



WORKING FOR A HEALTHY FUTURE

**Final report on Project CB01099
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REFNANO: Reference materials for engineered nanoparticle toxicology and metrology

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EXECUTIVE SUMMARY

Background

Over the last decade there has been increasing public and private investment in nanotechnology. One of the main outputs of this investment has been the development of new nanomaterials, in particular new nanoparticles (NP), that are already finding their way into a growing range of new consumer and industrial products. However, there is increasing recognition that nanoparticles may pose a risk to human health. Findings from recent toxicology studies have indicated that there are some fundamental properties of nanoparticles which drive their toxicity, and concerns over the safety of NP use by consumers and of release into the environment are significant areas of active research.

In the United Kingdom, the Nanotechnology Research Coordination Group (NRCG) was set up by the UK Government to coordinate publicly funded research into the potential risks presented by the products and applications of nanotechnologies. The “*Development of testing strategies and methods for human health hazard assessment of nanoparticles*” has been identified as an important research objective by the NRCG. Several of the task-forces taking this research forward identified the development of a bank of reference materials for toxicology and metrology as a critical objective. A consortium, led by IOM, was successful in responding to a UK Department of Environment, Food and Rural Affairs (Defra) call for proposals in 2006 on “prioritisation of reference materials for engineered nanoparticle toxicology and metrology”. This report describes the project which resulted (REFNANO) and its outcomes.

Methods

The REFNANO project was based on an informed discussion and opinion-gathering activity with representatives from the toxicology, metrology and nanomaterials producer / user communities. This was achieved through representation of the communities on the Project Management Group, the preparation of five topic briefing papers designed to inform discussion at two *by-invitation* workshops attended by key opinion-leaders in the field, and consultation with other internationally-recognised reference material initiatives.

Project Outputs

Based on the discussions and recommendations arising from the two workshops, we have developed a series of outputs from the project. These outputs include:

- a rationale and set of criteria for the selection of priority reference/test materials which give precedence to:
 - industrial nanomaterials produced in large volume;
 - materials which can be used in hypothesis testing;
 - materials which can be used for distributed analysis.
- a list of eight high priority reference/test materials or types to meet the needs of toxicology and metrology:
 - carbon black
 - TiO₂
 - ZnO
 - Polystyrene
 - SWCNT & MWCNT
 - Ag
 - Other key metals & metal oxides (e.g. Cu, Ni, Fe, Zn and their oxides)
 - Combustion-derived NPs

- a further eight lower priority materials to meet the needs of toxicology and metrology;
- information relating to the quantities of materials needed and the matrix in which they are present;
- a minimum set of six characteristics to be determined for the reference/test materials:
 - Aerodynamic equivalent diameter
 - Absolute length
 - Specific surface area
 - Number of particles per unit mass
 - Concentration of bulk and/or surface contaminants
 - Polymorphic composition
- a further ten lower priority parameters for characterisation;
- the appropriateness and availability of characterisation methods;
- a proposed development schedule for nanoparticle reference materials.

Conclusions and future requirements

The REFNANO project sought to provide a priority list of candidates for inclusion in a set of reference materials to support measurement, toxicology and risk assessment of engineered nanoparticles in the UK. Consensus has been reached between the toxicology and metrology communities on the rationale for reference materials, a list of priority candidates, their selection criteria and the suitability of existing instrumental techniques for characterisation. The prioritised candidates are toxicologically and industrially relevant at the nano-scale and focus on materials produced and used in the UK.

The REFNANO project has identified a series of requirements for the further development and promulgation of reference materials for nanoparticles. These requirements have been grouped according to the following themes:

- Existing reference & test materials;
- New reference & test materials;
- Measurement techniques;
- Guidance;
- Strategic developments.

These recommendations and proposed requirements are offered for consideration by other initiatives considering the development of reference materials, including on-going UK Government, OECD and international projects.

1 INTRODUCTION

1.1 INTRODUCTION

The term “nanotechnologies” refers to technologies of the very small, with dimensions in the range of nanometres. Nanotechnologies exploit the specific, novel and sometimes unpredictable properties that arise from structuring matter at this scale. They are concerned with developing new processes, products and materials based on these properties. Over the last decade there has been increasing public and private investment in nanotechnology. One of the main outputs of this investment has been the development of new nanomaterials and in particular, new nanoparticles (NP). These new materials are already finding their way into an increasing range of new consumer and industrial products, integrated into many aspects of our every-day lives (Woodrow Wilson Centre 2007).

Juxtaposed with this positive background however, there is increasing recognition that nanoparticles may pose a risk to human health. Recent findings from toxicology studies have indicated that there are some fundamental properties of nanoparticles which drive their toxicity. These properties include surface area, surface chemistry, size, shape, charge etc. The increasing development of novel formulations of nanoparticles in the nanotechnology industry and their increasing industrial usage poses the most immediate problem for hazard assessment, as many of them remain untested. At this time, initial concerns are mainly focused on issues surrounding occupational health and worker safety at manufacturing premises since these are the situations in which exposure is likely to be greatest. However, concerns over the safety of NP use by consumers and of release into the environment are also significant areas of active research. These concerns have been most clearly expressed in the 2004 review carried out by the Royal Society and Royal Academy of Engineering (RS/RAEng 2004), and a number of recent articles such as Maynard *et al.*, (2006).

In the United Kingdom, the Nanotechnology Research Coordination Group (NRCG) was set up by the UK Government to coordinate publicly funded research into the potential risks presented by the products and applications of nanotechnologies. The UK Department for Environment, Food and Rural Affairs (Defra) chairs the NRCG and the membership includes Government Departments, Regulatory Agencies and the Research Councils. In November 2005, the NRCG produced a first research report (Defra 2005) setting out a programme of research objectives to characterise the potential risks posed by nanotechnologies, with a particular focus on nanoparticles. The NRCG report described ongoing activities and funding mechanisms to address research priorities. The “*Development of testing strategies and methods for human health hazard assessment of nanoparticles*” was identified as one of the Research Objectives (RO16) in this report. The research objectives are being taken forward by five dedicated task-force groups. Several of the task-forces identified the development of a bank of reference materials for toxicology and metrology as a critical objective.

This led to a call for proposals on “prioritisation of reference materials for engineered nanoparticle toxicology and metrology” being issued by Defra in late summer 2006. A proposal to do this work was submitted by a consortium involving many of the collaborators from SnIRC (Safety of Nanoparticles Interdisciplinary Research Centre, www.snirc.org), to be led by IOM. SnIRC was well placed to carry out an extensive and authoritative review of nanomaterial toxicology issues due to its positioning as a leading group carrying out research in this area, their links with government and

industry (both in the UK and internationally), and their world-wide network of collaborators and experts.

Participants in the proposal and the project team which resulted were drawn from the various stakeholder communities (toxicology, metrology and users) identified in the call. We chose to take a broad definition of the “toxicology” community to include those involved in the ecotoxicology and in exposure and risk assessment. The team comprised IOM (toxicology, exposure and risk), Edinburgh University (toxicology), Napier University (toxicology and ecotoxicology), National Physical Laboratory (metrology and standardisation), Central Science Laboratory (ecotoxicology and reference materials) and Nanocentral (nanomaterial manufacture and use).

This proposal was accepted by Defra and this report describes the project which resulted (REFNANO) and its outcomes.

1.2 STRUCTURE OF THIS REPORT

The structure of this report is consistent with Defra contractual requirements. Section 2 describes the scientific objectives as set out in the agreement. Section 3 describes the methods used in the project. The main results are in Sections 4, 5 and 6. Section 4 provides a summary of the information collected to inform the two workshops. Section 5 describes the organisation of the two workshops. Section 6 describes the consolidated output of the two workshops including the main implications of the findings and a series of recommendations about next steps. Final conclusions are presented in Section 7.

2 OBJECTIVES

The aim of this project was to develop consensus views on the need and priorities for reference materials, relevant to nanomaterials, in the toxicology, metrology and user communities. The main objective stated in the original tender specification was:

To provide a priority list of candidates for inclusion in a set of reference materials to support measurement, toxicology and risk assessment of engineered nanomaterials in the UK.

The tender document also specified a series of tasks to be undertaken to achieve this objective. These were further elaborated through the proposal submission process and are reproduced below. They were to:

1. Prioritise potential NPs for inclusion in a Reference Library for toxicology studies;
2. Prioritise potential NPs for inclusion in a Reference Library for:
 - a. calibration and testing of physico-chemical measurement;
 - b. particle characterisation methods.
3. Choose a panel of candidate NP which:
 - a. are relevant at the nano-scale;
 - b. gives priority to materials produced and used in the UK;
 - c. will include carbon nanotubes (single and multi-wall), carbon black, metallic nanoparticles, metal oxides, quantum dots, fullerenes, polymers, quartz, polystyrene particles, uncoated super-paramagnetic iron oxide nanoparticles (SPION) and 100nm PLGA (poly(D,L-lactic co-glycidic acid)).
4. Organise and conduct two workshops with stakeholders from the UK toxicology, metrology, nano-material producer and user communities to scope:
 - a. the reference material needs of the professional community;
 - b. options for the provision of physico-chemical reference materials and metrology services.
5. Consult informally other international bodies which are developing or planning to develop reference materials. These will include NIST, NIOSH, IRMM and AIST;
6. Recommend a set of priorities for a practical and workable minimum of physico-chemical characterisation needs for the chosen candidate materials;
7. Report on key knowledge gaps, and provide advice on future research requirements.

3 METHODS AND PLAN OF WORK

This project required interaction and understanding between the toxicology (including exposure assessment), metrology and nanomaterials producer/user communities so that consensus views could be developed. We chose workshops as our main methodology to achieve this. To maximise the interaction between the various communities we:

- involved members of the communities represented in the Project Management Team who planned and executed this project;
- ensured, as far as possible, a process by which there was adequate exchange of information within each community and between these communities prior to the main workshop events;
- ensured that the core team plus additional members of each community participated in the main workshop events.

To provide a framework for the project and to inform the workshops we developed a series of concise state of the art reviews on:

- types of materials made or used in the UK and the potential exposures which may occur;
- characterisation needs for toxicology testing (particle types, characteristics which drive toxicity, quantities, matrices);
- characterisation needs for eco-toxicology testing (particle types, characteristics which drive toxicity, quantities, matrices);
- particle characterisation (metrology) capabilities and limitations, and the need for standardised materials for metrology;
- other international activities through organisations including NIST, NIOSH, IRMM, AIST, BSI, ISO and EU NMP.

These were provided to the participants prior to the workshops.

Based on these initial reviews, the Project Management Team developed ideas towards a candidate set of materials, characterisation processes and prioritisation criteria including relevance, needs and practicability.

Two workshops were organised. The first was held at Central Science Laboratory, York, and sought to scope and define the reference material needs for the toxicology community. The second was held at the National Physical Laboratory (NPL), Teddington, and was intended to provide solutions to the toxicology needs as well as prioritising and solving metrology needs. A list of the workshop participants is provided in Appendix 1. At the workshops we attempted to draw out consensus, particularly in relation to the list of priority materials but also in relation to the other issues discussed. In general a good level of consensus was achieved, but other than for the list of materials we did not specifically set out to test the level of consensus reached on specific statements or recommendations in this report.

The outputs from the workshop were synthesised subsequently by the project team and used to update and refine the proposed panel of reference materials and

characterisation processes, and to form the recommendations and conclusions. Although the final report was not reviewed again by the workshop participants, it was peer-reviewed by the UK Advisory Committee on Hazardous Substances.

4 PRELIMINARY REVIEWS AND ACTIVITIES

4.1 PRODUCTION AND USE

The project's first briefing paper on the production and use of nanoparticles was prepared by members of the Project Management Team and was based on a recent review of nanoparticle production and use in the UK (Aitken *et al.* 2006). The following text provides a short summary of the key points from that paper.

Due to their extremely small size, nanomaterials (NMs) have a much greater surface area than materials at the micrometre scale. At this scale, quantum effects also appear to be much more important in determining the properties and characteristics of NMs. This has led to the development of novel materials with distinctly different properties compared to their conventional chemically-identical forms. Of particular interest are nanoparticles (NPs) - particles with one or more dimensions at the nanoscale. They can be spherical, tubular, irregularly shaped, or can exist in fused, aggregated or agglomerated forms.

A number of NPs are already used in a variety of consumer products, for example:

- TiO₂, SiO₂, Ag, and quantum dots in paints and coatings;
- CeO₂ in fuel catalysts;
- TiO₂, ZnO, fullerene (C₆₀), Fe₂O₃, and Ag in cosmetics and personal care products such as sunscreen formulations;
- Fe, Fe-Pd, and polyurethane in water treatment and environmental remediation;
- Ag, nanoclay, and TiO₂ in food packaging.

The largest number of currently available nanotechnology products (over 60%) are in the health and fitness sector, which includes cosmetics and personal care products (Woodrow Wilson Centre 2007). This is followed by other applications including paints & coatings, electronics, food & food packaging. From the available information on nanomaterials used in the 356 currently available consumer products listed in the Woodrow Wilson inventory, the most commonly used nanomaterial is silver. This is followed by carbon nanomaterials (fullerenes and nanotubes), silica, zinc oxide, titanium dioxide, and cerium oxide. A number of other applications are anticipated, for example, for targeted drug delivery, gene therapy, stain-resistant coatings, self-cleaning glass, agrochemicals, industrial lubricants, advanced tyres and semiconductors. Also, other more ambitious uses of NPs are being projected that would involve intentional release of NPs in the environment, such as in-water treatment and remediation of contaminated environments.

Current applications for various classes of nanoparticles are summarised in Table 4.1. This illustrates the wide range of these materials that are now in commercial use.

Table 4.1 Current applications for various classes of nanoparticles.

Applications	Materials																					
	Fullerenes (C60, C70, C80, derivatized)	Metallofullerenes (with endohedral metals)	Multi-wall nanotubes	Single-wall nanotubes	Rods	Fibers	Whiskers	Metals and metal oxides	Ceramics	Colloids	Quantum dots	Non-Quantum dot semi-conducting materials	Silica	Polymers	Composites	Dendrimers	Intermetallics	Ion-beam processed materials	Thin films (nm thickness)	Biomolecules	Molecular motors	
Hydrogen storage	X		X	X											X							
Environmental remediation							X								X		X					
Catalysis		X					X			X					X		X					
Drug delivery	X	X	X	X			X							X	X						X	X
Medical imaging	X	X	X	X			X			X	X	X			X	X					X	X
Photovoltaics	X	X	X	X	X		X	X		X			X	X	X		X					
Textiles			X	X		X									X							
Therapeutics	X	X								X					X	X					X	X
Reinforced composites			X	X		X								X	X							
Electronics and electronic devices	X	X	X	X	X		X	X	X		X	X	X	X	X	X	X	X	X	X		
Optics and optical devices	X	X					X			X	X	X	X	X	X	X	X	X	X			
Coatings and pigments	X	X	X	X			X			X				X	X	X	X	X	X			
Cosmetics							X								X							
Ceramics applications								X														
Anti-oxidants								X														
Lubrication	X						X							X	X							
Sensors and sensing devices	X	X	X	X	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Absorbents							X			X				X	X		X					
Energetics and energetic materials			X	X			X								X		X					
Magnetics and magnetic devices							X								X	X						
Water purification and filtration media			X	X		X	X							X	X							
Air emissions reduction							X	X						X	X							
Natural and green products															X							
Quantum computing		X					X			X	X				X							
Masonry and building materials			X	X		X			X						X							
Photonics and photonic devices	X	X					X			X	X	X	X		X							
Surfactants															X							

4.2 RATIONALE FOR THE NANOPARTICLE REFERENCE MATERIAL BANK

Reference materials play an important role in particle toxicology and ecotoxicology. The rationale for a bank of materials was outlined in two of the project’s further briefing papers on particle toxicology and ecotoxicology. Particle toxicology is unique in that particles have a variable hazard. This is very unlike conventional chemical toxins where a solution concentration of a particular chemical, even obtained from different suppliers, is identical in any two laboratories (if prepared properly). In contrast, a sample of asbestos can vary in type, origin, chemistry, size distribution etc. yet still be called asbestos. For these reasons particle “reference materials” have been used historically in particle toxicology in order to try and bring some benchmarking into this challenging science. Some of the best known examples of particle reference materials are described in Table 4.2. It should be noted that the process by which they were developed was seldom straightforward, as indicated in the comments column of this table.

Quantitative structure activity relationships (QSAR) are one way that the toxicity and biological activity of chemicals and pharmaceuticals respectively can be predicted. These techniques rely on characterising the structure and relating it to activity and may be applicable to nanoparticle assessment. The adoption of a characterisation strategy for nanoparticles outlined here may inform the development of a QSAR model for NP.

Table 4.2 Reference particles used in the past.

Reference particle type	Reference	Source	Comment/problem
UICC asbestos	Timbrell <i>et al.</i> 1968	Union Internationale contre le Cancer	Crocidolite was short which gave rise to misleading studies.
DQ12 quartz	Robock 1973	German government	Much more active than workplace samples.
TIMA glass fibre bank	Bunn <i>et al.</i> 1993	Thermal Insulation Manufacturers of America	Some samples had excess 'shot' (particulate, non fibrous material).
NIST Urban Particulate Matter (NIST SRM 1648)	Don Porto <i>et al.</i> 2001	National Institute of Standards & Technology	25 years old and so volatiles lost.

As part of the REFNANO review of toxicology needs, a questionnaire survey of 22 opinion leaders in particle toxicology was carried out. The findings were described in the second of the project's briefing papers. There was virtually unanimous agreement that a reference bank was needed. There was also strong agreement that the bank should contain particles selected around 3 main criteria:

1. **Industrial Nanomaterials:** to select reference nanomaterials on the basis of scale of production and likelihood of exposure;
2. **Hypothesis Driven:** to select reference nanomaterials on the basis of how their physicochemical properties will interact with the living system, for answering particular toxicology (and eco-toxicology) questions, e.g. length distribution and its effect on nanotube toxicity;
3. **Distributed Analysis:** to select reference nanomaterials to be used in inter-laboratory comparative studies.

The survey also indicated that the bank should:

1. contain different classes of NP such as metal oxides, carbon-based NP and combustion-derived NP;
2. contain high aspect ratio nanoparticles;
3. contain 'marker' NP (attached to a stable label) so that they can be followed using microscopic or other techniques;
4. remain under regular review and that NP could be added or removed from the bank.

Suggestions about the types of NP to be included identified many of the common types of nanoparticles. These included fullerenes, carbon nanotubes (CNT), metals and metal oxides.

4.3 MEASUREMENT APPROACHES

Detection methods for nanoparticles can be broadly divided into two general categories: high resolution microscopy and spectroscopy. The prepared briefing paper described possible measurement approaches for carbon nanotubes that are also appropriate for other nanoparticles.

Techniques such as electron and scanning probe microscopies, can offer selectivity through image analysis and subsequent identification of structural features. However, they have an extremely small field of view and therefore are considered not to be cost effective for routine analysis. Even accepting the high cost, there are sampling and counting statistical limits to the accuracy with which the properties of a sample can be characterised. Spectroscopic techniques may offer more attractive options, as they have the potential of automated data collection from multiple point locations over a large area of sample, which may provide a more representative analysis of the sample. In terms of selectivity, spectroscopic techniques provide a chemical fingerprinting capability. With some techniques, this fingerprinting capability may not be selective enough to differentiate between particles and sample impurities.

For carbon nanotubes (CNT), this is very much the case with FTIR, in which the vibrational modes of graphite dominates the spectrum. The remaining spectroscopic techniques described (Raman, fluorescence and terahertz spectroscopy) have the potential to offer better selectivity with respect to CNT detection amongst other carbon impurities. Of these three, Raman shows most promise and the popularity of this technique is evident from the extensive literature on the use of Raman for characterisation of CNTs. Raman spectroscopy is also very sensitive, with a very low detection limit (capable of achieving single nanotube detection capability). Unlike fluorescence spectroscopy, Raman is capable of analysing different structural forms of nanotubes (individual strands, ropes, bundles, aggregates); this is useful as airborne CNTs are often a mixture of tubes in different structural forms. The high information content (through the analysis in the radial-breathing mode domain) that is offered by Raman spectroscopy for the specific detection of CNTs is very attractive. However, this high level of selectivity is only applicable to single and double walled carbon nanotubes, as the highly unique radial breathing modes in the spectrum often disappear in the case of most multi-walled carbon nanotubes. Furthermore, the resonance of these breathing modes were shown to be very dependent on tube diameter and so, if SWCNTs are produced with a wide distribution of diameters, it will be necessary to employ a Raman instrument with access to multiple laser lines. Currently, all of the techniques so far described are less than perfect for the detection of airborne CNTs.

The choice of instrument will be strongly dependent on assumptions concerning the relative toxicity of different nanoparticles to health. For example, on the assumption that all nanoparticles are 'hazardous', there is no shortage of techniques that can satisfy the minimum criteria for their detection. Conversely, if we assume that only certain types of NPs are hazardous to health, for example single and double walled CNTs, then Raman spectroscopy with multiple laser excitations is an attractive option for the analysis of this material. Continuing with CNTs, perhaps the most challenging scenario, as far as spectroscopic detection of CNTs is concerned, is to be able to measure all types of CNTs with sufficient selectivity to differentiate between the analyte of interest and other impurities. In this case, high resolution microscopic techniques, for example Atomic Force Microscopy (AFM), would be better diagnostic tools in

comparison to spectroscopic based instruments, albeit with the aforementioned disadvantages of cost and limited accuracy due to sampling statistics.

4.4 INTERNATIONAL ACTIVITIES

The last of the five briefing papers prepared by the Project Management Team, scoped existing activity conducted by international and government agencies in Europe, the United States and South East Asia. This was based on discussions with regulators and investigators in these regions. In general, although the need for reference materials was widely supported there were few actual initiatives taking place. The principal activity was through OECD.

The OECD Council has established a Working Party on Manufactured Nanomaterials (WPMN) as a subsidiary body of their Chemicals Committee. This working party has been established to address human health and environmental safety aspects of manufactured nanomaterials, in the chemicals sector. The 1st Meeting of the Working Party was held on 26-27 October 2006 in London, UK. The main objective of this meeting was to agree a programme of work on the safety of manufactured nanomaterials. This has now been established and comprises six projects for which draft operational plans are being developed. Each project has an identified steering group. Of particular interest is Project 3, entitled "*Safety testing of a representative set of reference materials*". This OECD project has similar objectives to the REFNANO project but has a broader scope and is developing a plan to not only to develop recommendations (as a first step) but also to implement these recommendations through a programme of testing.

The OECD project will be in two stages. Firstly, the project will seek to develop and agree on a priority list of candidate nanomaterials, representative of manufactured nanomaterials now or soon-to-be in commercial production, for inclusion in a set of reference materials to support measurement, toxicology and risk assessment of nanomaterials. The first stage will also include developing a general working definition of nanomaterials for use by the WPMN.

In the second stage, the project will develop a programme on the intrinsic properties that may be relevant for exposure and effects assessment of nanomaterials, by testing representative nanomaterials for human health and environmental effects, as well as environmental fate for a specified set of endpoints (including e.g., specific physicochemical properties, ecotoxicity).

The expected outputs of the OECD project are:

- a working definition of "manufactured nanomaterials" (MN);
- a description of the information on intrinsic properties that are relevant for exposure and effects assessment of different groups of nanomaterials (foundation data set) and the corresponding methods of measurements;
- the identification of a representative set of manufactured nanomaterials;
- testing of a number of representative nanomaterials using the foundation data set;
- identification of the combination of physical- chemical properties having a major impact on adsorption, distribution, metabolism and elimination (ADME) of MN.

We understand that OECD is aware of the REFNANO project and that the project is specifically identified in their plans as a source to inform their process for the identification of an agreed representative set of nanomaterials.

4.5 TERMINOLOGY

Information on terminology was provided by the Institute for Reference Materials and Measurements (IRMM) Geel, Belgium. The formal definition of a reference material is given in ISO Guide 35 (2006) as follows:

A material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process.

Note that:

1. 'reference materials' is a generic term;
2. properties can be quantitative or qualitative;
3. uses may include the calibration of a measurement system, assessment of a measurement procedure, assigning values to other materials, and quality control;
4. a reference material can only be used for a single purpose in a given measurement.

It is useful to consider to what extent we require reference materials or test materials noting the potential differences in application in Figure 4.1 below.

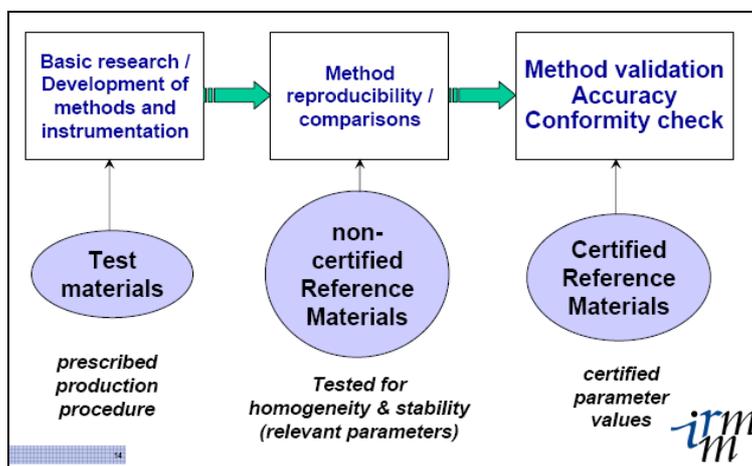


Figure 4.1 Applications of test materials and reference materials (courtesy of IRMM).

It is clear from these definitions that “reference materials” previously used in toxicology (Table 4.2) have really been test materials and that a material could be a *reference material* for particle size but could be a *test material* in, for example, an in-vitro test.

4.6 PROCESSES OF REFERENCE MATERIAL DEVELOPMENT

To make recommendations for action it is important to understand the process of developing reference materials. This is an evolving and iterative process that can take several years to complete if a fully certified reference material is required. The feedback from toxicologists and international governing authorities, such as the OECD, suggests reference materials are required to be developed quickly for nanotechnologies. This requires a coordinated approach between the material producers, metrology and toxicology communities.

For reference material development, there is generally a trade-off between the certainty and the authority of a measurement and the costs of production, analysis and certification. In order to develop competitively-priced products, sufficient financial investment is required to establish a reference material that can be accepted quickly. The usual organisations that produce certified reference materials are the national measurement institutes, such as NIST and NPL, however commercial organisations can produce 'test' materials quite readily if the analytical community are prepared to accept them. A further critical issue for consideration is the activity required to establish and maintain the standing of a test or reference material, at an international level of recognition. Considering the number of nanomaterials that might be required, the potential costs involved, and the views from the workshops, certified reference materials (CRMs) are not required for every instance of nanomaterial in the short term, as the costs to the toxicology community if required to use them would be prohibitive. It might be judicious therefore to use a well-characterised 'test' material that can be compared against a certified reference material in later years.

Production

The process of producing a reference or test material starts with identification of candidate materials. These must be able to be produced in a reproducible, homogenous and stable manner. A disadvantage of nanomaterials is that properties are often enhanced at the nanoscale, such as reactivity, due to the large surface areas involved. There is also the delivery matrix to consider. A candidate material could be delivered as a dry powder or as a suspension. The former may be used to constitute the latter under a careful preparation protocol. Also the longevity of samples maybe in question with enhanced reactivities invoking a 'shelf life' or special storage requirements such as passivation or storage under inert atmospheres. Once these considerations have been addressed, the candidate materials can be delivered to organisations for more precise characterisation.

Characterisation

Increasing the confidence in a measured value of a reference or test material is generally achieved by increasing the number of validation experiments conducted by appropriately accredited laboratories. In general, the greatest confidence is gained from performing an inter-laboratory comparison using very tightly controlled procedures that virtually eliminate the probability of operator or instrumental errors. Multiple measurement techniques are often employed to reduce technique bias in an attempt to make the values ultimately independent of the instrument and tied to the SI unit as the ultimate form of traceability.

Measurement Parameters and Bias

For nanomaterials, the stipulation and reporting of a specific measurement parameter is subject to biases associated with the measurement technique employed. Many current techniques operate close to or at the limit of their capabilities, causing this

effect. Numerous parameters for even simple measurements have been developed historically to help distinguish these effects. A selected list of these can be found in BSI PAS 71: *Nanoparticulate terminology* highlighting the proliferation of parameters. The main standards committee which has done work for several years in addressing these issues is ISO TC 24 (Sieves, Sieving and other sizing methods). One of the main recommendations in the forward of BSI PAS 71 is that the reported results specify the technique employed to help comparability of measurements.

The most common origins of error are in the simple measurement of size, using vacuum based and liquid based techniques. For example, the blurring of edges in scanning electron microscopy images and the presence of solvation and electric double layers, and electric double layers at different ionic strengths surrounding nanomaterials, often leads to variation in reported values in different matrices. The situation is further complicated by common nanomaterials often having well-defined shapes that are not spherical (i.e. rod or pyramidal). Common measurement parameters are derived from equations which often use spherical particle approximations, so different shapes can have different surface areas relative to a single measured dimension. Much metrological work is still therefore needed to develop satisfactory numerical models and validated procedures alongside new robust experimental techniques to account for these shape effects. The measurement of carbon nanotubes is an example where shape is a dominant factor.

Timescales

An estimate of a reference material development schedule is described below.

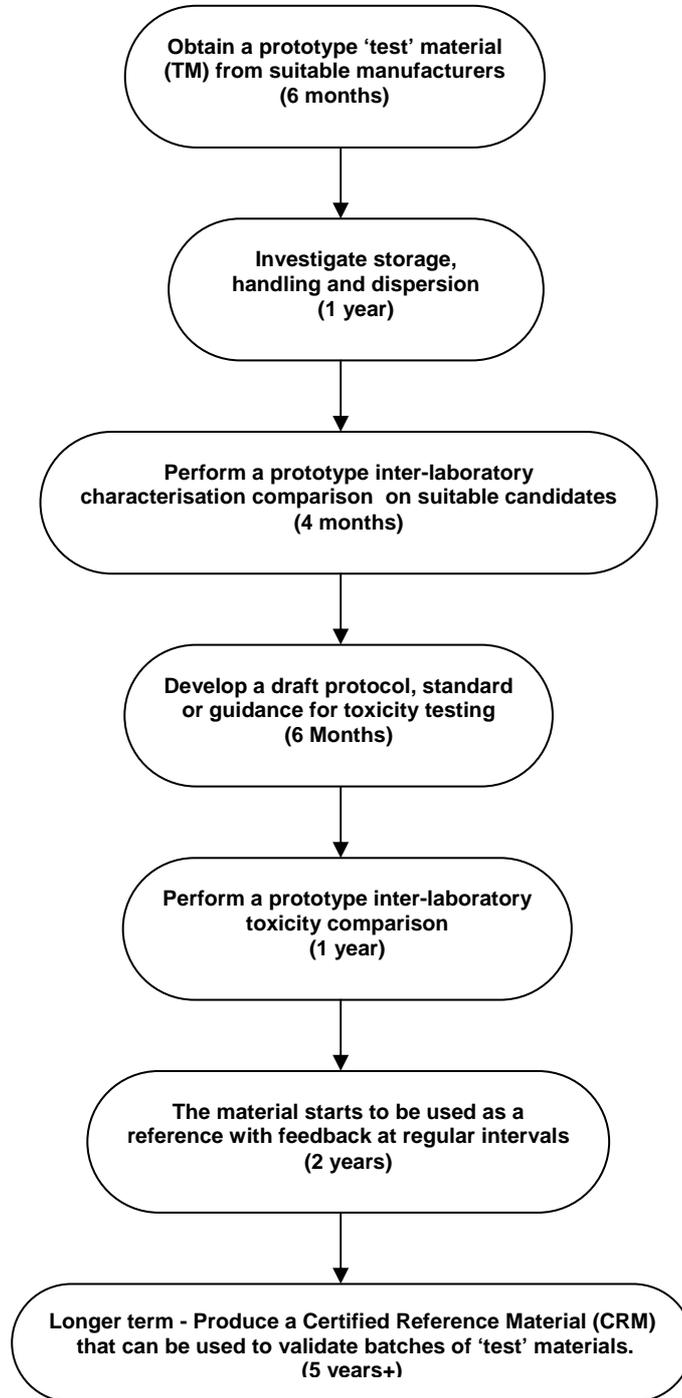


Figure 4.2 Reference material development schedule.

5 WORKSHOPS

5.1 WORKSHOP 1 AT CSL

The first workshop invited stakeholders from the (eco)toxicology, characterisation, industrial and regulatory communities, to scope and define the reference material needs for the toxicology community. The workshop was informed by the five state-of-the-art briefing papers that were provided to all participants prior to the workshop. The main objectives were to develop and prioritise the needs for toxicology in terms of:

- what particles should be included in a panel of reference materials?
- what characteristics and characterisation is required of the reference materials?
- what matrices should the nanomaterials be provided in?
- what quantity of materials do we need?
- how should a reference materials library be maintained and updated?
- how should reference materials be distributed?

The workshop was structured as follows:

- a facilitator introduced the meeting aims, objectives and protocol;
- core team members reprised the state-of-the-art briefing papers and prior information;
- a facilitated platform discussion took place;
- three break out groups were formed:
 - Industrial Nanomaterials;
 - Hypothesis Driven;
 - Distributed analysis.
- summaries and recommendations about the reference material panel and the minimum set of characterisation required were fed back.

The process informed the development of an indicative candidate list of reference materials which was further discussed and modified at the second workshop.

5.2 WORKSHOP 2 AT NPL

The second workshop was intended to provide solutions to the toxicology needs as well as prioritising and addressing metrology needs. It followed the same general format of platform sessions followed by breakout groups. It was informed by a presentation of the outcomes of the first workshop. Although the questions addressed were similar, the scope was different in that there was more emphasis on the needs for metrology. Hence the breakout groups were organised differently as follows:

- Prioritisation of the needs for reference materials for toxicology and ecotoxicology purposes;
- Minimum physico-chemical characterisation requirements;
- Identifying the existing and required characterisation techniques and documentary standards.

6 OUTPUTS

Based on the discussions and recommendations arising from the two workshops, we have developed a series of outputs from the project. These outputs include i) a rationale for the selection of priority reference/test materials; ii) a priority listing of reference/test materials to meet the needs of toxicology and metrology (including very valuable comments from workshop participants on key issues related to each particular material class); iii) information relating to the quantities of materials needed and the matrix in which they are present; and iv) a minimum set of characteristics to be determined for the reference/test materials.

6.1 A RATIONALE FOR SELECTION OF PARTICLE TYPES

In an earlier section we discussed the differences between test materials, reference materials and certified reference materials. Earlier materials used in toxicology have been closer to test materials rather than reference materials. A reference material has one or more “property” which can be measured. This property can be either quantitative (e.g. particle diameter) or qualitative (an example might be “toxicity”) and it is this property which must be measured when used as a reference material. For a property such as particle diameter, this is straightforward. The reference material is supplied with a reference value of diameter. It is used in a measurement process which gives a value of diameter which can be then compared with the reference value.

The property of interest of the toxicology reference materials used in the past was toxicity. However, no reference value of toxicity was supplied with the materials and in fact there was no agreement as to what the toxicity parameter should be, how it should be expressed or how it should be measured. At the time, the materials were used in a wide range of new and developing test methods. Although characterisation of some of the physical parameters was carried out (e.g. fibre length and diameters for UICC asbestos) what specified these materials was that they came from the same batch.

For toxicology, the new materials being requested have more of the characteristics of test materials, rather than reference materials. The property of interest of these materials remains their toxicity. However, to the best of our knowledge the scientific community has not yet reached agreement on what the measure of toxicity to be referenced should be and how that should be measured. It is also the case that the materials will be used in assays which produce differing measures of toxicity. Within the paradigm of developing reference materials for engineered nanoparticle toxicity and metrology, what is required are homogeneous, stable and generally insoluble materials, which could certainly have one or more physical properties well characterised, but would be used in various measurements of toxicity. The material could be a reference material in terms of particle size, but a test material for toxicology.

Whilst the need for reference materials has been expressed on many occasions, (e.g. Royal Society 2004) there has, to date, been little emphasis on what these reference materials would be used for or how specific types should be prioritised and developed. In this respect, the needs for the toxicology and metrology community are rather different.

For toxicology, there are two principal activities in which reference materials can have an important role. The first of these is to facilitate inter-laboratory comparisons. The current state of the art in particle toxicology is such that many different techniques can

be used to measure aspects of toxicity of particles. These include both *in vivo* and *in vitro* tests. While there is emerging agreement about interpretation of these tests, this does not yet extend (except in very few cases) to formally agreed standardised protocols for these tests. Nor are there established schemes in which inter-laboratory comparisons are routinely carried out.

This situation leads to differences between published results from different laboratories when using the “same” materials which may be attributed either to differences in the techniques used, or differences in the actual material. Hence, the first use of reference materials would be for inter-laboratory comparison broadly to remove the variability associated with the material. A further function of test materials is to ensure consistency in any assay as with within-laboratory ‘controls’ used longitudinally over time.

The second envisaged use is for “benchmarking”. This is an experimental design which involves comparison of the toxicity of an unknown particle against that of a known particle. In its most simple form, the toxicity of an unknown particle is compared with that of a particle for which no toxicity is expected to be measured based on wide experience of studying this particle. The standard or known particle is referred to as a negative control. In more complex experimental designs, a particle for which measured toxicity is expected is also added in (positive control). Ideally, a range of such positive control particles would be used to enable the unknown or test particle to be ranked in sequence with those of known toxicity. Benchmarking studies facilitate the investigation and explanation of effects and differences based on the defined characteristics.

For metrologists, the issue is more straightforward. Their interest is in the specific property or properties which will be referenced, for example the diameter or surface area. Their need is therefore for reference materials or certified reference materials having the reference value of the property of interest.

Consideration of these rationales and the potential uses of reference materials led to the proposal of three selection criteria (Table 6.1) and prioritisation in the choice of particle types. The process of particle selection and prioritisation started from their ability to fulfil these criteria and the underlying rationale.

Table 6.1 Selection criteria for Reference Materials.

Designation	Criterion	Rationale	Examples
A	Industrial	Particles in high volume production	CB; TiO ₂ ; CNT.
B	Hypothesis-testing	Particles possessing attributes that help understand mechanism of toxicity	Long and short CNT; nano and micro-sizes; coated and uncoated.
C	Distributed Analysis	Particles chosen as high and low toxicity standards or associated with well-established risks	Ni; diesel exhaust particulate; alumina.

6.2 THE PRIORITIES

6.2.1 Toxicology

Candidate materials were selected according to the rationale described in section 6.1. This resulted in an extensive list of potential materials for inclusion. Prioritisation of this initial list was largely the outcome from the second workshop at NPL. The materials were assessed according to their relative importance in relation to the rationale and their relevance to toxicology and ecotoxicology. Materials were prioritised on a scale of 1-5 with 1 being the highest priority. The Priority 1 candidate materials have been listed in Table 6.2, along with the selection criteria and additional comments.

Table 6.2 Priority 1 candidate materials for toxicology.

Material	Selection criteria*	Comments
Carbon black	A, B, C	B – should be available in nano and micro-sizes; C – already well-studied in humans and animals providing a good starting point.
TiO ₂	A, B, C	B – should be available in nano and micro-sizes, and in coated and uncoated forms; C – already well studied in humans and animals providing a good starting point.
ZnO	A, B	B - should be available in coated and uncoated forms.
SWCNT and MWCNT	A, B	B - straight rigid forms should be available along with bundled forms; long and short lengths should be available.
Polystyrene (Fluorescent)	B	Should be available with different surface modifications in any one size; should be available in fluorescein-labelled forms for tracking fate in cells; should be available in a range of different sizes in the nanometre scale.
Ag	A	Ag has increasing use for antibacterial properties including disinfectant sprays and wound dressings.
Other key metals and metal oxides	A (possibly B & C)	Priorities for ecotoxicology: Zn, Cu, Ni, Fe and their oxides.
Combustion derived NP	C	To be used as a control particle since there is already a significant body of literature, risk and toxicology information available.

* Defined in Table 6.1

This table details our view of the priority particles. No relative priority between them is implied by the order in the table. Candidate materials in this table represent those selected on the basis of all of the 3 criteria specified in Table 6.1. In several cases, more than one criterion is applicable. Those selected according to criterion A represent those materials that currently have (or are anticipated to have) the highest production volumes.

Almost all of the particles chosen have some utility in relation to hypotheses testing (criterion B) although they may also represent particles which may be used to test *different* hypotheses. For example, fluorescent polystyrene particles would support studies relating to (i) the role of particle size and (ii) the role of surface modifications on the ability of particles to cross cell boundaries. Hence, these materials are requested in a range of sizes (<100nm) and with a range of surface modifications. It was beyond the scope of this project to specify in detail the sizes and the surface modifications required.

The single-wall carbon nanotube (SWCNT) and multi-wall carbon nanotube (MWCNT) particles have applications in studies which address the question of whether nanoparticles with the characteristics of fibres (aspect ratio >3:1) also have toxicological characteristics similar to other fibres, such as asbestos. For these materials, 'long' implies a length greater than 15000nm and short implies a length less than 5000nm since these are the critical dimensions for harmfulness in the fibre paradigm.

The carbon black, TiO₂ and ZnO particles will have applications in studies investigating the effect of surface area on toxicity and will need to be available in two sizes, one <100nm and one greater than say 250nm.

Those particles selected on the basis of criterion C (Distributed Analysis) are all particles for which standards have appeared in the published literature. They exhibit a range of toxicities and therefore form the basis of establishing a scale of toxicity to facilitate benchmarking studies.

Nano silver was not originally identified as a Priority 1 material in the workshops. It was identified as Priority 2 for toxicology. The current entries in the Woodrow Wilson inventory suggest that the nanosilver is the most commonly used nanomaterial in consumer products. Examples of products containing nanosilver include soaps, tooth pastes, shampoos, skin creams, wound dressings such as Acticoat[®] (Smith & Nephew, UK), medical devices, fabric treatment, and food packaging materials. Given the widespread possibilities of exposure to humans and the environment from this material and its known antimicrobial properties, the authors have taken the view that nano silver merits inclusion in the priority list.

Other materials which were identified but at a lower priority are shown in Table 6.3.

Table 6.3 Lower priority candidate reference materials.

Material (and assigned priority)	Selection criteria*	Comments
Au (2)	B	Available in different sizes.
CeO ₂ , (2)	A	Used as a fuel additive, therefore has the potential for widespread chronic low level exposure.
SiO ₂ , (3)	A	
Ceramics, (5)	A	
Rods, cubes, horns, (5)	B	Included in the context of a 'shape standard'.
Isotope labelled, (5)	B	Priority for ecotoxicology only.
Nanoclays (5)	A	Not strictly a manufactured nanoparticle.

* Defined in Table 6.1

Organic particles (e.g. micelles, liposomes) were not included as priority substances by the toxicology community contributing to the project.

6.2.2 Quantities Required

It was the opinion of the workshops that relevant UK human toxicology laboratories would each need access to 1-5g of a test or reference material. A realistic estimate of 100 laboratories expressing interest in obtaining the test materials would require a supply of 500g of each material. The typical volume of a material required for human toxicological testing is 0.1-100ml whereas that required for ecotoxicology testing is 1 - 100L (e.g. to expose fish in a tank). To accommodate the potential ecotoxicology requirement therefore the initial estimate of 500g was multiplied by 20 yielding a required initial supply of 10kg per material.

6.2.3 Test Material Medium

The supply of the particles dispersed in a medium distinct from that which cells grow in, presents a problem for human toxicological studies. Biological systems in humans are very delicate and adding any extraneous agents can affect the cells and confound the study. There was a preference for materials to be available as "dry powders". Furthermore, a desire was expressed for test materials to be monodisperse, except in the case of materials which mimic industrial products. Whilst the stability of particles is likely to be greater in air than in any liquid (with some exceptions) and it is acknowledged that previous particle standards have been supplied dry (e.g. UICC asbestos, DQ12, NIST SRMs), some uncertainties exist about a nanoparticle reference material's stability / homogeneity in powder form that require further consideration by the materials production community. The only acceptable liquid would be ultra-pure water, but this would need to contain an antimicrobial preservative to prevent

bacterial/fungal growth, and the issues of possible aggregation and dispersion would remain extant.

6.2.4 Metrology

The priorities and requirements for metrological reference materials are significantly different. Where toxicology reference materials will often be chosen *because* detrimental physiological effects can be expected for those types of particle, metrology reference materials are often chosen to *avoid* such concerns. This reduces the cost of risk assessment and control measures. Particulate size standards, for example, are typically chosen from materials that are relatively chemically inert.

Discussion at the workshops highlighted that this has led to existing particulate standards being composed of a very narrow range of materials, e.g. hydrocarbon polymers. While it is unnecessary to develop particulate standards filling the entire matrix of possible sizes and compositions, a wider range of chemical composition at their surfaces would allow particle sizing instruments to be checked for any chemically-related systematic errors. There are some theoretical grounds for believing some instruments that are available commercially may be liable to such systematic errors.

Other factors affecting the metrology of reference and test materials in liquids include the aggregation and/or the flocculation of nanoparticulates over time. A complex interaction between dispersant medium, particle composition and the relative dilution can mean that averaging methods for particle sizing (for example dynamic light scattering) can become skewed by aggregation phenomena that can occur over the time scale of preparation and storage.

These behaviours are difficult to model currently, therefore careful protocols for preparation and analysis are needed in the future so that comparable measurements can be made.

6.3 REQUIRED CHARACTERISING PARAMETERS

6.3.1 Toxicology / ecotoxicology

Clearly, for any reference material the more characteristics which can be specified the more potential there is for describing a structure that is important for particle toxicology. However, increased characterisation leads to increased costs so that it is important to choose those characterisations that are necessary rather than those which are merely desirable. It is important for these measurements to be linked to the national measurement system through promulgated artefacts and/or documentary standards where appropriate. For materials considered as potential stable reference materials, the following minimum set of characterisation parameters are recommended:

- aerodynamic equivalent diameter; (as measured by electrical mobility or an equivalent technique);
- absolute length (in the case of high (>3:1) aspect ratio particles); (as measured by a length traceable (calibrated) microscopy);
- specific surface area, indicating the available reactive surface; (as measured by an agreed isotherm method and traceable mass measurement);

- number of particles per unit mass; (as measured either by a valid particle counting method or through validated indirect methods alongside a traceable mass measurement);
- concentration of bulk and/or surface contaminants/additives e.g. metals, soluble toxins; (as measured by elemental analysis and a validated surface chemical analysis);
- polymorphic composition (as measured by a validated crystallographic analysis).

In addition to this minimum set of parameters, the following have also been identified as being desirable:

- shape;
- zeta potential;
- surface charge;
- solubility;
- hydrophobicity;
- agglomeration state.

Furthermore, several semi-quantitative parameters useful for toxicology were also identified as desirable. No suggestions were made as to how they should be measured. These included translocation-potential, biopersistence, adsorptive ability (potential to adsorb substances to the surface of a particle that may influence its behaviour), and free radical action.

6.3.2 Metrology

The majority of the parameters listed in section 6.3.1 are physico-chemical characteristics for which reference materials would be useful to the metrology community, and not simply in support of toxicology studies. Prioritising the large number of characterisation parameters is important to meet as many of the critical requirements as cost-effectively as possible. An aspect particularly useful in metrology would be monodispersity (in conjunction with particular properties such as shape and surface charge). However, it is questionable whether the extra expense of producing such monodisperse reference materials beyond the needs of the toxicology community is really justified; this will depend on the details of the material and production method and in some cases it will be appropriate.

6.4 AVAILABILITY OF CHARACTERISATION METHODS

In principle, characterisation of NP may be carried out in gas, liquid or solid media. One of the workshop groups was tasked with reviewing the methods available to implement the characterisation requirements highlighted in section 6.3. In practice, little consideration was given to particles bound in a solid matrix, due to the inherent difficulties of this approach and the limited evidence for potential harm to health or the environment of such nanomaterials (although it is recognised that NP may be released from bound solid matrices during use or disposal). Strictly, gas based dispersions can be further refined into stable (powder form (designated as **S** in Tables 6.4 and 6.5)) and

unstable (finely dispersed as an aerosol (designated as **A** in Tables 6.4 and 6.5)) which again have different techniques applied. The final point to note from the metrology workshop was that even for simple measurements such as particle diameter, the media and the technique could skew results dramatically at the nanoscale. Therefore, reporting the measurement technique is recommended. The range of techniques that can be applied to the measurement of nanoparticles is bewildering to the non-specialist. Basically any analytical technique can be applied to the measurement of nanoparticles if modified correctly. However, there are favoured techniques that are used currently to estimate the basic parameters. Analytical methods for the minimum set of parameters recommended and additional optional parameters are summarised in Tables 6.4 and 6.5 respectively.

Table 6.4 Methods for the recommended minimum set of characterisation parameters.

Measurement parameter	Characterisation technique (Dispersion: A – Airborne, S- Solid, L – Liquid)
Apparent diameter	Scanning mobility analysis (i.e. SMPS) (7nm and above for airborne particles) coupled with Condensation counter or electrometer detection (A); Transmission (TEM) and Scanning (SEM) Electron Microscopy (offline solid samples) (S); Line broadening phenomena in spectroscopies (A, S, L); Dynamic light scattering (for liquids, but there are issues with non-spherical particles) (L).
Length	SEM and TEM (S).
Surface Area	BET methods (Gas Isotherm – solid samples only, but can be used to determine porosity). Difficulty with nanoporosity (<5nm) due to comparable size of probe molecules with pores (S); Surfactant BET methods for liquid based samples (more difficult) (L).
Number density	Indirect methods via size distribution (A, S, L)
Composition (Bulk & surface)	Elemental analysis, Inductively-coupled Plasma Mass Spectrometry (ICP-MS), Energy-dispersive X-ray Analysis (EDX), Electron Energy Loss Spectroscopy (EELS), dynamic Secondary Ion Mass Spectrometry (SIMS), 3D – Atom Probe (S).
Crystal Form	Powder X-ray Diffraction, High Resolution Transmission Electron Microscopy, Raman Spectroscopy (S).

Table 6.5 Methods for the additional suggested characterisation parameters.

Measurement parameter	Characterisation technique (Dispersion: A – Airborne, S- Solid, L – Liquid)
Total Charge	Electrometer measurements (difficult – no methods for mapping charge distribution on NPs) (A); Single particle Atomic Force microscope (not applicable to bulk analysis) (S); Voltammetry and potentiometric titrations (L).
Solubility	Traditional techniques (monitor solution and relate to residual mass of material or monitor opacity) (L)
Hydrophobicity Interfacial Measurements	Wetability studies (Surface energy/tension), contact angle measurements (difficult), organic/water partition methods (L).
Surface Composition	Surface Analytical Techniques (X-ray Photoelectron Spectroscopy, Auger, Secondary Ion Mass Spectrometry – in vacuo) (S) Radiation beam methods – (Infrared, Near infrared, Raman, Surface Enhanced Raman Spectroscopy) (S, possibly A) Electron microscopies coupled to EDX and EELS analysers. (S)
Agglomeration/ Aggregation	No easy methods – light scattering, small angle neutron and x-ray techniques. Critically dependant on surface charge/composition parameters) (L).
Zeta Potential	Electrophoretic mobility – laser light scattering (L).
Aspect Ratio	Electron microscopies (although very long CNT are very difficult to measure) (S).
Semi-quantitative or hybrid parameters	
Translocation	Tracing and imaging techniques (Optical/fluorescent microscopies), environmental TEM (S, possibly L).
Free Radical Action	Electron Spin Resonance (ESR) studies (L, possibly S).
Biopersistence	Imaging techniques – TEM/SEM. (No definitive technique for ‘in the field’ measurement due to relative mass of background and analyte) (A/S/L).
Adsorptive Ability	Porosimetry methods (related to BET) (S).

Three issues concerning the metrology aspects of developing and using reference nanomaterials emerged from the second workshop:

1. The need for **new measurement techniques** for sizing and characterisation of engineered NPs amid a background of a much larger number density of particles arising from incomplete combustion (e.g. diesel exhaust) having what may be a very similar size range;
2. **Methods of validating techniques** – for example particle sizing instruments, but including some others – for the much wider diversity of surface chemistry offered by engineered NPs compared to the types of particulate sources to which they have previously been applied;
3. The need for **surface chemistry characterisation** in general, since it is the biological activity of the surface that is physiologically important and needs physico-chemical parameters for correlation with toxicology studies.

6.5 CRITICAL PATH FOR REFERENCE MATERIAL DEVELOPMENT

In considering the preceding discussion of needs, it is apparent that some classes of reference material are better provided for than others. In some cases, reference or test materials are already available which may fulfil the necessary requirements. Therefore a cost effective strategy will require different actions for different classes of materials. It is therefore better to focus on providing consensus on analysis methods and possibly improving the status of 'test' materials to 'certified' reference materials. The discussions that have taken place as part of the REFNANO project have led to the proposal of actions required to develop the field of reference nanomaterials.

6.6 NEXT STEPS AND RECOMMENDATIONS

The REFNANO project has identified a series of actions which will facilitate the further development and promulgation of reference materials for nanoparticles. We have grouped these below by theme. Implementation of these actions is outwith the scope of the current project. However, we suggest that these proposed actions be considered for support by Defra, the UK Nanotechnology Research Coordination Group (NRCG), the UK Research Councils and international activities including OECD's Project "*Safety testing of a representative set of reference materials*".

Existing Reference & Test Materials

We have identified that certified reference materials are not required in all circumstances and that significant progress can be made with well characterised test materials. We have identified some examples of materials which could be used or developed in this way. We recommend that a programme of work is undertaken which will:

- scope the availability of existing sources of the candidate materials identified in REFNANO and map their suitability against the criteria specified in this report;
- review the status of currently available materials that may be suitable as reference / test materials and their appropriateness to go through the proposed development schedule;
- scope the options for conferring reference material status on existing materials.

New Reference & Test Materials

In some cases, it will be necessary to develop new materials *ab initio*. We have identified in Figure 4.2 a process by which this can be achieved. We recommend that an example is selected and developed through a programme of work which will:

- develop a full implementation plan for a single candidate material;
- progress that plan to the point at which a reference material becomes available;
- co-ordinate a limited set of inter-laboratory (round-robin) studies to test the practical usage of the material.

Toxicology

There are multiple assays which may be used to provide information concerning the potential toxicity of nanomaterials. While all have something useful to contribute, it would not be practical to initiate inter-laboratory studies on all of these. Therefore we recommend a study which will:

- select from the available assays a single or small set of assays which would be appropriate to form the basis of an inter-laboratory exercise;
- define in sufficient detail, the protocols for these assays, so as to facilitate the implementation of an inter-laboratory exercise;
- pilot the inter-laboratory exercise in a small number of laboratories with a single candidate material.

Measurement Techniques

While many relevant measurement techniques are available, there are no current “round-robin” activities. There are also multiple parameters of interest. Not all of these can be addressed at once but the need for information about particle size is almost universal. Therefore we recommend a programme of work to:

- scope out, develop and implement a round-robin study or studies for measurement techniques for particle size.

Guidance

While development of the materials themselves is undoubtedly important it is also critical that these materials are used in the same way. We therefore recommend:

- the development of standards for sample preparation and handling for metrology and toxicological analysis.

Strategic Developments

There is a clearly expressed need for reference nanomaterials to be developed, produced and distributed. Some progress can be made on a single sample or single issue basis (and perhaps this is necessary in the early stages). However, the ultimate outcome should be the development of a coordinated reference material library, considering issues of production, storage and distribution of reference nanomaterials. This will require a co-ordinated, preferably international approach and will need significant funding.

7 CONCLUSIONS

The advancement of our understanding of the effects of nanoparticles on health and the environment requires a hypothesis-driven approach underpinned by a weight-of-evidence with a high level of confidence. Well characterised, robust and reliable reference materials have a vital role to play in meeting this objective.

The REFNANO project sought to provide a priority list of candidates for inclusion in a set of reference materials to support the measurement and toxicology and risk assessment of engineered nanoparticles in the UK.

Consensus has been reached between the toxicology and metrology communities on i) the rationale for reference materials, ii) a list of priority candidates, iii) their selection criteria and iv) the suitability of existing instrumental techniques for characterisation. The prioritised candidates are toxicologically and industrially relevant at the nano-scale and focus on materials produced and used in the UK.

We consider the next important practical step to be the scoping of existing materials' suitability to meet the requirements of the toxicology and metrology communities detailed in this report, and options to confer reference material status in cooperation with aforementioned initiatives.

From the project's outset, it was acknowledged that the focussed work of REFNANO would be invaluable in informing the broader consideration of reference materials issues by other initiatives, including OECD Working Group 3, and we have highlighted a series of themed requirements for further consideration.

8 ACKNOWLEDGEMENT

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9 REFERENCES

- Aitken R.J., Chaudhry M.Q., Boxall A.B.A. and Hull M. (2006) 'In-depth review: Manufacture and use of nanomaterials: current status in the UK and global trends', *Occupational Medicine* **56**: 300-306.
- BSI PAS 71:2005 Vocabulary – Nanoparticles, BSI. (<http://www.bsi-global.com/Manufacturing/Nano/PAS71.pdf>).
- Bunn W.B., Bender J.R, Hesterberg T.W., Chase G.R., Konzen J.L. (1993) 'Recent studies of man-made vitreous fibers: Chronic animal inhalation studies', *J. Occup. Med.* **35(2)**: 101-113.
- Don Porto Carero A., Hoet P.H., Verschaeve L., Schoeters G., Nemery B. (2001) 'Genotoxic effects of carbon black particles, diesel exhaust particles, and urban air particulates and their extracts on a human alveolar epithelial cell line (A549) and a human monocytic cell line (THP-1)', *Environ. Mol. Mutagen.* **37(2)**:155-63.
- Defra (2005) 'Characterising the potential risks posed by engineered nanoparticles. A first UK Government research report'. (<http://www.defra.gov.uk/environment/nanotech/research/pdf/nanoparticles-riskreport.pdf>).
- ISO (2006) 'ISO Guide 35: Reference Materials – General and statistical principles for certification', International Standards Organisation.
- Maynard A.D., Aitken R.J., Butz T., Colvin V., Donaldson K., Oberdorster G., Philbert M.A., Ryan J., Seaton A., Stone V., Tinkle S.S., Tran L., Walker N.J., Warheit D.B. (2006) 'Safe handling of nanotechnology', *Nature* **444(16)**: 267-269.
- Royal Society (2004) 'Nanoscience and Nanotechnologies: Opportunities and Uncertainties', Royal Society Policy Document 19/04. (<http://www.royalsoc.ac.uk/document.asp?id=2023>).
- Robock K. (1973) 'Standard quartz DQ12 <5um for experimental pneumoconiosis research projects in the federal republic of Germany', *Ann. Occup. Hyg.* **16**: 63-66.
- Timbrell V., Gibson J.C., Webster I. (1968) 'UICC standard reference samples of asbestos', *Int. J. Cancer* **3**: 406-408.
- Woodrow Wilson Centre (2007) 'Woodrow Wilson Nanotechnology Consumer Products Inventory', (<http://www.nanotechproject.org/consumerproducts>).

APPENDIX 1 – WORKSHOP PARTICIPANTS

CSL Workshop Participants		
Margaret	Saunders	Bristol University
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Terry	Tetley	Imperial College
Lang	Tran	IOM
Rob	Aitken	IOM
Steve	Hankin	IOM
Del	Stark	ENTA
Dave	Gavin	Johnson Matthey
Herman	Stamm	JRC
Ben	Forbes	Kings College London
Malcom	Connah	Malvern
Stephen	Cash	Nanocentral
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Peter	Cumpson	NPL
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