HAZARDOUS SUBSTANCES ADVISORY COMMITTEE[1]

Nanomaterials: Physico-chemical properties and dose considerations required before assessment of biological behaviour

Background - At its June 2012 meeting the Advisory Committee on Hazardous Substances[2] (ACHS) was invited to discuss issues and difficulties around the need for particle characterisation in nanoscience, the outcome of which is this opinion paper.

Prior to assessment of biological uptake and effects, there is a need for higher, more consistent standards in nanoparticle (NP) characterisation, in dose measurement and in their reporting to a wider audience. Minimum requirements include the rationale for the choice and provenance of the nanoparticles, appropriate sample pre-treatment, physical and chemical characterization, appropriate formulation and measurement of actual, as opposed to nominal, dose. The dynamic nature of nanoparticles during exposure also needs to be taken into account. The details of the above considerations and the metrological analysis should be fully reported; without such information the assessment of biological effects is of doubtful value. The choice and response of biological systems is also important, but beyond the scope of this opinion.

[1] The Hazardous Substances Advisory Committee (HSAC) is an expert scientific committee of the Department for Food and Rural Affairs (Defra). The HSAC provides expert advice on the science behind hazardous chemicals. Further details can be found at: http://www.defra.gov.uk/hsac/. The views expressed in this statement are those of the HSAC and do not necessarily reflect the views or policy of UK Government. Comments are welcome, and should be directed to the HSAC Secretariat at: chemicals.strategy@defra.gsi.gov.uk

[2] The Advisory Committee on Hazardous Substances was abolished on 22 July 2012 by the Advisory Committee on Hazardous Substances (Abolition) Order 2012. The successor body to the ACHS – the Hazardous Substances Advisory Committee (HSAC), was automatically established on the same day as an independent expert scientific committee of Defra. Further details can be found at: http://www.defra.gov.uk/achs/. This opinion paper represents the views of Members of the ACHS/HSAC.

The exact definition of nanoscience is still open to question, but approximately it is the science of materials at sizes between 1 and 100 nm, which exhibit novel properties distinct from atomic and molecular scales and from larger particle sizes due to *inter alia* the material's high specific surface area and surface energy, the presence of large amounts of under-coordinated bonds and spatial constraint of electronic properties and systems. The current European Commission recommendation for a definition is an example of the on-going debate about definition (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:275:0038:0040:EN:PDF). Nanotechnology is the technological application of such novel materials. The importance of nanoscience and nanotechnology in social, economic, environmental and medical areas is acknowledged; there are current and future important applications in environmental remediation, clean energy production, drug delivery and medical imaging, for instance (Yang et al, 2012; Klostergaard and Seeney, 2012; Karn et al, 2009; Lee and Yang 2011). Total global investment in nanotechnology was estimated as ca $17 billion in 2008, with over 90 000 publications in 'nanotechnology' between August 2008 and July 2009 (Shapira and Wang, 2010), while there is an expected market of ca £81 billion in 2015 in nano-enabled products (http://www.bis.gov.uk/assets/BISPartners/GoScience/Docs/U/10-825-uk-nanotechnologies-strategy).

Nevertheless, there are significant knowledge gaps limiting our understanding of the human and environmental hazard and risks of nanotechnology and research is currently underway in order to reduce these uncertainties (Nystrom and Fadeel, 2012; Fabrega et

al, 2011). The *practical* importance of research (separate from the intrinsic good of curiosity-driven scientific research) into the potential hazards and risks of nanotechnology are three-fold: 1) it will help to protect human and environmental health; 2) it will help to ensure the benefits of the nanotechnology are utilized with minimum attendant costs and risks and 3) it will help to maintain and improve public understanding and support debate and discussion of this new technology. Taken together, hazard and risk research and its application is the only means to ensure the safety and sustainability of the very important nanotechnology industry. We strongly support the continued and increased support for this research.

Despite the importance of nano-hazard research, its literature has been criticized due to a lack of quantification of nanomaterials and experimental systems (Klaine et al, 2008). However, even from a cursory reading of the literature, it is clear that improvements have been made since the first reports in the mid-2000s, although the scale and rapidity of these improvements needs to be increased and made more consistent. Routinely, most current peer-reviewed journal articles will provide at least some information on particle procurement and particle characterisation, although often this is at a basic level. Given the positive but often limited developments, it is useful to revisit the limitations and requirements of the peer-reviewed scientific literature in relation to nanotoxicology and, in particular, the requirements necessary to allow data comparison and the future incorporation of currently produced data into hazard-based models which are being developed.

Conceptually and pragmatically, there is a difference in the purpose of characterisation and dose measurement for regulatory testing and for hypothesis-driven research; a more prescriptive approach is required in the former, for instance. Nevertheless, the requirements are similar for both areas and the critical discussion and recommendations below should be equally applicable.

1) *Choice and provenance of the nanoparticle*. This is frequently provided in the current literature but with little or no reason apparent for the choice and none explicitly provided; problem formulation and hypothesis generation is fundamental to material choice. Acceptable reasons for the choice of NP include the investigation of effects of NPs in current consumer goods, the investigation of the importance of size or other properties to biological hazard. However, for a large number of studies, the choice seems to be largely based on availability of materials, which fulfills little function. Further, there are issues related to the validity of sampling for NPs which are poorly addressed.

Recommendation: In addition to the details of procurement such as manufacturer details or description of the synthesis method, the specific choice of material should be justified and evidence of an appropriate choice should be presented. For instance, if the rationale is based on commercial importance, the particular sample investigated must show similar (range of) key features to that used commercially. It should be considered insufficient to say the materials are of the same core material, without other properties such as size, aggregation status, capping agents, crystal structure and others being matched to their commercial counterpart. As a second

example, if the effect of size on toxicity or environmental behavior is investigated, then nanoparticles with systematically varied size and other parameters kept constant should be used, with appropriate 'bulk' and dissolved controls. For other situations, similarly detailed justification and evidence should be fully reported.

2) *Exposure of NP to the organism.* Exposure in most published studies is via solution, often where the NPs were originally in powder form. Dispersion by stirring and by sonication are often used but without consideration of effects on NP surface properties, the possibility of agglomeration and aggregation once the treatment has stopped and any potential losses of material. This point is not to be confused with points 4 and 5 (below), relating to the pristine form of the NP or to its form in ecotoxicology or environmental media. A second issue is the metrics to be used; the utility of mass concentrations has been widely questioned and particle number and/or surface area concentrations put forward as alternatives. Nevertheless, accurate mass determination is relatively straightforward and well-established, while no methods are as yet fully validated for alternative metrics, especially in complex media.

Recommendation: The nature of the dispersion of powders and potential effects on surface chemistry, mass balance and agglomeration/aggregation need to be quantified and reported. Mass concentration should always be reported, ideally alongside suitably validated number or surface area concentration. Validation and uncertainty in all metrics should be discussed.

3) *Sample treatment.* Sample treatment prior to analysis is critical to producing accurate results, a prime example being electron and force microscopy. For instance, drying directly onto the substrate can result in high salt and particle concentrations leading to significant artifacts (Domingos et al, 2009). The majority of published studies use this method and largely do not perform any assessment of sample changes or do not report sample preparation.

Recommendation: Appropriate sample treatment must be used and validated within the study performed and the results reported.

4) *Non-quantitative measurement of size*. Size and aggregation are routinely measured usually by electron microscopy (EM) - either scanning electron microscopy (SEM) or transmission electron microscopy (TEM) - or dynamic light scattering (DLS). However, they are rarely quantified in a statistically meaningful manner, with EM providing qualitative or semi-quantitative assessment while the accuracy of DLS applied to polydisperse samples in complex media is infrequently assessed. Additionally, different methods measure different sizes, for instance X-ray diffraction (XRD) measures crystallite size, TEM measures core particle size and DLS measures hydrodynamic diameter after conversion from diffusion coefficient; microscopy produces a number-based size distribution while field flow fractionation (FFF) produces a volume-weighted distribution. The appropriateness of the technique and data interpretation is often questionable, especially in complex media, where for instance protein coronas might be formed.

Recommendation: Although valid and quantitative measurement of size is possible, there are a number of requirements:

i) There must be an appropriate use of metrology and analysis along with appropriate and valid interpretation.

ii) A multi-method approach is essential, which will provide both reduced uncertainty and extra detailed information.

iii) In a multi-method approach, the differences in 'size' data produced should be recognised. For instance, different methods will report number, volume or other average distributions; others will report crystal size, core size, hydrodynamic size or other measurements. These sizes and size distributions are not equivalent or identical.

iv) Application of any analysis methods, especially when applied to measurement in complex media, must be validated within the study and reported.

v) Appropriate statistical analysis of size should be conducted.

vi) Microscopy can be used quantitatively, but randomized imaging of a large number of particles (>> 100) is required.

vii) Data interpretation of polydisperse or aggregated samples by DLS must be done with caution. In general, data reported are not accurate estimates of size.

5) *Non-size properties*. While size and aggregation are routinely measured by SEM, TEM or DLS, and zeta potential is often measured, other properties are rarely

measured. The properties of NPs which are potentially important in hazard studies include crystal structure, dissolution, shape and nature of any surface coating (e.g. thickness, chemistry and lability). The impurities of either the core particle or the coating are almost never reported, although their importance in fullerene toxicity has been demonstrated (Henry et al, 2007).

Recommendation: More detailed, non-size characterization must be performed including measurement, quantification and reporting of agglomeration/aggregation, dissolution and solubility, shape and surface properties. A particular issue is that a number of properties such as oxidation state, shape and crystal structure are correlated with size changes and will strongly confound any size-based interpretation of NP biological effects.

6) *Dynamic nature of NP exposure systems (physico-chemical properties)*. Characterisation is performed on the powders or their dispersions in pure water, while exposure media usually contain high concentrations of salts and often organic materials such as proteins and vitamins, all designed to minimise organism stress. Particle changes caused by media components over time are rarely considered, although a number of studies have shown substantial changes in particle properties. Neutralisation of charge by salts followed by agglomeration, specific ion effects such as Ag(0) oxidation by sulfate or nitrate salts or interactions with proteins and other biomacromolecules forming a corona and resulting in surface chemistry alteration, agglomeration or deagglomeration are

very rarely reported in ecotoxicological studies. With such changes, the nature of the toxicant is radically altered during the exposure period.

Recommendation: The dynamic changes in NP physical and chemical properties must be monitored over the course of exposures, consistent with accurate interpretation of data and the demanding logistical requirements of such measurements. There must be an assumption that such changes are likely to occur once pristine NPs are put into exposure media, changing the nature of the toxicant and therefore changing bio-uptake and toxicity. However, the reporting of such changes is an inherent part of understanding nanotoxicology; journals should not publish studies which do not monitor such temporal changes. However, the observation of such changes should not be used as a reason not to publish, even if the toxicology is complicated by such results. The data is essential in interpreting dynamics in exposure media and therefore advancing the field.

7) *Dynamic nature of NP exposure systems (exposure concentration and dose).* Studies have reported losses of NPs due to dissolution possibly with re-precipitation, agglomeration and loss to container walls which may reduce the original (nominal) concentration by orders of magnitude. Nevertheless, the majority of studies report nominal concentrations only. Given the fundamental nature of dose to toxicology, the use of nominal dose only is worrying. In addition, in the majority of studies, nominal dose is extremely high and far higher than almost all likely exposure scenarios both to the environment and humans, making their relevance more questionable. In addition, the use of higher nominal

doses may lead to more rapid changes in the actual rather than nominal dose. For instance, aggregation and sedimentation are likely to be more rapid at higher NP concentrations, possibly leading non-dose dependent organism responses.

Recommendation: The recommendation in point 6 is largely applicable but for exposure concentration and dose, rather than physico-chemical characterisation. Detailed studies may be able to separate out dynamic effects of physico-chemical changes and dose changes.

8) *Use of non-validated methods or single methods*. The incorrect application of methods is rife in the literature, where single analytical methods are used with little or no assessment of uncertainties or errors. For instance, the use of DLS in polydisperse samples is frequently used, despite the known errors due to scattering dependence on size. Dissolution and solubility are measured by ultrafiltration, ultracentrifugation, equilibrium dialysis or other methods, followed by metal analysis. Different studies use different conditions; in ultrafiltration, for instance, membrane pore sizes are variable between studies, sorptive losses and contamination are not assessed and changes in membrane behaviour over time are not measured. After separation of the nominal dissolved and particulate phases has been performed appropriately, the more routine step of analysis by graphite furnace atomic absorption spectrometry (GFAAS) or inductively coupled plasma mass spectrometry (ICP-MS) must be performed.

Recommendation: The earlier recommendations apply: a multi-method approach is required, sample treatment and analysis methods must be appropriately validated and data must not be over-interpreted.

Overall recommendation: There is an urgent need to understand, quantify and report in detail dose and physico-chemistry before and during organism exposure. Data on bio-uptake and toxicity is meaningless without such suitable, reliable and detailed information. While there have been improvements in such reporting in recent years, rapid improvements in the majority of published studies are urgently needed.

[November 2012]

References

J. Fabrega, R, Tantra, A. Armer, B. Stolpe, J. Tompkins, J.R. Lead, C. R. Tyler, T. S. Galloway (2012). Sequestration of zinc from zinc oxide nanoparticles and life cycle effects in the sediment dweller and amphipod Corophium volutator. Environmental Science and Technology, 46, 1128-1135.

T. B. Henry, F-M. Menn, T. James J. T. Fleming, J.Wilgus, ER. N. Compton and G. S Saylor (2007). Attributing effects of C-60 nano-aggregates to tetrahydrofuran decomposition products in larval zebrafish by assessment of gene expression. Environemntal health Perspectives, 115, 1059-1065

B. Karn, T. Kuiken and M. Otto (2009). Nanotechnology and in Situ Remediation: A Review of the Benefits and Potential Risks. Environmental Health Perspectives,117, 1823-1831.

S. J. Klaine, P.J.J. Alvarez, G. E. Batley, T. F. Fernandes, R. D. Handy, D. Lyon, S. Mahendra, M. J. McLaughlin, and J. R. Lead (2008). Nanomaterials in the Environment: fate, behaviour, bioavailability and effects. Environmental Toxicology and Chemistry, 27, 1825-1851

J. Klostergaard and C. E. Sweeney (2012). Magnetic nanovectors for drug delivery. Nanomedicine – Nanotechnology Biology and Medicine, 8, S37-S50.

J-K. Lee, M. Yang (2011). Progress in light harvesting and charge injection of dye-sensitized solar cells. Materials Science and Engineering B – Advanced Functional Solid-State Materials, 176, 1142-1160.

A. Nystrom, Andreas M.; B. Fadeel (2012). Safety assessment of nanomaterials: Implications for nanomedicine. Journal of Controlled Release, 161, 403-408

P. Shapira and J. Wang (2010). Follow the money. Nature, 468, 627-628 2010

F. Yang, C. Jin, S. Subedi, C.L. Lee, Q. Wang, Y. J. Jiang, Y. Di and D. L. Fu. Emerging inorganic nanomaterials for pancreatic cancer diagnosis and treatment. Cancer Treatment Reviews, 38, 566-579.