Response to the public consultation on the SCENIHR report from UK Health Protection Agency, The Health and Safety Executive and the Department for Environment, Food and Rural Affairs

Opinion on: the SCENIHR documentation of the risk assessment methodology in accordance with the technical guidance documents for new and existing substances for assessing the risks of nanomaterials (Date 29 March 2007)

HEALTH PROTECTION AGENCY COMMENTS

General Comments

1. This is a detailed report compiled by a group including internationally recognised experts in the particle toxciology field. The report sets out the problems likely to be encountered in assessing risks to human health that are posed by exposure to materials of nanodimensions. The assessment is thorough and does not present any strikingly new thoughts: the field has been explored in detail in a number of reviews and the problems are well understood. Solving these problems is more difficult and the report could have said more about how this might be done.

2. In developing strategies to assess the risks that may be posed by nanomaterials we are both aided, and perhaps distracted, by two main areas of research:

    a) We know a little already about the possible effects of nanomaterials and about their possible mechanisms of activity. Work has shown that nanoparticles can behave in unexpected ways: they can, for example interact with the clotting processes of the blood and can be taken up and transported by nerve fibres. These are not effects generally associated with particles of larger size. These unexpected findings have led to speculation that nanoparticles could cross other physiological barriers, for example the placenta and could have further unexpected effects. These findings have caused doubt to be cast on standard approaches to toxicological testing: it is suggested that standard methods may not completely reveal the effects of nanoparticles and other nanomaterials. It is interesting that we seem to know more about the possible mechanisms of effects of some nanoparticles than we do about their actually toxicological effects. This is unusual in that we would generally expect toxic effects to be demonstrated first and mechanistic studies to follow. Extrapolating from the mechanistic evidence to possible toxicological effects is tempting: lung inflammation may lead to significant pathological changes, but it may also <u>not</u> lead to such changes. Viral infections, for example, cause marked inflammatory changes in the airways but, in general, do not cause longstanding pathological changes.

b) The second area of research that conditions thinking in the nanotoxicology field is particle research undertaken in the air pollution area. It is widely accepted that both short and long term exposure to the ambient aerosol is causally related to a range of effects on health. It was the association with effects on the cardiovascular system, at low mass concentrations, that led to the hypothesis that ultra-fine particles were playing a part. This remains unproven. That fine particles (PM2.5) are associated with a range of effects is certain but that the ultra-fine component of PM2.5 is responsible for these effects is not. This is sometimes forgotten and it is tacitly assumed that ultrafine particles are responsible for the many effects of the ambient aerosol. This assumption leads to the further assertion that we know that at least some types of nanoparticles at low mass concentrations have serious effects on health and thus to the assertion that other nanoparticles at low mass concentrations might also have serious effects on health.

3. These two strands of evidence have skewed the discussion of nanotoxicology and are reflected throughout the SCENIHR report and throughout other reports and reviews in this area.

4. A third line of thinking has also confused the area. This is to do with the findings that the toxicological properties of some nanoparticles seem to be dependent on their size. It stems from work by Gunter Oberdorster which showed that titanium dioxide, generally inert as particles of about 500nm diameter, appears to be toxicologically active when encountered as particles of 30nm diameter. These findings have been misinterpreted: Oberdorster has shown that the inflammatory response induced by titanium dioxide in both nanoparticle and larger particle form fits the same dose (exposure) response curve if surface area dose is plotted as the independent variable. It is thus not true to say that 500 nm diameter titanium dioxide is simply <u>not</u> toxic and that 30nm diameter titanium dioxide <u>is</u>. It does appear to be true that surface area dose is a better metric for reflecting the toxicity of titanium dioxide than is mass. Indeed, if mass dose were plotted one could be forgiven for thinking that nano TiO_2 and larger size TiO_2 were two different substances with different toxicological potencies. The assumption that all materials as presented in nano form will be more toxic than when presented in bulk form needs careful examination. It seems at least possible, that nanoforms may only appear more toxic when mass is used as the dose metric; when surface area is used as the dose metric, they may appear equally toxic with the larger forms.

5. The fact that some nanoparticles are insoluble and could thus be expected to persist for long periods in tissues accessible to them, has led to speculation about possible toxicological effects. The SCENIHR report emphasises recent work showing nanoparticles can reach the brain via the olfactory nerves. This is certainly a remarkable finding but evidence to show that a significant effect on the brain is produced by

such an update has not yet appeared. Unless the uptake system shows selectivity for particles capable of damaging the brain, and this seems unlikely, it must be accepted that exposure to ambient nanoparticles (many of which are insoluble) presumably leads to an accumulation of these in the brain. Whether or not this causes brain damage should be carefully considered. Calderon-Garciduenas has argued that exposure to the air pollution mixture in Mexico City does indeed cause damage to the CNS and maybe linked to Alzheimer's Disease. The author places stress on the possible uptake of particles via the olfactory nerve. This work is not mentioned in the SCENIHR report. Further discussion of this would be useful: it has not been followed up by other workers in the air pollution field.

6. It would clearly not be possible to investigate every nanomaterial in the detail outlined in the SCENIHR report and the flow chart produced is helpful in outlining a decision path for studies that are actually needed. The chart sets out a reasoned series of steps but when the stage of using in-vivo models to assess NOEL, TD_{10} PNEC levels of exposure, little advice on how this should be approached is provided. The section dealing with in-vivo studies (3.6.3.1, 2) seems very brief and no clear guidance is provided on how to begin: which species should be used for example. This may be deliberate as the group was asked to comment upon and not to rewrite, the guidance documents. The group has identified a number of gaps in the guidance documents, these now need to be filled.

Comments specific aspects of the document

Genotoxicity

7. We should question some of the conclusions of the report in this area. Coverage of this area appears somewhat contradictory. It is considered specifically in section 4.1.3.8 (page 42-3). This section starts by saying that there is reason to believe that any mutagenicity or genotoxicity shown by nanoparticles may be detected using current protocols...although there are several uncertainities. It then goes on to make the important point about the need to take into account the state of agglomeration (and relevance to the in-vivo situation) and the need for caution when extrapolating from in-vitro tests to the situation in vivo. This is fine.

8. The comment is then made that most mutagenicity test systems can be considered as black boxes without having recourse to underlying mechanisms. This needs to be challenged! The in-vitro studies most widely used namely gene mutation in bacterial, chromosome aberrations in mammalian cells and gene mutations in mammalian cells, have a very well defined underlying mechanism (more so than other in-vitro assays) This is one of the few areas where in-vitro studies are accepted as being validated. As regards the comments

about nanoparticles entering the nucleus or mitochondria and enhancing activity this should be covered by the mammalian assays (one would expect all 3 assays mentioned above to be carried out). Whilst the bacterial assays may not reflect this speculative , and probably indirect route to, DNA reactivity and mutation, they have the advantage of presenting very litter barrier to exposure of the nanomaterial to the DNA and are a sensitive approach to investigating the compounds mutagenic potential.

9. In the executive summary it states that there is a clear need for validated in-vitro assays for nanoparticle evaluation including meaningful endpoints for genotoxicity tests. I think we need to question what is meant here ..are not gene mutation and chromosome aberrations, meaningful endpoints?. In the answer to question 2 (page 61) it is stated that since it is not clear whether existing tests are sufficient to detect the mutagenicity of nanoparticles , further developments are required. This also needs to be clarified

10. In earlier discussion on in-vitro studies (s. 3 6 3 3 page 32, end of paragraph on cellular studies) it states that it is unclear whether bacterial or mammalian cell systems are appropriate to evaluate genotoxic effects…this seems to miss the point that we would expect results from both bacterial and mammalian cell tests to be available when assessing the potential mutagenicity of a chemical (or nanoparticle) by in-vitro studies.

11. Finally in section 4.3.3 concerning a staged approach to assessment of nanoparticles it is noted (second para page 53) that only a few of the in-vitro tests are sufficiently validated to be used in the framework at present, including mutagenicity, cytotoxicity and dermal uptake. This indicates (correctly) that mutagenicity tests are validated..and is at odds with the above!.

12. In summary the SCENIHR comments on mutagenicity are not clear and need to be revisited. We accept that special consideration needs to be given to dosimetry aspects, the appropriate dose metrics, with care to measure actual exposures (having regard to agglomeration/disagglomeration) but to say that the methods need validation for meaningful endpoints for genotoxicity is , in our view , incorrect.

Effects on the skin .

13. In section 3.6.3.1 (page 31) it is stated that following skin exposure (in animal models) the most likely effects are immunopathological…and methods are available to detect skin sensitization. This is also referred to in the earlier section on immunotoxicity (3.4.3.2 page 23) where most of this section relates to exposure via inhalation and the increased response in atopic individuals, with several references being quoted. It then states that similar questions have been raised

concerning nanoparticle exposure on the skin. No references are given anywhere to support this concern regarding skin sensitization and it does not appear to be a problem with the use of nanoparticles in sunscreens.

14. Section 3.6.3.1 then goes on to state that carcinogenic effects on the skin are also possible and chronic skin exposure studies in animals could be used to investigate this possibility. There is no mention of the use of mutagenicity studies to ascertain if the compounds have any mutagenic potential. This would provide useful information also regarding carcinogenic potential and would be much more practical than lifetime skin painting studies.

Repeated Dose Toxicity.

15. We accept that particular consideration needs to be given to neurotoxicity and cardiovascular toxicity (but are not so sure that this is also true for immunotoxicity). Some recognition of the ability of the animal studies to detecting functional changes in neurotoxicity eg from Functional Observation Battery and pathological changes in the CNS/PNS could be made. The relevance of the biochemical tests for neurotoxicity could be questioned as all the examples quoted in last para on page 41 (s.4.1.3.7) appear to relate to cardiovascular effects.

Reproductive Toxicity.

16. It is recognised that there are no data available on effects of nanoparticles as regards reproductive toxicity. Regarding test methods it is stated (s 4.1.3.10 page 44) that the OECD guidelines may need to be adapted for the evaluation of the reproductive toxicity of nanoparticles, but no further information is provided. Presumably there is a need for further guidance on issues relating to metrology and dose metrics , but it would have been helpful to state this.

Section 4.3.3 ; a staged approach to assessment of nanoparticles

Stage 3 To identify the hazardous properties of any forms of the nanomaterial to which significant human exposure is likely.

17. The document has, at least in places, been very cautious about the use of in-vitro data (particularly with regard to whether in vitro mutagenicity tests are appropriate). However this section goes too far the other way!. Even with the caveats that follow, the first sentence of the para on negative findings in vitro cannot be justified. (*If no effects are observed in a series of appropriate in-vitro tests that have been selected on the basis of the known physiochemical and biological properties the nanostructured material may be considered non-hazardous*).At most such studies can only provide approximate data on comparative toxicity particularly with regard to low toxicity bulk

material , not definitive data on hazard and this needs to be made clear. We could accept the final two sentences with the modifications in italics added namely ' Where there is a considerable body of knowledge on the toxicity of the bulk chemical *indicating that it has low toxicity*, the findings from a series of appropriate in-vitro tests on the nanostructured material should be compared with the bulk material. If the results are very similar there may be the need for only limited in-vivo testing on the nanomaterial, or *even* no testing at all.

Health and Safety Executive Comments

Background:

18. The Competent Authority (CA) Working Group on nanomaterials produced four recommendations, together with a draft manual of decisions (MOD) entry reflecting the new and existing substances positions, in April 2006. The final version of this document was presented to the CA meeting in May 2006, and the non-confidential version of the Notification of New Substances (NONS) MOD has been updated accordingly.

19. Recommendation 4 regarding the applicability of current testing strategies is particularly relevant to NONS, since we will have to try and apply the current OECD test guidelines to any 'new' nanomaterials, the potential example being fullerene. It should also be noted that under NONS we have to be sure that any testing, particularly substance characterisation and *in vivo* testing, are both suitably informative and justified for the type of material. We also need to evaluate whether any additional testing particularly relevant to nanomaterials is needed in addition to the standard notification dataset. Such issues will continue under REACH.

General Comments:

20. Against this background, overall we found the SCENIHR paper useful in providing the Committee opinion on the suitability of current testing methodologies. As we thought, it is clear that there is still significant uncertainty regarding whether the current guidelines will address all aspects of the potential toxicity, but the paper does provide some relevant information on specific investigations that may need to be considered. We note that OECD is also looking into this issue.

21. Section 4 of the document suggests potential text additions to the current TGD for NONS and ESR regarding nanomaterials. Whilst we agree that testing on nanomaterials will need to be carefully considered, as NONS/ESR has only 1 year left, we don't really see the mileage in updating the current TGD with this information. It's certainly useful and I'm sure that CAs would appreciate the information with which they can make their own judgements, maybe via a forum such

as the CA nano WG or NONS/ESR technical meeting. Indeed, given the timeframe, it may be better to feed this information into the REACH technical guidance discussions.

22. The suggested data being generated for nanomaterials need to eventually fit sensibly within the legislative framework for appropriate control of these materials and the suitability of any *in vivo* testing in this arena has also to be evaluated carefully in terms of national positions on this subject.

23. The suggested 'staged approach to assessment' in section 4.3.3 of the SCENIHR paper is an example of how proposed procedures to address nanomaterials do not fit well with current regulatory schemes such as NONS even though in principle the idea has merit. Whilst the *in vitro* methods suggested in this section may provide additional supporting information to a package, or investigate a specific effect, at present the evidence available does not support the replacement of standard testing in a NONS base-set package for example, with these *in vitro* methods. Even for the quoted 'contained systems' on p54, under NONS such substances often still require some standard *in vivo* testing at 1 tonne where no validated alternatives exist. In addition, whilst QSAR may be more utilised under REACH, its use in NONS is currently limited to supporting information when using a weight of evidence approach - it does not directly replace the *in vivo* testing.

Specific comments:

- We would agree with the comments by HPA in relation to negative in vitro test results (see comment 17 from HPA). Although beyond the remit of this report practical recommendations on how to address the gaps in knowledge and adapt the current test methods are urgently needed.

- We agree with the comments made by HPA relating to genotoxicity. Clarification on these points is required.

- Para 5 of Section 3.1 (page 12) refers to NPs as having at least 1 dimension of the order of 100nm or less however in the recommendations for the TGD (Section 4.1.1) NPs are referred to as having at least 2 dimensions at nanoscale. Clarification required.

- The recommendation for carcinogenicity (Section 4.1.3.9) makes an important point in relation to species differences but this is not discussed in the previous section on carcinogenicity (Section 3.4.3.5).

Defra Comments.

24. The OECD have been considering a working definition of manufactured nanomaterials to take forward the operational plans of the Steering Groups of the Working Party on Manufactured Nanomaterials (WPMN).

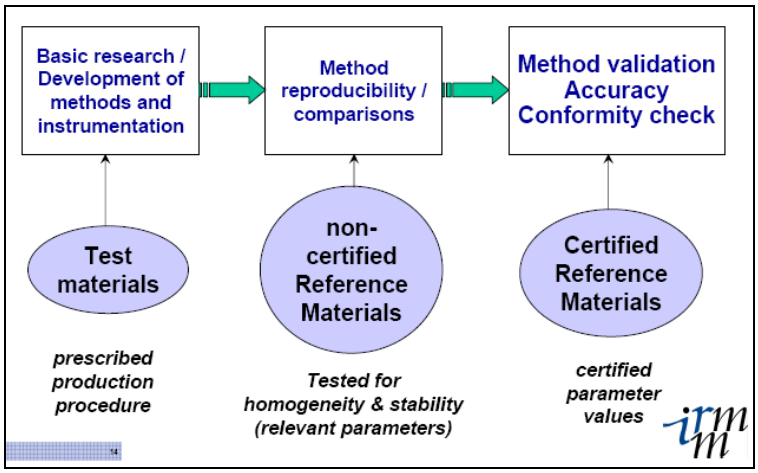
These are reproduced below for information. The key point is in the definition of nanoscale which introduces the term 'typically' so that particles above100nm in one dimension would not be excluded from consideration.

Manufactured nanomaterials: Nanomaterials intentionally produced to have specific properties or specific composition.
Nanoscale: The size range typically between 1 nm and 100 nm.
Nanomaterial: Material which is either a nano-object or is nanostructured.
Nano-object: Material confined in one, two, or three dimensions at the nanoscale.
Nanostructured: Having an internal or surface structure at the nanoscale.

SCENIHR might wish to comment on the working definition of OECD.

25. SCENIHR's attention is drawn to a research project funded by Defra and being carried out by the Institute of Occupational Medicine entitled 'Reference Materials for Engineered Nanoparticle Toxicology and Metrology'. This acknowledges the importance attached to this point by SCENIHR and attempts to take the issue forward. The project will be completed later in the year but a distinction has been made between various types of reference materials as shown in the following figure from IRMM:

Applications of test materials and reference materials



26. The Opinion considers the use of models for PEC estimations and considers that an evaluation of existing models and emission factors is required. Defra is currently funding a project undertaken by the Central Science Laboratory entitled 'Current and predicted environmental exposure arising from engineered nanomaterials' which includes a consideration of environmental, exposure models. This is due to report later in the year.

27. It is understood there there may be a need for new standardised ecotoxicity tests for nanoparticles but the basis for this opinion is not well defined. A sound evaluation of existing methods should be undertaken before developing new tests since this will be time consuming and data are required before any validated new methods could be introduced.. There is a project in progress in the UK which is looking at ecotox and other environmental tests from the TGD for their fitness to look at nanomaterials. This is being carried out by Watts and Crane associates and is entitled ‘An Assessment of Regulatory Testing strategies and Methods for Characterising the Ecotoxicological Hazards of Nanomaterials’ and is due to report later this year.

28. We support the emphasis on a ‘reverse risk assessment’ staged approach to the assessment of the human and environmental risks from nanomaterials which looks at the likelihood of exposure prior to embarking on hazard identification. An issue with such an approach is that in order to put in place appropriate occupational exposure protection, an assessment of hazard needs to be carried out. Also for requirements under Classification and Labelling legislation.

29. We can see no mention of the potential use of integrated testing strategies for risk assessment of nanomaterials which are strongly being advocated and developed, for example, under REACH.