrices, are immediately coated by proteins, leading to the formation of a protein ‘corona’. These coronas are complex and variable and of the approximately 3700 proteins in the plasma proteome, about 50 have been identified in contact with various nanoparticles. Their presence creates a molecular signature which is recognised by immune cells and determines the bio-distribution (reviewed in Aggarwal et al, 2009).

Significant differences can be observed in the binding of blood proteins and opsonins[22] to nanoscale particles, depending on surface properties of the particle (Moghimi et al, 2005). Whether nanoparticles bind proteins at all depends mainly on the surface characteristics, primarily hydrophobicity and charge. Other factors including core constituents, size, shape and curvature, are reported to mainly influence the amount of protein bound, but not protein identity (Aggarwal et al, 2009). Thus, differential opsonisation may account for differences in clearance rates and macrophage sequestration of particulates (Moghimi et al, 2005).

Shinohara et al (2013) administered Degussa P25 TiO_2 nanoparticles by intravenous injection to rats at a dose of 0.95 mg/kg bw. Blood concentrations of TiO_2 were 420 ng/mL and 19 ng/mL at five and 15 minutes after administration (equivalent to only 2.8 per cent and 0.13 per cent of the administered dose, respectively) indicating rapid clearance from the blood. At 6 h, the highest concentrations of TiO_2 were found in the liver (94 per cent of administered dose) followed by the spleen, lung, kidney, heart and blood with 2.0 per cent, 0.17 per cent, 0.023 per cent, 0.014 per cent and 0.026 per cent respectively. TiO_2 concentrations in the liver and spleen remained relatively constant over the 30-day experimental period. Elimination rates of TiO_2 in the faeces and urine did not differ significantly from controls.

Fischer et al (2006) administered QDs (cadmium-selenium; CdSe core; zinc sulfide; ZnS shell; coated with mercaptoundecanoic acid [QD-LM] or bovine serum albumin [QD-BSA]; hydrodynamic diameter (HD) 7–25 nm and 80 nm, respectively) intravenously to rats and measured blood and tissue levels, at time points up to 90 minutes. Faeces and urine were collected for up to 10 days. Plasma levels decayed according to first-order kinetics. Blood clearance of QD-BSA was 1.23 mL/min per kg compared to 0.59 mL/min per kg for QD-LM. The QD-BSA half-life was 39 min versus 58 min for QD-LM. The volume of distribution (Vd) for both QDs was approximately 65 mL/kg. Approximately 40 per cent of the administered dose of QD-LM was taken up by the liver 90 minutes following dosing, while virtually all QD-BSA was found in the liver at the same time point. There was no evidence that particles were excreted in urine or faeces over the 10-day period.

Yang et al (2007) administered QDs (cadmium tellurium; CdTe core; ZnS shell; methoxy-PEG-500 coating; approximately 13 nm diameter) by injection into the tail vein of ICR mice. The mice were killed at 1, 4, 24 h and at 3, 7, 14, and 28 days after dosing and plasma, red blood cells, liver, lungs, kidneys, spleen, muscle, fat, brain, skin and bones were collected for analysis. The plasma half-life of QD705 was approximately 18 h. The concentration of cadmium was highest in the spleen, liver and kidney, with kidney levels increasing over the 28-day study period. At day one, mass balance studies showed overall recovery as a percentage of dose was highest in the liver (29 per cent), blood (10 per cent), spleen (4.8 per cent) and kidney (1.5 per cent).
At day 28, recoveries were 40 per cent (liver), 0 per cent (blood), 5.2 per cent (spleen) and 9.1 per cent (kidney). QD was not detectable in faeces and only 0.01–0.04 per cent was recovered in urine.

Ballou et al (2004) administered QDs with four different coatings by intravenous injection to mice and monitored the localization of the QDs by fluorescence imaging, optical and electron microscopy. To determine the long-term stability and retention of QDs, mice were imaged at 15 minutes, one day, three days, seven days, 28 days and 133 days. Notably, fluorescence from QDs was decreased but visible in the liver, lymph nodes and bone marrow after one month, and persisted at 133 days in the axillary, inguinal, and lumbar lymph nodes.

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[22] An opsonin is an antibody or product of complement activation in blood serum that causes bacteria or other foreign cells to become more susceptible to the action of phagocytosis.

Choi et al (2007) intravenously administered a series of ^{99m}Technetium-labelled QDs with a CdSe core, a ZnS shell and zwitter ionic coating (cysteine) ranging from 4.36 to 8.65 nm HD to CD-1 mice. Blood was sampled from the tail vein intermittently and mice were killed 4 h after injection. The β-phase terminal half-life increased from 48 minutes to 20 h as the HD increased from 4.36 to 8.65 nm. QD515 (4.36 nm) was recovered primarily in the bladder at 4 h, with radioactivity also detected in the liver, kidney and intestine. The largest QD (QD574; 8.65 nm) showed increased uptake in the liver (approximately 27 per cent injected dose ID), lung (approximately 9 per cent ID) and spleen (approximately 6 per cent ID), and proportionally lower levels in the bladder. Excretion in urine was highest (approximately 80 per cent ID) for the QD515 and lowest for QD574 (approximately 20 per cent ID). An inverse relationship was observed for retention in the carcass.

5.6.5 Elimination

The renal elimination of particulates from the blood is relatively well understood and can at least partially be explained by renal anatomy and physiology. For particulates, glomerular filtration is dependent on the hydrodynamic diameter (HD) of the particle. For example:

- Inulin, which has an HD of about 3 nm, is completely filtered whereas for IgG, which has an HD of ca 11.0 nm, glomerular filtration is negligible.
- For non-biodegradable or slowly biodegraded materials such as QDs it has been suggested that a final HD ≤ 5.5 nm is needed to permit complete elimination from the body in urine (Choi et al, 2007). Similar findings have been reported for hydrophilic macromolecules such as dendrimer-based MRI contrast agents (Kobayashi and Brechbiel, 2005).

The hepatobiliary system represents the primary route of excretion for particles that do not undergo renal excretion. The weight-of-evidence to date suggests that elimination of non-biodegradable particles in the bile and faeces may occur at low levels, but this is likely to be a relatively slow and inefficient process. For example, following intravenous administration of 50 nm polystyrene spheres, approximately 4 per cent of the administered dose was excreted in the bile as intact particles in 24 h (Ogawara et al, 1999).

5.6.6 Adequacy of the existing OECD toxicokinetics guideline

The OECD WPMN considered that absorption/distribution studies are important when investigating the likely toxicity of nanomaterials. It was noted that studies will probably need to be designed on a case-by-case basis, and are likely to be technically challenging due to the analytical difficulty in tracking distribution in vivo using realistic exposure scenarios. The WPMN emphasised the need for care to ensure that the label remains with the particle, and that the label does not alter the toxicity of the particle (OECD, 2009a).

5.6.7 Conclusions from studies investigating the toxicokinetics of nanoparticles

- No classical toxicokinetic studies were found following inhalational, oral and dermal administration of nanoparticles which quantified bioavailability, metabolism, distribution or excretion. The lack of studies is probably due to the generally low absorption and tissue concentrations of nanoparticles.
- However, available studies suggest that the systemic absorption of insoluble nanoscale materials following inhalational, oral and dermal exposure is significantly restricted (compared to soluble small molecules) by the physiological barriers which exist to defend the body from particulate invasion.

- In studies in which insoluble materials have been administered by intravenous injection, the test material has typically been rapidly removed from the blood and sequestered into macrophage-like cells of the liver, spleen, lungs and bone marrow. Residence times in the blood were generally short which, depending on particle size, may reflect sequestration into MPS organs, rather than excretion in the urine.
- An important finding from oral ingestion and intravenous studies is that particulates taken up into MPS organs may be retained for extended periods, with little indication of elimination over extended investigation periods. Therefore, it is recommended that special attention be paid to organs of the MPS in toxicokinetic studies, and that extending the length of such studies should be considered.
- Particulate materials should be appropriately labelled and care taken to ensure that the label does not dissociate from the test material. Where appropriate, combination techniques may be required to differentiate dissolved and particulate material in biological matrices.

5.7 Toxicology

5.7.1 General considerations

Inhalational particle and fibre toxicology studies conducted in the 1980s and early 1990s are considered to be the first to examine the toxicity of nanoparticles. For example, it was understood in the 1990s that particle surface area correlated well to toxicity in certain cases, and that increased surface area provides an opportunity for a significant effect on toxicity through, for example, transition metal-mediated Fenton chemistry (Donaldson et al,1998). Similarly, Fubini (1997) observed that the toxicity of solid particles is not simply predictable from the chemical composition, but that the micromorphology determines the abundance of surface sites, which affects reactivity towards cells and tissues.

Since 2000 the field has expanded rapidly such that the term ‘nanotoxicology’ has been proposed to describe toxicology relating to nanoparticles and journals like Nanotoxicology have appeared to deal specifically with this area. Maynard et al (2011) noted that in 2005, there were an estimated 179 articles published on the potential environmental health and safety implications of engineered nanomaterials, and that by 2009 the number had risen to 791. A search of Pub Med using the terms ‘nanoparticle’ and ‘toxicity’ for the current (2014) review found more than 7000 results.

In response to the expanding literature and consumer concerns, the toxicity and potential human health risks of nanoparticles have been reviewed by a number of national and international expert groups, including Committee on Toxicity Mutagenicity and Carcinogenicity of Chemicals in Food Consumer Products and the Environment (COT) (COT, 2005; 2007), European Food Safety Authority (EFSA) (EFSA, 2009), FAO/WHO (2009), National Toxicology Program/National Institute of Environmental Health and Science (NTP/NIEHS) (NTP/NIEHS, 2004), Royal Society and Royal Academy of Engineering (Royal Society and Royal Academy of Engineering, 2004), SCENIHR (SCENIHR, 2005; 2007a), US Environmental Protection Agency (EPA) (EPA, 2005), Australian TGA (TGA, 2013), French Agence Française de Sécurité Sanitaire de l’Environnement et du Travail (AFSSET) (AFSSET, 2006), Canadian Institut de recherche Robert-Sauvé en santé et en sécurité du travail (IRSST) (IRSST, 2006), and an expert group of the European Chemical Industry’s European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) (Borm et al, 2006).

Many reviews focussing on nanoparticle toxicity have also been published by individual investigators over the past 10 years including: Balbus et al (2007); Bouwmeester et al (2011); Donaldson and Poland (2013); Hardman (2006); Hoet et al (2004), Hoet and Boczkowski (2008); Johnston et al (2013); Maynard et al (2006; 2011); Medina et al (2007); Nel et al (2006); Oberdörster (2005a; 2005b); and Stern and McNeil (2008).

5.7.2 Cellular uptake and mechanism of action

Many in vitro studies have employed a range of heterogeneous nanoparticles in different cell types and culture conditions to investigate the uptake and toxicity of nanoparticles.

Unfried et al (2007) considered it impossible to ascribe a unique common mechanism of cellular uptake for nanoparticles and that the mechanism depends upon the physicochemical characteristics of the material (eg size), the cellular environment (eg serum components) and the characteristics of the target cell (eg phagocyte versus non-phagocyte). A recent review by Treuel et al (2013) further discussed cellular uptake of nanoparticles and the effects of the protein corona on uptake efficiency. The structure and composition of the protein corona is influenced by the physicochemical properties of the nanoparticle, the nature of the physiological environment (eg culture medium) and duration of exposure. It alters both the size and composition of the nanoparticle at the interface, which can influence biological response, including cellular uptake and toxicity.

The generation of reactive oxygen species is a common finding with in vitro assays employing a diverse range of nanoparticles including fullerenes, single-walled carbon nanotubes (SWCNT), quantum dots (QD) and conventional ultrafine particles. (Donaldson et al, 2004; 2009; Oberdörster et al, 2005a; Nel et al, 2006; Balbus et al, 2007; Lewinski et al, 2008; Unfried et al, 2007). It has been proposed that oxidative stress may be due to the direct effects of particles in the inside of the cell, impacts on mitochondrial respiration, or depletion of antioxidant species within the cell (Donaldson and Seaton, 2012). Increased levels of reactive oxygen species may damage nucleic acid bases and membranes, and activate apoptosis and necrotic pathways.

This has led some investigators to propose that oxidative stress in cellular systems may serve as a relevant endpoint in determining the toxicity of these materials. However, others have cautioned that while the generation of reactive oxygen species may serve as a useful starting point for the toxicological investigation of nanomaterials, it should not be seen as a way to generalise the mechanistic toxicity of a diverse group of materials (Donaldson et al, 2009; Stern and McNeil, 2008; Unfried et al, 2007). For instance, Donaldson et al (2009) noted that while the conventional particles PM10, asbestos and quartz induce similar oxidative stress effects in cell culture, they induce quite different pathologies in vivo.

Lewinski et al (2008) comprehensively reviewed the cytotoxicity data for a range of nanoparticles including carbon-based nanoparticles (fullerenes, CNTs), metal-based nanoparticles and semiconductors (QDs). Dose and time-dependent cytotoxicity was observed for all these classes of nanoparticles. While the cytotoxicity of these nanoparticles is related to the core structure, the surface coating of the nanoparticle is an important determinant of this cytotoxicity. For instance, surface coating may prevent leaching of the toxic core constituents or increase water solubility of the nanoparticles. The authors note that a thorough comparison of results between studies is often limited by incomplete physicochemical characterisation of nanoparticles, and differences in cell lines and exposure conditions.

Unfortunately, there has been a tendency to over-interpret the results from in vitro studies in the literature, particularly where unrealistic concentrations of nanoparticles have been used. Also, cellular exposure for particulates in in vitro studies requires additional consideration because nanoparticles do not behave like soluble chemicals. Processes including settling, diffusion and aggregation may significantly affect the dose to which cells are exposed (Teeguarden et al, 2007). Furthermore, agglomeration and dispersion of nanoparticles can be expected to vary with each concentration within the test system (OECD, 2012b). These factors should be carefully considered in the design and interpretation of in vitro toxicity studies.

5.7.3 Inhalational toxicity

As mentioned above, the origins of nanotoxicology are derived from inhalation experiments with conventional nanoparticles and fibres. These studies are numerous but generally relate to a limited range of poorly-soluble particles such as TiO_2, carbon black, diesel soot, talc and asbestos. Collectively they identified that lung fibrosis and tumours can be formed in the lung of rats due to high particle concentrations of relatively low toxicity materials arising from either high exposure levels, or a failure to remove inhaled particles. These studies also provide the basis for an understanding of the importance of dose, dimension and duration, often referred to as the 'three Ds' (Oberdörster, 2002), which make up the fibre pathogenicity paradigm. An understanding of this historical work provides a framework for assessing the inhalational toxicity of potentially novel nanoparticles that may be used in agricultural or veterinary chemicals.

a) *Nanoparticles*

Much of the concern related to the inhalational toxicity of nanoparticles is derived from early studies which demonstrated that ultrafine particles elicited greater toxicity than larger-sized particles of the same material. These initial studies, conducted in the early 1990s, showed that ultrafine particles of TiO_2 and Al_2O_3 intra-tracheally instilled in rats, resulted in greater pulmonary inflammation and interstitial translocation than fine particles of the same material (Ferin et al, 1990; Oberdörster et al, 1990; Oberdörster et al, 2007). On the basis of later studies with TiO_2 and carbon black, it was suggested that lung toxicity correlates better with surface area than with mass, volume or particle number (Oberdörster et al, 1992).

A considerable body of evidence now suggests that lung fibrosis and tumours in rats develop as a consequence of a prolonged inflammatory response induced by high concentrations of poorly soluble particles, either due to high exposure levels or an inability to remove inhaled particles from the lung, which leads to rat lung overload. Rat lung overload has been defined as a "consequence of exposure that results in a retained lung burden of particles that is greater than the steady-state burden predicted from the deposition rates and clearance kinetics of particles during exposure" (ILSI, 2000).The sequence of events leading to the development of pathological effects in the lung in rat lung overload is generally considered to involve: failed clearance with accumulation of dose → inflammation and oxidative stress → altered particle kinetics with retention consequent on impaired clearance → fibrosis → proliferation → the development of benign and malignant lung tumours (Donaldson and Poland, 2012).

The mechanistic basis for these effects is believed to involve an overwhelming of the alveolar macrophage-mediated clearance of particles which defend the lungs from particulate invasion and oxidative stress.
Chronic recruitment of inflammatory cells into the alveolar compartment and their activation leads to oxidative stress which is believed to result in secondary genotoxicity, eventually leading to tumour development.
The concept of dose is extremely important because, as for non-genotoxic chemicals, low toxicity particulates in the lung can generally be expected to exhibit a dose-response relationship. That is, there is generally a dose (the threshold dose) below which the number of damaged alveolar macrophages is low enough not to cause appreciable inflammation. Therefore, a high enough dose of any particle may eventually cause lung injury, but it

can reasonably be assumed that it will require a lower exposure of a toxic material (Donaldson and Tran, 2002). While increased retention may enhance the potential for local effects and systemic redistribution, data from rats and other rodent species has not indicated adverse effects outside the respiratory tract and associated lymph nodes for poorly soluble particles deposited in the lung (ILSI, 2000). This is consistent with Section 5.6.1, which notes there is currently no convincing evidence for a significant redistribution of inhaled particulates outside the respiratory system and local lymph nodes, in the absence of lung injury.

Notably, rats appear much more susceptible to lung overload following exposure to poorly soluble particles than mice or hamsters. Inhalation exposures of hamsters and mice to talc, TiO_2, or diesel soot have not resulted in lung tumours, despite similar or greater lung particulate lung burdens than those that produced tumours in rats. As such, the relevance to human health risk assessment of lung tumours in rats following instillation or inhalation of poorly soluble particles of low toxicity has been a subject of debate (ILSI, 2000). Nevertheless, the concept of rat lung overload applies to poorly soluble particles of low toxicity, and other more toxic particles such as crystalline silica and synthetic fibres may actively damage alveolar macrophages. Thus, impairment of alveolar macrophage clearance should not be viewed as particle overload in all cases (Borm et al, 2004).

For experimental convenience most early studies were conducted using intra-tracheal administration which may lead to artefactual findings due to the formation of heavy, localised deposits of large particles, with ensuing localised inflammatory responses and increased permeability of the epithelium (reviewed in Pauluhn, 2009). This method of administration may also allow non-respirable materials to access the lung by avoiding nasal deposition of larger particles that would usually be filtered by physiological mechanisms (refer to Section 5.6.1). Furthermore, instillation techniques require dispersion with surfactants and sonication, which may also affect particle size and toxicity. Therefore, studies employing intra-tracheal administration should be viewed cautiously and accompanied by data indicating the type and fate of nanoparticles in the lung, since toxicological effects may reflect the methods of administration rather than the nature of the administered material.

Conversely, while it is recognised that generating stable, homogeneous, reproducible aerosols is technically difficult, inhalation is the physiological process by which nanoparticles are deposited in the respiratory tract and lungs. It has the technical advantage of not requiring carrier systems and the properties of the particles can be characterised and controlled (Pauluhn, 2009). In addition, it provides a more realistic exposure rate and an opportunity for normal clearance processes to occur (OECD, 2012c). The technical aspects of inhalation toxicology studies, including the use of nose-only inhalation systems, are addressed in current OECD guidelines for inhalational toxicity testing. Several investigators have also recently published methods describing the generation and characterisation of test atmospheres containing nanoparticles (Creutzenberg, 2012; Ma-Hock et al, 2007).

A growing number of inhalation toxicity studies on a diverse range of nanoparticles are becoming available in the public literature. For instance, Klein et al (2012) recently reported on short-term inhalation studies conducted in rats with nanoscale TiO_2, amorphous silica (Zeosil 45), silica gel (Syloid 74) and pyrogenic Cab-O-Sil M5, ZnO, barium sulfate, zirconium dioxide, carbon black and CeO_2. The protocol involved a 28-day study period, consisting of a five-day inhalation exposure (head-nose) in male Wistar rats and a three week post-exposure period. The available results have been compared against available information for those materials in the public literature to demonstrate the utility of the assay. The authors further note that short-term inhalation study results for more than 20 nanomaterials are available, including representative nanomaterials listed by the OECD WPMN.

At present, toxicological dossiers for approval of pesticides often only contain an acute inhalation study, which is used for classification and labelling purposes. In view of the observed effects in inhalation studies, there is a case for including repeated dose inhalation studies in the dossiers for approval of nanopesticides.

b) Nanofibres

Studies with asbestos fibres administered to experimental animals either by inhalation or intracavitary identified that the most important parameters to determine the toxicity and carcinogenicity of fibres are the 'three Ds'—dose, dimension and duration. The mechanism of toxicity is generally considered to involve frustrated phagocytosis which occurs when a macrophage fails to engulf a particle larger than itself, leading to the release of reactive oxygen species.

The three Ds describe what is referred to as the fibre pathogenicity paradigm, a criterion which a particle must meet if it is to present a fibre-type hazard. On the basis of this paradigm, it is reasonable to suspect that some high-aspect ratio nanoparticles such as carbon nanotubes (CNT) or nanowires might pose a hazard to the lungs, pleura and peritoneal mesothelium (Donaldson and Poland, 2012).

Indeed, the first published studies investigating pulmonary toxicity using instillation of CNTs appeared in 2004 (Lam et al, 2004; Warheit et al, 2004). Instilling high doses of short single-walled carbon nanotubes (SWCNT) into the lungs of rats led to the formation of multifocal granulomas with foreign body giant cells typical of a foreign body-induced reaction. However the high doses resulted in non-uniform distribution in the respiratory tract and approximately 15 per cent mortality due to asphyxiation, which limited the utility of the results for risk assessment (Warheit et al, 2004).

Since those early studies, the potential for CNTs to induce inflammation and fibrotic changes has been demonstrated in a number of inhalational studies. For example, Ma-Hock et al (2009) exposed rats to multi-walled carbon nanotubes (MWCNT) in a short-term inhalation study, and a 90-day inhalation study conducted according to OECD Test Guidelines 412 and 413. No systemic toxicity was observed, but a concentration-dependent increase in lung weights and granulomatous inflammation was observed. Inflammation was observed at the lowest exposure concentration tested (100 µg/m3), such that a NOAEC could not be established.

In another study Pauluhn (2010) exposed Wistar rats (nose-only) to 0.1, 0.5, 1.5 and 6 mg/m^3 Baytubes®[23] for 90 days (6 h/day, 5 days/week). Moderate inflammation with granulomatous appearance was noted only at 6 mg/m^3. Goblet cell hyper- and/or metaplasia were also observed. During the recovery period of six months, the effects were not fully reversible, but there was some evidence of regression over time. The NOAEC was 0.1 mg/m^3.

5.7.4 Oral toxicity

Humans have been exposed to nanoparticles and nanostructured food substances throughout evolution without significant adverse findings due to the particulate nature of the material. For example, homogenised milk contains oil droplets of 200–2000 nm, cow and human breast milk contain casein particles of around 50 nm, and silicon dioxide is commercially available as a food additive in a number of different sizes in the nanoparticle range.

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[23] Baytubes® are multi-walled carbon nanotubes (MWCNs)

Similarly, ferritin is a naturally occurring nano-particulate of around 12 nm that contains an iron oxide core of 6–8 nm, and is widely ingested in both meat and plant foods (Powell et al, 2010).

These observations demonstrate that being nanoscale does not necessarily imply additional safety concerns following oral ingestion. Soluble nanoscale materials of a conventional substance would be expected to exhibit toxicities largely attributable to the constituent ions and monomers. Conversely, relatively insoluble nanoparticles that remain particulate in nature in the final food, and following oral ingestion, may require additional regulatory consideration.

An understanding of the toxicity of insoluble nano-particulates following oral exposure is in its relative infancy, and mainly related to metals and metal oxides. Several of these studies are of questionable quality and of limited use for risk assessment purposes. Limitations include small group sizes, single and unrealistically high doses, examination of only a small number of biological parameters, and limited and occasionally unconvincing histopathology.

Chen et al (2006) investigated the toxicity of copper nanoparticles, copper microparticles and copper ions. Copper ions appeared to have higher oral toxicity than copper nanoparticles, which in turn had higher toxicity than microparticles. Dose-dependent findings were reported in the liver, kidney and spleen.

Wang et al (2007) compared the acute toxicity of nano-sized TiO_2 particles (25 and 80 nm) and fine TiO_2 particles (155 nm) following oral administration. The authors reported no overt signs of toxicity in mice administered doses of 5000 mg/kg for any particle sizes. Relative liver weight increased in female mice for 25 and 80 nm particles. Some changes in clinical chemistry were also reported in the serum of female mice for the 25 and 80 nm particles.

In an OECD guideline-compliant study, a single dose of TiO_2 particles (approximately 140 nm diameter) suspended in deionised water was administered by oral gavage to one female rat each at a dose of 175, 550, or 1750 mg/kg, and to three fasted female rats at a dose of 5000 mg/kg. No mortality occurred on the study. Grey coloured faeces were observed at the two highest doses. No gross lesions were observed at necropsy (Warheit et al, 2007).

Although the available data investigating the oral toxicity of nano-particulates are limited, findings from toxicokinetic studies indicate that nano-particulates may be retained in gut-associated lymphoid tissue (GALT), as well as in the liver and spleen for extended periods (refer to Sections 5.6.2 and 5.6.4). As such, these tissues should be carefully assessed as a part of oral toxicity studies. Careful attention should also be paid to characterisation of the nano-particulate in the feed since it is likely that aggregation/agglomeration (in the feed) will affect the bioavailability and toxicity of the test material. Where comparing the toxicity of a nanoparticle with a non-nanoscale material, control groups should ideally include the larger form of the particle, and where relevant, the soluble form of the material.

5.7.5 Dermal toxicity

While a large amount of literature is available investigating the potential dermal penetration of nanoparticles through skin (refer to Section 5.6.3), there is a relative dearth of in vivo studies investigating the systemic toxicity of nanoparticles following repeated dermal administration. The limited in vivo studies that have been conducted primarily address the issue of cutaneous toxicity, and have largely only identified irritation as an adverse effect following topical administration of nanoparticles (Stern and McNeil, 2008).

Where available, most of the literature on the potential dermal toxicity of nanoparticles has focused on the use of TiO_2 and ZnO in sunscreens. This subject has been comprehensively reviewed by the TGA on a number of occasions and will not be considered in further detail here. In August 2013, the TGA reaffirmed its scientific opinion that, based on current evidence, TiO_2 and ZnO nanoparticles in sunscreen are unlikely to cause harm. This conclusion was based on the current weight of evidence which suggested that TiO_2 and ZnO NPs do not reach viable skin cells or the general circulation, and a consideration of available toxicological data (TGA, 2013).

5.7.6 Chronic toxicity and carcinogenicity

There is a large database of existing studies with conventional carcinogenic particles, such as quartz and asbestos, and studies of exposure to diesel exhaust nanoparticles in the workplace (reviewed in Borm et al, 2004; Donaldson and Poland, 2012; Donaldson and Seaton 2012; ILSI 2000; Knaapen et al, 2004; Oberdörster, 2002; Oberdörster et al, 2005b; Pauluhn, 2009).

The existing work on conventional nanoparticles is likely to be useful in guiding investigations into the inhalational toxicity of potentially novel nanoparticles, or nano-particulates with a high-aspect ratio. For instance, it seems reasonable to predict that insoluble nanoparticles, particularly those with a reactive surface, may cause inflammation which could lead to secondary carcinogenesis where there is sufficient prolonged inhalational exposure (Donaldson and Poland, 2012). However, to date there are no published chronic inhalation or carcinogenicity studies investigating whether novel nano-particulates may deviate from the classical oxidative stress and inflammation mechanisms proposed for these conventional substances (refer to Section 5.7.3).

Similarly, on the basis of the fibre pathogenicity paradigm, it is reasonable to suspect that any insoluble high-aspect nanoparticle of suitable dimensions has the potential to be carcinogenic at the pleura and in the lung, given sufficient doses. Some data are available for MWCNT, however the relevance of these studies is difficult to assess due to limitations in experimental methodology and contrasting results. These issues were well addressed in the 2007–09 NICNAS review of the literature on toxicological and health-effects relating to six nanomaterials (Priestly, 2009). Specifically, the NICNAS report notes a study by Muller et al (2009) which showed a clearly positive peritoneal mesothelioma response to asbestos in rats following intraperitoneal injection, but did not show any mesothelioma response following a single administration of two dose levels of MWCNT. In contrast, another study by Sakamoto et al (2009) found that MWCNTs instilled into the scrotal sac of rats induced a peritoneal mesothelioma response, but asbestos failed to show a similar response.

In 2012, Safe Work Australia commissioned NICNAS to undertake an assessment of the health hazards of carbon nanotubes for classification under the hazardous chemicals regulations. The review (Safe Work Australia, 2012) found that multi-walled carbon nanotubes may cause damage to lungs through prolonged or repeated inhalation exposure. In the absence of specific data on single-walled nanotubes, the review took a precautionary approach and recommended that unless product-specific data suggested otherwise, all carbon nanotubes should be classified as hazardous with the following Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009) hazard statements:

- suspected of causing cancer
- may cause damage to lungs/respiratory system through prolonged or repeated inhalation exposure.

Classification of a chemical as hazardous means that there are mandatory requirements under Australian work health and safety laws, including requirements for workplace labelling and Safety Data Sheets.

The 2012 NICNAS report noted that MWCNTs can rapidly translocate to the pleura, enter the intrapleural space and reach the sub-pleural region after inhalation exposure. While it was not possible to definitively conclude the minimum length, thickness and aspect ratio of MWCNTs required to contribute to the carcinogenic potential of CNTs, the report noted that the ability to present as a fibre with pathogenic dimensions may involve either an individual fibre or an aggregate of fibres.

Further work is required in relation to the potential for MWCNTs to induce a mesothelioma-type response.

5.7.7 Genotoxicity

Although the mechanisms have not been fully elucidated, it is generally considered that nanoparticles may elicit genotoxic responses through direct interaction with DNA, indirectly as a result of induced intermediates such as reactive oxygen species, or as a result of ions released from soluble nanoparticles. Secondary mechanisms may also be relevant, whereby nanoparticles induce a chronic inflammatory response in tissues in vivo through the recruitment of macrophages and neutrophils to the site and the subsequent release of reactive oxygen species (Donaldson et al, 2010; Gonzalez et al, 2008; Landsiedel et al, 2009; Magdolenova et al, 2013; Oesch and Landsiedel, 2012; Warheit and Donner, 2010).

In a recent review, Magdolenova et al (2013) identified 94 in vitro and 22 in vivo studies published between 2000 and 2012; 67 genotoxicity studies used the comet assay (58 in vitro, nine in vivo), 44 used the micronucleus assay (31 in vitro, 14 in vivo), 11 used the chromosome aberration test (10 in vitro, one in vivo) and 13 used the bacterial reverse mutation assay. Investigated nanoparticles included TiO_2, iron, silver, fullerenes, silica, carbon black, zinc, gold, SWCNT, MWCNT, polymer nanoparticles, QDs, metals and metal oxides. The authors noted that results in the literature are often conflicting and attribute the variability to factors including the source of nanoparticles, the method of preparation and synthesis, the dispersion protocol and variables in experimental conditions (eg cell type, exposure time and concentration).

Doak et al (2012) observed that of 19 reviewed studies using the Ames test, 17 of the tests were negative for mutagenicity and the remaining two studies reported only weak mutagenic effects, despite several nanoparticles testing positive for genotoxic responses in other in vitro tests in mammalian cells. Considering the utility of bacterial assays, they concluded the Ames test was unsuitable for analysing particulate nanoscale materials, perhaps due to the presence of a cell wall in prokaryotes which prevents uptake into the cell.

The EFSA Scientific Committee (2011) considered a bacterial reverse mutation test inappropriate for nano-particulates, on the basis that nanoparticles may not be able to penetrate the bacterial cell wall and because bacteria cannot phagocytose particles. EFSA recommend an in vitro test for induction of gene mutations in mammalian cells, and a micronucleus assay. It noted that choosing the appropriate in vivo genotoxicity test(s) requires expert judgment based on all other relevant data, including toxicokinetics. However an in vivo micronucleus test, an in vivo Comet assay or transgenic rodent gene mutation assay may all be suitable.

Where adequate exposure cannot be achieved, conventional genotoxicity studies have little value, so it is essential to give due consideration to the toxicokinetics of the test material before conducting in vivo testing. Modified protocols should be adopted where it is evident that standard protocols will give a false negative result. This is not unique to nanoparticles. There are a number of compounds for which in vivo tests do not provide useful information because data on the toxicokinetics indicate that they are not available to target tissues. Some examples include radio-imaging agents, aluminium-based antacids, some compounds given by inhalation and some dermally, or other topically applied pharmaceuticals.

5.7.8 Reproductive and development toxicity

No adequate reproductive or developmental toxicity studies were identified in the literature, however there is limited evidence that nanoparticles may reach the foetus following dosing of maternal animals by intravenous and subcutaneous injection. Semmler-Behnke et al (2008) reported that nanoscale gold particles were transferred to the embryos of rats following intravenous injection. Similarly, Yamashita et al (2011) reported that silica and TiO_2 nanoparticles injected intravenously into pregnant mice at gestational day 16 crossed the placental barrier and were distributed to foetal tissues.

Takeda et al (2009) reported that TiO_2 (25–70 nm) administered subcutaneously to pregnant mice on gestation days three, seven, 10 and 14 was found in the brain and testes of six-week-old male offspring. Particles in the testis and brain were identified as TiO_2 using transmission electron microscopy (TEM) and field emission SEM. Aggregates of TiO_2 nanoparticles (100–200 nm) were present in Leydig cells, Sertoli cells and spermatids in the testes. Testicular morphology in TiO_2-exposed mice was abnormal; some seminiferous tubules appeared disorganised and disrupted and the Sertoli cells had large nuclei and nucleoli. Sperm morphology did not differ significantly from controls but daily sperm production and sperm motility were decreased. Nanoparticles of TiO_2 were also present in the olfactory bulb and cerebral cortex (frontal and temporal lobes) of the six-week-old mice.

While the utility of these studies is limited by a lack of detail in reporting, there is some evidence that following administration of large doses of nano-particulates intravenously or by subcutaneous injection, some test material may reach the developing foetus.

5.7.9 Adequacy of OECD guidelines for assessing the toxicity of nanoparticles

a) *Inhalational toxicity*

The OECD WPMN considered whether the current toxicity testing guidelines are adequate for determining the inhalational toxicity of novel nanoparticles (OECD, 2009a). It noted that Test Guideline 403 (adopted 1981) for acute inhalational toxicity includes only very limited histological examination at autopsy. The WPMN recommended that studies investigating the acute toxicity of nanoparticles by inhalation should include detailed examination of the respiratory tract, with consideration of the addition of broncho-alveolar lavage (BAL) and possibly pulmonary cell proliferation endpoints. Detailed histological examination of the entire respiratory tract would be expected when investigating the effect of nanoparticles following repeated exposure by inhalation, with consideration of the addition of BAL and possibly pulmonary cell proliferation endpoints.

The adequacy of OECD guidelines for inhalation toxicity testing was further considered in an OECD WPMN on inhalation toxicity testing of nanomaterials held in The Hague, the Netherlands in 2011 (OECD, 2012c). The meeting report contained suggestions for some minor technical revisions to the OECD Test Guidelines (TG 403, TG 412, TG 413 and TG 436), the Guidance Document on Acute Inhalation Toxicity Testing [ENV/JM/MONO(2009)28] (OECD, 2009b), and the Preliminary Guidance Notes on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials[ENV/JM/MONO(2010)25] (OECD, 2010b). The latter has been superseded by Guidance on Sample Preparation and Dosimetry for the Safety Testing of

Manufactured Nanomaterials [ENV/JM/MONO(2012)40] (OECD, 2012b). This guidance should be consulted for further technical discussion related to the design and conduct of inhalation experiments using nanoparticles.

b) Oral toxicity

The OECD WPMN concluded that Test Guidelines 420, 423 or 425 are appropriate for an initial investigation of oral toxicity. It recommended that specific attention should be paid to cardiovascular and inflammatory parameters and the mononuclear phagocytic system (MPS) in repeat dose studies. Furthermore, the WPMN anticipated that it might not be possible to strictly adhere to some OECD guidelines for in vivo tests requiring specifically high mass concentrations to be tested due to limited dispersability of some nanomaterials. In such cases it is recommended to test a dose range up to the dispersability limit (OECD, 2012b).

c) Dermal toxicity

OECD (2009a) considered it desirable to have enhanced pathology investigations in the current guideline for dermal exposure, Test Guideline 402, when assessing nanoparticles. It further recommended that the local lymph node assay (LLNA), Test Guideline 429, may be the most appropriate method for investigating skin sensitisation.

d) Genotoxicity

OECD (2009a) considered that Test Guideline 471 (‘Bacterial Reverse Mutation Test’), Test Guideline 473 (‘In vitro Mammalian Chromosomal Aberration Test’) and Test Guideline 476 (‘In vivo Mammalian Cell Gene Mutation Test’; with the mouse lymphoma assay being the preferred assay) are suitable for an initial investigation of the mutagenicity of a nanoparticle. It further recommended that positive results in vitro would need to be followed up in vivo using Test Guidelines 474, 475, or 486 if the bone marrow or liver were likely target organs.

5.7.10 Conclusions on studies investigating the toxicity of nanoparticles

- The literature on the toxicity of nanoparticles is growing rapidly and there exist numerous in vitro and in vivo studies in the literature investigating the toxicity of various nanoparticles including metals, metal oxides, fullerenes and CNTs.
- There appears to be a general consensus that as a result of an increased surface area, altered surface chemistry and increased potential for dissolution, there is a potential for nanoparticles to exhibit a toxicity profile that deviates from that of conventional materials of the same composition. However, while the available evidence supports the fact that there may be a difference in potency, there is as yet no convincing evidence to indicate that nanoparticles induce unique or unconventional toxicities not seen with conventional materials.
- Numerous in vitro studies have shown that some nanoparticles can induce the generation of reactive oxygen species, inflammation and cytotoxicity. However, this is not considered to be sufficient evidence to explain the toxicity of all nanoparticles and other mechanisms are likely to be involved. Furthermore, many of these studies should not be over-interpreted, especially where unrealistic exposure conditions have been used.
- Early studies on the inhalational toxicity of nanoparticles and fibres provide a framework for establishing the toxicity of potentially novel nanoparticles that may be deposited in the lung. Although no chronic studies have been conducted, it seems reasonable to predict that:
    - insoluble nanoparticles, particularly those with a reactive surface, may be able to cause inflammation which could lead to secondary carcinogenesis where there is sufficiently prolonged inhalational exposure.

  - any insoluble high-aspect nanoparticle of suitable dimensions has the potential to be carcinogenic at the pleura and in the lung given sufficient doses.
- A large number of genotoxicity studies are available in the literature. Bacterial cell assays are not recommended due to the inability of nanoparticles to penetrate the cell wall and the inability of bacteria to phagocytose particles. Rather, in vitro genotoxicity studies should be conducted in mammalian cells. Available toxicokinetic studies suggest that tissue distribution is likely to be limited compared to small molecules. As such, the choice of an in vivo genotoxicity assay should be carefully considered to ensure the test material is distributed to the target organ.

5.8 Residues of nanomaterials in food

5.8.1 Dietary exposure to nanomaterials in plant and animal commodities

At the time of writing this chapter in 2014, neither the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) nor the Joint FAO/WHO Expert Committee on Food Additives (Veterinary Drugs) (JECFA) has reported on the evaluation of residues in edible commodities derived from plants or animals following the application or administration of nanomaterials. This reflects the fact that the application of nanotechnology in food-producing plant and animal species is in its infancy and currently does not represent a risk to public health or a barrier to international trade. Nevertheless both FAO and WHO have turned their minds to residues of nanomaterials.

In 2007, the WHO noted that ‘neither the specifications nor the ADI for food additives that have been evaluated in other forms are intended to apply to nano-particulate materials’ (WHO, 2007). Later, an expert meeting noted that the current risk assessment approaches used by FAO/WHO and Codex for residues in food are suitable for manufactured nanomaterials; however, any additional safety concerns arising from the characteristic properties of nanomaterials would need to be addressed (FAO/WHO, 2009).

The discussion that follows is based on proposed use patterns of nanomaterials such as those described in Chapter 1 of this report. It addresses the residues evaluation and focuses on potential safety concerns arising from nanomaterials.

A literature review on nanopesticides undertaken by Kah and Hoffman (2014) highlighted many behavioural modifications compared with conventional pesticide formulations. These include controlled and/or targeted release, improved degradation profiles and increased apparent solubility of poorly soluble active ingredients. Based on this information, nanopesticide formulations must be regarded as separate formulation types. Similarly, nano-clay devices installed in drip irrigation lines that release agrochemicals on demand must also be regarded as separate application types from a residues perspective. Supervised field trials will be required to determine the residues left on agricultural commodities. Separate rat, plant and livestock metabolism studies may be needed for nanopesticides formulations to assess whether the residue is metabolised within rats, plants and livestock in the same way as with conventional formulations. If the apparent water solubility of the nanopesticide is altered, livestock metabolism studies may be needed to re-assess fat solubility and distribution within livestock. Where plants are treated with the nanopesticide, the nano-formulation per se or the feedstuff may need to be offered to livestock.

Other uses of nanotechnology in agriculture and animal husbandry may also require that specific metabolism studies and/or trials are conducted to generate data for analysis. For example, controlled release formulations will require that supervised field trials are conducted that cover the time-points when pesticide formulations are released. The inclusion of smart surface technology in pesticide formulations may completely change the chemistry on the leaf surface of a plant as well as distribution within the plant. In this scenario, specific metabolism studies on plants would be required. The impact of photolysis on residue levels in plants will need to be assessed. Supervised field trials conducted in regions with high and low sunlight potential will be needed to determine the possible impact on residue levels in plants. Therefore the global assessment strategy of using glasshouse trials from anywhere in the world, as introduced by OECD and JMPR, may need to be revisited in the case of nanopesticides. Finally, nanopesticides delivered to plants by novel routes or 'greener' nanopesticides may require specific metabolism studies and/or supervised residue trials.

When considering residues of nanomaterials in food of plant or animal origin, a relevant question is whether the nano-characteristic of the materials is retained long enough to be present in the edible portion. For example, the nano-delivery of a conventional pesticide or veterinary medicine may involve transient presence of the nano-characteristic and an absence of nanomaterial in the edible portion. Similarly a nano-emulsion may display a transient presence following application. By contrast, an insoluble nanoparticle may persist in the edible portions of plants and animals and warrant a detailed residues evaluation. As an extension of this concept, it needs to be assessed whether a nanopesticide consumed by livestock, and whose commodities are consumed by humans, still acts as a nanoparticle. If it is shown that the residue retains nano-characteristics, then nanoparticles may translocate to other (specific) locations within laboratory animals and this may result in a different toxicity profile compared to conventional formulations. If the nano-characteristics are not retained, then it needs to be shown that toxicity studies conducted with the nanoformulation represent the active substance as consumed by humans.

The characteristic properties of nanomaterials have two important implications for nanomaterial behaviour, both of which have potential implications for residues. Firstly, the potential for distribution of a larger number of smaller nanoparticles (for a defined weight of a substance) to more and different locations is increased. This increases the challenge for analytical laboratories as new analytical techniques may need to be developed for quantifying nanopesticides residues in rats, plants and livestock and residues of veterinary nanomedicines in animals (including fish), milk, eggs and honey. Secondly, increased chemical reactivity of nanomaterials may require separate toxicology studies with nano-formulations in rats. To assess the impact of the reactivity of nanopesticides once metabolised by plants, it might be necessary to feed plants treated with nano-pesticides to rats. Similarly, it might be necessary to feed livestock commodities to rats to assess the impact of the reactivity of nanopesticides once metabolised by livestock. It may be that any increased chemical reactivity of the nanopesticide is lost after the nanoformulation is metabolised by plants and/or livestock.

Certain behaviours of nanomaterials, eg their adhesion to the walls of vessels, demand particular attention in analytical method validation, storage stability tests and actual sample analysis in residue evaluations.

Since nanotechnology may involve targeted delivery to plants and animals, the distribution and residue composition within the plant or animal may need to be reassessed through specially conducted metabolism studies in plants and livestock for nanopesticides, and in animals for veterinary nanomedicines. In the case of nanopesticides, special care must be given to whether the residue composition in the edible portion of the crop used for human food is the same as the residue composition in the parts of the crop that are used as livestock feed, and whether these residue compositions are the same as for conventional formulations.

Similar considerations to those discussed here for nanopesticides apply also to veterinary nanomedicines.

5.9 Conclusions

It is the APVMA's view that current risk assessment and testing methodologies are generally appropriate for assessing the toxicity of nanoparticles. However, it is anticipated that some modifications to test methodologies will be required. Adequate characterisation of test materials is critical so that the test material lies within the range of specifications for the material for which approval is sought; it should address the physicochemical properties of the naked particle and the nanoparticle in the final product, as well as any changes that may occur through the life-cycle of the product.

An understanding of the toxicokinetics of nanoparticles as a key determinant of potential novel toxicities is critical to risk assessment. Special attention should be paid to organs of the mononuclear phagocyte system (MPS), since available toxicokinetic studies indicate that nanoparticles are generally rapidly sequestered into these organs and the test material may be stored for extended periods. Detection, quantification and characterisation of nanoparticles in biological media present additional challenges and are likely to require appropriately radiolabelled materials.

Genotoxicity assays in bacterial cells are considered inappropriate because nanoparticles may not be able to penetrate bacterial cell walls and they cannot phagocytose particles. As such, in vitro tests should be conducted in mammalian cells. It is essential that the toxicokinetics of nanoparticles are duly considered before in vivo genotoxicity testing and modified protocols adopted where it is evident that achieving adequate target tissue concentrations is unlikely.

The APVMA recognises that the state of the science in relation to nanoparticle toxicology has undergone rapid development in recent years so there may be instances where a novel nano-particulate material has toxicological endpoints that are not addressed through standard guidelines. It is the responsibility of the applicant to develop suitable methods and protocols to address particular toxicological concerns related to the material, based on its novel physicochemical properties. In such cases, the APVMA recommends that the applicant consult early with the Authority about the safety of the end use of the product.

The APVMA notes that most of the accumulated knowledge related to human health risk assessment of nanoparticles relates to relatively simple nanoparticles. It will be important to monitor and periodically revise the validity of the current conclusions as the development of nanotechnologies allows the manufacture of more sophisticated materials.

Because the application of nanotechnology to food-producing plants and animals is in its infancy, residues of nanomaterials in food commodities derived from them do not pose a risk to public health or jeopardise market access in international trade. However, this situation is expected to change in the very near future when modern food production systems exploit the potential benefits of nanotechnology. Such systems and many of the ways they use nanotechnology in agriculture and animal husbandry will require the generation of residues data, which is described in section 5.8.1 of this chapter.

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6 NANOMATERIALS IN AGRICULTURAL AND VETERINARY CHEMICALS: ENVIRONMENTAL RISK ASSESSMENT CONSIDERATIONS

Abstract

Nanotechnology has the potential to create nanoproducts with significant benefits in agriculture and animal husbandry. However, in many situations, their development as agvet chemicals will mean their intentional application in the environment: to crops, intensively raised animals, and in aquaculture. The fate of nanoparticles in water, air and soil, and their effects on biota require special attention from risk assessors and regulators since manufactured nanoparticles have the potential to behave quite differently from their normal-sized counterparts. Some nanoparticles will aggregate with natural colloids in terrestrial and aquatic environments and persist; while these nanoparticles are stable, it may not be problematic since they are not freely available to be taken up by biota. By contrast, nanoformulations such as nano-emulsion delivery systems will be short-lived in the environment.

There are no fundamental differences in the general risk assessment paradigms for chemicals and nanomaterials (Canady, 2010; OECD, 2012a). The general categories of information required to carry out a risk assessment for nanomaterials include:

- identity information
- physicochemical properties
- industrial and consumer uses and environmental releases
- environmental fate
- absorption, distribution, metabolism and excretion (ADME) and the potential toxicity of nanomaterials.

The steps in the risk assessment paradigm are the same as for other chemicals and include exposure assessment, effects assessment (hazard identification and classification), and risk characterisation (Figure 6.1).

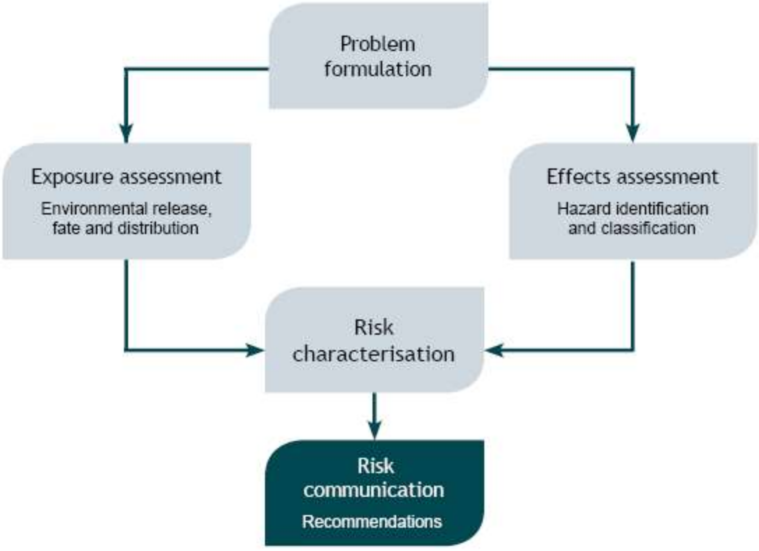


Figure 6.1: The risk assessment framework for nanoscale agricultural and veterinary chemicals in Australia.

In the case of nanotechnologies, the potential number of nanomaterials, which may each have different properties, uses and exposure pathways, requires that a range of important issues be considered or addressed to enhance their risk assessment. These issues relate principally to the physicochemical properties of nanomaterials and possible exposure pathways.

The problem formulation stage is a problem-scoping exercise. It identifies the relevant sources and targets of suspected harm and guides the remainder of the assessment. It may provide an opportunity to consider the physical and chemical properties of the nanomaterial upstream to reduce or avoid downstream risks. For example, if the problem formulation stage suggested a 'safe by design' approach for environmental sustainability reasons, the manufacturing process might be modified accordingly.

The exposure assessment analyses the physicochemical properties of the nanomaterial and possible exposure pathways whereas the effects assessment (hazard identification and classification) evaluates ecotoxicology information.

The risk assessment considers:

- the behaviour of nanomaterials in various media
- the persistence of nanomaterials (both chemical and physical persistence)
- transportation/distribution
- transformation products and impurities
- bioaccumulation.

It derives:

- the Predicted Environmental Concentration (PEC)
- the Predicted No Effect Concentration (PNEC).

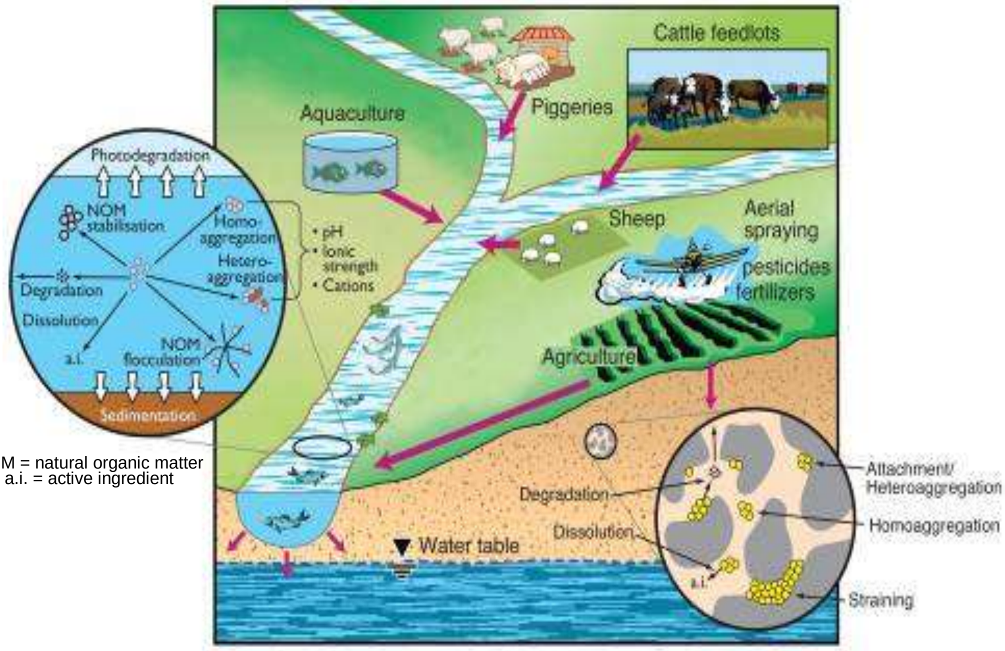
Risk characterisation draws together the various lines of evidence from the assessment of the nanomaterial. A weight of evidence approach is used when determining the potential for harmful effects.

The discussion that follows addresses the environmental risk assessment of potential agricultural and veterinary chemicals. The reader is also referred to the work of Kookana et al (2014), for which the APVMA provided financial support and scientific expertise.

6.1 Nanomaterials in environmental systems

Nanomaterials may have unique properties due to their size and/or shape that make them desirable for application in industry, consumer goods or in agriculture. There is increasing interest in the use of nanomaterials in agricultural systems as fertilisers, plant protection products and/or for animal husbandry. Nano-sized agricultural and veterinary products introduced (deliberately or unintentionally) into environmental systems may include metals and metal oxides, nanoclays, emulsions, nanopolymers, nanocapsules and nanocages (**Table 3.1**). This makes regulating nanomaterials a challenge for agencies applying existing frameworks designed to address the environmental safety of soluble chemicals and bulk-sized solids. The issue is complicated further by the range of natural nanoparticles already in the environment, including colloidal clays, iron and manganese hydrous oxides, metals such as silver, dissolved organic matter (comprising fulvic and humic acids), and fibrous colloids (exopolymers).

After dispersion in the environment, nanomaterials may have a very different fate compared to soluble or bulk-sized agricultural and veterinary chemicals (**Figure 6.2**). Nanomaterials can behave quite differently to soluble chemicals in natural waters, soils and sediments in that they may associate via particle-particle interactions with natural colloids such as clays or organic matter. Nanomaterials are also likely to be more mobile in the environment than the equivalent bulk-sized material due to greater filtration and straining of bulk materials in soils and sediments. The high surface area and small diameter of nanomaterials also means that interactions with biota may be unique so bioaccumulation and biomagnification may be different to soluble or bulk-sized materials.



NOM = natural organic matter
a.i. = active ingredient

Figure 6.2: **Potential reactions and fate of nanoscale agricultural and veterinary chemicals in the environment (modified from Batley et al, 2012). With permission from CSIRO, Copyright CSIRO 2013.**

The fate of nanomaterials in the environment is governed by both their route of entry into the environment (ie whether applied to land or water) and on their behaviour in environmental media (soils, sediments or waters) (**Figure 6.3**). A primary consideration for some nanomaterials will be solubility, as this dictates whether the material can be evaluated as a soluble chemical or as a particulate chemical. Transformation and degradation products of the nanomaterial need to be similarly evaluated. Transformation/degradation of the nanomaterial in the environment may markedly affect its bioavailability, bioaccumulation and potential toxicity (**Figure 6.3**). Distribution of the nanomaterial (and its transformation/degradation products) between air, water and soil/sediment will depend on physicochemical properties of the nanomaterial as well as the physicochemical properties of the receiving media (**Figure 6.3**).

This document reviews the intrinsic properties of nanomaterials that need to be considered for evaluating the risk of agriculture and veterinary nanomaterial products to the environment, as well as properties leading to unique interactions with environmental media (**Table 6.2**), and with biota that need to be considered when evaluating environmental risk

Table 6.1: Examples of nanomaterial products on overseas markets for use in the agricultural and veterinary medicines sector.

Function	How this can be achieved	Current examples
Enhance apparent solubility of active ingredient (a.i.)	Nano- and micro-emulsions	Emulsion based registered pesticides Banner MAXX® of Syngenta (Observatory NANO 2010)
Enhance uptake/efficacy of a.i.	Nano and micro-emulsions, nanospheres	Nanopermethrin (Anjali et al, 2010); nanosphere insecticides (Boehm et al, 2003)
Targeted delivery of a.i	Nanocapsules	Nanoenapcsulated glyphosate or sulfonylurea herbicide (Perez-de-Luque and Rubiales, 2009)
	Nano-liposomal drug formulation	Delivery of adriamycin (anticancer agent) via hyaluron-bound liposome to target hyaluron receptors on tumour cells (Yoo and Park, 2004)
	PLGA-loaded drugs	PLGA-loaded growth hormones for pigs (Wang et al, 2011)
Controlled release of a.i.	Nanocapsules, nanospheres	Polymeric stabilised bifenthrin (Liu et al, 2008); Nanocomposite 2,4-D (bin Hussein et al, 2005); Porous hollow Si-encaged validamycin (Liu et al, 2006)
Enhance toxicity of a.i. to target organism (lower dose)	Nanodispersion, nanosuspensions	Nanodispersed triclosan (Zhang et al, 2008)
Protect a.i. from immediate metabolism and excretion	Nanospheres	Nano spheres using PLGA for controlled delivery of porcine somatotropin (pST) hormone (Kuzma, 2008)
Protect a.i. against premature degradation	Nanocapsules with catalyst a.i. conjugate	TiO_2-M262 polymer metaflumizone (Ishaque et al. 2009); Porous hollow Si encaged validamycin (Liu et al, 2006)
Enhance decomposition of a.i. in soil and/or plant	Nanocatalyst conjugated a.i. in microcapsules	SDS-modified TiO_2/Ag conjugated with a.i. such as dimethomorphv (Yan et al, 2005), imidacloprid and avermectin (Guan et al, 2011; Guan et al, 2008)
Nanoparticle as a.i.	Nanometals and nanoclays	Nano-Ag biocide (USEPA, 2011); Nano-Si (Nair et al, 2010)
Early disease detection	Quantum dots	Quantum dots with specific surface coatings to target cancer cell (Mattoussi et al, 2012)

Abbreviations: a.i. = active ingredient

PLGA = poly(lactic-co-glycolic acid)

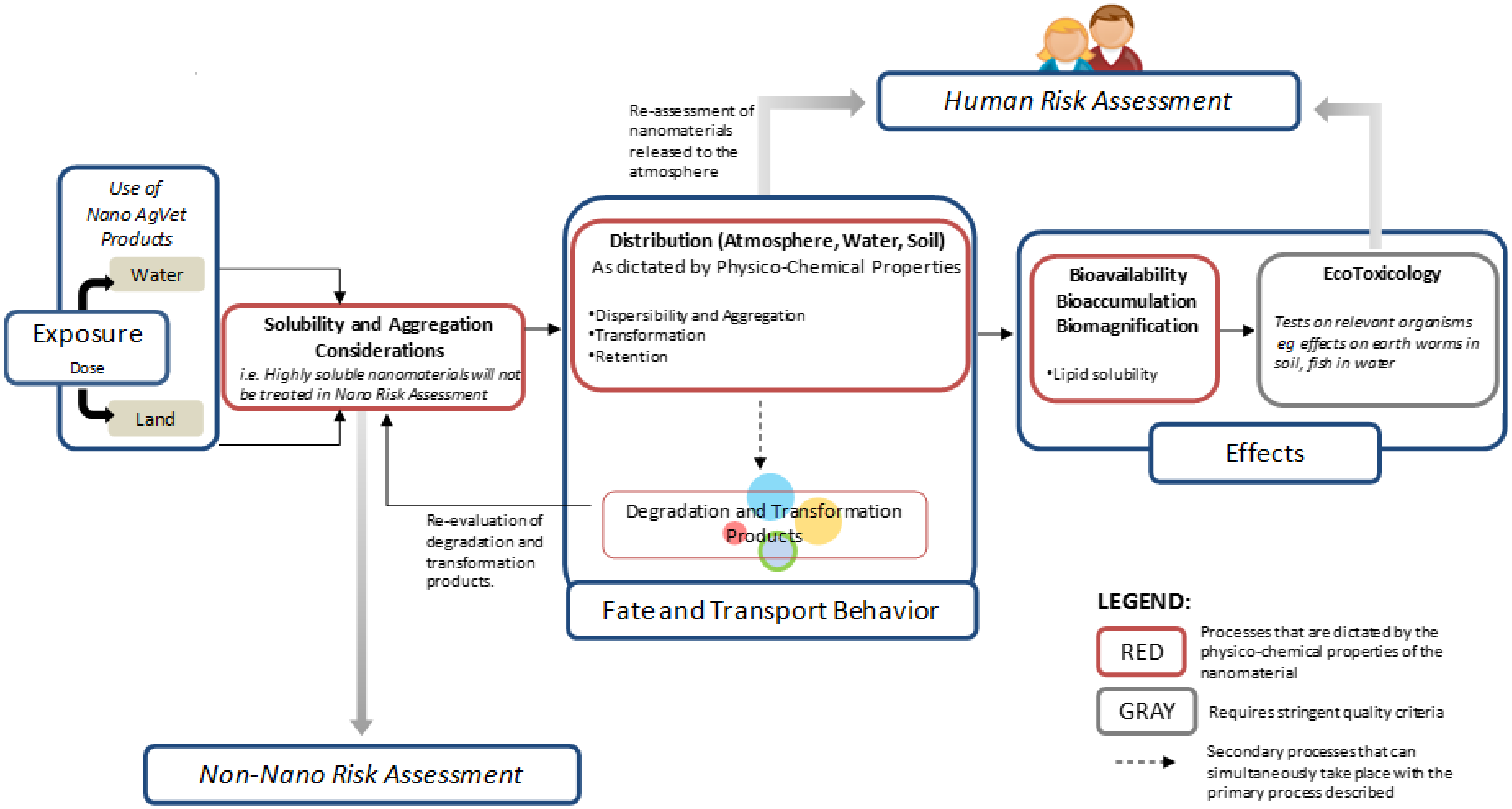


Figure 6.3: Framework for environmental risk assessment (exposure, fate/transport behaviour and effects) of agriculture and veterinary products containing nanomaterials. Note that degradation and transformation products will require re-evaluation based on solubility and aggregation considerations. For nanomaterials that reach the atmosphere and/or accumulate in edible plants, assessment of human risks must be undertaken.

Table 6.2: Important environmental considerations for some potential nanomaterials in agricultural and veterinary products based on mechanism of action.

Product Category	Function	Forms	Details	Environmental consideration
Delivery systems				
Nanoformulation of existing product	Enhance solubility (loading capacity) of otherwise insoluble substances	Oil/water nanostructure emulsions	o/w emulsions are labile	May be instantly destroyed in the environment
		Biodegradable polymers (biopolymers)	Degradability is influenced by the type of polymer used, formulation and mechanism of degradation (bio, oxo-bio, and hydro-bio-degradation)	Degradation process may take a few weeks to years
Controlled release carrier	Nanomaterials are used as template for loading large amounts of substances	Mesopouros silica	Good chemical and thermal stability	Will likely persist and can become sorbents for other contaminants
			High sorbing capacity	Movement through soil will be dependent on the hrydrophilicity of the silica surface
			Biocompatible	Low toxicity
Nanoencapsulation	Protect agvet ingredients from adverse reactions, loss, or against light	Polymer-clay composites	Excellent absorbents—clay is known for their swelling properties	Can act as sorbents for other contaminants
		Nanoclays		Movement through soil will be dependent on the hydrophilicity of the silica surface
		Mesoporous silica	Good chemical and thermal stability	Will likely persist and can act as sorbents for other contaminants
			High sorbing capacity	Movement through soil will be dependent on the hydrophilicity of the silica surface
			Biocompatible	Low toxicity

Product category	Function	Forms	Details	Evnironmental consideration
Product Additives				
Catalyst	Enhance degradation of pesticide to reduce pesticide residues	TiO_2 + pesticides	TiO_2 is a good photocatalyst; anatase form considered more superior than the rutile form	Persistence could be a threat as it can facilitate the generation of toxic reactive oxygen species Likelihood for transformation of the TiO_2 core is low as uncoated TiO_2 has poor solubility in water.
Protective material	Protect active ingredients that are prone to degradation	TiO_2 or AlO_2-silicon + a.i.		For composites like Al_2O_3-silicon, though Al_2O_3 is typically presumed to be insoluble, dissolution and release of Al^{3+} is still possible
Nanomaterial as active material				
Fungicide	To kill and inhibit growth of fungi or fungal spores; eg control downy blight disease occurring on plants and powdery mildew	TiO_2	TiO_2 is a good photocatalyst; anatase form considered more superior than the rutile form	Persistence could be a threat as it can facilitate the generation of toxic reactive oxygen species Likelihood for transformation of the TiO_2 core is low as uncoated TiO_2 has poor solubility in water
		Ag	Agnanoparticles (and Ag ions) are known antibactericidal	Persistence of intact Ag nanoparticless may be low since it is vulnerable to transformation—it oxidises in O_2 and forms more stable silver salts (sulphides, chlorides, oxides, etc.) Antibacterial property of Agnanoparticles (and Ag^+) could pose threat to microbial communities

Product category	Function	Forms	Details	Environmental consideration
Photoprotective material	As a superspreading material to protect leaves from UV light—almost like a plant sunscreen	TiO_2/ZnO	TiO_2 is a good photocatalyst; anatase form considered more superior than the rutile form	Persistence could be a threat as it can facilitate the generation of toxic reactive oxygen species Likelihood for transformation of the TiO_2 core is low as TiO_2 bulk has poor solubility in water Persistence of intact ZnO nanoparticless will be low as uncoated ZnO nanoparticless have high water solubility and is susceptible to dissolution
Plant growth improver	Enhance plant growth **enhanced growth in spinach was related to photocatalytic reduction of* N_2 *to* NH_3 *in leaves*	TiO_2	TiO_2 is a good photocatalyst; anatase form considered more superior than the rutile form.	Persistence could be a threat as it can facilitate the generation of toxic reactive oxygen species Likelihood for transformation of the TiO_2 core is low as TiO_2 bulk has poor solubility in water
	Enhance germination **attributed to mechanical penetration of the seed coat which then improves water uptake*	Carbon nanotubes	Excellent sorbents; can come in different lengths and surface functionalisation	Can act as sorbents and carriers for other contaminants Mobility will be dependent on the surface functionalization
			Preparations of carbon nanotubes often contain metallic impurities (eg Fe)	Long pristine CNTs have been shown to be more toxic than shorter and functionalised CNTs Metallic impurities have been reported to contribute to the toxicity of CNTs due to its redox activity
Dust insecticides	Protect from insects—where the nanoparticles mode of action is to block the trachea or impair the digestive tract	SiO_2, Al_2O_3, ZnO	SiO_2 is typically considered inert Al_2O_3 is typically presumed poorly water soluble	Persistence of uncoated SiO_2 and Al_2O_3 nanoparticless is high since both have poor solubilities in water—some dissolution of Al_2O_3 can occur under highly acidic conditions
			ZnO is photocatalytically active	Persistence of uncoated SiO_2 and Al_2O_3 nanoparticless is high since both have poor solubilities in water—some dissolution of Al_2O_3 can occur under highly acidic conditions

Product category	Function	Forms	Details	Environmental consideration
Devices				
Precision farming and residue determination	Enhance productivity in agriculture by providing accurate information (i.e. on soil conditions)	Monitoring systems Smart sensors Nanochips	Typically in solid-state form	Release of nanoparticless in devices will be slower Persistence in the environment may be high, but would likely be in embedded forms.
Genetics				
Nanoparticle-mediated gene transfer	Crop improvement and developing new insect resistant varieties		This process will be conducted in the laboratory, not the field	

6.2 Exposure to nanomaterials derived from nano agvet chemical products

While the unique properties of nanomaterials offer a range of potential benefits for agvet chemical products, the same properties could lead to unintentional or un-targeted release to the environment and expose non-target biota to these materials or their transformed or degraded products.

Using agvet chemical products containing nanomaterials may expose terrestrial and aquatic environments to materials that behave differently from their normal-sized counterparts. The majority of agvet chemical applications will involve products being directly discharged to land, in the form of fertilisers and plant protection products for agriculture, to soil through manure, or to water in the form of pharmaceuticals for aquaculture or animal husbandry (Gogos et al, 2012). It is expected that through their use and weathering, nanomaterials will eventually be released from these products (Nowack et al, 2012). Their intentional use will inevitably increase the concentration of nanomaterials in the environment up to levels that may be significantly higher than those initially predicted (**Table 6.3**).

Table 6.3: Modelled fluxes of different manufactured nanomaterials and application rates of plant protection products or fertilisers from scientific literature and patents. Reproduced with permission from Gogos et al, 2012. Copyright 2012 American Chemical Society.

Nanomaterial	Modelled flux into soil[a] (high exposure and realistic exposure scenarios)	Application rate and calculated flux from plant protection products and fertilisers[a,b]	Flux ratio[c]
TiO_2	HES: 4.8 µg $kg^{-1}y^{-1}$ RES: 0.4 µg $kg^{-1}y^{-1}$	For AR of 4.5 kg ha^{-1}, CF: 1607-5357 µg $kg^{-1}y^{-1}$ For AR of 7.5 g ha^{-1}, CF: 2.7 µg $kg^{-1}y^{-1}$	334-1116 0.56
Ag	HES: 0.1 µg $kg^{-1}y^{-1}$ RES: 0.02 µg $kg^{-1}y^{-1}$	For AR of 15 g ha^{-1}, CF: 5.4 µg $kg^{-1}y^{-1}$	54
Carbon Nanotubes	HES: 0.02 µg $kg^{-1}y^{-1}$ RES: 0.01 µg $kg^{-1}y^{-1}$	For AR of 3-12 g ha^{-1}, CF: 1.1-4.3 µg $kg^{-1}y^{-1}$	55-215

[a] Sources for each data could be found in the review by Gogos and co-workers (Gogos et al, 2012). [b] Assumes an application volume of 300 L ha^{-1}, 20 cm plough depth, a soil bulk density of 1.4 g cm^{-3} and 1 application per year. [c] Calculated as flux from plant protection products/fertilizers divided by the value of the highest modeled flux. HES = high exposure scenario; RES = realistic exposure scenario and AR = application rate; CF = calculated flux

Environmental risks associated with exposure to nanoparticles from agvet chemical products will be governed by processes that control fate (form and chemistry), transport, and consequent concentrations of nanomaterials in the environment (**Figure 6.2**). Given the dynamic nature of nanoparticles, the first forms that will likely enter the terrestrial and aquatic environments will be dictated by the agvet chemical product formulation, such as whether they are solid or non-solid, their particle/aggregate size and their surface composition/modification—all of which will be different from the pristine nanoparticles used in manufacturing.

Proper nanomaterial characterisation in the product mix, for example size/size distribution and morphology, mass concentration in the applied dose, charge characteristics, water dispersibility and reactivities, will be instrumental in providing a preliminary assessment of their potential fate and behaviour.

6.2.1 Physicochemical properties and nanomaterial behaviour

The physical and chemical properties of nanomaterials are important factors influencing their fate and eco-toxicity in aquatic and terrestrial environments (Batley et al, 2012). The properties of nanomaterials recognised as relevant to toxicological testing have been compiled by a number of organisations, including the Organisation for Economic Co-operation and Development (OECD) Working Party on Manufactured Nanomaterials (WPMN) and the International Organization for Standardization (ISO, 2008c; OECD, 2012a). Recently, a tiered approach for reporting nanomaterial properties in different nanotoxicology studies, for example in vitro and in vivo, was proposed by members of the Australian consortium which participated in the OECD Sponsorship Programme on Safety Testing of Manufactured nanomaterials (McCall et al, 2013). **Table 6.4** lists the properties of nanomaterials identified as important in their fate and toxicity and the multiple techniques available for characterisation. McCall et al highlighted the importance of the characterisation of nanomaterials throughout their lifecycle from the pristine state through to formulations, coatings and ecotoxicity test media (McCall et al, 2013).

Table 6.4: Nanomaterial properties most commonly identified as being important in fate and toxicity

Nanomaterial property	Characterisation technique
Particle size, size distribution	Transmission electron microscopy, scanning electron microscopy, x-ray diffraction
Shape and aspect ratio	Transmission electron microscopy, scanning electron microscopy
Specific surface area	Brenauer-Emmett-Teller (N_2-BET) adsorption method
Solubility	Dialysis, centrifugation, membrane filtration
Aggregation and agglomeration	Dynamic light scattering, cryo-transmission electron microscopy, field flow fractionation, disc centrifugation
Surface chemistry	Spectroscopy, thermogravimetric analysis
Surface Charge	Electrophoretic mobility
Elemental and chemical composition	X-ray diffraction, inductively-coupled plasma mass spectrometry and inductively-coupled plasma optical emission spectroscopy, gas and liquid tandem mass spectrometry, liquid chromatography-ultra violet and liquid chromatography-florescence detection

Size

The size of nanoparticles will significantly affect their dissolution, aggregation, surface area and reactivity (Batley et al, 2012). Nanomaterials with smaller size/dimensions could potentially have enhanced mobility through soil pores, reactivity (ie increased surface area), biological uptake via passive transport and toxicity. Kim et al found in zebra fish (*Danio rerio*) embryos that 20 nm Ag nanoparticles were more toxic than 110 nm Ag nanoparticles (Kim et al, 2013). Ag nanoparticles coated with polyvinylpyrrolidone (PVP) that remain unagglomerated in suspension were also observed to be more toxic than Ag nanoparticles coated with citrate, despite similar sizes of the core nanoparticles. Soil retention of Ag, CeO_2, and ZnO nanoparticles has been shown to be dependent on particle size, with significantly higher numbers of nanoparticles retained compared to micron-sized (bulk) particles (Cornelis et al, 2010, 2011, 2012; Milani et al, 2012).

Smaller <u>sized</u> nanomaterials are more likely to have unique properties and therefore require more careful evaluation

Morphology/shape/surface area

The morphology/shape of nanomaterials is recognised as an important property that can significantly affect the surface area and reactivity of the nanomaterials (Batley et al, 2012). Higher surface area-to-volume ratios suggest an increased number of available sites for interaction that may increase adsorption and enhance reactivity, cellular uptake and toxicity. Pal et al (2007) investigated the effect of nanomaterial shape on the antibacterial properties of Ag nanoparticles against the gram-negative bacterium *Escherichia coli.* The authors found that truncated triangular silver nanoplates with a {111} lattice crystallographic plane as the basal plane displayed the strongest biocidal action, compared with spherical and rod-shaped nanoparticles or with soluble Ag (in the form of $AgNO_3$)—emphasising the importance of the nanoparticle surface. Xiong et al (2013) investigated the effect of surface area on the photoactivity of TiO_2 particles and found those with a larger specific surface area induce higher cytotoxicity (UV absent) and phototoxicity (UV-activated) to cells after 24h incubation. A large number of hydroxyl radicals were detected from TiO_2 particles with larger surface areas. The authors suggested this was due to their increased interaction with biomolecules.

In the case of inert and porous materials used in controlled-release applications, their high pore volumes could enable sorption and facilitate transport of other contaminants that enter and are retained in their pores, especially once the active ingredient (ie pesticide) has been delivered.

High <u>aspect ratios</u> are more likely to lead to greater retention in soils and sediments

Effects of <u>shape</u> on toxicity are not well understood at this stage

High <u>surface areas</u> are likely to lead to greater reactivity with environmental media and biota

Solubility

'Solubility' is often loosely applied in nanomaterial studies, especially in relation to carbon-based nanomaterials, often meaning 'forming stabilised suspensions' rather than truly dissolving (Batley et al, 2012). Hydrophobic nanomaterials are virtually insoluble, for example fullerene solubility has been calculated as 10^{-18} mol L^{-1} (Abraham et al, 2000), and require the addition of organic solvents to form stabilised dispersions.

Interactions of hydrophobic nanomaterial with different aqueous/soil components, such as dissolved organic matter, may result in a variety of nanoparticle transformations, including partial/complete degradation, chemical transformation, and surface modification that can promote nanomaterial solubility/dispersibility. Hydrophobic fullerenes (C_{60}) show increased dispersion in the presence of dissolved organic matter (possibly due to changes in surface chemistry via hydrophobic or pi-pi interactions) and increased solubility through transformation into more water soluble poly-oxygenated/hydroxylated C_{60} derivatives (ie oxygenation and hydroxylation via photoactivation or ozonation) (Hwang and Li, 2010; Klavins and Ansone, 2010; Pycke et al, 2012).

Most metal-based nanoparticles are hydrophilic with solubility dependent on size, shape, composition and reactivity. In many studies solubility is not measured, despite the soluble ionic metal fraction being the most toxic to aquatic and terrestrial biota. Franklin et al (2007), while investigating the biological impacts of ZnO nanoparticles, found nanoparticulate ZnO rapidly dissolved to produce 6 mg L^{-1} of dissolved Zn within 6 h and 16 mg L^{-1} in 72 h in a buffered pH 7.5 soft water, in excess of the 5 mg Zn L^{-1} that would be toxic to most aquatic biota (Franklin et al, 2007). By contrast, nanoparticulate CeO_2 has a very low solubility (ng L^{-1}), so the effects of nanoparticle versus micron-sized particle toxicity could be readily investigated (in the absence of the confounding influence of dissolution). Rogers et al observed higher toxicity to algae exposed to nanoparticulate CeO_2 compared to its micron-sized equivalent (Rogers et al, 2010).

> High nanomaterial solubility means that nanoparticle-specific behaviour is less relevant for fate and ecotoxicity considerations

Aggregation and agglomeration

Aggregation (homo- and hetero-) and agglomeration are important properties that determine the fate and toxicity of nanomaterials in the environment (Batley et al, 2012). Aggregation results when the repulsive forces that keep colliding particles apart are weakened (or when the attractive forces are enhanced). This process is heavily influenced by the properties of the suspension such as pH, ionic strength, and the presence of other colloids. Because nanoparticles can aggregate with each other (homoaggregation) and with other natural particles (heteroaggregation), the main route for the removal of most nanoparticles in the environment will be through aggregation, followed by sedimentation. Aquatic and terrestrial environments contain a wide range of natural particles, including colloidal clays, iron and manganese hydrous oxides, and dissolved organic matter (comprising fulvic and humic acids) and fibrillar colloids (exo-polymers) that are exudates from algae and other microorganisms (the exudates are largely polysaccharides and some proteins) (Wilkinson and Lead, 2007). Heteroaggregation of artificial nanomaterials with natural colloids is therefore likely to control the fate of most nanoparticles. Despite this, most studies have focused on investigations of homoaggregation (Batley et al, 2012). The aggregation process may change the overall size of the nanomaterials, surface area and reactivity that will affect their mobility and ecotoxicity in the environment.

In a study by Quik and co-workers (2012), CeO_2 nanoparticle (20 nm) aggregation in river water containing natural organic matter (NOM) followed first-order kinetics towards a residual concentration of the nanoparticles in the water phase. The authors suggested heteroaggregation with, or deposition onto, the solid fraction of natural colloids was the main mechanism causing sedimentation. Cornelis et al (2011), in an investigation of the retention of CeO_2 nanoparticles (nominal particle size 20 nm) in soils, found a positive correlation with clay content and not with parameters that increase the homoaggregation rate of CeO_2 nanoparticles. This finding in natural soils suggests the negatively charged CeO_2 nanoparticles were adsorbed preferentially by clay surfaces at positively charged sites.

The uptake sites in/on organisms such as fish, invertebrates, plants and microorganisms consist of cell surfaces (eg gill epithelial cells and root epidermis cells) that contain polysaccharides, proteins, and other ligands with an overall slight negative charge. Nanomaterials with a positive charge can be attracted through electrostatic attractions to negatively charged cell surfaces that can result in shear forces causing either particle aggregation or higher concentrations of the nanoparticles at the surface of the organism. In contrast, negatively charged nanoparticles are electrostatically repelled from the cell surfaces/membrane and are less likely to be taken up.

Homoaggregation is less important than heteroaggregation for environmental fate of agvet chemical products

Heteroaggregation is likely to be a key property controlling environmental fate and effects

Surface chemistry

The surface chemistry of a nanomaterial has implications for its dissolution, aggregation, reactivity and toxicity. In agvet chemical products, the nanomaterial surface could be expected to be coated/modified with multiple organic/inorganic coating combinations in the different formulations. These modifications are typically done for the following reasons: to passivate the surface of the nanoparticles (remove free electrons and dangling bonds), control the size of the nanoparticles, render the nanoparticles dispersible in a particular solvent/formulation, and, in some cases, to improve specificity (ie plant uptake via biologically recognisable ligands).

Nanomaterials with hydrophobic coatings can render the nanoparticles dispersible in hydrophobic formulations, while hydrophilic (charged) coatings make nanomaterial dispersible in hydrophilic formulations.

In the same manner, the hydrophobicity/hydrophilicity of the nanoparticle surface can influence its overall soil retention, where transport and mobility within soil may be promoted by more hydrophilic (typically charged) surface chemistries. This property is exploited in some agvet chemical products, where oil-water-based emulsions are used to contain active ingredients that are otherwise poorly water soluble. Loss of surface coatings (ie biodegradation, hydrolysis etc) may lead to nanoparticle aggregation and/or dissolution (Kirschling et al, 2011).

In water, a practical measure of the potential stability of a colloidal system can be obtained from the zeta potential. By convention, it has been used as a measure of surface charge. This parameter is affected by surface chemistry as well as solution properties and composition, such as pH, ionic strength and dissolved organic matter. It provides an indication of the degree of repulsion between adjacent, similarly charged particles, ie low values indicating poor stability and potential aggregation of particles. Zeta potential also has implications for other environmental processes such as adsorption, complexation (eg with organic matter), and interaction with biological systems such as cell membranes. In aquatic and soil environments (Milani et al, 2012; Stebounova et al, 2011), nanoparticle interaction with other materials is more likely. For heteroaggregation, the polarity of the zeta potential will be more important than the magnitude of the charge. Nanomaterials having negative zeta potentials are less likely to heteroaggregate than those with positive zeta potentials and hence will likely have greater mobility in the environment.

Zeta potential is an important property of nanomaterials as it governs interactions with environmental media and biota

High zeta potential impacts greater stability of particles to remain in suspension and to resist homoaggregation BUT may lead to stronger interactions by heteroaggregation with clays and organic matter

The influence of surface chemistry on uptake and toxicity has been demonstrated in a number of studies. For example, Chompoosor et al (2012) investigated the effect of surface hydrophobicity on toxicity, such as cytotoxicity and consequently DNA damage, using gold (Au) nanoparticles (2 nm core) featuring quaternary ammonium functionality with varied hydrophobic alkyl chain. These workers found increasing hydrophobicity on the surface of the nanoparticles resulted in higher cytotoxicity with concomitant reactive oxygen species (ROS)[24] production. In a study by Kim et al (2013), silver nanoparticles coated with PVP were found to be more toxic to zebra fish (*Danio rerio*) embryos than silver nanoparticles coated with citrate at the same particle core size (20 nm or 110 nm). The effect of surface charge (as dictated by surface coatings) on the uptake and dissolution of Au nanoparticles by four plant species (rice, *Oryza sativa*; ryegrass, *Lolium perenne L*; radish, *Raphanus sativus*; pumpkin, *Cucurbita mixta cv. white cushaw*) was investigated by Zhu et al (2012). In this study, positively charged Aunanoparticles were most readily taken up by plant roots which are predominantly negatively charged. On the other hand, negatively charged Aunanoparticles were more efficiently translocated into plant shoots, including stems and leaves, from the roots, indicating surface chemistry-controlled behaviour. Feswick et al (2013) reported that carboxyl-modified (negatively charged) cadmium selenide/zinc sulfide quantum dots (QDs) were taken up to a greater extent by *Daphnia magna*than either the amino-terminated (positively charged) or polyethylene glycol (uncharged) QDs.

Surface coatings control the behaviour of nanomaterials through changing hydrodynamic size, surface charge, and hydrophobicity

Concentration and composition

The concentration and composition of manufactured nanomaterials (and their impurities) can affect their fate (eg dissolution, adsorption and aggregation) and ecotoxicity in environments. Xia et al (2011), found changing the composition of ZnO nanoparticles by doping with iron (1–10 wt%) decreased ZnO nanoparticle dissolution and reduced toxicity to zebra fish embryos. Quantum dots, such as CdS, CdSe, Cd/Te and CdSe/ZnS, contain toxic components (eg cadmium and zinc) that can adversely affect organisms and biological processes (Bottrill and Green, 2011; Gagne et al, 2008). The observed toxicity may be directly related to the nanoparticles or release of the toxic component ions, such as Cd^{2+}. Quantum dots containing Cd are more toxic than those containing only Zn due to the higher toxicity of Cd^{2+} (Li et al, 2011). Metallic impurities in CNTs (resulting from synthesis) may lead to

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[24] Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen, eg oxygen ions and peroxides. They are formed as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis. ROS levels increase significantly in a process referred to as oxidative stress.

toxicity which is unrelated to the CNT (Hull et al, 2009). Nanomaterials have been shown to display dose-response relationships to aquatic and terrestrial organisms (Handy et al, 2008b, 2012; Tourinho et al, 2012). However, ecotoxicity testing of nanomaterials can be complicated and often difficult to interpret due to changes in the actual dose (dissolution, aggregation/agglomeration, precipitation, adsorption, and complexation, for example, onto gill tissue surfaces) during the test period (Gagne et al, 2008). Cornelis et al found the retention (K_r) of CeO_2 nanoparticles onto solid phases in soils to significantly increase with the rate of CeO_2 nanoparticle addition (Cornelis et al, 2011). The authors suggested higher CeO_2 nanoparticle concentrations may have increased the collision efficiency and thus the fraction of larger aggregates.

Impurities in nanomaterials may change behaviour or toxicity

Concentration may affect NM behaviour due to particle-particle interactions (aggregation/agglomeration)

Reactivity

Evaluating nanomaterial reactivities—catalytic activity, redox potential, radical formation potential—is important, particularly when considering potential ecotoxicity (ie nanomaterials facilitating the formation of toxic ROS). Reactivity is closely associated to other fundamental properties such as size, surface area, composition, and crystalline structure. For example, TiO_2, in its anatase form, is known for its photocatalytic activity (ie TiO_{2}- nanoparticles used to enhance pesticide degradation) (Khot et al, 2012), while it is photostable in rutile form (ie TiO_2 nanoparticles used to protect a system from photodegradation) (Gogos et al, 2012). When doped with different metals (ie Ce-doped TiO_2 used as a fungicide), the photocatalytic response of TiO_2-anatase is changed significantly, shifting from UV to the visible region. When surface coated with other materials (ie $Al(OH)_3$ or SiO_2 as in sunscreen formulations (Auffan et al, 2010), surface reactions, such as generation of ROS, are diminished. Nanomaterials known to produce these toxic species would then be of environmental concern, especially if fate and transport data suggest them to be unintentionally active and available to non-target organisms.

Reactivity is currently difficult to assess from nanomaterial physicochemical properties

6.2.2 Preliminary considerations for risk characterisation

A detailed framework of stages required for the environmental risk characterisation (exposure, fate/transport and effects) of agricultural and veterinary nanomaterials can be found in **Figure 6.4**. The first step toward the environmental risk assessment of an agvet chemical product that contains nanoparticles is to determine whether or not a special nanomaterial risk assessment must be applied (**Figure 6.4,** A). Aside from the size range (1–100 nm) that initially separates nano agvet chemical products from traditional products, another important consideration for preliminary assessment would be nanomaterial stability—does the nanomaterial remain in the nano-scale in the formulation and when delivered? Nanomaterial dissolution and aggregation are two processes that would directly affect nanomaterial size and can significantly affect the nanomaterial activity.

Dissolution refers to the release of individual ions and/or component molecules that are soluble in water. Homoaggregation refers to the aggregation/attachment of two similar particles forming larger units. Homoaggregation of nanoparticles would be important in areas such as formulation stability and ecotoxicity testing. However, heteroaggregation of nanoparticles with natural colloids is likely to control their fate and ecotoxicity in aquatic and terrestrial environments.

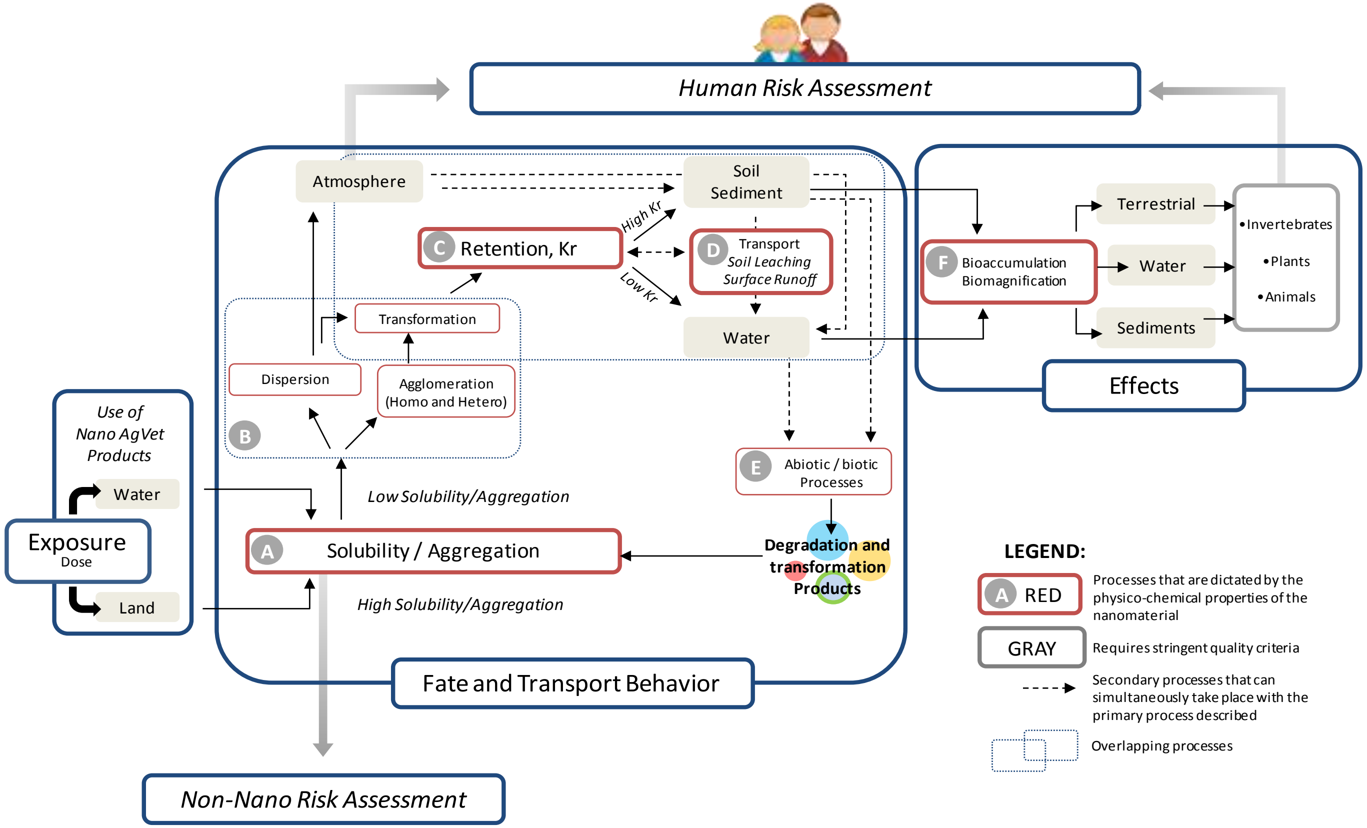

Figure 6.4: Framework for environmental risk characterisation of agricultural and veterinary nanomaterials, highlighting different processes that can control their exposure, fate, transport and effects. Note that degradation and transformation products will require re-evaluation based on solubility and aggregation considerations. For nanomaterials that reach the atmosphere and/or accumulate in edible plants, assessment of human risks must be undertaken.[25]

Abbreviation: The retention coefficient (Kr) is the ratio of the concentration of nanomaterial in the solid phase to that in the aqueous phase.

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[25] Figure 6.4 is an extended version of Figure 6.3

Initial screening would require a characterisation of the durability/rate of dissolution/agglomeration of nanomaterials. This will depend heavily on the general functions (ie active ingredient or additive) and tasks (ie controlled release carrier or dispersing agent) of the nanomaterial in these products, as well as the formulation. Materials with strong evidence for complete dissolution or formation of large aggregates (eg >100 nm) in the product formulation, and upon delivery, may already be suitable to traditional risk assessments of chemicals or bulk-sized materials.

Below are some examples of how dissolution and homoaggregation can be used in the initial screening of nano-agvet chemical products for risk assessment.

Dissolution of components

For metallic nanoparticles, the potential for dissolution could be initially assessed based on the solubility product (K_{sp}) of its bulk form. This constant is dependent on the ionic strength, ligand availability, pH and temperature of the surrounding media (Batley et al, 2012). For example, oxide materials such as ZnO, CeO_2 and TiO_2, have K_{sp} values that are in the order of 10^{-17}, 10^{-48} and 10^{-29} respectively, which indicate that ZnO will dissolve in water more than CeO_2 or TiO_2. In the nanoscale, this solubility is expected to increase even more. Indeed, uncoated ZnO nanoparticles have been observed to rapidly dissolve. In the work by Franklin et al (2007), 19% (16 mg L^{-1}) of the nominal Zn available was dissolved after 72 h. On the other hand, uncoated CeO_2 nanoparticles have very poor water solubilities (ng L^{-1}) and an almost negligible tendency for dissolution. For commercial products containing nanoparticles such as ZnO in sunscreen formulations or CeO_2 in fuel additives, it is therefore expected that the lifetime of ZnO as a nanoparticle could be potentially shorter than CeO_2 as a result of likely dissolution. Under this scenario, ZnO nanoparticles in agvet chemical products may be evaluated through traditional risk assessment (for Zn^{2+}), while CeO_2 nanoparticles may have to undergo nanomaterial-specific risk assessment.

However, uncoated nanoparticles that are known to exhibit fast dissolution rates, such as ZnO, may be intentionally surface-modified during the manufacturing process by adding coatings or suitable surfactants to decrease dissolution. For coated particles, dissolution will be a function of coating desorption, degradation (organic coatings), and/or dissolution (inorganic coatings). Polymer coatings such as PVP are typically more stable than coatings prepared from small molecules such as citrate. Weakly bound and degradable coatings will eventually expose the nanoparticle surface and promote dissolution. In cases where ZnO nanoparticles demonstrate stability against dissolution (dissolution rates are low, thus nano-lifetime is high) in the nano agvet formulation (and upon delivery), evaluation should still proceed through nanomaterial-specific risk assessment. Note that new synthetic procedures for producing more stable nanoparticles are constantly being developed, such as ultra-stable Ag nanoparticles (Desireddy et al, 2013). With the different synthetic methods available, the same type of nanoparticles, such as ZnO from different sources, may not necessarily exhibit the same dissolution rates. Hence, to evaluate the nano- agvet chemical product properly, relevant conditions for the stability of the nanoparticles must be provided.

For non-metallic nanoparticles, dissolution may refer to the breakdown of the nanostructure into its components (polymers, emulsions or lipids). Some nanomaterials in agvet chemical products are synthesised with biocompatible materials, which potentially increases their biodegradability. In extreme cases, breakdown would constitute complete mineralisation.

NP dissolution must be evaluated using the agvet formulation and in the relevant application scenario. Due to the dynamic character of nanoparticulate systems, information on dissolution rates would be more instructive than dissolution state. Dissolution rates may have to be established in both abiotic and biotic conditions (or where relevant), depending on product usage. This must not be confused with “nanopesticide degradation” as this terminology often refers to the degradation of the pesticide a.i. and not the nano-delivery medium that encapsulates the pesticide.

Aggregation of primary particles

Aggregation of particles in aqueous suspensions may occur either with other nanoparticles (homoaggregation) or with natural nanoparticles (colloids) (heteroaggregation). Aggregation is controlled by the nanoparticles’ characteristics such as size, shape, and surface charge, and by the solution/formulation conditions such as pH, ionic strength and cation composition. Nanomaterials that acquire a near-neutral charge homoaggregate rapidly, such as when pH and ionic concentrations are close to or at the point of zero charge (PZC) and critical coagulation concentration (CCC) respectively. Consideration of nanoparticle aggregation in the preliminary assessment is significant for agvet chemical products that would be used as dispersions, including products where powder formulations are redispersed for application. This preliminary screening also assumes that nano-specific activity/behaviour is lost with the formation of larger and closely-packed aggregates. For example, the ability of C_{60} to produce ROS is significantly reduced when in the form of closely-packed aggregates (Kong and Zepp 2012). Nano-Agvet chemical products whose components readily aggregate (homoaggregation rates are high, thus nano-lifetime is low) may be amenable to traditional risk assessment. However, manufacturers are likely to ensure aqueous suspensions of nanoparticles remain dispersed in the formulation and that little or no homoaggregation takes place (otherwise nano-specific properties would be lost before use). It must be noted that once dispersed in the environment, heteroaggregation with natural colloids is much more important—homoaggregation is unlikely due to the very low concentrations of nanoparticles expected in aqueous environmental media.

6.3 Environmental fate and transport

Nanomaterials that are released from agvet chemical products are likely to go through the different environmental processes highlighted in **Figure 6.4**. These include:

- physical and chemical transformation during use (B)
- retention/partitioning between soil and water (C)
- transport and remobilisation from soil to water (D)
- abiotic/biotic degradation/transformation of nanoparticles in soil and water (E)
- uptake by terrestrial and aquatic organisms (F).

While these processes are not unique, because of their dynamic nature the responses of nanomaterials to these processes may differ from those of traditional chemicals.

6.3.1 Physical and chemical transformations

Release of nanoparticles from matrix

The use and eventual weathering of an agvet chemical product will result in the release of nanoparticles from the originally introduced product matrix. Following design principles that are dictated by the desired agvet function, such as delivery systems and additives, nanoparticle release rate will depend on the complexity of the matrix and how the nanoparticle is incorporated, that is whether or not the nanoparticles are loosely or tightly bound to the product matrix (**Table 6.5**). For example, it could be expected that nanomaterial delivery systems (ie mesoporous nano-SiO_2 emulsions) in agvet chemical products will be released readily from the product matrix via simple hydrolysis. On the other hand, nanoparticles that are contained in cages, capsules or devices may be released only after exposure to the environment (continued exposure to light, air, and water) via photochemical, oxidation-reduction, and/or dissolution reactions, resulting in a controlled release rate that occurs over an extended period. During this release process, it could be expected that the physicochemical properties of the nanoparticles would have been modified. For example, in other types of products, TiO_2 nanoparticles released from paints via natural weathering, and ZnO nanoparticles released from surface coatings via abrasion, could still be surrounded by the matrix. On the other hand, Ag nanoparticles released from socks (detected in washings as AgCl) and TiO_2 nanoparticles released from sunscreens could undergo considerable transformation. In particular, for the TiO_2-based nanocomposite used in sunscreens (**Figure 6.5**), the surface coatings that are designed to reduce ROS formation and impart hydrophobicity have reportedly altered with ageing (as a function of light and time). The observed desorption of the outer hydrophobic layer (PDMS) could lead to increased dispersion of this material in aqueous environments. These examples were described in a review paper by Nowack et al (2012).

Product-weathered nanoparticles that are released from the main product matrix will then undergo further physical and chemical transformations which will be controlled by the receiving matrix (plant or soil).
These are alterations that become increasingly significant over time.

Transformation processes

The different transformation processes which can occur before and after the release of nanoparticles from a product matrix, and which affect (or are affected by) nanoparticle form, are described below. These changes may involve transformation of the nanoparticle core, nanoparticle surface, or both. The likelihood of each type will depend on the application protocol (initial form—as solid, dispersion, device or receiving matrix), agricultural practices employed (soil tilling, irrigation or addition of other chemicals) and natural weathering (wind, rain or heat).

Table 6.5: Some nano-enabled products illustrating release behaviour of nanomaterial components as described by Nowack et al, 2012.

Nanomaterial components	Release potential	Examples	Summary
Solid-bound	Nanomaterials incorporated in the solid matrix will be released from the product at a much slower rate than if they were in liquid dispersed form.	TiO_2 in paints (Kaegi et al, 2008)	Use of the product involves nanomaterial embedded onto surfaces. Release of nanomaterials may occur with weathering, and as particles that are heteroaggregated with the paint matrix.

Nanomaterial components	Release potential	Examples	Summary
	Nanomaterials that would leach from this product may be altered significantly in the product even before it reaches the environment.	CNTs in composite materials	Nanomaterials in the product are directly embedded in a composite material. These composites are typically durable and can withstand physical and chemical transformations. Hence, release and alteration of nanomaterials may occur over longer periods.
Loosely-bound	Nanomaterials that are loosely-bound will be readily released as nanomaterials with very few modifications.	TiO_2 in cosmetics (Auffan et al, 2010)	Nanomaterials could be released with washing of skin. Nanoparticles could be readily released as matrix-modified materials. Change in composition is not expected given the stability and insolubility of TiO_2.
		Ag in textiles (Benn and Westerhoff, 2008)	Use of the product involves addition of a copious amount of water which will potentially facilitate release of component nanomaterials. Application (washing) results in significant alteration of the nanoparticles (ie detergents, water).

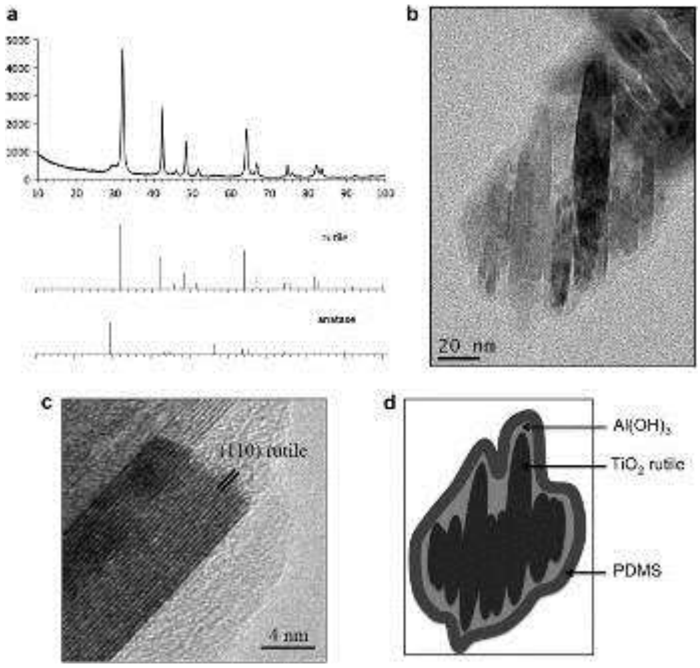


Figure 6.5: An example of a TiO_2-based nanocomposite used in sunscreens, characterised by: (a) XRD, (b) TEM, and (c) High Resolution TEM. (d) A schematic view of the formulation highlights the TiO_2 core, as well as the $Al(OH)_3$ and polydimethylsiloxane (PDMS) layers that surround the TiO_2 nanoparticles. Reproduced with permission from Labille et al, 2010. Copyright 2010 Elsevier.

Photochemical Transformation

Description: Photoexcitation facilitates in situ generation of (highly reactive) ROS via transfer of excited electrons from the nanoparticles to H_2O, O_2 and OH^- ions, or other nearby electron acceptors. The ROS generated can then oxidise compounds adsorbed onto the nanoparticle surface.

Relevant materials: Photochemical transformation is a process that is most applicable to products which will be exposed to significant levels of light, such as AgVet chemical products sprayed directly onto plants or soil surfaces, and/or are generally photoreactive, including nano AgVet chemical products containing TiO_2-anatase, ZnO and Al-SiO_2.

- Ubiquitous natural organic matter (NOM) could also act as a photosensitiser and could absorb light to initiate a photochemical reaction.

Reaction specifics: The extent of the photochemical transformation will be influenced by (a) the wavelength of incident light (ie TiO_2-anatase is active in the UV region, while Ce-doped TiO_2 is active in the visible region of the electromagnetic spectrum), (b) the capacity of the incident light to penetrate and reach the photoreactive nanoparticles—past surface modifications and aggregation, (c) reactive surface area of the nanoparticles and (d) product composition.

- Other components in the product formulation, such as antioxidants, fillers, and solvents, could also participate in photochemical reactions by forming or neutralising ROS (**Figure 6.5**). Products of photoexcitation could also participate in other oxidation-reduction reactions, such as degradation of other organic species in soil (a process similar to how photoactivated nanoparticles are used to degrade pesticides) (Thomas et al, 2011).

Nanoparticle transformation: This process may or may not result in alteration of the nanoparticle core. Coating ligands could be oxidised by ROS, such as disulfides ←→ thiols, alcohols ←→ carboxylic acids, thereby changing the surface properties of the nanoparticles.

Examples: Photoreactive nanoparticles like TiO_2 (anatase) facilitate generation of ROS. While ROS may not be sufficient to further oxidise Ti^{4+}, ROS generation can facilitate oxidation (or degradation) of other species, such as nanoparticle organic coatings, and change the overall surface properties of the nanoparticles. This is the same principle as used with TiO_2 for degradation of pesticides (Carp et al, 2004; Thomas et al, 2011).
On the other hand, Ag nanoparticles are susceptible to photochemically-induced changes. ROS generation could oxidise Ag to Ag^+ (Gorham et al, 2012). For nanoparticles like fullerenes (C_{60}), photochemical transformation may lead to formation of hydroxylated/oxygenated products (Pycke et al, 2012) that have been reported to be more toxic, (ie generate more ROS – Chae et al, 2010).

Oxidation and reduction

Description: Oxidation and reduction are reactions that involve transfer of (valence) electrons and are typically manifested by changes in oxidation states. This type of transformation requires the presence of oxidising and reducing agents, whose rate is determined by the reduction potential (E^0_{red}) of the component ions.

- E^0_{red} constant is influenced by pH (determines favourability of reaction), temperature (kinetics of the reaction), the presence of other species essential for reaction to occur (increases the likelihood of the reaction occurring), and the presence of ligands adsorbed onto the nanoparticles' surface (reduces the rate of reaction).

The majority of data from the literature have reported oxidation of nanoparticles. Excluding reports of formation of nano-sized particles resulting by reduction, that is, Ag nanoparticles formed by reduction of Ag^+ by humic acids (Akaighe et al, 2011), data on the reduction of nanoparticles in the environment has yet to be reported.

Relevant materials: Nanoparticles in AgVet chemical products are susceptible to oxidation or reduction as long as the reaction is thermodynamically favourable. Some of the most common oxidising and reducing agents in soil are:

a) Oxidising agents: O2 (present in air and could be dissolved in a water/soil-solution; it could also facilitate the formation of ROS), Fe3+ and Mn4+ (present in soil, part of clay), and SO42- and NO3- (present in soils/soil solution).

b) Reducing agents: NOM (present in soil and in soil solution).

- In soil, nanomaterials will be mostly exposed to an oxidising environment as a result of agricultural practices, such as soil tilling or the adding of H2O2, which keeps the soil well-aerated.

Reaction specifics: The extent of this oxidation/reduction process relies on the availability of the nanoparticle surface, the capacity of oxidising/reducing agent to move through surface modifications and the reactive surface area. Nanoparticle coatings reduce the rate of nanoparticle oxidative/reductive transformation. Poorly passivated/coated nanoparticles that often have partially exposed surfaces will be susceptible to oxidation/reduction of the core nanoparticle.

Nanoparticle transformation: Oxidation/reduction significantly alters the nanoparticle core. Coating ligands could also undergo oxidation/reduction, such as disulfides ←→ thiols or alcohols ←→ carboxylic acids, changing the surface properties of the nanoparticles.

Example: As described in a review by Levard et al (2012), the most commonly-used Ag nanoparticles will not persist under relevant environmental conditions due to oxidation. Surfaces of Ag nanoparticles (oxidation state = 0) can be readily oxidised by O_2 to form a layer of Ag_2O (oxidation state = +1). Note that other nanoparticles, such as the ultra-stable Ag nanoparticles synthesised by Desireddy et al (2013) have been suggested to be more stable against oxidation.

- Depending on the surrounding conditions, partially oxidised Ag_2O-Ag species may completely convert to Ag_2O, form other stable silver compounds (Ag_2S in the presence of sulphides) or remain partially oxidised and acquire new surface coatings, such as organic matter.

Dissolution and precipitation (for metallic nanomaterials)

Description: Dissolution refers to the release of component ions in solution. The dissolution process starts either with a hydrolysis or oxidation reaction at the nanoparticle surface resulting in a complete/incomplete release of its component ions/molecules. Precipitation refers to the formation of an insoluble material from dissolved ions.

- Dissolution is governed by the solubility product constant (K_{sp}) and the standard reduction potential (E^0_{red}) of its components (nanoparticle and surface coatings).
- Precipitation is governed by the solubility product constant (K_{sp}) and complex formation constant (K_f).

Relevant materials: Dissolution and precipitation will mostly apply to agvet chemical products based on metallic nanoparticles that are highly water soluble and/or are easily oxidised. In soil, precipitates could be formed with ions such as Cl^-, CO_3^{2-}, NO_3^-, SO_4^{2-} and natural colloids such as clay, organic acids, organic thiols, and NOM.

Reaction specifics: Dissolution relies on the availability of the nanoparticle surface and the reactive surface area. Nanoparticle coatings reduce the rate of nanoparticle dissolution. Precipitation relies on the availability of dissolved ions. Concentration gradients can also drive or inhibit dissolution and precipitation (hydrolysis/precipitation: NP-X ←→ NP + X; oxidation: NP^0←→ NOP^+ + e').

Nanoparticle transformation: Dissolution and precipitation directly affects the core nanoparticle. In the absence of precipitation, slow dissolution of metallic nanoparticles would result in them gradually becoming smaller in size, whereas fast dissolution of metallic nanoparticles would result in their rapid disappearance. Otherwise, dissolved ions could form insoluble ionic compounds (ie free Ag^+ forming Ag_2O), complexes (ie M^{x+}-dissolved organic matter) or attach to suspended material and promote settling of nanoparticles out of solution. The loss of ions also changes the nanoparticle surface charge, which can influence its aggregation state and concomitant reactivity.

Examples: Some nanoparticles that have been shown to release component ions/molecules even under mild conditions include ZnO nanoparticles (via hydrolysis), Ag nanoparticles (via oxidation) and polymeric nanoparticles (via hydrolysis). For ZnO and Ag nanoparticles, toxicity has often been associated with released ions. Work by

Franklin et al (2007) on aquatic toxicity of ZnO nanoparticles has demonstrated that toxic levels of Zn could be readily released (6 mg L^{-1} within 6h) from suspensions in soft water (pH 7.5) . Some nanoparticles which are relatively resistant to dissolution of ionic components include TiO_2, CeO_2, and SiO_2 nanoparticles. For these nanoparticles, toxicity is related to properties apparent at the nanoscale—CeO_2 nanoparticles were observed to be more toxic to algae compared to CeO_2 bulk (Rogers et al, 2010).

6.3.2 Phase partitioning processes (adsorption/desorption or attachment/retention)

One of the important processes that determine the fate of an active ingredient (a.i.) in conventional agvet chemicals is the (ad)sorption/desorption on the solid phases in soil and water environments. The extent of partitioning of mass between the solid (sediment, soil) and water phases is governed by these processes which take effect as soon as the contact between the a.i. and the solid phase occurs. Phase partitioning processes determine if the a.i. would predominantly reside in solid or solution phases as well as the pathways of exposure to the a.i. (via sediment, soil or water). In addition, processes such as transformations, degradation, bioavailability and transport are dependent upon the partitioning (adsorption/desorption) processes. The phase partitioning into air may also be important for agvet chemicals, such as through drift. For example, if the nanoparticles get airborne, there may be implications for human health. However, this aspect has not been covered here (**Figure 6.4**).

For nanoparticles, the phase partitioning between soil/sediment and water may not strictly depend on adsorption/desorption processes but on other physicochemical interactions such as homoaggregation or heteroaggregation (as described later in this section). However, for nanoformulations of agvet chemicals such as nanopesticides that are likely to contain conventional a.i. together with a nano-delivery mechanism, these processes still remain relevant, especially in terms of interaction of a.i. with the nano-delivery system. Adsorption/desorption processes have been well understood for conventional a.i. and are not discussed here. For further details on these, the reader is referred to the book by Cheng (1990). However, the effect of adsorption/desorption of nano- agvet chemical products may also arise through transformations and changes in speciation in nanoparticles in the receiving environment. These interactions in the context of transformations that could influence partitioning behaviour of nanoparticles are discussed below.

Adsorption of substances onto nanomaterials

Description: Adsorption involves attachment of substances onto the nanoparticle surface (and/or nanoparticle surface coating, adsorbents) via Van der Waals attraction forces (physisorption), electrostatic interactions (ionic), and/or chemical bonding (chemisorption). Adsorbing species (adsorbates) can attach to the nanoparticles surface by (a) ligand exchange, following desorption of existing ligands, (b) Van der Waals interaction with the partially exposed nanoparticles surface and/or other ligands that surround the nanoparticles, or (c) by both mechanisms.

- This is the same process that applies to nanoparticles that acquire coatings during their synthesis and formulation.
- Compared to precipitation, adsorption does not require dissolution of the nanoparticle surface and does not necessarily involve formation of an insoluble solid.

Relevant materials: All nanoparticles will be susceptible to this transformation. In the environment, soil components viz Cl^-, CO_3^{2-}, NO_3^-, SO_4^{2-}, clay, NOM and other naturally occurring colloids, biological secretions (ie root exudates) organic acids, organic thiols and enzymes could sorb onto nanoparticle surfaces. Soil solutions typically have high concentrations of these natural colloids (especially in comparison with the concentration of nanoparticles in soil) that can form (hetero) aggregates with the nanoparticles (Batley et al, 2012). Porous SiO_2 nanoparticles and carbon nanotubes that are known to have very large pore volumes and adsorptive properties could serve as adsorbents for other contaminants (Pan and Xing, 2008).

- Humic substances have highly complex molecular structures and composition which vary depending on their origin. They can further be subdivided into fulvic acids, humic acids (HA), humins—different fractions that vary in their molecular weights. These fractions could be expected to adsorb onto nanoparticles (and vice versa) and influence nanoparticle surface chemistry to varying degrees.

Reaction specifics: The extent of adsorption will be highly dependent on the surface area available for interaction. For nano-agricultural products that will mostly be applied to soil, the nanoparticles released from the formulations will be readily subjected to this adsorption process.

Nanoparticle transformation: This transformation will result in nanoparticles that are heteroaggregated, which has implications for nanoparticle dispersibility (ie charge). While adsorption with small colloids may or may not result in nanoparticles stable in suspension, adsorption onto charged surfaces or large molecules (ie humic substances) will essentially immobilise the nanoparticle (formation of larger aggregates, and sedimentation) (Stebounova et al, 2011).

Example: Interaction between nanoparticles (Fe_2O_3, Al_2O_3, TiO_2, Au, Ag, C_{60} and CNTs) and NOM (specifically HA) have been shown to facilitate dispersion of the nanoparticles (Batley et al, 2012). Stabilisation of the nanoparticles was attributable to both the steric separation and electrostatic repulsion imparted by the natural colloids. The amphiphilic characteristic of HA allows for both coordinative and Van der Waals interactions. Note that these heteroaggregated species (NP-HA) can further adsorb onto other natural colloids (a heterogeneous mixture of NOM and inorganic binding phases) and form unstable clusters.

Desorption of substances from nanomaterials

Description: Desorption involves the detachment of substances adsorbed onto the nanoparticle surface and/or nanoparticle surface coating.

Relevant materials: All nanoparticles will be susceptible to this transformation.

Reaction specifics: The process relies on the nature of the surface binding (type and strength of the interaction) between the adsorbent and the adsorbate. Weakly bound species (ie bound by Van der Waals interaction) could be expected to desorb easily compared to those that are more strongly bound (ie bound by chemical bonds). For chemisorbed species, desorption can occur when a substance that has higher affinity for the adsorbent (nanomaterial) surface (related to K_{sp}, K_f or bond strength), promotes displacement of the original coating, then attaches to the nanoparticle surface (ligand exchange). Concentration gradients can drive or inhibit this process (nanoparticle-adsorbate $\leftarrow\rightarrow$ nanoparticle + adsorbate).

- Ligand exchange is a common procedure in preparing water-dispersible nanoparticles from hydrophobically-coated nanoparticles.

Nanoparticle transformation: This process does not necessarily cause significant alteration of the nanoparticle core composition but directly affects nanoparticle surface properties.

Example: For nanoparticle formulations dispersed in water, loss of nanoparticle coatings (desorption following hydrolysis process) may result in destabilisation of nanoparticles in the formulation itself. Desorption of nanoparticle coatings has been demonstrated for CdSe QDs (coated with hydrophobic ligands) upon interaction with HA; the process facilitated stabilisation of the hydrophobic nanoparticles in aqueous solution (Navarro et al, 2009).

Nanoparticle retention (soil/sediment-water distribution)

Given the different types of transformations that can occur within and outside the formulation matrix (before and after application of an agvet chemical), nanoparticles in the environment can exist in many different forms. One of the main drivers for transport of these nanoparticles will be how they partition between soil and water ($NP_{water} \leftrightarrow NP_{soil}$)—in relation to their retention in soil/sediment. Conventional parameters such as partitioning coefficients (K_d or K_{oc}) may not be directly applicable to nanoparticles. Instead, other surrogate parameters such as attachment coefficients are being proposed (Westerhoff and Nowack, 2013). Others have proposed using the retention coefficient (K_r), defined as the ratio of the concentration of the nanoparticle in the solid phase and the aqueous phase, which provides an estimation of the nanoparticle's mobility (Cornelis et al, 2010, 2011; Milani et al, 2012).

- K_r is distinguished from sorption coefficients (K_d) that are traditionally used to describe partitioning of solutes as it accounts for potential dissolution of metal-based nanoparticles.

Conventional partitioning coefficients (egK_d or K_{oc}) may not be directly applicable to nanoparticles. However, they may still provide some useful information until surrogate parameters such as attachment efficiency or retention values (K_r) become acceptable.

Examples of processes that may be involved in the retention of nanoparticles onto soil include adsorption, precipitation, and solid-state diffusion, which are all dependent on the characteristics of the nanoparticle surface and of the receiving matrix. Hence, K_r values would be expected to vary for different soil (sorbent) types, water chemistry conditions, as well as different nanoparticle surface chemistry. These factors are described in some of the examples given below.

- Note that, overall, studies on the behaviour of nanoparticles in soil have been significantly hampered by the limited techniques used to distinguish nanoparticles in complex systems. The influence of these factors (soil and nanoparticle properties) on nanoparticle retention has not been studied systematically. In most cases, comparisons were made between nanoparticles of different composition, which may intrinsically already have different retention behaviours, and without control of nanoparticle surface chemistry, such as size, shape and coatings. Examples below have been derived from both batch and column studies, with reported deposition from column studies treated as examples of retention.

Soil composition

- **High K_r** would generally be expected for soils or sediments that have the capacity to retain the nanoparticles and/or promote conditions that facilitate nanoparticle destabilisation. These retaining soils would typically have high clay, and high organic matter contents.

Example: Significant retention of nanoparticles in soils rich in organic matter and/or in clay content has been consistently observed for many of the metallic nanoparticles (uncoated or coated)—Ag nanoparticles (Cornelis et al, 2010; Coutris et al, 2012), CeO_2 nanoparticles (Cornelis et al, 2011); and TiO_2 nanoparticles (Fang et al, 2009) compared to sandy soils (Cornelis et al, 2010; Cornelis et al, 2011). Interaction of C_{60} with different clay types has also been investigated and was found to be a function of the available surface charge on the clay relative to the net negative surface charge of C_{60}. A synthetically produced sorbent (layered double hydroxide) exhibited faster association with C_{60} than to montmorillonite and kaolinite (Fortner et al, 2012). Zhang et al (2012) noted greater retention of C_{60} in freshwater sediments than model porous media. Some interactions also serve as important retention mechanisms to specific nanoparticles, ie formation of insoluble AgCl upon oxidation of Ag nanoparticles when Ag nanoparticles are subjected to high levels of Cl^- in the soil (Sagee et al, 2012).

- Note that while different retention behaviours have been observed for different soil types, very few comparisons of retention behaviour of one nanoparticle in several soils in a single study have been reported.

Water chemistry

- **High K_r** would be observed during long term heteroaggregation of nanoparticles' natural colloids.

Example: Deposition as a result of changes in ionic strength of the leaching medium has been reported for (uncoated) Fe_3O_4, TiO_2, CuO and ZnO nanoparticles in column experiments using porous glass beads, and for CNTs in a sandy loam soil (Ben-Moshe et al, 2010; Jaisi and Elimelech, 2009). Suspensions with higher ionic strengths and valency of constituent ions exhibited faster deposition rates. This aggregation and sedimentation behaviour has also been observed independent of nanoparticle surface modification for Ag nanoparticles in high ionic strength media (Stebounova et al. 2011). When compared to fullerenes, effective retention of CNTs (carboxyl-functionalised) by the soil matrix was associated with large aspect ratios and its highly bundled aggregated state in aqueous solutions (Jaisi and Elimelech, 2009). Clay (montmorillonite) has also been shown to destabilise nanoparticles of varying surface charges (negatively-charged Ag and positively-charged TiO_2) at relevant environmental conditions (pH 5-8) (Zhou et al, 2012). Indeed, heteroaggregation with suspended particulate matter could facilitate efficient removal of nanoparticles with sedimentation, as demonstrated for TiO_2 nanoparticles in a river system (Praetorius et al, 2012).

- No data are available on the combined effects of ionic strength and natural colloids on retention and deposition.

Nanoparticle surface chemistry

(Nanoparticles of different surface characteristics but the same core composition and soil type).

- **High K_r** would be expected for nanoparticles that have highly hydrophobic (less charged) surfaces, like nanoparticles coated with long chain alkyl groups, such as octylamines and phosphonic acids. Nanoparticles that have hydrophilic coatings with high positive zeta potentials may also result in high K_r following removal or desorption of bound ligands.

Example: The retention behaviour of Ag nanoparticles coated with citrate, PVP and HA varies with respect to the size and hydrophilic/hydrophobic characteristics of the coating material (Navarro et al, unpublished data)—Ag nanoparticles K_r citrate<PVP<HA—though the contribution from potential particle dissolution cannot be excluded. A similar observation was reported for CdSe QDs that were coated with mercaptopropionic acid and hydrophilic

polymers, where the polymer-coated nanoparticles exhibited less retention, and was suggested to be potentially due to the higher density of hydrophilic (OH) groups on its surface (Navarro et al, 2011). In the case of fullerene nanoparticles, retention was also significant for C_{60}, which is intrinsically hydrophobic, compared with its hydroxylated form (fullerol) (Lecoanet et al, 2004).The effects of surface charge have also been demonstrated, where positively-charged Al nanoparticles were preferentially retained compared to negatively-charged nanoparticles (Darlington et al, 2009).

- Indeed, when compared to uncoated nanoparticles, nanoparticles coated with hydrophilic ligands are poorly retained in soil. This was observed by Coutris et al (2012) for Ag nanoparticles.
- Studies that specifically investigate the effect of size or shape on nanoparticles' retention were not found.

Nano vs bulk forms

Example: When compared to ionic (soluble) controls, retention behaviours vary for different nanoparticles. K_r values for Ag nanoparticles and Ag^+ (performed in five soils) were reported to be in the same order of magnitude, whereas Kr values for coated CeO_2 nanoparticles dispersed in citrate were significantly lower than Ce^{3+} and Ce^{4+}. When compared to bulk controls, retention of nanoparticles is still lower (Cornelis et al, 2010, 2011).

Measurement of retention parameters

The applicability of batch sorption tests used for conventional active ingredients of agvet chemicals for nanomaterials is currently being debated in the literature. For some carbon-based nanomaterials (CNTs) as well as metal-oxide nanomaterials, batch sorption tests have been successfully used to predict behaviour under soil and wastewater treatment plant conditions (Westerhoff and Nowack, 2013). However, since the nanoparticles do not behave like dissolved chemicals, their properties may change based on the conditions used in the protocol. Size, surface charge, particle density or other properties such as the capping agent used or interactions with NOM need to be taken into consideration. Some workers suggest that since the nanoparticles form thermodynamically unstable suspensions in the aqueous phase, their retention should be considered in terms of heteroaggregation. Hence the attachment efficiency of heteroaggregation would be a suitable measure of retention (Praetorius et al, 2012). Column tests may be more appropriate for measuring the retention of nanoparticles under conditions more realistic of the receiving environment. Certain parameters used for conventional active ingredients such as K_{oc} are not suitable for use with nanoparticles. Indeed, the search for global parameters that are appropriate for nanoparticles requires much more research (Westerhoff and Nowack, 2013).

6.3.3 Transport and remobilization

Although environmental inputs of nanoparticles from agvet chemical products may be heavily concentrated to soil, agvet nanoparticles can indirectly enter water bodies through soil pores. Nanomaterial surface chemistry will influence this transport process. In general, transport through porous media will be likely for nanoparticles that have a **low K_r** and are poorly retained in soil. Enhanced soil mobility of nanoparticles may be observed with increased flow rates, and in the presence of species that could stabilise nanoparticles. Conversely, soil mobility could be limited when the nanoparticles enter small pore spaces (straining), and/or as a result of destabilising interactions between the nanoparticles and soil components (ie clays and soil organic matter) (Mcdowellboyer et al, 1986). The mobility of nanoparticles has been observed to be reduced with increasing aggregate size (not primary nanoparticles size—Darlington et al, 2009). For the heteroaggregated nanoparticles in the environment, this suggests that temporal changes in nanoparticle surface characteristics will likely impede their transport (Batley et al, 2012).

Studies on the remobilisation of retained nanoparticles have been fairly limited. Nonetheless, in theory, nanoparticles that are initially retained in soil could be remobilised and redispersed when interactions that facilitate retention are weakened or disturbed, often resulting in some surface transformation. For agvet chemical products, the process could be promoted as a consequence of agricultural practices, such as adding biosolids, fertilisers, treatments with hydrogen peroxide, tilling, and intermittent wet-drying cycles. In addition, the sodicity of soils, prevalent in Australia, could also be a factor leading to dispersion of nanoparticles from the soil matrix.

- Mixing biosolids that are rich in organic and inorganic compounds with soil could release species that potentially restabilise retained nanoparticles. The ability of dissolved organic matter to stabilise nanoparticles could essentially enhance nanoparticle mobility (Fang et al, 2009). In a recent study on the behaviour of C_{60}-spiked biosolids in soil, the levels of C_{60} released following water leaching correlated with the levels of dissolved organic carbon (DOC) released from the system (Navarro et al, 2013).
- Adding phosphate (from fertilisers) has also been reported to decrease retention (which potentially increases mobilisation) of (uncoated) CeO_2 nanoparticles in soils that have low colloid concentration. Poor retention was attributed to attachment of negatively charged phosphate onto the nanoparticles resulting in nanoparticle stabilization (Cornelis et al, 2011). Hence, for retained nanoparticles, remobilisation via this route is possible.

Processes such as soil erosion and surface run-off could also facilitate the transport of soil-adsorbed nanoparticles to water bodies where it is subjected to further transformation and potential remobilisation.
In water, soil-adsorbed nanoparticles will again be subjected to a change in surrounding conditions. High surface area properties of the nanoparticles could make them effective sinks for some organic compounds and the nanoparticles may act as vectors for certain contaminants.

6.3.4 Abiotically and biotically-mediated processes

The abiotic and biotic processes may involve degradation (and/or transformation) of the nanoparticles, leading to breakdown of the chemical or nanoparticles to non-toxic elements, compounds or building blocks such as C, H and O. Degradation is one of the major processes that determines the persistence and fate of a product in the environment. For conventional active ingredients a measure of persistence, such as half-life, would be a crucial parameter determining their risk in the environment. However, the situation with nanomaterials is slightly more complicated in that they undergo a range of transformations that may have a bearing on the toxicity profile of the nano-product. These are discussed under abiotic and biotic categories of processes below.

Abiotic processes

Abiotic processes include photochemical transformations, oxidation-reduction reactions, dissolution/precipitation and hydrolytic transformations. All of these processes have the potential to transform and/or breakdown the active ingredient or other constituents in the nano agvet chemical products to render them non-toxic and contribute to their loss from the environment. The details of such processes were provided earlier in this report. They enhance the environmental friendliness of nano agvet chemical products. For example, nano-formulations of conventional agvet chemicals such as imidacloprid, avermectin and chlorfenapyr have been developed that incorporate different proportions of nano-Ag and nano-TiO_2 (see reviews by Kah et al, 2012 and Gogos et al, 2012). Due to the presence of these photo-catalysts in the formulations, the persistence of these agvet chemical products on plant surfaces and in soil were found to be shorter than that of conventional products (see reviews by Kah et al, 2012 and Gogos et al, 2012).

Biotic processes

Biodegradation and biotransformation are critical processes that determine the persistence of toxicants such as pesticides and other organic compounds in soils. During the last forty years, much research has been published on conventional active ingredients of pesticides, demonstrating the ability of a wide range of microorganisms in soils to mineralise these compounds and use them as a source of carbon and energy (see reviews in Cheng et al, 1990). Repeated applications of pesticides and other organic molecules to soils have been reported to result in adaptation of microbial populations leading to accelerated degradation or much shorter persistence of the same compound. For nano- agvet chemical products, the biological processes leading to transformations or mineralisation of active ingredients or other constituents of formulated product remain highly relevant. Biotic transformations of nano-agvet chemical products may occur at several levels. For example, the carrier, such as a nanocapsule, may be broken down by biological processes in the receiving environment and thus influence the rate of release of the active ingredient. The active ingredient released may be transformed and/or mineralised by microbial processes. Similarly, nanoparticles in the formulation, such as an active ingredient or a catalyst, may undergo biotransformation through biological processes, as described earlier. Some of the processes discussed above under abiotic processes, such as oxidation/reduction, hydrolysis and other transformations may indeed be biologically mediated, as has been observed in the case of conventional chemicals (Bollag 1990).

Differences between conventional and nano agvet chemical products

Several aspects of nano agvet chemical products are expected to be different in comparison with the conventional agvet chemicals.

- The stability of the nano-active ingredient complex, such a polymer-active ingredient capsule, may be a significant rate-limiting step that may determine the rate of biotransformation/biodegradation of the active ingredient.
- Similarly, the capping agent used in the case of a nanoparticle as an active ingredient may be biologically removed and the properties of the particle may change.
- A pristine nanoparticle may be modified through interactions with organisms by adsorption of organic ligands and biological materials.
- Microorganisms may transform a nanoparticle into species that are insoluble, such as the conversion of $AgNO_3$ to Ag_2S.
- Unlike conventional organic compounds, metal oxide and other novel actives such as Si in agvet chemicals may not be able to support microbial processes through the supply of C or energy.
- The nano constituents of the formulation may inhibit biological activity in the receiving environment. Conversely, the targeted delivery of the active ingredient through nano-formulations may protect the beneficial functions of the microorganisms.

Currently there is limited understanding of the role of microbial processes in determining the fate of nanoformulation constituents or nano-agvet chemicals, or the effect of these on microorganisms in the environments. However, studies on metal oxide and carbon-based nanoparticles have shown some effects on soil microbial communities (Dinesh et al, 2012). The wide recognition of the antibacterial properties of metallic nanoparticles has led to the development of metal-based nanopesticides such as Ag-based biocides. In terms of nanoproduct biodegradation, Fukushima et al (2010) studied the biodegradation of poly(ε-caprolactone) and its nanocomposites and reported that the polymer was effectively degraded in composts but that nanoclay delayed the process. In a study using an OECD protocol on the ready biodegradability of C_{60}, Hartmann et al (2011) noted negligible transformation in 28 days. However, it has been reported that fungi can biodegrade the photolytic transformation product of C_{60} (fullerol) but not the parent compound (Schreiner et al, 2009).

Transformation products

The biotic and abiotic processes discussed above may lead to the production of transformation products or metabolites that retain toxic properties. This has been recognised for conventional agvet chemicals and therefore for several products the parent and the transformation products are generally considered together as a total toxic residue in the environment, for example atrazine and its transformation products and organophosphates such as aldicarb and fenamiphos and their thioxidation products. For nanomaterials, the transformation products need to be considered for their environmental fate and toxicological properties (as shown in the conceptual diagram—**Figure 6.3**).

For example, transformation products of the nano-Ag biocide Ag_2S are known to have very different toxicological properties and environmental fate. Ag_2S, which is produced when nano-Ag undergoes oxidative sulfidation, has very limited solubility and thus its mobility in the environment is expected to be very different from the $AgNO_3$ biocide. Peterson et al (2012) studied the adsorption and breakdown of penicillin in the presence of TiO_2 (anatase) nanoparticles in water. They found that a range of products such as penicilloic acid, penilloic acid and related de-ammoniated by-products were formed by degradation of amplicilin on TiO_2 surface. The degradation process was found to be pH dependent. Nano- agvet chemical products may be deliberately designed to have nanoparticles such as TiO_2 in the formulation. It is therefore important to understand the transformations that may be induced by the coexistence of nanoparticles and conventional active ingredients and the potential fate and effects of the by-products in the environment.

6.4 Ecotoxicological effects

6.4.1 General considerations

For conventional agvet chemical products there is a clear and broadly accepted approach for ecotoxicological effects assessment. However, this is based on assessing the effect of the active ingredient, and where it exerts its toxicological effect through its molecular interactions with the organism as a toxicant rather than as a particle. The ability of nanomaterials to enhance penetration or bioavailability and toxicity to the target organisms is among the main drivers for some of the emerging nano-formulations of agvet chemicals. Therefore it is crucial that such properties of nanoformulations are given adequate consideration in effects assessments on non-target organisms. Also the nano-constituent of the formulation (active ingredient or the excipient) may in some cases have potential to exert a toxicological effect in its own right. However, other formulations may be designed to reduce the toxicological impact or risk of the product in the environment. Regardless of the toxicity profile of the active ingredient in nano- agvet chemical products, the effects assessment may need to be on a formulation basis rather than on an active ingredient basis.

The toxicity of nano- agvet chemicals may arise either individually or from a combination of the active ingredient (either as a nanomaterial or a conventional molecule) and/or from the carrier or the nano-active ingredient complex and/or the nanoparticles themselves that have been added to the formulation. In the case of nanomaterials, an understanding of particle chemistry and interactions in the receiving environment is crucial in the context of bioavailability and ecotoxicology (Handy et al, 2008b, 2012; Klaine et al, 2008).

Consequently, methods of assessing the ecotoxicological impact of engineered nanoparticles may require considerable adaptation and careful control during the tests (eg Crane et al, 2008; Handy et al, 2012). Indeed, the challenges that ecotoxicologists face during effect assessment of nano- agvet chemical products are significant.

The next section identifies some of the differences between conventional approaches and those that may be suitable for nano agvet chemicals. It also identifies some of the aspects deserving extra care or caution.

6.4.2 Chemistry considerations for uptake and toxicology

Lipid solubility

For a conventional active ingredient, the aqueous and/or lipid solubility (often measured by octanol-water partition coefficient—K_{ow}) is one of the fundamental properties that determines the fate, bioavailability, uptake, bioaccumulation and effects of the chemical in organisms. However, depending on the nature of the nano-active ingredient complex for nano- agvet chemicals, lipid solubility alone may not be an appropriate indicator of nanoparticle phase transfer potential and the presence of nanoparticles can influence the partitioning behavior of organic compounds. For hydrophobic organic compounds (HOCs), conventionally K_{ow} has been a robust predictor of bioaccumulation. A number of studies examining nanoparticle accumulation in organisms (Hou et al, 2013) have shown that partitioning processes as measured by K_{ow} may not be relevant for predicting nanoparticle bioaccumulation. The uptake and accumulation of nanoparticles has been noted to be independent of factors influencing partitioning processes, such as the lipid content of organisms and organic carbon content of receiving environments. Indeed, the accumulation of nanoparticles in daphnids was found to be higher than that in fish, reflecting more their feeding mechanism than a partitioning process, unlike HOCs (Hou et al, 2013). For example, the accumulation of particles at the organ surfaces may be more important than their solubility (aqueous or lipid) for uptake and toxicity. Similarly, due to the tendency of particles to accumulate at the interface of octanol/water phases, depending on the pH of the system, measuring the K_{ow} of nanomaterials is fraught with difficulty.
A suitable adaptation of the conventional approach may be desirable to identify a surrogate parameter suitable for predicting the interaction of nanoparticles with biological or environmental interfaces, such as attachment/deposition efficiency (Westerhoff and Nowack, 2013).

Speciation

Interactions and transformations, such as dissolution, functionalisation, redox process, and photo-transformation that nanoparticles can undergo in the environment (waters, sediments, soils, and in or on biological organisms) may change their speciation, charge and other surface characteristics substantially, thus altering their fate, transport, bioavailability and ecotoxicology.

Hydrophobic fullerenes (C_{60}) have been shown to have increased dispersion in the presence of dissolved organic matter and their solubility may also increase due to the transformation into more water soluble poly-oxygenated/hydroxylated products such as fullerols (Hwang and Li, 2010; Klavins and Ansone, 2010; Pycke et al, 2012). Adding Suwannee River humic acid (HA) has been shown to greatly enhance the dispersion of multi-walled CNTs (Hyung et al, 2007). HAs have also been demonstrated to stabilise iron oxide, alumina, titanium dioxide, gold, and Ag nanoparticles (largely dependent on surface charge) (Akaighe et al, 2011; Baalousha, 2009; Diegoli et al, 2008).

Metallic nanomaterials can undergo dissolution and transformation processes in the environment as has been reported for Ag nanoparticles and ZnO nanoparticles (Kaegi et al, 2011; Lombi et al, 2012). The major pathway for Ag nanoparticles into the environment, such as through their use as an antibacterial agent, is believed to occur through the application of sewage sludge/biosolids to land. Kaegi et al (2011) reported the majority of Ag nanoparticles added into a pilot waste water treatment plant were converted to silver sulfide (Ag_2S). The authors concluded that physical and chemical transformations of Ag nanoparticles in waste water treatment processes would control the fate and toxicity of Ag nanoparticles and therefore need to be considered in future risk assessments.

Assessing the fate and ecotoxicity of ‘as-supplied’ nanomaterials may not provide a true understanding of the potential risks of agvet chemical products. A life-cycle history of agvet chemical products is needed for a true understanding of their potential risk, such as primary and secondary species, that may undergo speciation/transformations changes in the environment. For nano- agvet chemical products, changes in the characteristics of an active ingredient or nano-active ingredient complex may occur due to interactions with the constituents of the formulation. From an operational standpoint, the stability of the formulated product may govern the solubility and reactivity of the active ingredient. As mentioned earlier, nanoparticles are expected to behave very differently than the soluble molecules of the active ingredient. Speciation would be particularly relevant for those formulated products designed to enhance efficacy by facilitating their solubilisation through nanoparticulate size, such as the controlled particle size of bifenthrin by Liu et al (2008).

Dispersion and aggregation

What makes nano agvet chemicals different to conventional active ingredients is their dispersion (in natural waters, sediments or soils), their surface chemistry and reactivity, and especially their aggregation and colloidal chemistry. The physicochemical properties of the receiving environment are likely to play an even greater role for nanoparticles than appreciated for conventional chemicals. Environmental factors such as pH, salinity, divalent ions, and the presence of NOM, have been identified as important factors that alter the ecotoxicological impact of (Handy et al, 2008b; Klaine et al, 2008). The different nature of interactions of nanoparticles, such as aggregation chemistry, in various environmental matrices (air, fresh water, seawater, sediment or soil) may result in different ecotoxicological effects. Dispersion and aggregation are very important among different processes impacting ecotoxicology of nanoparticles (Handy et al, 2008b), namely:

- manufactured nanoparticles may aggregate or form stable dispersions
- aggregation chemistry and ecotoxicity may be affected by the particle and surface properties such as size, shape, surface area and surface charge
- manufactured nanoparticles may sorb on surfaces in soil, suspended sediment and on organism cell walls
- environmental conditions such as pH, salinity, water hardness, and the presence of NOM, may affect nanoparticle chemistry.

6.4.3 Uptake of nanoparticles by organisms

Bioavailability

Not only the molecular chemistry of the active ingredient (as in the case of conventional agvet chemicals), but also its particle chemistry, or the carrier, or active ingredient nano complex all assume major importance in relation to the biovailability and ecotoxicology of nanoparticles. The ecotoxicological effects of nanoparticles may either follow the same exposure pathways as conventional agvet chemicals or, in some cases, may expose the non-target organisms through unconventional routes. The nature of effects may also be different, such as the particulate nature of the active ingredient, or the excipient may elicit different forms of toxicological impact. For example, fullerene nanoparticles can potentially inflame and injure the gut walls of fish or earthworms, leading to poor feeding efficiency (Pakarinen et al, 2011).

Fish and vertebrates

The mechanisms and processes of uptake and toxicology are likely to be different for nano agvet chemical product active ingredients or excipients compared with conventional active ingredient molecules. For example, the mechanisms of absorption, distribution, metabolism and excretion (ADME) for nanoparticles in comparison with conventional chemicals in fish were considered by Handy et al (2008a). They noted that adsorption of nanoparticles on the gill surfaces of fish are likely to be similar for nanoparticles as other chemicals but their uptake in epithelial cells are more likely to occur via vesicular processes, such as endocytosis, than via diffusion or membrane transporters. This may make fish more vulnerable than mammals since fish guts are able to take up much larger materials across the cell membrane; indeed the oral delivery of fish vaccines has exploited this phenomenon (Handy et al, 2008b). The inflammation or injury caused by nanoparticles to the gut wall may facilitate direct uptake of nanoparticles in the blood, if nanoparticles do not aggregate in high ionic strength body fluids oozing out of injured tissue. Even low concentrations of nanoparticles such as TiO_2 and CNTs have been reported to cause inflammation of gills and injury to gut walls (Handy et al, 2008b). Similarly in terms of metabolism and excretion processes, they observed that rather than renal or bronchial excretion, the hepatic excretion into bile is likely to be the main mechanism of efflux for nanoparticles (**Figure 6.6**).

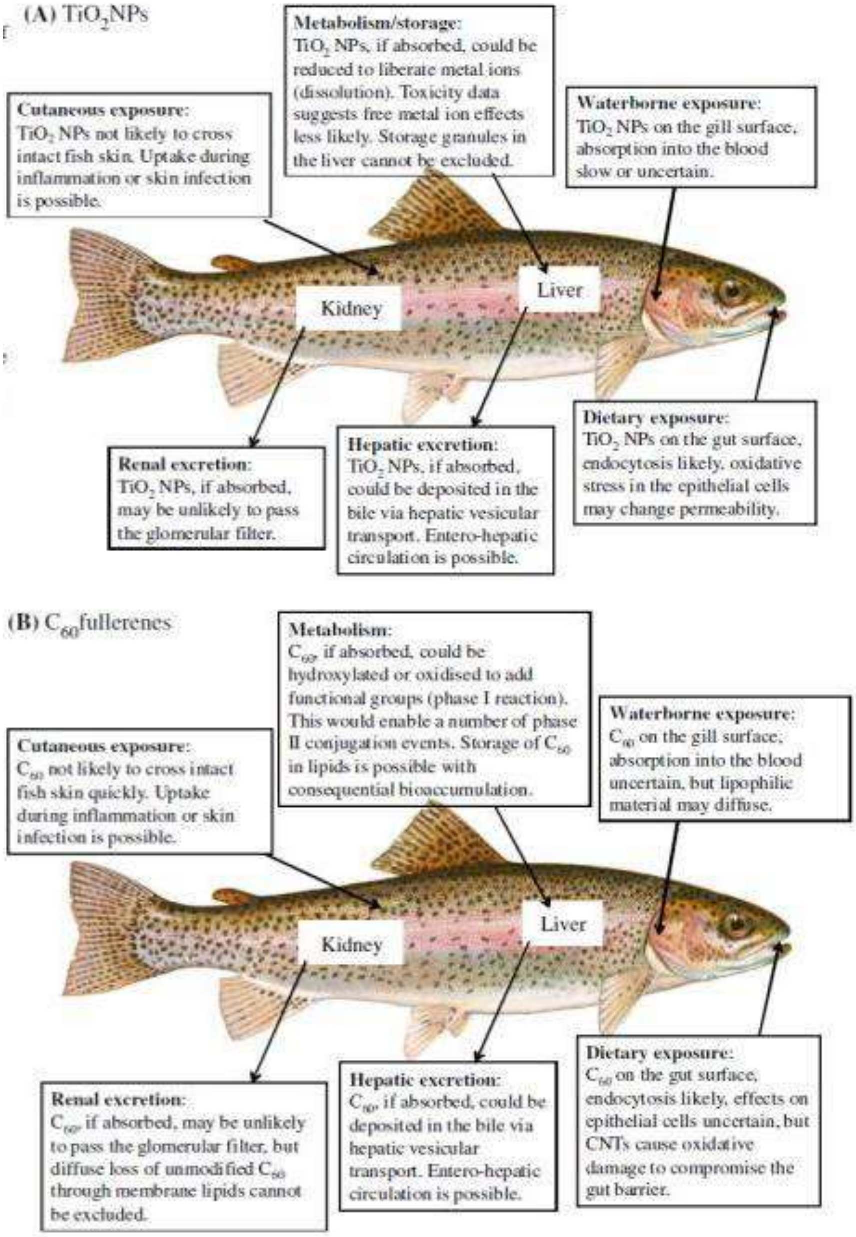
Invertebrates

In invertebrates also, the major route of entry for nanoparticles may be through endocytosis, ie penetrating through the semi-permeable cell wall (consisting of cellulose in algae and plants and chitin in fungi) and then through the bilayer lipid plasma membrane through active (ion channels or protein carriers) or passive mechanisms (Handy et al, 2008b). The nanoparticles may subsequently bind to organelles in the cell, produce toxic ions or ROS and interfere with the metabolic processes.

Plants

Plants have also been observed to uptake nanoparticlesvia endocytosis (Ovecka et al, 2005). The surface area (leaf area index) as well as the nature of plant surfaces, such as waxy leaves, may have an effect on the accumulation, bioavailability and uptake of nanoparticles by plants. The accumulation of nanoparticles and blocking of stomata may influence gas and heat exchange in plants and consequently change their physiology (Da Silva et al, 2006). Photo-induced electron transfer caused by nanoparticles may affect photosynthetic surfaces. Adsorption to cell surfaces in organisms can interfere with the uptake of essential nutrients.

It is well established that root exudates can enhance the bioavailability of metals through organo-metal complexes. However, it is not clear if these can influence the distribution, diffusion and bioavailability of nanoparticles to plants. Soil properties such as pH, salinity, nature of cations, clay mineralogy, and organic matter content are likely to affect the nanoparticle chemistry and thus are expected to affect the bioavailability of nanoparticles to soil organisms. It is therefore imperative that these soil properties are adequately considered in terrestrial ecotoxicological assessments.



(A) TiO_2NPs

Metabolism/storage: TiO_2 NPs, if absorbed, could be reduced to liberate metal ions (dissolution). Toxicity data suggests free metal ion effects less likely. Storage granules in the liver cannot be excluded.

Cutaneous exposure: TiO_2 NPs not likely to cross intact fish skin. Uptake during inflammation or skin infection is possible.

Waterborne exposure: TiO_2 NPs on the gill surface, absorption into the blood slow or uncertain.

Dietary exposure: TiO_2 NPs on the gut surface, endocytosis likely, oxidative stress in the epithelial cells may change permeability.

Renal excretion: TiO_2 NPs, if absorbed, may be unlikely to pass the glomerular filter.

Hepatic excretion: TiO_2 NPs, if absorbed, could be deposited in the bile via hepatic vesicular transport. Entero-hepatic circulation is possible.

(B) C_{60}fullerenes

Metabolism: C_{60}, if absorbed, could be hydroxylated or oxidised to add functional groups (phase I reaction). This would enable a number of phase II conjugation events. Storage of C_{60} in lipids is possible with consequential bioaccumulation.

Cutaneous exposure: C_{60} not likely to cross intact fish skin quickly. Uptake during inflammation or skin infection is possible.

Waterborne exposure: C_{60} on the gill surface, absorption into the blood uncertain, but lipophilic material may diffuse.

Dietary exposure: C_{60} on the gut surface, endocytosis likely, effects on epithelial cells uncertain, but CNTs cause oxidative damage to compromise the gut barrier.

Renal excretion: C_{60}, if absorbed, may be unlikely to pass the glomerular filter, but diffuse loss of unmodified C_{60} through membrane lipids cannot be excluded.

Hepatic excretion: C_{60}, if absorbed, could be deposited in the bile via hepatic vesicular transport. Entero-hepatic circulation is possible.

Figure 6.6: An illustration of the different routes for uptake, excretion and metabolism of TiO_2 nanoparticles compared to C_{60}fullerenes.Reproduced with permission from Handy et al, 2008a. Copyright (2008) Springer.

Soil organisms

The type of soil matrix may have profound effects on the fate, behaviour, and bioavailability of the test material. Although this is also well known for traditional HOCs, the effect of soil type on nanoparticle bioavailability may be through different, little known, mechanisms. However, studies on the bioavailability of nanoparticles to earthworms indicate that in contrast to HOCs (where soil properties such as organic carbon content determine the partitioning to soil and moderate the bioavailability of the contaminants to organisms) the uptake of CNTs was found to be independent of soil OC content (Petersen et al, 2011b). The likely soil factors that would affect particle chemistry directly (and thereby the bioavailability of nanoparticles to organisms depending on the exposure route) include soil pH, salinity, dissolved organic matter and other factors affecting fate in soil. Given the antimicrobial properties of nano Ag and TiO_2, it is expected that soil microbial processes may be sensitive to certain nanoparticles. However, there is limited information in the literature about this.

Bioaccumulation and biomagnification (trophic transfer)

The bioconcentration of HOCs for conventional agvet chemical active ingredients is well studied and represents the absorption (passive uptake) of chemicals by organisms from the environment, such as water or air phases, through dermal or respiratory pathways, excluding dietary uptake. The bioconcentration factor (BCF) is the ratio of chemical concentration in the organism to that in the environmental media—water, soil and sediment. The term bioaccumulation is used where food as an exposure pathway contributes to the accumulation of a chemical in organisms. Where the chemical gets concentrated in organisms of higher trophic level in the food chain, the term biomagnification is used. The accumulation through these processes has been the subject of recent studies on nanoparticles and has highlighted how nanoparticle behaviour can be very different to that expected of HOCs.

A review by Hou et al (2013) of ecotoxicological studies and compilation of data on the accumulation of nanoparticles in aquatic (mainly daphnia and fish) and terrestrial organisms (mainly earthworms) have shown that mechanisms explaining accumulation behaviour of conventional HOCs do not apply to nanoparticles. For example, BCF values for the accumulation of a range of nanoparticles, such as TiO_2, Ag nanoparticles and $nC_{60,}$ daphnids, were generally large enough (log BCF ranging from 3.16 to 5.64) to fall into the category 'very bioaccumulative substances' (for example USEPA criteria of >= 3.7). In contrast, the log BCF values for fish nanoparticle accumulations were much lower, (ranging from 1.27 to 2.87) the converse of that expected of HOCs, implying that the underlying mechanism of nanoparticle accumulation is different. The filter feeding behaviour of daphnids, and their ability to filter particles with size 0.4 to 40 µm (including nanoparticle aggregates), may be responsible for this. Furthermore, a lack of dependence on nanoparticle composition, particle size, aspect ratio and surface coating was noted. The uptake of CNTs by daphnids was reported by Petersen et al (2011b) to be unaffected by their surface modification with polymers with different charge characteristics (positive, negative or neutral). The current literature suggests that the bioaccumulation potential of nanoparticles in fish is relatively low and the major route of uptake may be oral (direct ingestion of nanoparticles) or via food (Hou et al, 2013). Bioaccumulation of several nanoparticles in earthworms has been found to be relatively lower than HOCs for non-nanoscale materials such as polyaromatic hydrocarbons. The organic carbon content of soil (from 1.6 to 5.7%) was found to have little effect on bioaccumulation in earthworms, which is in sharp contrast with HOCs (Petersen et al, 2011a). Similar to daphnids, the surface properties (coating with polyetheyleneimine polymer) had a minimal effect on bioaccumulation.

Trophic transfer, such as from 'algae to daphnid' and 'daphnids to fish', has been reported for QDs and TiO_2 nanoparticles (Hou et al, 2013). Similarly, biomagnification of QDs from contaminated bacteria to ciliated protozoa has been reported by Werlin et al (2011). In terms of terrestrial food chain effects, Judy et al (2011) reported tobacco hornworm caterpillars (*Manduca sexta*) bioaccumulating Au nanoparticles after ingesting plant tissue surfaces contaminated with nanoparticles, and found biomagnifications factors ranging from 6.2 to 11.6. However, they found no dependency on the Au particle size (from 5–15 nm). Clearly the plant uptake of nanoparticles also raises the possibility of human health exposure to nanoparticles through the food chain.

In summary, the bioaccumulation of nanoparticles in organisms has been demonstrated through several studies now. It is apparent mechanisms and routes of exposures are different for nanomaterials than for conventional HOC agvet chemicals. Currently no robust measure of the potential bioaccumulation potential of nanoparticles exists (such as K_{ow} for HOCs), although some attempts are being made to adapt conventional approaches in search of a suitable surrogate parameter (Hou et al, 2013).

6.4.4 Ecotoxicological test systems and their characterisation

Characterisation of formulated product and associated nanoparticles

One of the major weaknesses in several of the published studies on the effects of nanomaterials on organisms thus far has been poor control on the exposure to, and inadequate characterisation of, nanoparticles during the ecotoxicity testing. Nano agvet formulations are likely to range from nanoemulsions (with essentially no particle chemistry involved) to nano-active ingredient complexes doped with a nano catalyst, TiO_2 for example, and pristine metal oxide nanoparticles such as Nano Ag and Nano ZnO). It is therefore crucial to have a very clear understanding of the constituents of the formulation so that an appropriate test protocol can be designed. In this regard, even the minor constituents of the formulations, such as nanoparticles, or the impurities present in the formulation and/or indeed used in delivering the nanoparticles in test systems, may become a source of toxicity (**Table 6.6**). Some of the early work on ecotoxicity of nanoparticles such as fullerenes was seriously compromised by the presence of solvent impurities, for example tetrahydrofuran used in stabilising the suspension, as highlighted by Fortner et al (2005). Appropriate nomenclature and details of manufacturing processes can help establish the validity of the comparison with published work. Here particle size, shape and surface area to volume ratio for nanoparticles must be included, given their important role in determining particle chemistry, as discussed earlier.

Considering the currently available techniques and their practicality, a minimum set of characteristics for nano agvet chemical products used in ecotoxicity tests have been suggested (**Table 6.6**). Further details on some aspects of ecotoxicity test methods may be found in Crane et al (2008) and Handy et al (2008b).

Test organisms, organs and endpoints

Ecotoxicological tests on conventional agvet active ingredients have been conducted on aquatic organisms, mostly in aqueous media. However, for nanoparticles toxicity may arise from particles and therefore different organisms, such as mammals, as well as different endpoints, respiratory health and inflammation for example, may be necessary, depending on the environmental compartment being studied. Fairly well developed experience on particle toxicity in mammals could be valuable in guiding nanoparticle ecotoxicology. Lethal toxicity endpoints commonly used for conventional active ingredients for fish may be difficult to achieve for nanoparticles as they may aggregate more readily at high concentrations (Handy et al, 2008b). Therefore, sub-lethal effects, especially on target organs for nanoparticles, may be more appropriate. Target organs where nanoparticles are likely to adsorb, aggregate and accumulate, such as on gill surfaces, gut tissues, liver and brain in fish, may be the focus of ecotoxicological investigations (Kashiwada 2006; Smith et al, 2007). Oxidative stress in the developmental toxicity of fish is relevant for nanoparticles (Smith et al, 2007). Improved understanding of ADME of nanoparticles is required and the research and development on this in coming years may guide the selection of test organs and endpoints. For aquatic invertebrates, the water flea (*Daphnia magna*) and scud (*Hyallela azecta)* have been studied for carbon-based nanoparticles and lethal and sub-lethal effects such as moulting, mobility or feeding behaviour have been reported (Handy et al, 2008b). There is a lack of information on terrestrial invertebrates, though some studies on earthworms are available in the literature. For example, as stated above, inflammation and injury to the gut wall of earthworms could lead to poorer feeding efficiency (Pakarinen et al, 2011). Given the antimicrobial properties of nano Ag and TiO_2, it is possible that soil microbial processes may be sensitive to certain nanoparticles, however, there is limited information in the literature on this aspect.

Exposure conditions and their environmental relevance

Substantial uncertainty in current ecotoxicological studies of nanoparticles arises from the lack of control on nanoparticle exposure during testing. This is because the particle chemistry and particle stability may be changing during the test period. Even nanoparticles in a stable suspension may aggregate with time. Also, often it is not clear if a toxicological response is due to the particles, or the dissolved form, or both. Nanomaterials tend to aggregate and settle out of the water column depending on the test conditions imposed. For example, particle chemistry in seawater and freshwater is very different because the aggregation behaviour of nanoparticles is strongly influenced by changes in salinity (Stolpe and Hassellov, 2007). Therefore it is crucial that during test exposures, factors affecting the particle dispersion and aggregation (such as pH, salinity, water hardness, and the presence of NOM) are controlled and are maintained at environmentally relevant levels.

Use of reference materials, positive and negative controls

The availability of reference materials for nanoparticles is improving and their use in ecotoxicological studies should facilitate greater clarity on sources of toxicity. For example, in mammalian respiratory toxicological studies, particles with known toxicity to rodents, such as quartz or carbon black, have been used both as a reference material and as a positive control (Oberdorster et al, 1992). Reference materials of a known inert nature could be used as a negative control but such materials are currently lacking for aquatic and terrestrial ecotoxicological assessments. However, experience with suspended particles in the aquaculture literature may provide a starting point (Crane et al, 2008). Simultaneous measurements of reference material may provide a crucial insight as to the suitability of the ecotoxicological test method employed for the nanoparticles.

Table 6.6: Key characterisation and other details required for ecotoxicological tests (adapted from Crane et al, 2008; Handy et al, 2008b)

Parameter	Property	Reasons	Comments
Clear nomenclature and manufacturers information	Formulation make up and concentration of individual component (especially nano)	To design the test method appropriately and to know what to expect in terms of impurities	
Surface charge	Zeta potential	To assess electrokinetic potential and stability of the suspension	Surface charge is often dependent on pH of the system and hence a good control on pH is needed
Particle concentration or surface area by volume	Number or specific surface area by volume	To capture particle chemistry-driven toxicological effect	
Particle size	Mean particle size + SD	To understand nano-particle related effects	Measure particle size in both the stock and the test solutions. Use at least two different methods
Aggregation in test solution	Mean particle size + SD	To take into account any aggregation during the test	
Particle stability (eg dispersion) at various dilutions	A spectroscopic image	Visual evidence of dispersion/aggregation	Cover various dilutions used in the test system
Particle shape	Aspect ratio	Particle shape may affect toxicological impact	
Presence of the impurity in the formulation	Concentration	To assess potential contribution of toxicity due to impurities	
Impurity profile	Residual concentrations of impurity	To eliminate potential contribution of toxicity due to impurity	Details of washing procedure employed
Adsorption to vessels	Loss of particles or decreased concentration in control	To eliminate detoxification due to adsorption on test containers	Details of how the loss on test containers was eliminated
Appropriate controls	Effect assessment (eg EC50)	To isolate any experimental artifacts and clearly identify the toxicity source	Both positive or negative control may be useful
Relevance of test organism	Effect assessment (eg EC50)	To ensure an appropriate test organism is chosen for the target environmental compartment	
Relevance of test endpoint	Effect assessment (eg EC50)	To ensure an appropriate endpoint is chosen for the effect of nanoparticles	

Parameter	Property	Reasons	Comments
Environmentally relevant conditions	Effect assessment (eg EC50)	The test condition should reflect the environmental conditions	For example, the environmentally relevant concentrations of DOC, salinity, pH can influence aggregation of nanoparticles
Comparison of formulation versus a.i.	Effect assessment (eg EC50)	To compare the contribution of formulation constituent with that of a.i.	

SD = Standard deviation

6.5 Concluding remarks

The application of nanotechnology to agvet chemicals may span a wide range of products considered to be nanoformulations (some examples are given in **Table 6.1**). These may vary from nanoemulsions, nanoencapsulations, such as polymer-active ingredient complex, to pristine nanoparticles like nanometals and nanoclays. The literature discussed in this chapter is generally more relevant to nanoparticles as active ingredients because most of the literature is based on metal oxide or fullerenes particles. Since nanoemulsion formulations are metastable and do not contain nanoparticles, they may not need to be treated any differently than the conventional agvet chemicals. The category of nano-active ingredient complex (nano encapsulation with TiO_2 catalyst) is perhaps the most complex formulation where the approach to be adopted is quite different. For this category a pragmatic approach, based on the durability of formulated product, may be the way forward.

6.5.1 Need for a pragmatic approach

For nanometal oxides or nanoclays, the nanoparticles are expected to be persistent. However, the changes in their surface characteristics (either during product formulation or in the environment) may have implications for their fate and potential effects. For the nano-active ingredient complex involving complex formulations, information on the durability of a product, ie the fate of the product in soil or water, may become crucial in making decisions as to what material (conventional active ingredient, nano-active ingredient complex or free nanoparticles) should be tested and analysed in aquatic/terrestrial fate studies and in aquatic/terrestrial ecotoxicity studies. For example, if the polymer cage or capsule does not persist in the environment, and readily releases the active ingredient without releasing any persistent nanoparticles, the conventional risk assessment approach may be appropriate. Conversely, if the formulation results in the release of a significant number of nanoparticles, such as TiO_2 catalyst, the product has to be treated differently and assessed for risk as a nanomaterial.

6.5.2 Special considerations for fate and effect assessment

There are some major challenges that nanoparticles present during fate and effect assessment and they require a very different approach to that for conventional agvet chemicals. The key considerations for fate and effect studies are:

- The fate and effects of nanoparticles are likely to be governed by particle chemistry and their interactions, heteroaggregation for example, rather than the traditional molecular interactions with the environmental media and organism surfaces used for solutes.
- The aggregation chemistry and ecotoxicity of nanoparticles may be related to particle and surface properties such as size, shape, surface area and surface charge.
- Environmental conditions such as pH, salinity, water hardness and the presence of NOM may modify the physico-chemistry of nanoparticles and consequently their fate and effects.
- Conventional measures of fate and bioaccumulation potential such as sorption coefficient (Koc) or octanol-water partition coefficient (Kow) may not be directly relevant to nanoparticles. New measures such as attachment efficiency and retention coefficient (Kr) may be more relevant. However, these new indices are still under development and require further testing and standardisation.
- A critical weakness in current ecotoxicological studies in the literature is the lack of characterisation of nanoparticles during the test exposure time. Due to continuously changing properties (aggregation, deposition) with time, the exposure conditions must be controlled for a proper assessment of ecotoxicological effects.

6.5.3 Descriptors for fate and transport of nanomaterials

As discussed earlier, some of the global parameters commonly used by regulatory agencies to assess fate and transport may not be directly applicable to nanomaterials. Recently, some good discussion papers have considered the issue of suitability or adaptability of these parameters. For example, Westerhoff and Nowack (2013) considered the utility of a set of commonly used global parameters, such as K_{ow}, K_d and K_{oc} to predict the distribution of nanoparticles between environmental compartments. They suggested that, while a number of the existing parameters have considerable potential to be adapted for nanomaterials, the crucial need is the measurement techniques that are appropriate for nanomaterials and colloids. They presented a set of testing schemes that may be considered as a potential strategy for predicting the fate and transport of nanomaterials (**Table 6.7**).

6.5.4 Recommended requirements for characterisation of nano agvet chemicals

Given the above discussion and the importance of certain properties for fate and effects assessment, a minimum set of characterisation criteria are essential for nano- agvet chemicals. These have been listed in **Table 6.8** for both environmental fate and effect studies. Generally speaking, the properties that determine the stability of particles in suspension or their aggregation behaviour, namely, size, shape, zeta potential and specific surface are a minimum set that is needed for both fate and effects assessments. Aggregation state is a crucial parameter that determines the fate, as it provides an indicator of partitioning into different environmental compartments and the extent of exposure in, for example, the sediment or the water column in aquatic systems. However, the aggregation state is a function of ambient conditions such as pH, salinity, the presence of DOC and other complexing agents. Therefore, characteristics such as zeta potential and aggregation should be presented as a function of environmental parameters. Where possible, more than two methods based on different scientific principles should be used in the characterisation of nanoparticles.

Table 6.7: Testing schemes suggested for predicting fate and transport of nanomaterials. Reproduced with permission from Westerhoff and Nowack, 2013. Copyright (2013) American Chemical Society.

Testing approach	Example schemes	Potential fate and transport outcomes	Global descriptor
Solvent exchange	Measuring the distribution of nanomaterials between water containing nanomaterials and a solvent phase (octanol)	Single coefficient (K_{ow}, % hydrophobicity) indicating potential to interact with hydrophobic phases (eg soil, lipids, tissue)	K_{ow}
Surface affinity	Dynamic column tests using media coated with different surfaces (eg silica, iron oxide, hydrophobic material	Single coefficient (α) from 0 to 1 indicating tendency to interact with different environmental surfaces (soils, suspended sediment)	α_D
Sorption	Interaction of ions or NOM with nanomaterials	Changes in zeta potential, sorptive capacity factors	Freundlich isotherm K and 1/n values *OR* Ligand binding constants (L_1) and conditional stability constants (K_1)
Sediment retention	Measuring the retention of nanomaterials from water or natural soils or sediment	Single coefficient (K_R or K_D) indicating tendency to interact with environmental surfaces	K_R or K_D
Self-aggregation	Measuring aggregation kinetics of nanomaterials with themselves in water matrices with different ionic composition or NOM	Single coefficient (α) from 0 to 1 indicating the effect of water composition on the tendency of nanomaterial to aggregate into larger particles that could settle out of a water column	α
Electrostatic repulsion	Measuring the zeta potential as an indicator of particle stability	Zeta potential of nanomaterials can be binned into likely to be stable or likely to aggregate	E_{NET} (net energy barrier)
Multidimensional parameter or high-throughput testing	Automated chemical addition of salts, organics, acids, or suspended sediment to quickly assess nanomaterial stability	3D contour plots of key parameters (zeta potential, turbidity) to understand nanomaterial stability in a series of water chemistries	STIFF diagram plotting parameters
Dissolution kinetics	Measuring dissolution of metallic nanomaterials as a function of dissolved oxygen, pH, and redox conditions	Thermodynamic conditional stability coefficients and surface area-dependent kinetic rate constants; solubility limits	$k_{dissolution}$
Weathering	Simulated photolysis, dissolution, and biodegradation of nanomaterials and/or their coatings	Changes in size, zeta potential, composition, coatings, etc.	k_i (multiple rate constants for different “i” mechanisms)

Table 6.8: Recommended minimum characterisation requirements for agvet chemicals.

Property/parameter	Environmental fate	Effects	Techniques available	Comments
Chemical and/or elemental composition including capping agents, impurities (especially nano)	Capping and functionality may have a major impact on fate. Fate of impurity only important if shown to be toxic in nature	Essential to assess the potential toxic impact on non-target organisms, especially of the nano component and any impurities	Combination of conventional (egchromatography) and nano-specific techniques listed below in this column	Appropriateness and robustness of method should be demonstrated
Solubility	Determines mobility and phase transfer	Conventionally toxicity is linked with solubility	Dialysis membrane, centrifugation	Toxicity may arise from both soluble and particulate fractions
Primary particle size, size distribution	Fate and transport is dependent on particle size	Essential for ecotoxicological effects assessment where size is seen as an important determinant of toxicity	TEM, SEM, Cryo-TEM, XRD	Size distribution is more appropriate. At least two methods based on different principles to be used
Aspect ratio	May impact fate and transport (desirable but not essential)	Shape may have a major impact on toxicology	TEM, SEM, cryo-TEM	Morphology may elicit unique toxicity responses for inhalation exposures, eg asbestos
Specific surface area (SSA)	Particle chemistry-driven fate processes are dependent on SSA	High specific surface area may induce toxic effects	BET	Surface interactions with organisms and in environment are influenced by SSA
Surface charge	Determines the stability of suspension and mobility of nanoparticles; will determine mobility in the environment	Stability of suspensions during ecotoxicity testing is linked to zeta potential	Electrophoretic mobility	Surface charge distribution at different environmentally-relevant pH values is needed
Aggregation behaviour	Governs fate and behaviour but heteroaggregation may be more important in this case	Homoaggregation may be more important for ecotoxicity testing. It is crucial to maintain suspension stability during the ecotoxicity testing.	Light scattering, cryo-TEM, FFF, disc centrifugation	Data relating to important environmental parameters, eg pH, salinity, dissolved organic matter, is needed
Surface chemistry	Any product-modified transformations as well as those due to weathering in environment	Toxicity depends on surface chemistry (eg capping on nanoparticles)	Spectroscopy, TGA	Including any transformations that are expected to occur in the formulated product or in the environment

Property/parameter	Environmental fate	Effects	Techniques available	Comments
Speciation	Fate of nanoparticles is markedly influenced by speciation (e.g. $AgNO_3$ versus Ag_2S)	Different species (including transformation products) may have different toxicity than the parent particle	Synchrotron, FFF, Chromatography	Speciation may change with time in product or in the environment
Stability of formulation	The information about the stability of nano-a.i. complex is crucial for appropriate assessment of environmental fate	The decision about appropriate ecotoxicity assessment can only be made based on the stability of the product	Conventional plus light scattering, TEM, Cryo-TEM	The fate and ecotoxicity assessment of the product needs to be compared with a.i. to ensure there is no added toxicity due to the formulation

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APPENDIXES

APPENDIX 1 – PARTICLE CHARACTERISATION—INSTRUMENTS AND TECHNIQUES USED TO MEASURE THE SIZE AND SHAPE OF NANOPARTICLES

Particle characteristion techniques are classified under three broad headings: (i) ensemble techniques; (ii) single particle analysis techniques; and (iii) separation techniques. The instruments and techniques relating to each of these classifications are discussed below. Aerosol characterization and the measurement of nanoparticle surface area are also discussed in this Appendix.

A1.1 Ensemble techniques

Ensemble techniques average over a large number of particles and measure an average for the system as a whole. These techniques provide good statistical representation of the particle system but are often unable to resolve contributions from individual particles or from small parts of a broad particle size distribution.

A1.1.1 Dynamic light scattering

Dynamic Light Scattering (DLS), also known as photon correlation spectroscopy (PCS) or quasi-elastic light scattering (QELS), is a method in which particles undergoing Brownian motion in a liquid suspension are illuminated by a laser. Analysing the time-dependent intensity of the scattered light yields the translational diffusion coefficient and hence the particle size as the hydrodynamic diameter via the Stokes-Einstein relationship (see Figure A1.1).

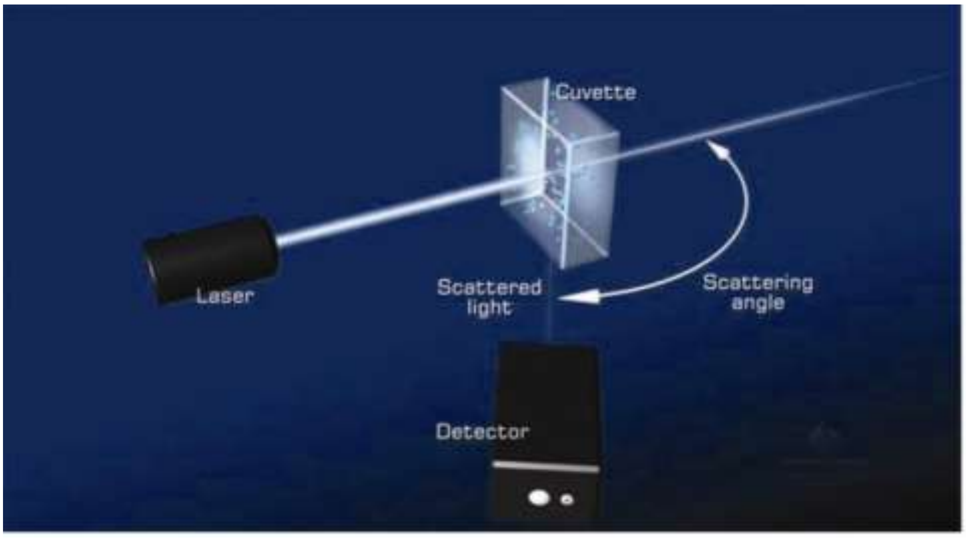

Figure A1.1: Typical DLS configuration: a laser illumination source, a sample under measurement in the cuvette and a detector (Jamting and Miles, 2013).

The time dependence of the intensity fluctuation is most commonly analysed using a digital correlator. Such a device determines an intensity autocorrelation function that can be described as the ensemble average of the product of the signal with a delayed version of itself as a function of the delay time. The ‘signal’ in this case is the number of photons counted in one sampling interval. At short delay times, correlation is high and, over time as particles diffuse, correlation diminishes to zero and the exponential decay of the correlation function is characteristic of the diffusion coefficient of the particles. Data are typically collected over a delay range of 100 nanoseconds to several seconds depending upon the particle size and viscosity of the medium.

Analysing the autocorrelation function in terms of particle size distribution is done by numerically fitting the data with calculations based on assumed distributions (Morrison et al, 1985; Ruf 1993). A monodisperse sample would give rise to a single exponential decay, to which fitting a calculated particle size distribution is relatively straightforward. In practice, polydisperse samples give rise to a series of exponentials and several quite complex schemes have been devised for the fitting process.

DLS can provide accurate Particle Size Distributions (PSDs) for samples that approximate a monodispersed system. The technique is fast and easy to use, and can measure particles with diameters in the range from approximately 1 nm to 5 µm, depending on particle density. The sample concentration is typically limited to 10^7–10^{11} particles/mL. DLS requires only small sample volumes (typically less than 1 mL of suspension at ~0.1% particle mass fraction). A range of suspendants can be used, keeping in mind that the viscosity and the refractive index of the suspension medium, as well as the temperature of the system, have to be known. Due to its ease of use and quick turnaround, DLS is commonly used in research and for quality control purposes. This technique is non-invasive and the samples can be fully recovered after analysis.

DLS measurements are sensitive to the quality of the suspension, as the intensity of the scattered light is proportional to the sixth power of the particle size. This means the scattering signal due to the presence of dust or agglomerated or aggregated particles will obscure the scattering signal from smaller particles. This also makes DLS less suitable for accurate measurements of broad PSDs. For low particle concentrations, there is a risk of number fluctuations due to a low number of particles in the measurement volume. If the particle concentration is too high, errors can occur due to multiple scattering events. For analysis, it is assumed that the particles are spherical and that the sample composition is homogenous. Information on particle shape can only be obtained in instruments equipped with multi-angle or goniometric detector configuration.

To ensure the best possible measurement results the DLS instrument performance should be checked regularly. The ISO standard (ISO, 2008b) recommends that a verification procedure be performed by measuring 100 nm polystyrene latex spheres with a narrow size distribution. Intermittent checks should be performed as required using suitable reference materials.

A1.1.2 Laser diffraction

Laser diffraction (LD) is a well-established ensemble technique that allows measurements of particle suspensions over a wide range of particle sizes from 100 nm up to several mm. The technique is easy to use and fast.

A collimated laser beam is passed through a sample suspension of particles and an array of detectors is located at different angles to the transmitted beam. The particles in the sample scatter the light at angles related to their size. The larger particles in the sample scatter light with strong intensity at small angles and smaller particles scatter light with lower intensity at larger angles. Assuming that the particles are spherical, and that the refractive index (both real and imaginary) of the particles and the optical properties of the suspendant are known, Mie theory can be used to convert the scattering pattern from all sizes of particles into a volume-weighted PSD. For large particles (diameter $\gtrsim$50 µm) the Fraunhofer approximation can be used, which assumes that the particles are opaque, that particles of all sizes scatter light with the same efficiency and that the particle size is much larger than the wavelength of the light (Bohren and Huffman 1983). Particles with diameters much less than the laser wavelength will scatter light uniformly in all directions and LD systems are often equipped with a backscattering detector to capture the signal from the smaller particles.

The detection range of the LD technique excludes size measurements of very small particles. But it can be a very useful tool when assessing complex particle systems containing agglomerates and/or aggregates. In most cases it is possible to recover the samples after analysis.

LD measurements require the sample concentration to be optimized to avoid multiple scattering effects or particle-particle interactions. Since LD is an ensemble technique, the sample is assumed to be of homogenous composition. One of the requirements when applying Mie theory to model the results is that the optical properties of the particles and of the suspension medium must be known, and that assumptions about the particle shape have to be incorporated into the model. The technique is limited in its ability to discriminate between different particle populations with closely spaced mean diameters.

To ensure the best possible measurement results the instrument performance should be checked regularly. The ISO standard (ISO, 2009) recommends performing measurements on traceable, spherical certified reference materials (CRMs) to ensure accuracy.

A1.1.3 Small Angle X-ray Scattering

Small Angle X-ray Scattering (SAXS) is a method which measures the elastically scattered intensity of X-rays for small-angle deflections. The angular scattering is usually measured within the range 0.1° to 10°, providing structural information on macromolecules as well as periodicity on length scales, typically larger than 5 nm and less than 200 nm, for ordered or partially ordered systems.

The technique is non-destructive for most materials and does not require complex sample preparation. The instruments use a collimated, monochromatic X-ray beam, and the sample is rotated through a range of angles generating a scan of scattering intensities versus angle. The resulting scattering signal is modelled and the results can provide specific information about the sample, such as the radius of gyration, particle shape, and size and shape distributions. By using advanced data analysis software, it is possible to resolve complex multimodal size distributions.

Traditional SAXS instruments are very expensive and even more so when considering the synchrotron sources that are now providing SAXS beam lines. Measurements and data analysis can be very complex. Sample preparation is important as this is a scattering technique and the presence of large particles or aggregates may suppress the signal from smaller particles. Both the experiments and the analysis can be time consuming.

The ISO standard (ISO, 2001c) recommends verifying instrument performance at regular intervals using CRMs or particles with a known size distribution. There are nanoparticle reference materials available that can be used for instrument verification.

A1.1.4 Particle tracking analysis

Particle Tracking Analysis (PTA) uses a laser to illuminate particles undergoing Brownian motion in a liquid suspension and the change in position of individual particles determines particle size.

The instrument dynamically tracks individual particle positions in real time, and records the resulting length of the particle track as well as the scattered intensity. The particle size is derived from analysing the track length and time by determining the diffusion coefficient, which can then be related to the hydrodynamic diameter via the Einstein-Stokes equation (ISO, 2008b).

The detectable size range is ~20 nm–1000 nm, the absolute limits of which depend on the scattering properties of the particles, their size, and the instrument configuration. A PSD measured using PTA is a number-weighted distribution of the hydrodynamic diameters. Different laser wavelengths allow studies of small-sized particles and/or fluorescent particles.

As the particle size measurement is based on the tracking of individual particles, the results are not greatly affected by size-dependent scattering intensity. It is possible to resolve particle sizes in multimodal mixes to a moderate degree. The technique can be used for in situ studies of particle aggregation (Montes-Burgos et al, 2010). The technique is also suitable for use with a range of suspendants, although the viscosity and temperature of the liquid must be known. Being a single-particle measuring technique, PTA can be used to determine particle number concentration.

The PTA analysis model for hydrodynamic diameter assumes that the particles are spherical. The suspendant needs to be optically transparent and the viscosity and measurement temperature must be known. The particle motion is tracked in a two-dimensional focal plane, and an approximation is made to fit the diffusion behaviour for a particle undergoing three-dimensional Brownian motion. Only a limited number of particles are analysed, providing limited statistical relevance. Even if analysis is carried out for extended periods of time to generate more data, there is a chance that the same particles will be analysed repeatedly. The data analysis is susceptible to user interpretation and requires a high level of understanding to interpret correctly.

PTA is a newly developed technique and thus no standards for this type of instrument currently exist. However, there is a range of reference materials of different composition available that can be used for instrument verification.

A2.1 Single particle analysis techniques

Single particle analysis techniques measure the properties of individual particles and can resolve particle size distributions in great detail but are limited by small sample sizes. Although it is possible to increase the number of particles that are measured, the time and expense involved are prohibitive.

A2.1.1 Electron Microscopy (EM)

Electron microscopes use electrons for illumination rather than visible light. This is because the maximum resolution (ability to discriminate features) of a microscope is approximately equal to the wavelength of the illumination used. This is 300 to 600 nm for visible light but for electrons the wavelength, and hence the potential resolution, is 0.002 to 0.1 nm depending on the electron energy.

The transmission electron microscope (TEM) produces magnified images or diffraction patterns of a sample by passing the electron beam through a very thin sample and interacting with it. The spatial variation in this image is then magnified by a series of magnetic lenses until it is recorded by hitting a fluorescent screen, photographic plate, or light-sensitive sensor such as a charge-coupled device (CCD) camera. The image detected by the CCD may be displayed in real-time on a monitor or computer.

New generation TEMs have overcome spherical and chromatic aberration and produce images with very high resolution. The ability to determine the positions of atoms within materials has made high resolution TEMs an indispensable tool for nanotechnology research and development in many fields.

Unlike the TEM, where electrons are detected by beam transmission, the scanning electron microscope (SEM) examines and analyses the physical information (such as secondary electrons, backscattered electrons, absorbed electrons and X-ray radiation) obtained by generating electron beams and scanning the surface of a sample to determine the structure, composition and topography of the sample.

Generally, the TEM resolution is about an order of magnitude better than the SEM resolution. However, because the SEM image relies on surface processes rather than transmission it is able to image bulk samples and has a much greater depth of field, producing images that are a good representation of the three-dimensional structure of the sample.

EMs are very useful tools in nanoparticle characterisation, though they are very expensive to purchase and require great expertise to operate and maintain properly. They can provide representative images of nanoparticles, as well as measurements on a single particle basis. For particle sizing, the measurand in EM is a diameter such as a projected area diameter, x_a or a Feret‘s diameter, x_F. The number-weighted particle size distribution (PSD) can be constructed by analysing a large number of particles. However, it can be very time consuming to generate results that are statistically relevant and representative of the entire sample.

The TEM requires an ultra-high vacuum and a high voltage. To be imaged the sample needs to be transparent to the electron beam, small enough to be placed on a copper support grid (about 3 mm in diameter) and inserted into a suitable holder. The electron optical column in the SEM is shorter than in the TEM, as there are fewer lenses involved in generating the beam. The column typically houses gun alignment coils, lenses that condition the beam into a fine spot on the sample surface and the scan coils. In the SEM, the focused electron beam is scanned in a raster pattern across the sample using a set of scan coils. As the beam scans across the specimens, different interactions between the beam and the sample occur. A range of detectors in the chamber above the specimen detect the signals from these interactions. The most commonly used detectors pick up the signal from secondary electrons; that is, electrons that have been knocked from their positions by the scanning focused beam. Different interactions of the beam and sample give images based on topography, elemental composition (x-rays), density variation or crystalline structure of the sample.

TEMs can have resolution limits as low as sub-nm, and can be used to analyse both large numbers of nanoparticles at lower magnifications and individual particles at higher magnifications. The technique can be suitable for characterisation by image analysis, as the darker areas in the formed image represent areas where fewer electrons have passed through the sample due to higher electron density, such as nanoparticles.

The TEM beam is quite wide in normal imaging mode but, due to the sophisticated system of lenses, it can also be formed into a very fine (sub-nm) focused electron probe. Combined with the thin, electron-transparent sample it enables the generation of diffraction patterns for crystallographic information.

The resolution of an SEM depends on the size of the beam, but ranges from a few nm up to several mm. The SEM can be used to study almost any kind of sample, as long as it is conductive. This makes sample preparation for SEM much less complicated than for TEM. For particle sizing, the measurand in SEM is a number weighted distribution of diameters, such as a projected area diameter, x_a or a Feret’s diameter, x_F.

The accelerating voltages used in SEM are much lower than in TEM since the beam does not need to penetrate the specimen. As the imaging does not depend on the density of the sample, it is possible to image low-density samples. The sample chamber in an SEM is considerably larger than in a TEM and is often capable of housing many different samples at the same time. The SEM has a substantial depth of field, which allows a large amount of the sample to be in focus at one time and produces an image that is a good representation of the three-dimensional sample. Using EDS (Energy-dispersive x-ray spectroscopy) allows for elemental detection, but as the beam-sample interaction volume in SEM is quite large, the element detection is less sensitive than for TEM.

The sample stage for a TEM is invariably only capable of holding one sample grid at a time, so if multiple samples are to be examined the process lengthens considerably. Preparing samples for TEM can be very time consuming and it requires expertise to get good and reproducible results. For nanoparticle suspensions, the sample has to be thoroughly dried and this may alter the appearance of the particles. The drying process itself can generate artefacts such as apparent aggregation. Also, for nanoparticle suspensions, the particle concentration needs to be low which may lead to a poor sampling ratio. It can also be very time consuming to generate results that are statistically relevant and representative of the entire sample.

An SEM usually requires a conducting sample. If the sample is non-conducting, a conductive coating may be applied to prevent charge from being built up in the sample. But carrying out dimensional measurements has to be done carefully, as any measurement will show the applied coating as well as the feature of interest. Particles suspended in a liquid have to be deposited onto a suitable substrate and thoroughly dried before inserting into the vacuum chamber. This may cause significant changes to the sample. Also, as the scanning motion of the electron beam in an SEM gives a topographical image, this often leads to some edge distortion caused by strong beam interaction.

To ensure the best possible measurement results, electron microscopes should be checked, verified and calibrated as required. For TEM a typical particle measurement is usually based on image analysis, making the image calibration crucial. For SEM there is an ISO standard (ISO, 2004b) on image/instrument calibration at a range of magnifications using a purpose-made artefact. There are also ranges of reference materials of different composition (Au, SiO_2, PS latex) that can be used to calibrate images.

A2.1.2 Atomic force microscopy

Scanning probe microscopy (SPM) is a method of imaging surfaces by mechanically scanning a probe over the surface under study and measuring the response of a detector. This generic term includes many methods such as atomic force microscopy (AFM), scanning near field optical microscopy (SNOM), scanning ion conductance microscopy (SICM) and scanning tunnelling microscopy (STM). AFM is one of the most common techniques where a solid tip measures the Van der Waals forces between the tip and the surface. The tip is on the end of a flexible beam or cantilever whose displacement is measured using a laser beam.

The AFM instrument can be operated in three modes: contact, non-contact and tapping. The first involves the tip staying in contact with the surface at all times. This can result in the tip scratching the surface for very soft materials and excessive wear of the tip for hard materials, changing its original shape. The non-contact mode uses an oscillating cantilever with high stiffness. The tip is brought into such close proximity with the surface that the oscillation frequency changes. The change in frequency is measured during scanning and produces a map of the surface. The tapping mode also uses an oscillating cantilever but the tip is brought closer to the surface than in non-contact mode, so that the tip touches (taps) the surface intermittently. At the point of contact, the oscillation of the cantilever is reduced and this change can be used to detect features of the surface.

AFM has several advantages over the EM. Samples viewed by an EM require special treatment that is often destructive and need an expensive vacuum environment to work properly. AFMs work perfectly well in an ambient or even liquid environment.

The resolution of an AFM is very high. Features down to 0.1 nm can easily be detected in the *z* direction. The resolution in *x* and *y* direction is directly linked to the sharpness and shape of the tip, but features as small as a few nm can be imaged with a standard tip. The AFM can generate three-dimensional maps of surfaces and image individual nanoparticles. Some information about size and shape can be deducted from AFM images and it is possible to make particle size measurements based on height measurements.

The main disadvantage of the AFM is the image size. The SEM can show an area in the order of millimetres by millimetres and a depth of field in the order of millimetres. The AFM can only show a maximum height in the order of micrometres and a maximum area of around 150 by 150 µm. Also, the AFM cannot scan images as fast as an SEM. It may take several minutes to scan a typical region with the AFM, while an SEM is capable of scanning at near real time.

The information generated using AFM strongly depends on the tip shape (Villarrubia 2004). The illustration in Figure A1.2 shows a nanoparticle on a substrate being scanned with a) an ideally thin and sharp tip, b) a more realistic AFM tip and c) the result from a 100 nm polystyrene particle scanned with a typical AFM tip. The image illustrates the complexity in using AFM for nanoparticle sizing, where the tip may cause severe distortion to the shape of a feature in the *x* and/or *y* directions.

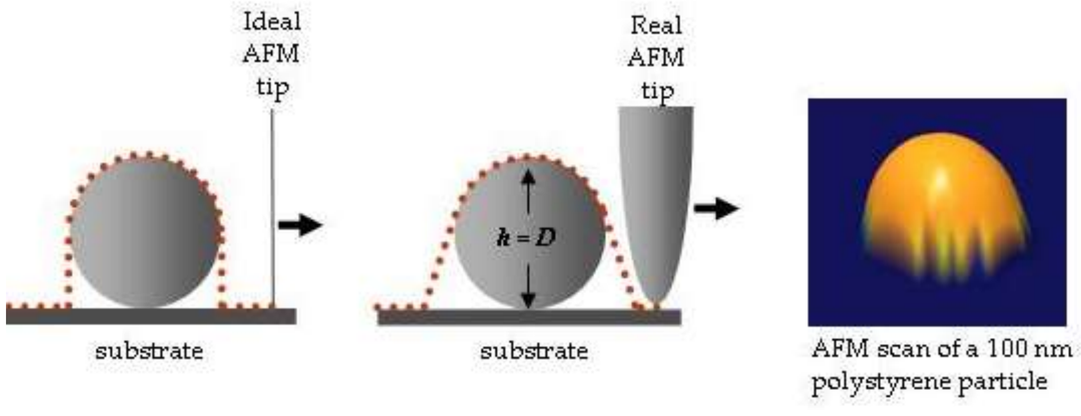

Figure A1.2: A schematic on a substrate being scanned with a) an ideally thin and sharp tip, b) a more realistic AFM tip and c) the result from a 100 nm polystyrene particle scanned with a typical AFM tip (Jamting and Miles, 2013).

Preparing a sample for AFM measurement can be complex and the process of placing a particle suspension onto a substrate may change the properties of the particles. Like the other microscopy techniques, it can also be very time consuming to generate results that are statistically relevant and representative of the entire sample.

To calibrate an AFM, the preferred method is to perform a set of measurements on an appropriate physical standard, which should be chosen according to the requirements. There are ranges of suitable artefacts with regular periodic structures of well-known dimensions in one, two or three dimensions, a comprehensive list of which can be found on the German NMI website (Physikalisch-Technische Bundesanstalt—PTB). There are also ranges of nanoparticle reference materials available with different composition (Au, SiO_2, PS latex) that can be used to evaluate performance.

A3.1 Separation techniques

Separation techniques are based on a separation step before applying detection and measuring techniques. Fractionation allows the sample to be separated into smaller volume fractions which can be detected with either an ensemble technique now capable of detecting contributions to the measurement from each fraction, or further analysed using single particle analysis techniques.

A3.1.1 Differential Centrifugal Sedimentation

Differential Centrifugal Sedimentation (DCS) is a method in which a sample is separated based on size and density using a rotating disc filled with a fluid containing a density gradient. The instrument consists of a hollow spinning disk into the centre of which a sample is injected. The disk is mounted on a drive shaft that rotates at a known speed. The particles sediment radially from the centre of the disk through the fluid after injection. A detector beam (usually a laser beam) passes through the liquid near the outside edge of the disk, and as the particles pass through the beam, the intensity is reduced as the particles obscure the beam proportionally to the concentration and particle size. The measurand is the sedimentation time, which can be converted to diameter using Stokes' Law and to a volume-weighted PSD using Mie theory, if the optical properties of the particles are known (Jamting and Miles, 2013).

The disk centrifuge is capable of measuring a wide range of particle sizes, from 5 nm to 30 μm, depending on the rotation speed and the density of both the particles and the gradient fluid. It is possible to achieve very good size

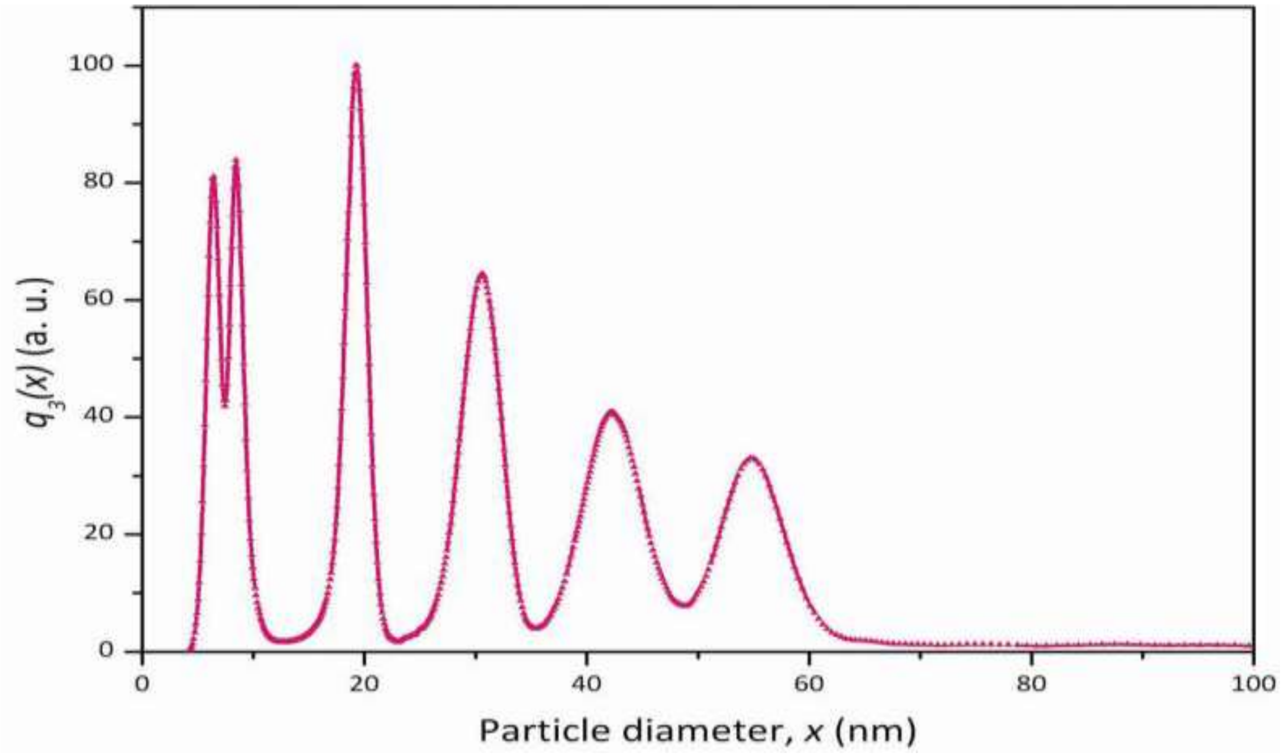

Figure A1.1: Volume-weighted PSD of a 6 modal mix of Au nanoparticles, measured by DCS. The plot illustrates the ability of the technique to clearly separate each of the particle populations in the 6-modal Au suspension (nominal diameters: 5 nm, 10 nm, 20 nm, 30 nm, 40 nm and 50 nm) (Jamting and Miles, 2013).

discrimination, as illustrated in Figure A1.3, which shows the results from a typical DCS measurement of a 6-modal gold nanoparticle suspension, with nominal particle diameters ranging from 5 nm to 50 nm.

Typical gradients can be created using sucrose and water, which can easily be adjusted to change the density of the gradient. Other suspendants, such as oil or cell culture media, can be used to create the gradient so particles can be measured in an environment representative of typical applications, such as cosmetics or toxicological studies.

The requirement that the particles have a higher density than the gradient is the most limiting factor of the technique. The DCS analysis for the Stokes diameter assumes that the particles are spherical and that the sample’s composition is homogeneous. The densities and optical properties of both the particle material and the gradient fluid have to be known. Only dilute samples can be measured. Low-density particles require a low-density gradient and high rotational speed. It may take a long time to measure complex samples and it may not be possible to keep measurement conditions constant during the experiment.

The sedimentation velocity can be calibrated directly preceding each measurement run using reference particles of known diameter and density. A range of reference materials of different composition is available, though currently there is a lack of these materials for such calibration. Several ISO standards provide information about best practices in DCS measurements (ISO, 2001a, 2004a, 2007a).

A3.1.2 Field Flow Fractionation

Field Flow Fractionation (FFF) is a separation technique where a field is applied to a liquid suspension passing along a narrow channel in order to separate the particles present in the liquid, depending on their differing mobility under the force exerted by the field. The field can be, for example, gravitational, centrifugal, a liquid flow, electrical or magnetic. Using a suitable detector after or during separation allows determination of the size and size distribution of nano-objects.

Once the external field is applied, the particles in the suspension are forced into a narrow layer along one wall of the flow channel. The particles in this compressed layer interact with both the axial channel flow and the external field. Separation occurs depending on the particle size, density, diffusion coefficient or thermal diffusion coefficient, and on which type of field is applied.

The mode of fractionation depends on the size of the particles in the suspension, as shown in Figure A1.4a.
For small particles undergoing Brownian motion, the particle diffusion coefficient determines the elution sequence. The smaller particles diffuse at a higher rate and are eluted first, followed by the larger-sized particle fractions.

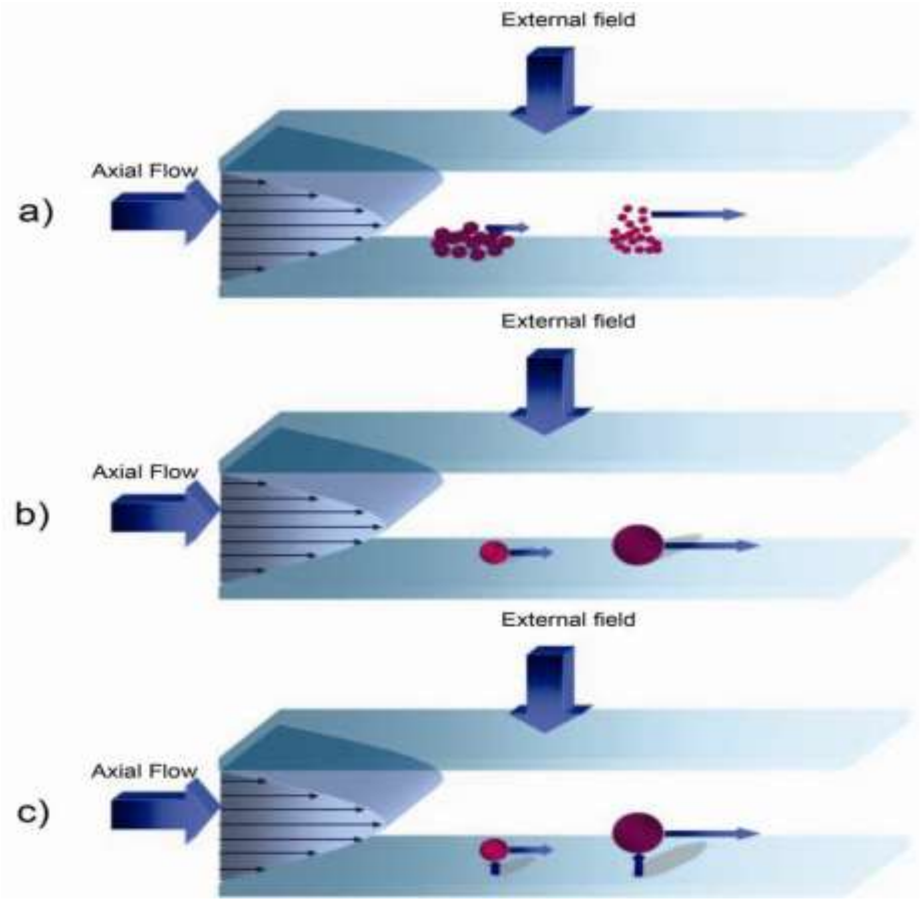

Figure A1.2: Schematic diagram illustrating the typical operation of FFF, showing the mechanism of separation for particles of different size. a) the separation sequence based on particle diffusion coefficients, b) particle separation in steric mode and c) the particle separation in hyperlayer mode (Jamting and Miles, 2013).

For larger particle systems with diameters above ~1 µm a different principle occurs, often denoted by the steric mode (see Figure 1A.4b). In this mode the particle sizes are large enough to no longer interact with the channel wall by diffusion but instead are forced by the external field into a thin layer close to the opposite channel wall. The parabolic channel flow now interacts more directly with the particles, and the elution sequence is reversed: larger particles elute earlier than smaller particles.

Another wall interaction mode is also possible. It's called the hyperlayer mode, where the particles are subjected to flow-induced hydrodynamic forces and form thin layers some distance away from the wall (see Figure A1.4c).

The dynamic range of FFF is very broad allowing particle sizes from nanometres up to several micrometres to be measured. FFF is capable of continuously separating out PSDs with diameters spanning the range from 1 to 1000 nm. The particle size discrimination is very good; narrow peaks in the PSD with as little as 5% difference in mean diameter can be resolved. The technique can be used stand-alone or integrated with auxiliary methods for further characterisation. It is possible to collect the eluted fractions for further analysis, for example by AFM or EM.

The technique requires extensive method development. Without external detection systems, the algorithms for determining the size separation are very complicated. Some of the FFF techniques, such as Sedimentation Field Flow Fractionation, may require very long experimental run times to separate PSDs of very small particles or broad distributions (Jamting and Miles, 2013).

A4.1 Aerosol Characterisation

The characterisation of nanoparticles in an aerosol is not particularly relevant to nanomaterials in pesticides or veterinary medicine so this field of characterisation will only be briefly addressed.

A4.1.1 Condensation Particle Counter

A Condensation Particle Counter (CPC) is an instrument that measures the particle number concentration of an aerosol. Aerosol particles in the nanoscale range are grown by condensation to a size of 10–12 µm allowing easy detection and counting using laser scattering, normally by counting individual pulses of scattered light.

A4.1.2 Differential Electrical Mobility Classifier

A Differential Electrical Mobility Classifier (DEMC) is able to select aerosol particles according to their electrical mobility. This is accomplished by balancing the electrical force on each aerosol particle with its aerodynamic drag force in an electrical field. Classified particles are in a narrow range of electrical mobility determined by the operating conditions and physical dimensions of the DEMC. They can have different sizes due to differences in the number of their charges.

A4.1.3 Differential Mobility Analysing System

A Differential Mobility Analysing System (DMAS) is a system used to measure the size distribution of submicrometre aerosol particles consisting of a DEMC interfaced with a detection and analysis system.
The DEMC transmits particles within a narrow size range and a detector, often a CPC, counts the number of particles within that differential size interval.

A5.1 Surface Area Measurement

A5.1.1 Brunauer, Emmet, Teller method

The Brunauer, Emmet, Teller method (Brunauer et al, 1938) determines the total specific external and internal surface area of disperse powders and/or porous solids by measuring the amount of physically adsorbed gas, using the model developed by Brunauer, Emmet and Teller for interpreting gas adsorption isotherms. The method is only available for dry powders.

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SYMPOSIUM ISSUES AND DISCUSSIONS

The Australian Pesticide and Veterinary Medicines Authority’s Chief Executive Officer, Kareena Arthy, launched the Nanotechnology Regulation Symposium.

Ms Arthy welcomed delegates and briefly discussed the role of the APVMA, recent reforms it has carried out and the challenges it has faced. Looming large among these challenges is nanotechnology and the need for the Authority as the responsible regulator to keep pace with nano developments. Ms Arthy said the aim of the Authority’s draft report on regulatory considerations for nanopesticides and veterinary nanomedicines is to guide discussion.

The symposium was divided into three sessions. In the first, APVMA Chief Regulatory Scientist Dr Phil Reeves set the scene for the speakers and delegates by introducing the draft report. He said it is one of the first of its type in the world and has attracted global interest. Even before its launch scientists from the US and Europe, who had received advance copies of it, expressed interest and asked to be kept in the loop.

Health and medical journalist Dr Norman Swan, as convenor for the symposium, then opened the symposium for general discussion, asking how scientists know a new nanoproduct might be dangerous. Dr Reeves said that existing techniques for assessing risks of conventional medicines and pesticides apply just as well to nano ones. He added that while nano is big and exciting news, such is the commitment to thorough testing and risk assessments of any new products, it will be sometime before any are given the regulators tick and can be commercialised.

In separate questions, representatives of the National Toxics Network and Friends of the Earth asked about existing uncertainties in the regulatory system and whether it will be capable of dealing with nanomaterials. Dr Reeves and Ms Arthy explained how the system works very conservatively through a number of checks and balances, that it is adequate and that controls already in place and new ones to be worked out will effectively control the technology. Other issues raised by the National Toxics Network and Friends of the Earth are in their submissions, which are included in the final report.

Professor Neena Mitter, University of Queensland, asked whether the size of a nanomaterial alone would affect a safety assessment. Dr Reeves said size is just a guide and safety is gauged by thorough risk assessment.

In the second session, an Introduction to Nanomaterials, Dr Jan Herrman spoke about the measurement characteristics of nanomaterials and the challenges of measuring them, Professor Terry Turney revealed the details and difficulties of nano manufacturing that ensured a stable and safe product, and Mr John Hughes discussed nano chemistry and how, by knowing all about a product’s manufacture, characteristics and physicochemical properties, a regulator can properly assess its risk.

The speakers responded to questions from delegates about the challenges of monitoring and controlling the behaviour of nanomaterials over their life cycle in the environment, and how scientists use very sophisticated techniques to do this and continually pool their knowledge to reduce the risks and improve the technology.

There were concerns about product safety assessment processes and regulators having to rely of product information from a manufacturer. The speakers assured delegates that if a regulator was not satisfied about an issue the manufacturer would need to explain it adequately or have the product rejected.

Session three examined the human health considerations of nanotechnology. Professor Brian Priestly spoke about toxicology and how scientists have a range of tests for ‘normal-sized’ chemicals and know how they behave. But he questioned whether toxicity changes at the nano scale and suggested scientists need to expand their existing knowledge to be able to predict chemical behaviour at the nano scale. Professor Mike Roberts said when studying the safety of nano chemicals and conducting tests, while in vitro studies are important, only in vivo studies can be relied on. Dr Andrew Bartholomaeus said size is not the primary determinant of toxicity and that simply being nano does not equate to being harmful—that depends on its novelty and what it has been specifically engineered to do.

In the ensuing plenary session the speakers said new paradigms will need to be developed for assessing nanoparticles for toxicity but this was the case for all novel materials, not just nano ones. They acknowledged community concerns, expressed also by OECD researchers, about the need for regulators to develop new guidelines to assess the effectiveness of toxicity testing for nano chemicals.

Session four centred on the effects the products of nanotechnology might have on the environment. Dr Glen Walker said the risk assessment paradigm used by the Department of the Environment, where he is a team leader, is constantly evolving and represents the interests of individuals, communities, ecosystems, land and soil quality, the air and climate. Dr Rai Kookana’s interest is in environmental fate and how durable nanoparticles, for which the driving forces are different from those affecting conventional particles, will be in the environment. Dr Graeme Batley said the challenge is to come up with sufficient data for risk assessments, particularly formulation-specific toxicity data, so a regulator knows when a novel material affects an organism and by what pathway.

In questioning that followed, the speakers agreed it would be a good idea to set up a nano register because to date scientific surveys have relied on people supplying information of variable quality. However, Dr Priestly said if there is no certainty about what to test, there is no point in having a register. All agreed there is a need to engage more with one another.

Dr Swan completed the session saying transparency and sunlight are the best mediums for clarifying issues and exposing doubt. He thanked the delegates, and not least the consumer groups, for participating and congratulated the APVMA for a ‘fantastic and honest dissection of the issues’. He added that he believed no one regulator should be expected to handle the nano issue by itself. Agencies need to cooperate more to ensure adequate resourcing.

Dr Reeves brought the symposium to a close with thanks to all participants for their comments and ideas, which have been very helpful, especially for the APVMA. He said the authority has already received much valuable comment but would like more. What happens next is for the authority to convert its draft Regulatory Considerations nano report into a final document.

Because it is the first such document written it carries some risk, he said, but hopes it will be a catalyst for other organisations around the world.

LIST OF REGISTRANTS FOR THE APVMA NANOTECHNOLOGY REGULATION SYMPOSIUM

This is a list of people who registered their names to attend the Symposium in Canberra in October 2014, though not all of them were able to attend.

ABC	Rural Reporter	Sarina	Locke
ABC	Rural Reporter	James	Bennett
ACCC	Assistant Director	Dixie	Lim
Animal Medicines Australia	Director Regulatory Policy	Michael	Wright
APVMA	Chemistry Evaluator	Rongwei	Teng
APVMA	Evaluator	David	Lin
APVMA	Veterinary Residues	Kyeelee	Driver
APVMA	Product Evaluator	Isaac	Ugwumba
APVMA	Residues Evaluator	Michelle	Bulbrook
APVMA	Director	Jason	Lutze
APVMA	Senior Evaluator	Jun	Wang
APVMA	Senior Evaluator	Zuzanna	Rajczyk
APVMA	Research Librarian	Garry	Hall
APVMA	Project Officer	Colin	Byrnes
APVMA	International Coordinator	Caroline	Harvey
APVMA	Chief Regulatory Scientist, Vet Medicines	Phil	Reeves
APVMA	Chief Regulatory Scientist, Pesticides	Les	Davies
APVMA	Spray Drift Project Manager	Dave	Rumbold
APVMA	Senior Risk Manager	Susan	Hanns
APVMA	Registration Manager	Adriana	Garcia-Londono
APVMA	Risk Manager	Erica	Malcolm
APVMA	Risk Manager	Lisa	Vlahos

APVMA Audit Committee	Member	Claude	Gauchat
Attorney-General's Department	Referendar	Tobias	Schulz
Food and Grocery Council	Advisor, Policy and Regulation	Fiona	Fleming
Australian Research Council	Executive Director	Fiona	Cameron
AUSVEG	Minor Use and Agronomy Coordinator	Scott	Kwasny
BASF	Regulatory Affairs	Melissa	Palviainen
Bayer CropScience Pty. Ltd.	Regulatory Affairs Manager	David	Gregor
Callington Haven P/L	Group Technical Manager	Matthew	Wilkinson
Checkbox 3D Pty Ltd	Managing Director	Noelene	Davis
Costco	Compliance/Supply Chain Manager	Laura	Altarac
Crop Protection Australia	Managing Director	Rohan	Rainbow
CropLife Australia	Policy Manager	Alastair	James
CSIRO	Senior Research Scientist	Jason	Kirby
CSIRO	Research Scientist	Megan	Osmond
Department of Industry	Policy Officer	Dharini	Kethesparan
Department of Agriculture	Research Officer to Chief Scientist	Justyna	Paplinska
Department of Agriculture	Senior Policy Officer	Scott	McKenzie
Department of Defence	Senior Physician	Ian	Gardner
Department of Industry	EL1	Hema	Indrasamy
Department of Industry	Manager	James	Pitman
Department of Industry	Policy Officer	June	Fan
Department of Industry	Policy Officer	Josephine	Kwok
Department of Industry	Assistant Manager	Sharon	Ding
Department of Industry	Manager	Alison	Hemmings
Department of Environment	Senior Evaluator	Farzad	Jahromi
Department of Environment	Director	David	Swanton
Department of Agriculture	Director	Marc	Kelly

Department of Environment QLD	Chief Scientist	Faiz	Khan (Dr)
Dow AgroSciences	Government and Reg Affairs	Colin	Sharpe
Dow AgroSciences	Regulatory Specialist	Emilia	Cieslak
Elanco Animal Health	Senior Regulatory Advisor	Robert	Pottie
Elanco Animal health	Regional Regulatory Manager	Anne	Wary
Elanco R&D	Associate Director	Kim	Agnew
EPA NZ	Senior Advisor	Christophe	Rosiers
EPA NSW	Head of Operations	Sandra	Jones
Eurofins Agrisearch	Regulatory Leader	Kathryn	Adams
Friends of the Earth	Campaigner	Jeremy	Tager
FSANZ	Senior Food Scientist	Barbara	Butow
FSANZ	SSIS	Nick	Fletcher
Futureye	Senior consultant	Lydia	Buchtmann
Gene Ethics	Exec Director	Bob	Phelps
GRDC	Senior Manager Plant Health	Ken	Young
Health	Director	Alan	Philp
Health Protection NSW	Manager	Adam	Capon
Laural Consulting	Founder	Sam	Bruschi
Lonza	R&D Manager	David	Humphrey
Merial Australia Pty Ltd	Head of Regulatory Affairs	Pip	Hannan
Monsanto	Regulatory Affairs Manager	Quynh	Jewell
MPI New Zealand	Veterinary Adviser	Pauline	Calvert
MSD Animal Health	Technical and Site Manager	Neil	Sammons
MSD Animal Health	R&D Scientist	Emmanuel	de Asis
Nanotech Industry Assn	Director of Advocacy	David	Carlander
National Measurement Institute	Project Leader	Victoria	Coleman
National Toxics Network	Senior Researcher	Dr Rye	Senjen

National Toxics Network	Coordinator	Joanna	Immig
NICNAS	Senior Regulatory Scientist	Nicola	Hall
NICNAS	Senior Regulatory Scientist	Mark	Horsham
NICNAS	Regulatory Scientist	Sarah	Robinson
NICNAS	Senior Regulatory Scientist	Nobheetha	Jayasekara
Nufarm	Group Executive	Mike	Pointon
Department of Health	Principal Scientist	Daniela	Leonte
Office of the Gene Technology Regulatory	Assistant Director	Maryanne	Shoobridge
Office of the Gene Technology Regulator	Evaluator	Markus	Koeck
Pacific Environment Limited	Senior Consultant	Maria	Davoren
Pesticide Manufacturer and Dist	Manager	Alexander	Soeriyadi
Propharma Australia Pty Ltd	Project Officer	Debra	Mooney
Public	Consultant	Maxine	McCall
Redcap Solutions	Regulatory Affairs	Stephen	Burman
Ruralco	National Category Manager	Peter	Walters
Starpharma	Director Business Development	Scott	Carpenter
Starpharma	Vice President Research	David	Owen
TGA	Ingredients Database Mngm	Anna	Wieczorek
TGA	Senior Toxicologist	Rhian	Cope
TGA	Senior GMP Inspector	Hongxia	Jin
TGA	Principal Toxicologist	Martin	Fryer
TGA	Regulatory Officer	Mohammad	Refki
TGA	Senior Toxicologist	Manuel	Navarro-Gonzalez
TGA	Senior Toxicologist	Anne	Field
TGA	Business Analyst	Abby	Robinson
TGA	Senior Toxicologist	Claire	Larter

TGA	Senior Toxicologist	Anthony	Oldfield
TGA	Departmental Officer	Anna	Hart
ToxNous Pty Ltd	Director	Gary	Buffinton
UNSW	PhD candidate	Georgia	Miller
UQ	Associate Professor	Neena	Mitter
Veterinary	Safety manager	Mary	Smal

ABBREVIATIONS

ACRONYMS	LONG FORMS
3D-APT	Three-dimensional Atom Probe Tomography
a, b, c, d	Dimensions of sides and cross-sections of regularly shaped particles
ACC	American Chemistry Council
ADME	Absorption Distribution Metabolism and Excretion
AES	Auger Electron Spectrometry
a.i.	Active Ingredient
AFM	Atomic Force Microscopy
AFSSET	Agence Française de sécurité sanitaire de l'environnement et du travail
APVMA	Australian Pesticides and Veterinary Medicines Authority
APTMS	Aminopropyltrimethoxysilane
AR	Application Rate
ASTM	American Society for Testing and Materials
AUC	Analytical Ultracentrifuge
BAM	German Federal Institute for Materials Research and Testing
BCF	Bioconcentration Factor
BET	Brunauer-Emmett-Teller
BAuA	Federal Institute for Occupational Safety and Health (Germany)
BCR	Community Bureau of Reference
BFTEM	Bright Field Transmission Electron Microscopy
BIPM	International Bureau of Weights and Measures
BSA	Bovine Serum Albumin
CCC	Critical Coagulation Concentration
CCD	Charge-Coupled Device
CEN	European Committee for Standardization
CIPM	International Committee for Weights and Measures
CLP	Classification, Labelling and Packaging (European Union Regulation)
CLS	Centrifugal Liquid Sedimentation
CNT	Carbon Nanotube
COM	Confocal Optical Microscopy

COT	Committee on Toxicology (United States)
CPC	Condensation Particle Counting
CRM	Certified Reference Material
CSIRO	Commonwealth Scientific and Industrial Research Organisation (Australia)
D	Diameter of a sphere
d	Diameter of circular cross-section
DCS	Differential Centrifugal Sedimentation
DEMC	Differential Electrical Mobility Classification
DG	Directorate-General of the European Commission
DGENV	Environment Directorate-General
DLS	Dynamic Light Scattering
DNA	Deoxyribonucleic Acid
DMAS	Differential Mobility Analysing System
DMSO	Dimethyl Sulfoxide
DOC	Dissolved Organic Carbon
DSC	Differential Scanning Calorimetry
EBSD	Electron Backscatter Diffraction
EC	European Commission
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECHA	European Chemicals Agency
EDDS	Ethylenediamine-*N*,*N′*-disuccinic acid
EDS	Energy Dispersive X-ray Spectroscopy
EELS	Electron Energy Loss Spectroscopy
EFSA	European Food Safety Authority
EFTEM	Energy Filtered Transmission Electron Microscopy
EHS	Environment Health and Safety
EM	Electron Microscopy
EPA	Environmental Protection Agency (United States)
EPA	Environment Protection Authority (Australia)
EPR	Enhanced Permeability and Retention
ET	Electron Tomography
EU	European Union

FAO	Food and Agriculture Organization (United Nations)
FCS	Fluorescence Correlation Spectroscopy
FDA	(United States) Food and Drug Administration
FFF	Field-Flow Fractionation
FIB	Focussed Ion Beam
FM	Fluorescence Microscopy
FSANZ	Food Standards Australia New Zealand
FSP	Flame Spray Processing
FTIR	Fourier Transform Infrared Spectroscopy
GIT	Gastro Intestinal Tract
GALT	Gut-Associated Lymphoid Tissue
HA	Humic Acid
HD	Hydrodynamic Diameter
HES	High Exposure Scenario
HOC	Hydrophobic Organic Compound
HRTEM	High Resolution Transmission Electron Microscopy
HSE	Health Safety and Environment
ICCA	International Council of Chemical Associations
ICCR	International Cooperation on Cosmetic Regulation
ICP-AES	Inductively Coupled Plasma-Atomic Emission Spectroscopy
ICPMS	Inductively Coupled Plasma Mass Spectrometry
ICRP	International Commission on Radiological Protection
ID	Injected Dose
IEC	International Electrotechnical Commission
IGC	Inert Gas Condensation
IHCP	Institute for Health and Consumer Protection (one of seven institutes of the JRC)
ILC	Inter-Laboratory Comparison
ILSI	International Life Sciences Institute (United States)
IRMM	Institute for Reference Materials and Measurements (Belgium)
IRRST	Institut de recherche Robert-Sauvé en santé et en sécurité du travail (Canada)
ISCOM	Immune Stimulating Complex
ISO	International Organization for Standardization

ISOTS	International Organization for Standardization Technical Specification
ITS	Integrated Testing Strategy
IUPAC	International Union of Pure and Applied Chemistry
JRC	Joint Research Centre (European Union)
LD	Laser Diffraction
LED	Light-Emitting Diode
LDH	Layered Double Hydroxide
LLNA	Local Lymph Node Assay
MLS	Manufacturing Licencing Scheme (of the APVMA)
MLV	Multilamellar Vesicle
MPS	Mononuclear Phagocyte System
MRI	Magnetic Resonance Imaging
MWCNT	Multi-Walled Carbon Nanotube
NATA	National Association of Testing Authorities (Australia)
NGO	Non-Government Organisation
NICNAS	National Industrial Chemicals Notification and Assessment Scheme (Australia)
NIEHS	National Institute of Environmental Health Sciences (United States)
NIST	National Institute for Standards and Technology (United States)
NM	Nanomaterial
NMI	National Measurement Institute (Australia)
NMR	Nuclear Magnetic Resonance
NOAEC	No Observed Adverse Effect Concentration
NOM	Natural Organic Matter
NSOM	Near-field Scanning Optical Microscopy
OC	Organic Carbon
OECD	Organisation for Economic Cooperation and Development (34 member countries)
WPN	(OECD) Working Party on Nanotechnology
WPMN	(OECD) Working Party on Manufactured Nanomaterials
OEL	Occupational Exposure Limit
PDMS	Polydimethylsiloxane
PEC	Predicted Environmental Concentration
PEG	Polyethylene Glycol

PGSS	Particle from Gas-Saturated Solutions
PI	Polydispersity Index
PLGA	Polylactic-co-Glycolic Acid
PNEC	Predicted No Effect Concentration
PNT	Polymer Nanotube
PP	Peyer’s Patches
PSA	Particle Size Analysis
PSD	Particle Size Distribution
PTA	Particle Tracking Analysis
PVA	Polyvinyl Acetate
PVP	Polyvinylpyrrolidone
PZC	Point of Zero Charge
QCM	Quartz Crystal Microbalance
QD	Quantum Dot
REACH	Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (European Union)
RES	Realistic Exposure Scenario
RESS	Rapid Expansion of Supercritical Solution
RFID	Radio Frequency Identification Display
RIVM	National Institute for Public Health and the Environment (Netherlands)
RM	Reference Material
ROS	Reactive Oxygen Species
SANS	Small Angle Neutron Scattering
SAS	Supercritical Anti Solvent
SAXS	Small-Angle X-ray Scattering
SCCS	Scientific Committee on Consumer Safety (of the European Commission)
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCF	Super Critical Fluid
SDS	Sodium Dodecyl Sulphate
SEC	Size Exclusion Chromatography
SECO	Swiss State Secretariat for Economic Affairs
SEM	Scanning Electron Microscopy
SI	International System of Units

SICM	Scanning Ion Conductance Microscopy
SIMS	Secondary Ion Mass Spectroscopy
SLN	Solid Lipid Nanoparticle
SNOM	Scanning Near-field Optical Microscopy
SPM	Scanning Probe Microscopy
SRA	Society for Risk Analysis
SSA	Specific Surface Area
STM	Scanning Tunneling Microscopy
SVHC	Substance of Very High Concern
SWCNT	Single-Walled Carbon Nanotube
SWD	Staff Working Document (of the European Commission)
TC	Technical Committee
TEM	Transmission Electron Microscopy
TGA	Therapeutic Goods Administration
TNT	Titanium Dioxide Nanotube
TR	Technical Report
TS	Technical Specification
UBA	German Federal Environment Agency
ULV	Unilamellar Vesicle
USOPP	United States Office of Pesticide Programs (part of the EPA)
UV	Ultraviolet
VAMAS	Versailles Project on Advanced Materials and Standards
VCI	German Chemical Industry Association
VICH	Veterinary International Cooperation on Harmonization
VSSA	Volume Specific Surface Area
WHO	World Health Organization
XPS	X-ray Photoelectron Spectroscopy
XRD	X-ray Diffraction

GLOSSARY

Adsorption	The process occurring when a gas or liquid solute accumulates on the surface of a solid or liquid to form a molecular or atomic film
Agglomerate	Collection of weakly bound particles or aggregates or mixtures of the two where the resulting external surface area is similar to the sum of the surface areas of the individual components
Aggregate	A particle comprising strongly bonded particles in an irreversible attachment. The external surface area may be significantly smaller than the sum of calculated areas of its individual components
Allotropes	One or more forms of an element; graphite and diamond are both allotropes of carbon
Aspect ratio	Ratio of length of a particle to its width
Atom	The smallest known unit of a chemical element measuring a third of a nanometre in diameter
Atomic force microscopy	Method for imaging surfaces by mechanically scanning their surface contours, in which the deflection of a sharp tip sensing the surface forces, mounted on a compliant cantilever, is monitored
Auger electron spectroscopy	Method in which an electron spectrometer is used to measure the energy distribution of Auger electrons emitted from a surface
Biological nanomaterials	Materials that make up biological molecules (such as proteins, carbohydrates, nucleic acids, lipids and hormones) manufactured to a specific design for a specific technology in the nanoscale
Bionanotechnology	Application of biology to nanotechnology, ie the use of biological molecules in nanomaterials, nanoscale devices or nanoscale systems
Bottom up	Building up organic and inorganic structures atom by atom or molecule by molecule, often using materials such as colloidal suspensions that have a tendency to organise themselves into ordered arrays
Brunauer-Emmett-Teller (BET) method	Method for the determination of the total specific external and internal surface area of disperse powders and/or porous solids by measuring the amount of physically absorbed gas utilising the model developed
Buckminsterfullerene	This is the allotrope of carbon consisting of 60 atoms arranged in 12 pentagonal and 20 hexagonal faces to form a hollow sphere, commonly referred to as C60, first artificially generated in 1985. It is the most common naturally occurring fullerene molecule and is named after Richard Buckminster Fuller, the inventor of the geodesic dome, because the dome is similar in design to a C60. It is commonly referred to as a Buckyball
Carbon nanomaterials	Naturally occurring or manufactured nanostructures of carbon including fullerenes, tubes, fibres, needles, and cones
Carbon nanotubes	Allotropes of carbon that are graphene sheets rolled into a cylindrical shape (single-walled nanotubes) or multiple tubes of increasing diameter (multiwalled nanotubes). Carbon nanotubes have a large length-to-diameter ratio and exhibit extraordinary strength and unique electrical properties, making them attractive for many materials applications
Chemical vapour deposition	Deposition of a solid material by chemical reaction of a gaseous precursor or mixture of precursors, commonly initiated by heat on a substrate

Colloid	A mixture in which a homogeneous, noncrystalline substance that is divided into minute particles called colloidal particles and dispersed throughout a second substance. A colloidal system may be solid, liquid, or gaseous. Colloids may range in size from 1 to 1,000 nm
Confocal microscopy	Technique used to increase image contrast and/or to reconstruct 3-dimensional images by using a spatial pinhole to eliminate out-of-focus light or flare in specimens that are thicker than the focal plane
Dendrimer	An artificially engineered or synthesised molecule, comprised of many smaller molecules, built up from branched units called monomers
Differential scanning calorimetry	Method in which the difference in energy inputs into a substance and a reference material is measured as a function of temperature while the substance and reference material are subjected to a controlled temperature programme
Dispersion	A distribution of particles in a medium
Engineered (manufactured) nanomaterials	Particles with at least one dimension that is less than 100 nm that have been manufactured and not created through environmental processes
Field flow fractionation	Separation technique where a field is applied to a liquid suspension passing along a narrow channel in order to cause separation of the particles present in the liquid, dependent on their differing mobility under the force exerted by the field
Fullerenes	Allotropes of carbon consisting of 60 (C60) or 70 (C70) carbon atoms arranged into a hollow cage of atoms
Graphene	An allotrope of carbon arranged as a single-layer planar sheet of sp2 bonded carbon
Inductively coupled plasma mass spectrometry	Method in which a high-temperature discharge, generated in flowing argon by an alternating magnetic field induced by a radiofrequency load coil that surrounds the tube carrying the gas, is detected using a mass spectrometer
Lab on a chip	A laboratory procedure in miniature combining a number of processes
Ligand	Anion, a molecule, or a molecular group that binds to another chemical entity to form a larger complex
Metal oxide nanomaterials	Nanomaterial that has a core composed of metal oxides
Microencapsulation	Individually encapsulated small particles
Molecule	A group of atoms held together by chemical bonds, a molecule is the typical unit manipulated by nanotechnology
Multi-wall carbon nanotube	Carbon nanotube composed of nested, concentric or near-concentric graphene sheets with interlayer distances similar to those of graphite
Nanocrystal	Solid material whose constituent atoms, molecules, or ions are arranged in an orderly repeating pattern extending in all spatial dimensions, with at least one of these dimensions being less than 100 nm
Nanofabrication	The design and manufacture of devices with nano dimensions

Nanolithography	The science and art of etching, writing or printing at a nano level
Nanomaterial	A material with at least one of its dimensions being less than 100 nm
Nanometre	A unit of measure equal to 1×10^{-9} metres (one billionth of a metre)
Nanoparticle	A substance having all three dimensions less than 100 nm
Nanoparticle tracking analysis	Method where particles undergoing Brownian motion in a liquid suspension are illuminated by a laser and the change in position of individual particles is used to determine particle size
Nanoscale	The nanoscale refers to the size influence on the physical and chemical properties of materials as they are reduced from bulk material to particles having at least one dimension less than 100 nm. One example is the catalytic property of gold that only emerges as the particle size becomes nanoscale.
Nanostructures	See *Nanoscale*
Nanotechnology	The study of engineering materials on the atomic or molecular scale that have at least one critical dimension on the nanometre length scale
Nanotoxicology	The study of the adverse effects of nanomaterials to organisms, populations, communities and ecosystems
Nanotubes	A nanometre-scale cylindrical structure made out of carbon, inorganics, phospholipids, nucleic acids, or proteins
Natural nanomaterials	Nanoscale materials that are not produced through anthropogenic activities
Photobleaching	The progressive loss of fluorescence signal intensity due to exposure to light. This can result in a decreased signal to noise ratio
Physical vapour deposition	Process of depositing a coating by vaporising and subsequently condensing an element or compound, usually in a high vacuum
Protein corona	A particle coating consisting of adsorbed proteins effectively masking the core chemistry of the particle
Quantum dots	Nanometre-sized semiconductor crystals that, when surface-modified to be water soluble and biocompatible, can transform the colour of light. They can be attached to targeting molecules and used as fluorescent probes. The wavelength of light from quantum dots is largely controlled by their size and material composition, so an entire spectrum of distinct colours can be generated by the same material.
Scanning electron microscopy	Method that examines and analyses the physical information (such as secondary electron, backscattered electron, absorbed electron and x-ray radiation) obtained by generating electron beams and scanning the surface of the sample in order to determine the structure, composition and topography of the sample
Scanning near field optical microscopy	Method of imaging surfaces optically in transmission or reflection by mechanically scanning an optically active probe much smaller than the wavelength of light over the surface whilst monitoring the transmitted or reflected light or an associated signal in the near-field regime
Scanning probe microscopy	Method of imaging surfaces by mechanically scanning a probe over the surface under study, in which the concomitant response of a detector is measured

Scanning tunneling microscopy	Scanning probe microscopy mode for imaging conductive surfaces by mechanically scanning a sharp, voltage-biased, conducting probe tip over their surface, in which the data of the tunnelling current and the tip-surface separation are used in generating the image
Self-assembly	Autonomous action by which components organise themselves into patterns or structures
Single-walled nanotubes	A tube made from a single sheet of graphene
Size distribution	A list of values or a mathematical function that defines the relative amounts of particles present, sorted according to size
Small angle x-ray scattering	Method in which the elastically scattered intensity of x-rays is measured for small-angle deflections
Surface area	A two-dimensional description of the exposed area of a solid object
Surface chemistry/composition	The physical and chemical phenomena occurring at the interface of two phases. The surface chemistry of a nanomaterial significantly influences its reactivity as well as its stability in an aqueous suspension
Surface modification/ functionalization	Chemical or physical modification of a nanomaterial that changes its surface chemistry, appearance, or reactivity
Top down	Making nanoscale structures by machining and etching a material layer by layer
Transmission electron microscopy	Method that produces magnified images or diffraction patterns of the sample by an electron beam which passes through the sample and interacts with it
Volume specific surface area	Absolute surface area of the sample volume
X-ray diffraction	Method to obtain crystallographic information about a sample by observing the diffraction pattern due to an x-ray beam hitting a sample
X-ray photoelectron spectroscopy	Method in which an electron spectrometer is used to measure the energy distribution of photoelectrons and Auger electrons emitted from a surface irradiated by x-ray photons
Zeta potential	The electric potential in the interfacial double layer at the location of the slipping plane versus a point in the bulk fluid away from the interface. It is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed nanomaterial

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