



Response

by

The Dr Hadwen Trust for Humane Research

to

**House of Lords Science and Technology Select Committee Call for
Evidence: Nanotechnologies and Food**

March 2009

Introduction

The following comments on nanotechnologies and food are submitted by the Dr Hadwen Trust for Humane Research.

The Dr Hadwen Trust is the UK's leading medical research charity that funds and promotes exclusively non-animal techniques to replace animal experiments. Our vital work benefits humans with the development of more relevant and reliable science whilst also benefiting laboratory animals. We believe that excellence in medical research and testing can and should be pursued without animal experiments. Our organisation has 38 years' experience of funding high-quality, peer-reviewed and innovative research aimed both at advancing medical progress and replacing procedures on animals.

We very much appreciate the opportunity to comment on this paper, and believe that as a research organisation dedicated to replacing animal tests (as well as the use of animals for other experimental purposes), our specific scientific expertise in the fields of toxicology and human health are relevant to this topic.

We hope that our comments will be considered useful and constructive.

Dr Hadwen Trust Comments

1. The current uncertainties for risk assessment of nanotechnologies and their possible applications in the food and feed area, as well as in other areas of use, arise due to the presently limiting information in characterisation, detection and toxicology data. This view was also echoed in the recent scientific opinion produced by the European Food Safety Authority on the potential risks arising from nanoscience and nanotechnologies on food and feed safety¹. We are also aware of the lack of knowledge surrounding the current usage of engineered nanomaterials (ENM) and therefore exposure to such products is an area requiring immediate attention.

2. Whilst recognising that the currently used risk-assessment paradigm is applicable for ENM our concerns regarding proposed guidance for risk assessment of ENM in food and feed area centre around the acceptance of conventional toxicity testing methods to be used in identification of ENM hazards. We are of the opinion that additional issues specific to ENM need to be addressed due to the different properties displayed by ENM when compared to

¹ EFSA (2009) The potential risks arising from Nanoscience and Nanotechnologies on Food and Feed Safety. Scientific opinion of the Scientific Committee.

the bulk-form material², as well as the differences likely to arise when storage and production methods are different, and this will require use of new test methods as well as new criteria by which the validity of such methods should be assessed. We also believe that assessment of ENM should be based on a case-by-case approach and that current testing strategies are not adequate for ENM and do not represent the most scientifically robust methods to employ. As with the cosmetics sector, it will be extremely difficult in the food and feed industry to characterise ENM and current guidelines do not specifically address ENM. Until methods are in place to properly determine the behaviour of ENM in living organisms and make careful and informed risk assessments, it is hard to see how regulators or companies are in a position to assert that ENM in food or feed products are safe.

3. We feel that it is more appropriate in the case of nanomaterials for companies to take a precautionary approach by avoiding exposing workers, consumers or the environment to these forms of substances. We do not believe clear commercial and societal drives to produce and market the many new and exciting nano-containing applications should overtake the fundamental requisite to protect human and environmental health and safety.

4. In a recent scientific opinion produced by the European Food Safety Authority on the potential risks arising from nanoscience and nanotechnologies on food and feed safety they concluded that engineered nanomaterials should be assessed on a case-by-case basis. It was also concluded that current toxicological methods may need methodological modifications which may include observing additional toxic effects and endpoints as well as developing, improving and validating *in silico* and *in vitro* test methodologies³. We wholly agree with the recommendations into furthering the currently limited knowledge and understanding of ENM behaviour and toxicokinetics through *in silico* and *in vitro* methodologies, as suggested by EFSA. However, we do not support the assumption that *in vivo* studies can be modified so that they accurately predict effects of ENM on human health or the environment for reasons set out below.

For these reasons the Dr Hadwen Trust recommends that for non-essential, non-medical applications (including cosmetic and household products, sporting equipment, textiles, food, feed and paints), ENM manufacture and use is prohibited immediately until relevant non-animal and nano-specific safety testing and risk assessment protocols are in place.

² SCENIHR (June 2007) The appropriateness of the risk assessment methodology in accordance with technical guidance documents for new and existing substances for assessing the risks of nanomaterials.

³ EFSA (2009) The potential risks arising from Nanoscience and Nanotechnologies on Food and Feed Safety. Scientific opinion of the Scientific Committee.

5. In a recent publication by the Royal Commission on Environmental Pollution it was acknowledged that "...the scientific basis to fully understand all properties and risks of nanomaterials is not sufficiently available at this point in time"⁴. In accordance with this the Dr Hadwen Trust further believes that animal testing of nanomaterials is scientifically highly questionable. We would prefer to see an acknowledgement that, in concordance with the mention that some specified *in vitro* methods are not yet validated, existing animal tests are also not validated for this application (indeed, in some cases, existing animal tests have not been formally validated to modern standards for any application), and greater emphasis to be placed on the development, validation and use of non-animal test methods.

6. Animal tests have limited value because of their inherent uncertainties⁵. These include the difficulties of extrapolating test data between species, genders and breeds of animals including humans (due to anatomical, physiological, biochemical, metabolic and pharmacological differences). There are major uncertainties in interpreting information from high-dose animal tests with single chemicals in ways that are relevant to low-dose human exposures to chemical cocktails. There are also problems with mimicking human routes of exposure in animal tests, and with scaling up from small animals with a short lifespan to larger humans who may be exposed to chemicals over decades. Even for data-rich chemicals, these uncertainties often delay rather than facilitate regulatory decision-making, prolonging risks of damage to human health and the environment.

7. With a new field such as nanomaterials, the full range of potential toxicities is not known. Using standard animal toxicity tests, which are little more than 'black box' methods, would risk overlooking novel unwanted effects. Human cell-based assays, in contrast, would allow the study and elucidation of a range of molecular and cellular mechanisms of toxicity. For example, human cell culture assays can be used to monitor the oxidative stress responses of cells exposed to nanoparticles.

8. There are a number of non-animal techniques currently being developed that represent a potential for nanomaterial safety testing. For example, perfusable 3D cell-matrix chambers for testing nanoparticle permeability and transport through tissues⁶; and the H μ REL device⁷, which allows the toxicity of nanomaterials to be tested on several cell types in a multi-chambered microchip with a microfluidic channel, represent promising *in vitro* methods.

⁴ Royal Commission on Environmental Pollution (2008) Novel materials in the environment: the case of nanotechnology.

⁵ Langley, G (2004) Chemical Safety and Animal Testing: A Regulatory Smokescreen? BUAV report, 35pp. Report available from the Dr Hadwen Trust.

⁶ Ng, C and Pun, SH (2007) A perfusable 3D cell-matrix tissue culture chamber for in situ evaluation of nanoparticle vehicle penetration and transport. *Biotechnology and Bioengineering* 99:1490–1501.

⁷ www.hurelcorp.com

9. Human cell culture techniques have provided useful information on specific cellular responses to nanomaterials by measuring chemical responses⁸ or responses at the DNA level using biomarkers and genomic techniques⁹. The feasibility of analysing *in vitro* nanomaterial activity in a general, systemic fashion has also been demonstrated using a multidimensional profiling approach with multiple cell types and assays reflecting different aspects of cellular physiology¹⁰. The data are then clustered using computational methods to identify nanomaterials with similar patterns of biological activity across a broad sampling of cellular contexts, as opposed to sampling from a single assay. This approach yields robust and detailed structure-activity relationships. Additionally, interesting alternative tests are already being developed by EU-funded Joint Research Centre projects such as Nanotox, which involves human cell culture techniques.

We hope that the House of Lords Select Committee will recommend that funding be made available for further research intended to result in creation of nano-specific non-animal test methods that have a clear regulatory applicability. Sadly, it appears to be the case that some potentially useful research in this field is not sufficiently targeted to meet genuine regulatory needs, and we would suggest that regulators or researchers with knowledge of the regulatory environment are required partners in any publicly funded research intended to produce new test methods.

10. In summary, human-relevant non-animal assays offer several advantages: using human cells or sub-cellular components they avoid species differences, and high-throughput systems allow the very rapid and cost-effective testing of multiple chemicals and multiple toxic endpoints, including novel ones. A moratorium should be introduced on all non-essential uses of ENM. This will ensure the protection both of human health and environmental safety, as well as fulfilling citizens' wishes to maintain high animal welfare standards¹¹ and prohibit unnecessary laboratory animal use, especially with inhumane and misleading methods.

⁸ Lin W, Huang Y, Zhou X, Ma Y (2006) In vitro toxicity of silica nanoparticles in human lung cancer cells. *Toxicology and Applied Pharmacology* 3, 252-259.

⁹ Papis E, Gornati R, Ponti J et al (2007) Gene expression in nanotoxicology: A search for biomarkers of exposure to cobalt particles and ions. *Nanotoxicology* 1, 198-203.

¹⁰ Shaw SY, Westly EC, Pittet MJ et al (2008) Perturbational profiling of nanomaterial biologic activity. *PNAS* 105, 7387-7392.

¹¹ http://ec.europa.eu/environment/chemicals/lab_animals/pdf/results_citizens.pdf