

Discussion paper on nanotechnology standardisation and nomenclature issues



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Executive Summary

Friends of the Earth Australia recommends defining nanoparticles as 'particles having one or more dimensions measuring approximately 0.3 nanometres (nm) to 300 nm, or particles which have structures that exist at this scale' for the purposes of health and safety assessment.

Soluble nanoparticles (eg micelles, nano-liposomes and nano-encapsulated active ingredients) must be included within the definition of 'nanoparticles'. Soluble nanoparticles must be subject to new nanotechnology-specific safety assessments and exposure metrics given the large gaps in our understanding of how their potentially far greater bioavailability, solubility and potency will influence their biological behaviour and toxicological significance (Chaudhry et al. 2008).

Agglomerates and aggregates whose primary particles are nanoscale or which possess nano-structures must also be included within the definition of 'nanoparticles' and subject to nanotechnology-specific risk assessment and exposure metrics. The poor understanding we have of de-agglomeration processes and the early evidence that aggregates and agglomerates may share both the surface characteristics and toxic properties of the primary nanoparticles that compose them demand that we treat these particles as nanoparticles for the purposes of health and safety assessment.

Finally, we support the recognition of 'substances with nanomaterial properties'. These are substances that fall outside the size range used to define 'nanomaterials' but which nonetheless exhibit nano-specific behaviour which warrants their assessment using safety testing procedures and metrics appropriate for nanomaterials.

Why size matters – the significance of a size-based definition for nanoparticles

The International Standards Organisation (ISO) and other international bodies have recently agreed on a size-based definition for nanoparticles (ISO 27687), which is scheduled to be adopted by many governments. The size range within which the ISO has defined nanoparticles will have significant implications for health and safety regulation at a national level. However we consider the definition in ISO 27687 as now adopted as something preliminary and temporary and urge further and continued discussion on the matter.

In many countries, it is anticipated that existing health, safety and environmental regulatory systems will eventually be amended to better manage the novel properties and different risk profiles of nanoparticles compared to larger particles of the same



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substance. If this occurs, it is possible that manufactured nanoparticles will be treated as 'new chemicals', which will trigger new health and safety assessments prior to manufactured nanoparticles being allowed in commercial products. It is hoped that exposure and commercial use will be measured using nanoparticle-appropriate metrics (eg number of particles or particle surface area) rather than mass as per conventional materials, and that safety assessments will also be tailored to the special requirements of nanoparticles (eg full physico-chemical characterisation of particle properties such as surface characteristics, size and shape that are known to influence the toxicity of nanoparticles).

However the use of an arbitrarily defined size range to act as an index of novel properties is problematic. Particles that fall outside the size range deemed to encompass nanoparticles – even if they are not much bigger and also exhibit novel, nano-specific behaviour - will not be assessed as new chemicals. These particles will not trigger new health and safety assessments where substances have previously been approved for use in larger particle form. Inappropriate metrics that apply to larger particles will be used to measure exposure or commercial use quantities. This makes it particularly important not to set too narrow a size-based definition of nanoparticles.

Unsurprisingly, many industry proponents have argued for a narrow size-based definition of nanoparticles (1-100nm). However given early evidence that some particles up to a few hundred nanometres in size also behave like nanoparticles (eg Cedervall et al. 2007, Garnett and Kallinteri 2006; Linse et al. 2007), civil society groups have called for the size-based definition to be larger. The United Kingdom's Soil Association's new organic standard excludes particles <200nm in size; Friends of the Earth Australia, Europe and United States have called for particles <300nm to be treated as nanoparticles for the purposes of health and safety assessment.

Reflecting the considerable uncertainty around what size-based definition is most likely to capture the range in which novel nano-specific properties are seen, different government agencies, research institutions and scientists have used different size-based definitions. In its 2006 voluntary industry notification scheme, the British government defined nanoparticles as "having two or more dimensions up to 200nm" (U.K. DEFRA 2006). In a 2006 report the Chemical Selection Working Group of the U.S. Food and Drug Administration (FDA) defined nanoparticles as "particles with dimensions less than micrometer scale [i.e. less than 1,000nm] that exhibit unique properties not recognized in micron or larger sized particles" (U.S. FDA 2006). Food scientists from Australia's Commonwealth Scientific and Industrial Research Organisation (CSIRO) have also defined nanomaterials as measuring up to 1,000nm (Sanguansri and Augustin 2006). In a 2007 report on nanomaterials FDA chose not to offer a size-based definition at all (U.S. FDA 2007).

Friends of the Earth Australia recommends defining as nanoparticles particles measuring 0.3nm to 300nm in size

Friends of the Earth Australia recommends defining nanoparticles as 'particles having one or more dimensions measuring between 0.3nm and 300 nanometres (nm)' for the purposes of health and safety assessment. That is, we recommend that 300nm be the particle size at which nanoparticles are considered to be new



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chemicals and requirements for new health and safety assessments are triggered. This definition of nanoparticles must include soluble particles, and also aggregates and agglomerates composed of nanoscale particles or which have nanostructures.

Particles up to a few hundred nm in size share many of the novel biological behaviours of nanoparticles than <100nm in size, including very high reactivity, bioactivity and bioavailability, increased influence of particle surface effects, strong particle surface adhesion and strong ability to bind proteins (Cedervall et al. 2007; Garnett and Kallinteri 2006; Linse et al. 2007). As with even smaller particles, particles <300nm in size have the capacity to be taken up into individual cells (Garnett and Kallinteri 2006). Particles up to a few hundred nm in size may also pose similar health and environment risks to particles <100nm.

Recent studies finding that carbon nanotubes can cause the same disease as asbestos fibres received world wide attention (Poland et al. 2008; Takagi et al. 2008). Yet many of the nanotubes in the studies measured >100nm and so would not be considered to be 'nanomaterials' using a <100nm size-based definition. Poland et al. (2008) found that two samples of long, tangled multi-walled carbon nanotubes caused asbestos-like pathogenicity when introduced into the stomachs of mice. One of their two samples had a diameter of 165nm and a length of greater than 10 μ m. Similarly, Takagi et al. (2008) found that in a long term study, more mice died from mesothelioma following exposure to multi-walled carbon nanotubes than died following exposure to crocidolite (blue) asbestos. In this study >40% of sample nanotubes had a diameter >110nm.

Several studies have also reported nanomaterial-like biological behaviour in particles 200nm in size - suggesting strongly that even 200nm is not an appropriate upper limit for defining nanoparticles. In an *in vitro* study Ashwood et al. (2007) found that 200nm particles of titanium dioxide adsorb bacterial fragments to their surface and 'smuggle' these into human intestinal tissue where they mimic invasive pathogens and can provoke inflammation. Linse et al. (2007) found that in an *in vitro* study, along with smaller nanoparticles, the large surface area and surface charge of 200nm nanoparticles catalysed protein fibrillation (mis-folding). Protein fibrillation is involved in many human diseases, including Alzheimer's, Creutzfeld-Jacob disease, and Type 2 diabetes. Cedervall et al. (2007) also found strong interactions between proteins and 200nm particles.

Given the early evidence of novel, nano-specific behaviour, bioavailability and potential to cause harm, it would be reckless to exclude particles 100-300nm from new nanotechnology-specific safety testing requirements and nanoparticle-appropriate exposure metrics.

Friends of the Earth Australia also recommends recognition of 'substances with nanomaterial properties'

Friends of the Earth Australia recommends recognition of 'substances with nanomaterial properties'. These are substances that fall outside the size range used to define 'nanomaterials' but which nonetheless exhibit nano-specific behaviour – eg very high reactivity, bioactivity and bioavailability, increased influence of particle surface effects, strong particle surface adhesion and strong ability to bind proteins.



We recommend that if a material is recognised as a ‘substance with nanomaterial properties’ it must be assessed using safety testing procedures and metrics developed for nanomaterials. For example in an *in vitro* study Magrez et al. (2006) found that flake-like carbon black particles of different sizes <1,000nm reduced cell proliferation, led to cell death and were consistently more cytotoxic than carbon nanofibres or carbon nanotubes. These carbon black particles should be subject to nano-specific safety testing and exposure and commercial use metrics, rather than being treated as the equivalent of bulk carbon.

Recognising ‘substances with nanomaterial properties’ that fall outside the size-based definition of nanomaterials will be especially important if the more narrow definition of nanomaterials measuring <100nm in at least one dimension is adopted. As noted, some of the nanomaterials about which the most serious safety concerns exist – for example multi-walled carbon nanotubes that produce mesothelioma in test mice – measure greater than 100nm in their smallest dimension. It is essential that we have a mechanism to refer such substances for appropriate, nano-specific safety assessment.

Soluble nanoparticles (eg micelles, nano-liposomes, nano-emulsions and nano-encapsulated active ingredients) must be assessed as nanoparticles

Friends of the Earth Australia emphasises that soluble nanomaterials (eg micelles, nano-liposomes and nano-encapsulated active ingredients) must be included within the definition of ‘nanoparticles’ and subject to nanotechnology-specific risk assessment and exposure metrics. We reject the proposal from some quarters to leave soluble nanoparticles subject to conventional risk assessment processes and conventional mass metrics to measure exposure.

Nano-sizing or nano-encapsulating food additives including vitamins, enzymes or preservatives results in greater bioavailability, improved solubility and increased potency of these substances compared to larger or micro-encapsulated form (Mozafari et al. 2006). These novel of these nanomaterials are already being exploited commercially. For example AquaNova markets its nanoscale micelles for use in foods and cosmetics because they deliver “significantly higher bioavailability” of enclosed active ingredients once ingested or applied to the skin (AquaNova undated). Omega 3 food additives have in the past been added to food in 140-180,000 nm micro-capsules, for example micro-encapsulated tuna fish oils used by Nu-Mega Diphorm® to fortify Australia’s Tip Top bread line (Personal communication with Nu-Mega representative 2007). However to increase the Omega 3 potency, companies such as Aquanova and Zymes are now selling 30-40nm nano-capsules of Omega 3 – an incredible 4,000 times smaller than the Nu-Mega range (Halliday 2007).

If nano-nutritional additives and supplements provide an excessive dose of some vitamins or nutrients these may have a toxic effect or interfere with the absorption of other nutrients. Dr Qasim Chaudhry who leads the nanotechnology research team at the United Kingdom’s Central Science Laboratory warns that nanoparticle and nano-encapsulated food ingredients “may have unanticipated effects, far greater



absorption than intended or altered uptake of other nutrients, but little, if anything, is known currently” (Parry 2006).

Given the poor understanding we have of how the far greater bioavailability, solubility and potency of nano-formulated soluble substances will influence their biological behaviour and potential toxicity, it is essential to subject these nanomaterials to new nanotechnology-specific safety assessments and exposure metrics.

Aggregates and agglomerates whose primary particles are nanoscale or which possess nano-structures must also be assessed as nanoparticles

Friends of the Earth Australia emphasises that agglomerates and aggregates whose primary particles are nanoscale or which possess nano-structures must be included within the definition of ‘nanoparticles’ and subject to nanotechnology-specific risk assessment and exposure metrics. We reject the proposal from some quarters to exempt nano-structured agglomerates and aggregates from nanoparticle-specific risk assessment processes and to continue using mass metrics to measure exposure.

If nanoparticles fuse together, they form aggregates which are hard to separate. These nano-structured aggregates may be larger than 100nm – or even larger than 300nm. However in many instances aggregates will have close to the same surface area as the nanoparticles they are made from and will have ‘hooks and crannies’ on their surface structure that are nano-sized (Maynard 2007). Where toxicity is driven by surface characteristics, the toxic properties of aggregated nanoparticles may be very similar to that of the primary nanoparticles that compose them. In fact some early studies exposing animals to large nanoparticle aggregates showed effects that appeared to be associated with these primary particles, although the primary particles were more potent in many respects (see reviews in Maynard and Kuempel 2005, Oberdörster et al. 2007). In other instances, nano-structured aggregates may result in greater damage than that associated with the primary nanoparticles. In an inhalation study using mice Shvedova et al. (2005) found that aggregates of single walled carbon nanotubes were the focal point of granulomatous inflammation.

Nanoparticles that form clusters but do not adhere so strongly together are called agglomerates. Agglomerates have similar structures and surface properties to aggregates and so may also share the toxicity risks associated with the primary nanoparticles that compose them. Additionally, in principle agglomerates can also change shape or come apart (Maynard 2007). If particles do not de-agglomerate, their size could reduce their bioavailability relative to that of their primary nanoparticles (Limbach et al. 2005). However this may not necessarily reduce their toxicity. For example Muller et al. (2005) found that 2 months after intratracheal installation of multi-walled carbon nanotubes in rats, pulmonary lesions were caused by the accumulation of large carbon nanotube agglomerates in the airways.

It is still unknown to what extent aggregates and agglomerates will break down into smaller particles in our bodies, eg after inhalation. Researchers routinely use surfactants to ‘debundle’ single and multi-walled carbon nanotube samples for physicochemical investigation (Blackburn et al. 2006, Lisunova et al. 2006). Biological fluids, eg the lung’s epithelial lining fluid which contains both surfactants and proteins, may similarly promote de-agglomeration (Maynard 2007, Oberdörster



et al. 2007) or even break up of aggregates (Donaldson et al. 2006) into smaller particles or even the primary nanoparticles or fibres. For example Maynard (2002, cited Maynard 2007) found that larger agglomerates of titanium dioxide broke into smaller agglomerates with a diameter around 100nm when exposed to a synthetic lung surfactant. Vigorous agitation also leads to disaggregation of nanotube clumps and the production of particles smaller than 100nm (Maynard et al. 2004).

The poor understanding we have of disaggregation and de-agglomeration processes and the early evidence that aggregates and agglomerates may share both surface characteristics and toxic properties with the primary nanoparticles that compose them demand that we treat these particles as nanoparticles for the purposes of health and safety assessment.



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