WORKING FOR A HEALTHY FUTURE

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EMERGNANO: A review of completed and near completed environment, health and safety research on nanomaterials and nanotechnology

Defra Project CB0409

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SAFENANO

EMERGNANO was carried out by IOM as part of its SAFENANO activities (www.safenano.org). SAFENANO is one of the UK Micro and Nanotechnology (MNT) Centres, and is focussed on collecting, interpreting and disseminating emerging scientific evidence on nanoparticle risks.

Our collaborators in this project were Napier University, the University of Edinburgh, the Central Science Laboratory – all partners in the SnIRC collaboration (www.snirc.org) – plus the University of Leeds, Cranfield University and the Woodrow Wilson International Center for Scholars Project on Emerging Nanotechnologies (PEN).

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EXECUTIVE SUMMARY

In 2004, the Royal Society and the Royal Academy of Engineering published, at the request of the UK Government, a major review of the opportunities and uncertainties of nanotechnologies (RS/RAEng, 2004). This was one of the first reports to highlight the potential risks to health and the environment which may arise from exposure to nanoparticles. Since then, more than 50 national and international reviews carried out by government departments, industry associations, insurance organisations and researchers have considered nanoparticle risk issues. These reviews have provided a consistent view about the nature and the potential risks of nanoparticles, which may be summarised as follows:

- There are potential risks to health and the environment from the manufacture and use of nanoparticles;
- There is a lack of knowledge about what these risks are and how to deal with them;
- As more processes and products containing NP were developed, the potential for exposure of people and the environment will increase;
- More information about the toxicity, exposure and risk is required;
- All of the stakeholders (regulators, companies) need to start to address these risks now.

Since publication of the Royal Society / Royal Academy of Engineering report, there has been a significant increase in research activity in the UK and internationally intended to fill these gaps.

In the UK, the Nanotechnology Research Coordination Group (NRCG) was set up by the Government in 2005 to determine priorities and 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 membership includes Government Departments, Regulatory Agencies and the Research Councils. Its first research progress report (Defra, 2006) set out a programme of 19 Research Objectives (ROs), across five main scientific areas, to characterise the potential risks. The five areas were:

- Metrology, Characterisation, standardisation and reference materials;
- Exposures – Sources, pathways and technologies;
- Human health hazard and risk assessment;
- Environmental hazard and risk assessment;
- Social and economic dimensions of nanotechnologies.

The NRCG set up five Task Forces, one for each area, to take forward the 19 objectives outlined in its first research progress report.

In this project we have carried out a detailed review and analysis of research carried out worldwide on Environment, Health and Safety aspects of engineered nanoparticles, (NP) including issues relating to hazard, exposure and risk assessment and regulation, and made an assessment of how far 18 of the ROs have been met and which gaps still remain to be filled (RO01, Nanotechnologies and public engagement, was specifically excluded from the contract). As far as practicable, we have carried out an appraisal of the research results with a view to highlighting any new information that may trigger a consideration for the need for regulation of nanomaterials, assessed the possibility of a qualitative risk assessment and considered whether there is sufficient information to

invoke the precautionary principle for one or more nanomaterials. Finally, we have made recommendations for new research to fill gaps.

The approach used in our review and analysis was to:

- Develop a comprehensive and categorised list of potentially relevant studies, active since 2004, starting with those on the Woodrow Wilson Project on Emerging Technologies database (www.nanotechproject.org), and adding to this through the knowledge and personal contacts of the authors plus information from national and agency contacts, existing project listings compiled at a national or agency level, and a focussed review of the peer-reviewed literature;
- Compile information about status, duration, funding, objectives, methods and output relating to these studies through dialogue with project leaders;
- Based on preliminary review, allocate (map) the studies to the 18 ROs being considered;
- Through a multidisciplinary panel of expert reviewers (the authors of this report), chosen to cover the range of scientific disciplines represented in this activity, carry out an appraisal of the contribution of each study in relation to 18 ROs, the extent to which the RO is likely to be met, and the gaps remaining;
- Undertake a risk assessment appraisal identifying the need for control or management of risk, including an appraisal of whether there is sufficient information to invoke the precautionary principle for one or more nanomaterials;
- And through a workshop and dialogue reach a consensus view about the remaining gaps and future priorities.

Since 2004, work in this area has been funded by both national government activity and research programmes within each country and at an international level, for example within the European Union. Internationally we have identified and assessed more than 650 projects. More than half of the projects identified in the first pass were only of marginal relevance to nanoparticle risk issues, were related to activities such as funding for a conference, or were duplicates. These were eliminated in the preliminary assessment. There was a wide disparity in the information available for the remaining studies, but sufficient to allow a preliminary allocation across the four task force areas and 18 ROs. Study allocation to the ROs was verified as part of the detailed review activity carried out by the expert panel. We found a widespread imbalance in the work being carried out (in terms of numbers of studies) between the four main task force areas and between the eighteen research objectives. The largest number of studies was in the Human Health area, followed by the Exposures area. Numbers in both the Environment and Characterisation areas were substantially lower. The distribution by RO was even more striking. RO14 (Research to establish a clear understanding of the deposition, distribution, toxicity, pathogenicity and translocation potential and pathways for nanoparticles in the airways and lung and their potential impacts on the cardiovascular system and brain) had 44 studies identified as relevant whereas RO9 (Optimisation, development and application of technologies that enable the measurement of exposure to nanoparticles in soil and water) had only one study.

As might be expected, there are large regional variations in the type of study funded in different countries or economic areas, perhaps reflecting different national priorities or capabilities. The largest number of studies was found in the US, followed by the UK.

It was noted that there was substantial overlap between many of the ROs. This may be quite appropriate from a scientific perspective but made the analysis quite challenging, both in study allocation and in the review activity where individual studies

had to be reassessed several times (or assessed by different assessors) from (slightly) different perspectives.

We were unable to identify useful output from many of the studies involved in the programme, including studies which had already been completed. It is unsatisfactory when publicly funded studies do not result in information being released into the public domain. It also made undertaking of this EMERGNANO project much more challenging.

In Task Force Area (TFA) 1, (Metrology, characterisation, standardisation and reference materials), the reviewers found that none of the studies could be said to be conclusive in providing new approaches or guidelines to characterising and measuring nanomaterials. Nor was the selection and/or development of exposure metric or metrics addressed well. Studies were focussed on addressing mainly the relevance and practicality of using surface area which, whilst important, is unlikely to be relevant for all NP. Progress has been made in identifying candidate materials which may be used to develop characterised reference nanoparticles for toxicology. Candidate lists, along with minimum characterisation specifications, have been developed and some commercial reference materials are beginning to emerge. However, there is little evidence that issues such as storage, distribution or protocols for use are being addressed. Only two studies were identified as addressing potential risk of explosion of NP and only one study was identified as addressing the issue of measurement of exposure to nanoparticles in soil and water. Overall the specific objectives within this RO appear to have been very sparsely addressed. A great deal of work remains to be done.

In TFA2, (Exposures, sources, pathways and technologies), some work has been undertaken on establishing inventories of nanoparticle use and application, and on trying to map out some of the potential exposure pathways. However, there are many complex exposure pathways and only a few have been considered. Little is known about NP in relation to consumer exposure and work in relation to NP in food seems to be entirely missing. Use of a life cycle assessment approach is missing. Little progress has yet been made in relation to development of measurement technologies for nanoparticles in air. Although there is some evidence that ongoing studies may produce devices, such as personal samplers, and approaches for some types of nanoparticles, major questions remain. These include discrimination between NP and the background particles and the evaluation of whether fibre counting methods can be applied to high aspect ratio nanoparticles. It now seems clear that filtration systems will be effective against nanoparticles and several studies have found improving collecting efficiency as particle size decreases. Studies have not thus far specifically addressed the performance of engineering controls as they are implemented in practical settings. Issues of leakage from filtration systems and the effectiveness of skin protective equipment are also under-researched.

In TFA3, (Human health hazard and risk assessment), there is an absence of studies aiming to describe the accumulation of particles in a variety of organs after inhalation. There are no specific studies on whether carbon nanotubes and other high aspect ratio nanoparticles behave like asbestos with respect to whether they translocate to the pleural mesothelium. In general there is no attempt to try to identify potential structure-activity relationships that govern penetration at any of the important boundaries. Many studies are addressing the issue of oxidative stress – inflammation. Several studies are also attempting to address structure- activity relationships in relation to this but, as yet, little progress has been made. There are few *in vivo* studies being carried out, making comparison between *in vitro* and *in vivo* data problematic. Few studies are using NP exposures at or near plausible exposure levels and also few studies are

addressing genotoxicity. Dermal uptake is not being addressed to any great extent and as yet toxicological testing strategies have not evolved to any level of agreement.

In TFA4, (Environmental hazard and risk assessment), studies have improved the understanding of kinetics of nanoparticle uptake in invertebrate and vertebrate models and have related this to toxicity. In addition, there are now a few studies focussing on microbial organisms and these provide information on effect assessment at both individual and community level. However, studies only cover a limited range of species and material types (metal oxide, fullerenes, CNT). There is some effort to relate study design and interpretation to human toxicology of nanoparticles, but there remains much more to be done in this area. Only one project is addressing bioaccumulation and bio-concentration of nanoparticles.

We have identified 260 relevant studies which are either completed or already underway. In projects which are just starting or have just started there is some evidence to support the view that the work in these projects will deliver much more in terms of output than the projects which are currently just closing. At this point in time, based on the evidence we have been able to collect regarding these studies, progress thus far has been disappointing. Whilst many studies are undoubtedly contributing in an incremental way to the advancement of knowledge, few of the key questions have been resolved. We conclude that the programme of research activity has yet to deliver step changes in the knowledge base on these issues.

In assessing quality and completeness for the purpose of carrying out a risk assessment, we did not identify a sufficient body of evidence in any case to make a risk assessment feasible. However, three different nanomaterials have been identified that give rise to sufficient concern from the results presented within the RO reports. There is evidence that carbon nanotubes may have an adverse effect on human health; and that silver nanoparticles and titanium dioxide nanoparticles are detrimental to the environment. In these specific cases, further investigation as to the need to invoke the precautionary principle is required, taking into consideration all available data.

The EMERGNANO project has been a unique attempt to identify and assess *worldwide* progress in relation to nanotechnology risk issues. On an international basis we have identified and assessed more than 260 unique, relevant projects completed, close to completion or in progress. We have observed a wide disparity in quality and quantity of the information available for these studies. We have also mapped these projects against the eighteen ROs set in the UK by the NRGC/DEFRA. This has been achieved over a period of six months. We cannot be certain that we have identified all of the relevant studies or that we have assessed all of these studies using all of the publicly available information. However, we consider that what has been achieved has been successful in identifying the overwhelming majority of important studies and having these studies assessed as to their output and relevance by some of the leading researchers currently working in this area. We have, as part of the project, achieved a comprehensive listing of projects and produced detailed comments and assessment of their outputs. It is our view that EMERGNANO represents the best available picture currently available of current strategic research. As such, EMERGNANO presents an excellent basis for assessing progress of these and other studies in the future.

1 INTRODUCTION

1.1 BACKGROUND

In 2004, the Royal Society and the Royal Academy of Engineering published, at the request of the UK Government, a major review of the opportunities and uncertainties of nanotechnologies (RS/RAEng, 2004). This was one of the first reports to highlight the potential risks to health and the environment which may arise from exposure to nanoparticles. Since then, more than 50 national and international reviews carried out by government departments, industry associations, insurance organisations and researchers have considered nanoparticle risk issues. These reviews have provided a consistent view about the nature and the potential risks of nanoparticles, which may be summarised as follows:

- There are potential risks to health and the environment from the manufacture and use of nanoparticles;
- There is a lack of knowledge about what these risks are and how to deal with them;
- As more processes and products containing NP were developed, the potential for exposure of people and the environment will increase;
- More information about the toxicity, exposure and risk is required;
- All of the stakeholders (regulators, companies) need to start to address these risks now;

Since publication of this report, there has been a significant increase in research activity in the UK and internationally intended to fill these gaps.

In the UK, the Nanotechnology Research Coordination Group (NRCG) was set up by the 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 membership includes Government Departments, Regulatory Agencies and the Research Councils. The NRCG produced a research progress report (Defra, 2006) setting out a programme of 19 research objectives (ROs), across five main scientific areas, to characterise the potential risks. The five areas are:

- Metrology, Characterisation, standardisation and reference materials;
- Exposures – Sources, pathways and technologies;
- Human health hazard and risk assessment;
- Environmental hazard and risk assessment;
- Social and economic dimensions of nanotechnologies.

The NRCG set up five Task Forces, one for each area, to take forward these ROs. These 5 areas became the Task Force Areas (TFA).

Each Task Force has members from a wide range of stakeholders including academic institutions, independent research organisations, industry, Government departments, their Agencies and the Research Councils. The 19 research objectives and their distribution across the five task forces are shown in Table 1.1.

Table 1.1: Defra Task Forces and NRGC Research Objectives

Defra Task Force	NRCG Research Objective (RO)	
1. Metrology, Characterisation, Standardisation and Reference Materials	RO 02	To identify the most suitable metrics and associated methods for the measurement and characterisation of nanoparticles.
	RO 03	To develop standardised, well-characterised reference nanoparticles.
	RO 04	To understand the properties of nanoparticles in the context of their ignition and explosion potential, and assess/develop methods for evaluating this.
	RO 09	Optimisation, development and application of technologies that enable the measurement of exposure to nanoparticles in soil and water.
2. Exposures – Sources, Pathways, and Technologies	RO 05	Further identification of sources of nanoparticles.
	RO 06	Optimisation and development of technologies that enable the measurement of occupational and environmental exposure to nanoparticles *via* air.
	RO 07	Understanding the fate and behaviour of nanoparticles in air.
	RO 08	Development of exposure control devices.
	RO 10	Research to understand the environmental fate, behaviour and interaction of nanoparticles in soils and water.
3. Human Health Hazard and Risk Assessment	RO 11	Research to establish a clear understanding of the adsorption of nanoparticles *via* the lung, skin and gut and their distribution in the body (i.e. toxicokinetics), identifying potential target organs/tissues for toxicity assessment.
	RO 12	Research to establish a clear understanding of inter- and intracellular transport and localisation of nanoparticles and their cellular toxicity.
	RO 13	To establish a clear understanding of whether oxidative stress, inflammatory effects and genotoxicity apply to nanoparticles.
	RO 14	Research to establish a clear understanding of the deposition, distribution, toxicity, pathogenicity and translocation potential and pathways for nanoparticles in the airways and lung and their potential impacts on the cardiovascular system and brain.
	RO 15	Given the current use of nanoparticles in consumer products there is a need to further our understanding of dermal uptake, penetration and toxicity in the skin.
	RO 16	To develop testing strategies for human health hazard assessment and assess how fit for purpose current test methods are as applied to nanoparticles.
4. Environmental Hazard and Risk Assessment	RO 17	Research to establish the uptake, toxicity and effects of nanoparticles on groundwater and soil microorganisms, animals and plants, especially in the context of remediation.
	RO 18	Research to establish the mechanisms of toxicity, toxicokinetics and *in vivo* effects of nanoparticles to key ecological groups (including invertebrates, vertebrates (e.g. fish) and plants). A key aspect of such work should be the facilitating of knowledge transfer from human toxicological studies to inform ecotoxicology.
	RO 19	Define endpoints to be measured in ecotoxicological studies and assess how fit for purpose current standard tests for persistence, bioaccumulation and toxicity are when considering nanoparticles. This should lead to the defining of a suite of standard PBT protocols for use in environmental hazard assessment.
5. Social and economic dimensions of nanotechnologies	RO 01	Nanotechnologies and public engagement.

Work to realise these objectives has comprised of both information-gathering activities (such as reviews and workshops), and, increasingly, new research activity intended to address the fundamental questions about the risks. This work has been most recently reported in "Characterising the Potential Risks posed by engineered Nanoparticles – A second UK Government Research Report" (Defra, 2007).

Many national and international organisations have developed similar research strategy documents and have initiated programmes of research in order to achieve the objectives described therein. For example, the European Framework (FP) Programme has funded several projects under the 6th Framework Programme (FP6), e.g. NANOSAFE2, PARTICLE_RISK and has a number of projects starting or under final negotiation resulting from calls in the 7th Framework Programme, under the NMP theme 4.1.3 Health, Safety and Environmental Impacts in FP7. In Europe, several countries including Germany, Denmark and Switzerland have developed their own national programmes. In the US, research has been funded principally by National Institute for Occupational Safety and Health (NIOSH), National Institutes of Health (NIH) and the Environmental Protection Agency (EPA).

While many of these agencies and organisations have developed and published research strategies, and although attempts are being now made to link up, for example through EU/US joint calls in the EU Framework Programme and OECD, until now there has been little effective international co-ordination on research activity. As a result, funded projects are unlikely to provide coherent or comprehensive coverage of the issues.

Many of the funded projects are only at the early stages of their work and as yet have not begun to publish their outputs. Such is the range of ongoing work, it is a significant challenge to track progress (or the promise of progress) in this complex landscape. Attempts have been made to collate information about ongoing studies, for example the Woodrow Wilson Project on Emerging Nanotechnology's Research Inventory, (www.nanotechproject.org) and the recent EU inventory of research and development activity on nanotechnology health and safety (European Commission, 2008). However, these inventories are not necessarily complete and do not provide an interpretation of the value of the work being carried out, or how the findings collectively contribute to resolving the uncertainties.

Research is currently underway across the spectrum of risk management for NP, including hazard, characterisation, exposure and risk assessment. This research is international in nature, multidisciplinary and recognised as a high priority by government and international organisations. In the UK context, NRCG needs to understand the extent to which the research objectives are being met on an international basis, and what gaps remain. To address this need, Defra commissioned the EMERGNANO project. EMERGNANO aims to capture and critically appraise the rapidly emerging evidence through a review of research projects concerning the health and environmental risks of nanomaterials, to inform the future prioritisation of research.

1.2 OBJECTIVES

The proposal for this work was developed in response to an invitation to tender published by Defra. The objectives stated in that tender, on which the current project is based, were as follows:

i) a detailed review and analysis of research carried out worldwide on Environment, Health and Safety aspects of engineered nanomaterials including issues relating to hazard, exposure and risk assessment and regulation;
ii) an evaluation of how far research objectives have been met and to identify which gaps still remain to be filled;
iii) an appraisal of the research results with a view to highlighting any new information on hazards and risks to human health and/or the environment from nanomaterials that may trigger a consideration for the need for regulation of nanomaterials;
iv) come to an interim position regarding the magnitude of risk and associated uncertainty given the evidence to date (and where the largest uncertainties lie), noting that this will almost certainly be a qualitative risk assessment process that is under review with respect to fitness-for-purpose;
v) consider whether there is sufficient information to invoke the precautionary principle for one or more nanomaterials;
vi) to make specific recommendations for new research to fill gaps in the understanding of the potential risks posed by engineered nanomaterials taking into consideration, as far as practicable, work currently in progress.

1.3 STRATEGIC APPROACH

We proposed the following approach, in line with the stated objectives of the call, to review completed and near-completed environment, health and safety research on nanomaterials and nanotechnology.

Our approach was to:

- Develop a comprehensive and categorised list of potentially relevant studies, active since 2004, starting with those on the Woodrow Wilson Project on Emerging Technologies database (www.nanotechproject.org), and adding to this through the knowledge and personal contacts of the authors plus information from national and agency contacts, existing project listings compiled at a national or agency level, and a focussed review of the peer-reviewed literature;
- Compile information about status, duration, funding, objectives, methods and output relating to these studies through dialogue with project leaders;
- Based on preliminary review, allocate (map) the studies to the 18 ROs being considered;
- Through a multidisciplinary panel of expert reviewers (the authors of this report), chosen to cover the range of scientific disciplines represented in this activity, carry out an appraisal of the contribution of each study in relation to 18 ROs, the extent to which the RO is likely to be met, and the gaps remaining;
- Undertake a risk assessment appraisal identifying the need for control or management of risk, including an appraisal of whether there is sufficient information to invoke the precautionary principle for one or more nanomaterials;
- And through a workshop and dialogue reach a consensus view about the remaining gaps and future priorities.

1.4 PROJECT TEAM

The proposal for this work was submitted as a consortium bid by the SnIRC collaboration (www.snirc.org) led, for this study, by the Institute of Occupational Medicine (IOM). SnIRC is uniquely placed to carry out such an extensive and authoritative review of nanomaterials toxicology issues due to its positioning as the leading group undertaking research in this area, our work and links with governments and industry and, our world-wide network of collaborators and experts. Partners in this project have a track-record in review activities and hosting workshops as well as undertaking fundamental research. All of the core participants have previous experience in working together. The capabilities of the core participants are outlined below in Table 1.2.

Table 1.2: The EMERGNANO Project Team

Name	Expertise	Institution
Dr Rob Aitken	Director of Strategic Consulting, IOM. Director of SAFENANO. Principle areas of expertise include exposure assessment, manufacture and use of nanomaterials.	IOM
Dr Lang Tran	Director of Nanotechnology Risk Research. Leading UK quantitative toxicologist.	IOM
Dr Steve Hankin	Consultant Chemical Toxicologist and Director of Operations, SAFENANO. Principle areas of expertise are the characterisation, toxicology and risk assessment of chemical hazards.	IOM
Bryony Ross	Research Scientist. Editor of SAFENANO Website (www.safenano.org).	IOM
Prof Ken Donaldson	Professor of Toxicology. One of Europe's leading particle toxicologists. Editor in Chief of Particle and Fibre Toxicology.	Consultant
Dr Roger Duffin	Particle Toxicologist.	Consultant
Prof Vicki Stone	Professor of Toxicology. Director of Toxicology, SAFENANO. Editor in Chief of Nanotoxicology. Leading UK particle toxicologist and ecotoxicologist.	Napier University
Dr Teresa Fernandes	Reader in ecotoxicology of nanoparticles and environmental chemicals.	Napier University
Dr Andrew Maynard	Chief Science Advisor to the Wilson Center Project on Emerging Nanotechnologies. Leading expert in identifying and addressing potential impacts of nanomaterials.	Woodrow Wilson International Center for Scholars
Prof Terry Wilkins	Professor of Nanomanufacturing. 30 years experience in nanostructured materials manufacturing.	University of Leeds
Dr Simon Wilkins	Enterprise Officer, Keyworth Institute.	University of Leeds
Prof Len Levy	Professor of Environmental Health. Author of occupational and environmental risk assessments on many types of substance.	Cranfield University
Dr Sophie Rocks	Toxicologist with background in materials science.	Cranfield University
Dr Qasim Chaudhry	Senior Environmental Chemist.	Central Science Laboratory

The Steering Group for the project consisted of Defra and the heads of the NRCG Task Forces 1-4.

1.5 REPORT OUTLINE

In this report we describe the outcomes of the work of the project. The report comprises the following sections:

- A description of the methodology adopted (Chapter 2);
- Quantitative assessment of the research including information about the distribution of research projects, in terms of number, value, and status, by RO and by country (Chapter 3);
- A synthesis of the key studies, highlighting the contribution considered to be being made to each RO, and the extent to which the RO has been met (Chapter 4);
- A summary of what has been achieved and the remaining gaps (Chapter 5);
- An assessment of additional relevant activities which are not within the scope of the ROs (Chapter 6);
- An assessment of the extent, based on the evidence collected, to which it is feasible to undertake a risk assessment and whether the evidence suggests that the precautionary principle should be applied (Chapter 7);
- Discussion of the above (Chapter 8);
- Conclusions (Chapter 9).

2 METHODOLOGY

To achieve the stated objectives of the Invitation to Tender (ITT), namely "to review completed and near-completed environment, health and safety research on nanomaterials and nanotechnology", the EMERGNANO project adopted a structured approach to identifying, collating and appraising research studies on the basis of the available information. This involved:

- Developing a comprehensive and categorised list, focussing on the emerging evidence of studies conducted or ongoing since 2004. This timeframe is appropriate given the publication of the RS/RAEng report, the typical 3-4 year lifetime of a major research study and recent projects commissioned to review earlier work;
- Identifying the completed and near completed studies (UK, Europe, US, Rest of World) primarily through sponsoring agency contacts and existing lists of studies;
- Collecting and compiling core and evidential information about these studies and outputs through dialogue with Project Leaders;
- Identifying the relevance of each study in relation to the NRCG's 18 Research Objectives;
- Appraising the quality and contribution of each study through expert assessment (using a Weight-of-Evidence approach, discussed below) by internationally renowned scientists;
- Undertaking a risk assessment appraisal identifying the need for control or management of risk, including an appraisal of whether there is sufficient information to invoke the precautionary principle for one or more nanomaterials;
- Presenting the evidence to a workshop of national and international experts;
- Reaching a consensus view about the remaining gaps and future priorities.

A series of tasks was specified by Defra in the published ITT. These are identified in Table 2.1 and have been mapped to the sequence of activities conducted in the EMERGNANO project and described in more detail below.

Table 2.1: EMERGNANO activities enabling the Defra Tasks identified in the ITT

Defra Task No.	Task Description	EMERGNANO Activities
1	Nanotechnology Research Coordination Group (NRCG) and the four relevant task forces, identify environment, health and safety research on nanomaterials and nanotechnologies that supports the appropriate 18 objectives of the NRCG.	i. Identify, list and compile information on completed and near completed studies. ii. Preliminary assessment to identify the relevance of each study in relation to the 18 Research Objectives. iii. Weight-of-Evidence appraisal of the quality and contribution of each study through expert assessment by internationally renowned scientists. iv. Risk assessment appraisal. v. Draft report.
2	Carry out a review and evaluation of completed and near-completed national and international research work and compile a summary of results.	
3	Review all research data within the scope of each Task Force and identify new information on hazards and risks posed by engineered nanomaterials.	
4	Consider whether there is sufficient information to identify and quantify risks to human health and/or the environment from manufactured nanomaterials that might lead to a need for control or management of the risk, including an appraisal as to whether there is sufficient information to invoke the precautionary principle for one or more nanomaterials.	
5	Conduct a workshop of national and international experts to discuss the findings of the project.	vi. Present the evidence to a workshop of national and international experts.
6	Identify and prioritise remaining gaps in EHS research on nanomaterials taking into consideration, as far as possible, ongoing UK and international research programmes.	vii. Reach a consensus concerning about the remaining gaps. viii. Finalise report

2.1 IDENTIFICATION, SELECTION AND COMPILATION OF INFORMATION ON STUDIES

We developed a comprehensive and categorised list, focussing on the emerging evidence, of studies since 2004. Information on projects funded in the UK was requested from the Nanotechnology Research Coordination Group (NRCG) and the four relevant task forces. Information on European studies was obtained from liaison with relevant European Commission Project Officers and the recent publication "EU technology R&D in the field of health and environmental impact of nanomaterials" (European Commission, 2008). Information on US and other international studies was gathered using the Woodrow Wilson Centre's Research Inventory (www.nanotechproject.org/) and selected contacts from agencies including National Institute for Occupational Safety and Health (NIOSH), National Institutes of Health (NIH), and the Environmental Protection Agency (EPA).

Studies for assessment under EMERGNANO were selected in an unbiased manner, in accordance with the following selection criteria:

- Study is relevant to at least one of the 18 NRCG Research Objectives;
- Study commenced or reported in or after 2004 (including "commissioned but not yet started" studies);
- Study was funded by recognised body, or nominated by an EMERGNANO expert assessor;
- Study outcomes are published in English (the project timescale and budget did not allow provision for translation).

From those studies meeting all of the aforementioned criteria and selected, a categorised list was collated and subsequently managed using a tailor-made Microsoft Access database. A list of the studies considered in the EMERGNANO project is contained in Appendix 1.

Core Information gathered for each project included the start date, duration, budget, objectives, and links to project websites as appropriate. We augmented this information, through an email dialogue with the principle investigators with available evidential data including project reports, publications, citations and other relevant outputs, as appropriate.

Information requests were distributed from a dedicated EMERGNANO email account, and their status and response managed from within Microsoft Access.

Where relevant information was received, its type was recorded (abstract, objectives, reports and publications) and then saved into designated project folders. Reminder emails were distributed to those who indicated they would provide information at a later date, those who were previously out of office and those who did not initially respond. Responses to this second batch of emails were monitored and any information received dealt with accordingly.

This compilation was also supplemented by recommendations of additional studies from the expert assessors within EMERGNANO's project team.

2.2 DATA MANAGEMENT

Information collated was managed throughout the project's duration within Microsoft Access. Initially data was recorded into an uncategorised database, where each study

was assigned a unique key by which it could be identified, based on the ISO standard country coding and a sequential three digit number. Additional studies suggested by the Assessors are distinctly coded using an "X" (e.g. UKX01). Subsequently, Core Information about the study was collated. A summary of Core Information fields within this initial dataset is outlined in Table 2.2. In addition, the availability of supplementary evidential information on each study was recorded. This information was held within folders labelled according to study key, and was categorised according to whether it was a Statement of Objectives, Study Abstract, Report or Publication. This key information was then used by the EMERGNANO project team to organise studies according to area of relevance, and assign them to the Research Objective(s) for assessment.

Table 2.2: Fields for storing Core Information on studies

Study ID	Funding Source(s)
Study Title	Funding Sector
Short Name	Funding Body Project Reference
Start Year	Budget (native)
End Year	Budget (£)
Duration	Contact Name
Status	URL
Currency	Summary
Country	

2.3 PRELIMINARY ASSESSMENT TO MAP EACH STUDY TO THE 18 RESEARCH OBJECTIVES

Following collation of all available information, duplicates were removed from the study database. Studies within the final pooled list were assigned a nanomaterial category and area of relevance (summarised in Table 2.3) using the available Core Information.

Table 2.3: Nanomaterial categorisation and area of relevance

Nanomaterial Category	
Engineered Nanomaterials	Generic
Incidental Nanomaterials	Unassigned
Natural Nanomaterials	
Area of relevance	
Exposure	Control
Hazard	Characterisation
Response	Risk Assessment
Generation	Risk Management
Safety	

A preliminary assessment to map the studies and their outputs to the appropriate Research Objective(s) was carried out as a centralised process and recorded in MS Access (Figure 2.1), based on inspection of the study title and objectives.

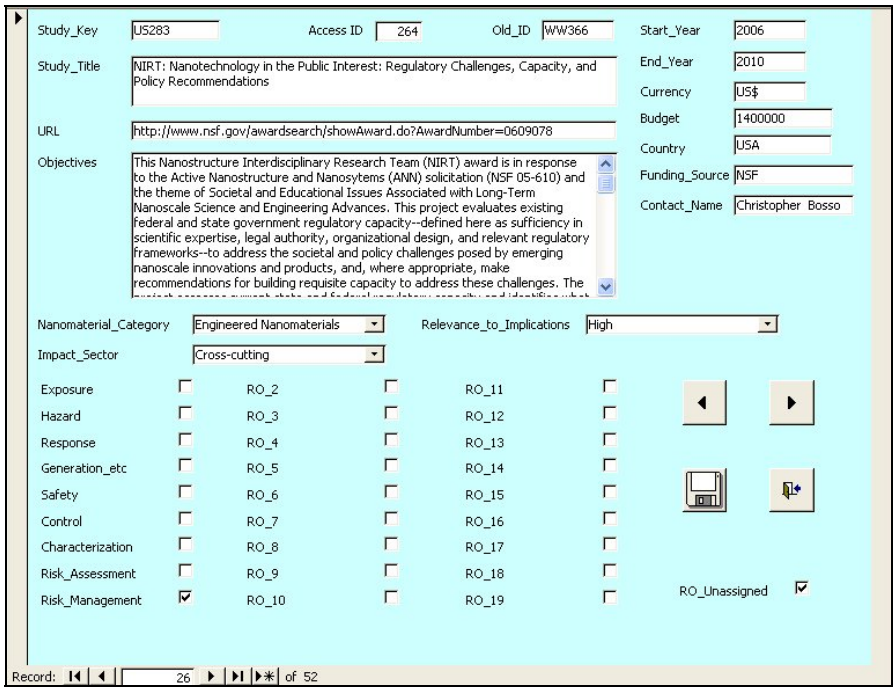

Figure 2.1: Assigning study category and RO

Any errors in the preliminary assignment of studies to the ROs were identified by the expert assessors during their detailed appraisals and corrected. Studies which the project team could not assign to a Research Objective at this time were considered separately, as were studies which were identified to be Enabling Activities, Observatories or Networks. A package of information was then provided to the expert assessor, selected on the basis of their expertise, to appraise studies for the ROs as shown in Table 2.4. This included RO-specific databases of the studies to be assessed, a Weight-of-Evidence appraisal template and the supplementary evidential information.

Table 2.4: Allocation of Research Objectives

Task Force Area	Research Objective	EMERGNANO Assessor	
Metrology, Characterisation, Standardisation and Reference Materials	RO 2	Andrew Maynard	**Lead: Rob Aitken**
	RO 3	Steve Hankin	
	RO 4	Simon Wilkins and Terry Wilkins	
	RO 9	Simon Wilkins and Terry Wilkins	
Exposures – Sources, Pathways, and Technologies	RO 5	Rob Aitken	**Lead: Rob Aitken**
	RO 6	Rob Aitken	
	RO 7	Qasim Chaudhry	
	RO 8	Rob Aitken	
	RO 10	Qasim Chaudhry	

Task Force Area	Research Objective	EMERGNANO Assessor	
Human Health Hazard and Risk Assessment	RO 11	Ken Donaldson and Rodger Duffin	**Lead: Ken Donaldson**
	RO 12	Ken Donaldson and Rodger Duffin	
	RO 13	Ken Donaldson and Rodger Duffin	
	RO 14	Ken Donaldson and Rodger Duffin	
	RO 15	Qasim Chaudhry	
	RO 16	Lang Tran	
Environmental Hazard and Risk Assessment	RO 17	Teresa Fernandes, Vicki Stone and Qasim Chaudhry	**Lead: Vicki Stone**
	RO 18	Teresa Fernandes and Vicki Stone	
	RO 19	Teresa Fernandes and Vicki Stone	

2.4 WEIGHT-OF-EVIDENCE APPRAISAL

An appraisal of the relevance, quality and contribution attributes of each study to its assigned Research Objective(s) was carried out by the designated Assessor using a Weight-of-Evidence (WoE) approach.

Four WoE Frameworks, tailored to the NRCG Task Force areas - Human Health, Environment, Metrology and Exposure, were developed by the EMERGNANO project team with input requested from Defra and the project's Steering Group. The WoE approach was developed to assist the assessors' appraisal of the studies. The purpose was to provide the EMERGNANO Assessors with a standardised and transparent means to appraise and compare studies consistently, within an RO but not across ROs (due to variable weighting as evident in Appendix 2), whilst still providing them with a degree of flexibility and latitude to judge the impact and contribution of each study towards the RO.

The WoE criteria and their component categories for the four frameworks are shown in Appendix 2. Each of the frameworks was piloted with representative studies and comment was sought from the EMERGNANO project's Steering Group (independent of the partner organisations conducting the project).

To streamline the process of carrying out the WoE assessment and reporting, a Microsoft Access database with a form-based front-end was populated for each of the 18 ROs under consideration (Figure 2.2) and distributed to Assessors on memory sticks along with a set of folders containing any supplementary study information. The WoE appraisal form was designed to present assessors with a standardised set of criteria in a set of drop-down menus. Assessors were provided with guidance on using the database.

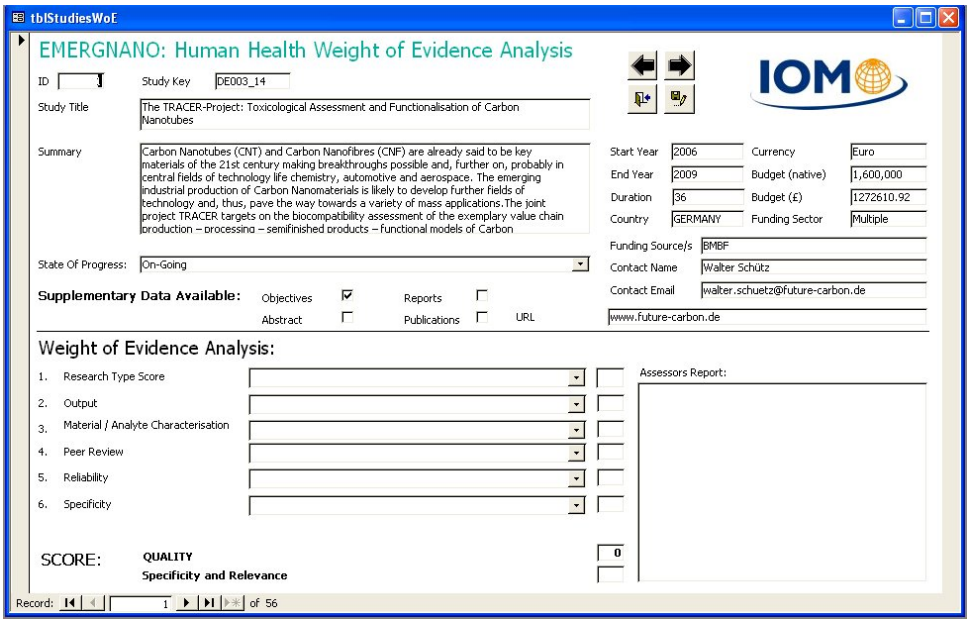

Figure 2.2: Example Weight of Evidence Assessment Form

The most appropriate criterion was selected on the basis of the assessors' expert judgement. In selecting the most appropriate criterion characterising the study, the associated score for the individual category was displayed and summed scores of i) research type, output, material characterisation, peer review and ii) reliability, specicificity, were automatically calculated.

Not all studies could be assessed systematically using the descriptive terms in the WoE frameworks, so the expert assessors used their judgement in a limited number of circumstances to score the study appropriately to reflect its quality and contribution to the RO. A study's WoE score was intended only to be used by the assessor to help rank the studies when considering their impact and contribution to the RO. The tailored design of the four WoE frameworks means that the possible range of scores is a function of the particular framework and therefore the interpretation of WoE scores is not equivalent across all ROs and WoE scores for studies cannot be compared outwith the boundaries of a particular RO. In a limited number of circumstances, not all categories could be assessed for a study, either because the study had just started, there was lack of information, or it was considered that the study did not fit the stated criteria. Thus, on the basis of the available information, some studies may have low WoE scores yet may ultimately contribute substantially to the RO. This is an inherent limitation of any review activity conducted at a fixed point in time.

The WoE form provided space to record comments and remarks on the attributes and contribution of each study considered.

Based upon the WoE score and the commentaries, the Assessors prepared a synthesis report for each of their assigned ROs, using a template provided. Where a large number of studies were to be considered, the assessor used their discretion to introduce a score threshold, to focus their subsequent discussion of studies. Any thresholds introduced are indicated in the histograms of the WoE score distributions for the ROs in Appendix 3.

The synthesis report included a summary of the RO, an overview of the relevant studies, comments on the merits of the key studies in meeting the objective of the RO

and the value of their contribution towards the emerging evidence. Specifically, the synthesis report template requested:

- the key features of the studies;
- the types of materials used in the studies;
- the extent to which each of the studies contributes to the RO;
- identification of studies considered to be of highest quality and impact (backed up by the WoE scoring);
- remaining gaps.

Once completed, the WoE data and synthesis report(s) were returned to IOM and the completed WoE databases were concatenated into a single database to enable analysis of the evidence for trends, and preparation of the descriptive statistics on the full dataset.

2.5 RISK ASSESSMENT APPRAISAL

Taking into consideration all the available information and data generated, the project team formed an opinion on whether there was sufficient information to identify and quantify risks to human health and/or the environment from manufactured nanomaterials, that might lead to a need for control or management of the risk. The appraisal is limited to the information collected and presented in the RO synthesis reports and the authors are aware that there may be more information within the published literature that has not been identified within the RO reports. The authors are also aware that there is available published generic advice on the control of exposure to nanomaterials (e.g. BSI, 2007) and also generic advice on health surveillance to workers who may be exposed to nanomaterials (e.g. NIOSH, 2009). In addition to considering the data generated from the Weight-of-Evidence appraisal of the quality and contribution of each study through expert assessment, the risk assessment appraisal also considered whether there is sufficient information to invoke the precautionary principle for one or more of the identified nanomaterials using the information collected in the EMERGNANO project.

It should be noted, however, that the risk assessment appraisal is not a risk assessment per se and the authors have only considered the information and expert opinions within the ROs and not taken into account the wider body of literature that may exist for some of the longer established nanomaterials.

2.6 PRESENTATION OF THE EVIDENCE AT A WORKSHOP

A workshop to present an overview of the project's findings and facilitate a discussion with stakeholders to identify critical issues was held at the Central Science Laboratory in Yorkshire on October 20-21, 2008. Stakeholder representatives invited included key members of the toxicology, regulatory, industry and consumer communities. The workshop participants were provided with a draft collation of the RO synthesis reports. The workshop was structured as follows:

- Introduction to the project's aims, objectives and approach;
- Overview of the studies and gaps from the RO appraisals;
- Chaired discussion and break-out groups to identify critical missing studies and reach a consensus on the extent to which research has met the objectives of the ROs.

Four break-out sessions discussed the findings of the RO appraisals grouped according to the Task Force Area. The relevant RO assessors facilitated the discussion and used the contributions in the preparation of the final version of their synthesis reports.

3 QUANTITATIVE ASSESSMENT

Using data collated on projects included in EMERGNANO, and based on performance of studies within the Weight-of-Evidence appraisals, the following section provides a short quantitative analysis of the studies considered. More detailed information on the distribution of Weight-of-Evidence scores and project financial values as a function of RO, is presented in Appendices 3 and 4.

3.1 OVERVIEW OF STUDIES CONSIDERED

In total, 673 studies were identified in an initial data search and collated in a common format into a Microsoft Access based database. Following removal of 315 studies found to be duplicates or which failed to meet the selection criteria, 358 studies remained. Of these, 19 studies were classified as Enabling activities, Networks or Observatories and thus were considered discretely in Chapter 6, and a further 46 did not fall within the scope of any of the 18 ROs and were therefore not included in the assessment. A final count of 293 studies were allocated for expert assessment under RO 2-19; this is represented graphically in Figure 3.1

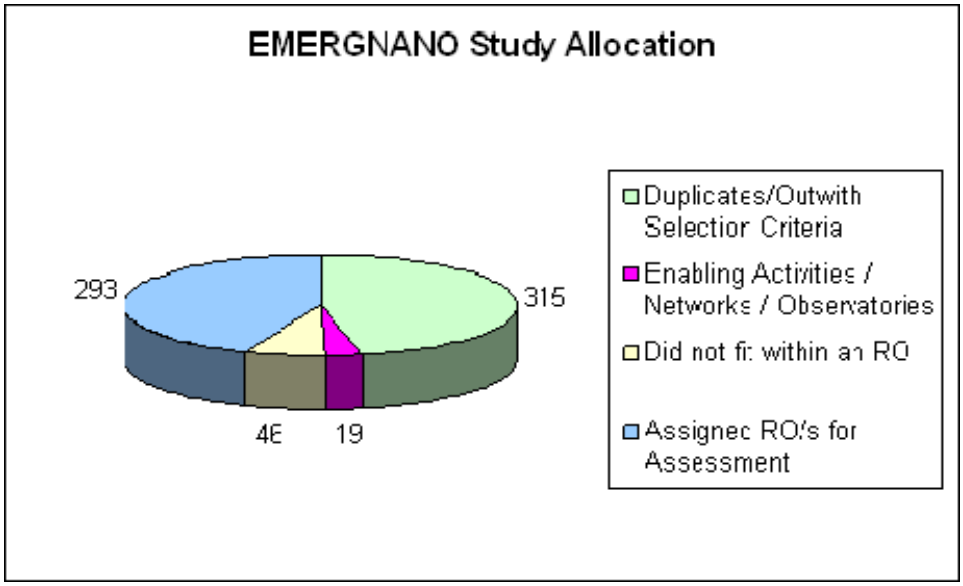

Figure 3.1: Selection and allocation of studies for assessment

Of the initial 293 studies considered, just under one third of these studies were complete (88). Seventy were near completion (i.e. completion was expected to be within 2008), 106 were ongoing and 13 were just commencing. For the remaining 16 studies, a status was not obtainable.

Taking into consideration a further five additional studies identified and thirty-eight studies rejected subsequently by assessors during WoE assessment, the final number of unique studies considered within the EMERGNANO project was 260. The distribution of studies across the ROs is shown in Table 3.1.

Table 3.1: Summary of studies allocated and analysed per Research Objective

Research Objective	No. studies allocated to RO	Additional studies identified	No. studies excluded by assessor	Total no. studies assessed within RO
2	28	0	0	28
3	6	2	0	8
4	3	2	2	3
5	30	1	6	25
6	25	0	6	19
7	13	0	1	12
8	21	0	5	16
9	1	0	0	1
10	38	0	3	35
11	44	0	18	26
12	48	0	9	39
13	41	0	5	36
14	53	0	9	44
15	8	0	0	8
16	18	0	0	18
17	45	0	2	43
18	26	0	4	22
19	12	0	0	12

3.2 WEIGHT-OF-EVIDENCE SCORE DISTRIBUTIONS

Due to inherent differences in the criteria used to conduct Weight-of-Evidence appraisal for each Research Objective, interpretation of scores awarded and their distributions is not comparable across ROs. With respect to preparation of synthesis reports, the threshold above which studies were deemed to be of the highest scientific value also varied according to the RO analysed; some assessors not electing to set a threshold, and others setting the threshold within the highest quartile of scores awarded. Table 3.2 provides a summary of score distribution per RO. A more detailed graphical representation showing the distribution for each RO is provided in the Figures of Appendix 3.

Table 3.2: Weight-Of-Evidence Score Distribution

Research Objective	No. Studies	Minimum Score	Maximum Score	Threshold	No. Studies over Threshold
2	28	5	23	15	18
3	8	4	20	Not assigned	8
4	3	9	22	Not assigned	3
5	24	7	26	17	16
6	19	7	25	16	16
7	12	3	25	Not assigned	12
8	16	4	26	20	5
9	1	18	18	Not assigned	1
10	35	11	28	16	11
11	26	4	22	12	14
12	39	3	22	11	15
13	36	3	23	18	13
14	44	0	21	13	20
15	8	6	20	18	4
16	18	8	20	14	17
17	43	0	21	11	20
18	22	5	24	11	15
19	12	5	18	11	9

3.3 STUDY FUNDING

Tables 3.3 provides a summary of the total funding value of the studies considered for each RO. A detailed breakdown is provided in Appendix 4.

Table 3.3: Summary of Value Study Budget

		State of progress					
		Unknown	**Starting (+/- 3 months)**	**On-Going**	**Near Completed (expected 2008)**	**Completed**	**Total**
RO02	**No. Studies**	4	2	10	5	7	**28**
	Value / £M	-	-	14.06 (6)	4.87 (5)	1.02 (4)	**19.94 (15)**
RO03	**No. Studies**	1	0	1	4	2	**8**
	Value / £M	-	-	-	0.24 (3)	0.17 (1)	**0.41 (4)**
RO04	**No. Studies**	0	0	1	1	1	**3**
	Value / £M	-	-	5.57 (1)	0.31 (1)	0.01 (1)	**5.88 (3)**
RO05	**No. Studies**	1	0	11	4	9	**25**
	Value / £M	-	-	11.57 (8)	1.31 (4)	0.74 (8)	**13.62 (20)**
RO06	**No. Studies**	5	1	5	3	5	**19**
	Value / £M	-	-	5.97 (2)	0.52(3)	4.81 (4)	**6.97 (9)**
RO07	**No. Studies**	0	0	7	3	2	**12**
	Value / £M	-	-	1.23 (5)	1.10 (3)	0.81 (2)	**3.15 (10)**
RO08	**No. Studies**	1	0	7	1	9	**16**
	Value / £M	-	-	5.57 (1)	0.20 (1)	1.16 (6)	**6.93 (8)**
RO09	**No. Studies**	0	0	0	1	0	**1**
	Value / £M	-	-	-	0.06 (1)	-	**0.06 (1)**
RO10	**No. Studies**	0	1	12	14	9	**35**
	Value / £M	-	-	1.51(9)	2.95 (14)	1.46 (9)	**5.92 (32)**
RO11	**No. Studies**	1	1	11	4	9	**26**
	Value / £	-	0.60 (1)	1.9 (9)	0.87 (3)	2.46(7)	**5.83 (20)**
RO12	**No. Studies**	1	2	14	6	11	**34**
	Value / £	-	-	15.97 (13)	2.36 (10)	1.07 (12)	19.41 **(25)**
RO13	**No. Studies**	1	4	14	6	11	**36**
	Value / £	-	-	10.12 (13)	3.80 (6)	1.38 (10)	**15.31 (29)**
RO14	**No. Studies**	5	4	13	9	13	**44**
	Value / £	-	5.96 (1)	16.39 (11)	1.60 (8)	2.99 (12)	**21.58 (32)**
RO15	**No. Studies**	0	0	2	1	5	**8**
	Value / £	-	-	8.15 (2)	0.06 (1)	1.90(4)	**10.11 (7)**
RO16	**No. Studies**	2	3	5	3	5	**18**
	Value / £	-	2.38 (1)	3.94 (3)	0.88 (3)	0.69 (5)	**7.90 (12)**
RO17	**No. Studies**	0	6	14	15	8	**43**
	Value / £	-	0.24 (5)	1.89 (11)	1.99 (15)	1,48(8)	**5.60 (39)**
RO18	**No. Studies**	0	3	4	9	6	**22**
	Value / £	-	0.13 (2)	0.70 (4)	0.70 (9)	0.91 (5)	**2.44 (20)**
RO19	**No. Studies**	0	1	8	1	2	**12**
	Value / £	-	-	4.07 (7)	0.18 (1)	0.51 (2)	**4.75(10)**

3.4 GEOGRAPHIC DISTRIBUTION OF STUDIES ASSESSED

Table 3.4 summarises geographic distribution of studies assessed under the Weight of Evidence appraisal. The majority of studies (56%) were conducted in the USA. The UK presented the second highest number of studies (15%) and Switzerland the third (7%). European Commission EU-wide collaborative projects accounted for 19 of the studies assessed. As it was not possible to obtain a value for every study assessed, the number of studies summed to obtain the total value for each country is in brackets.

Table 3.4: Geographical distribution of studies

Country	Completed	Near Completed	On-Going	Starting	Unknown	Total No. Studies	Value / £
Belgium	0	0	1	0	0	1	7,953,818 (n=1)
Canada	10	0	0	0	0	10	496,914 (n=10)
China	0	0	1	0	0	1	None listed
Czech Republic	0	0	1	0	0	1	None listed
Denmark	0	2	9	0	0	11	7,336,689 (n=11)
EU*	4	3	7	5	0	19	25,914,791 (n=13)
Finland	1	0	1	0	0	2	836,741 (n=2)
France	1	0	12	0	0	13	340,423 (n=1)
Germany	0	0	4	0	0	4	7,397,050 (n=4)
Switzerland	2	8	9	1	0	20	3,120,126 (n=19)
Taiwan	2	0	0	0	0	2	186,917 (n=2)
UK	10	25	5	4	0	44	3,292,976 (n=43)
USA	58	32	56	3	16	165	36,983,288 (n=115)
Total No. Studies	88	70	106	13	16	**293**	**93,859,738 (n=221)**

* Trans-boundary projects funded by the European Commission

Figure 3.2 presents an overview of the state of progress of studies, according to geographical location.

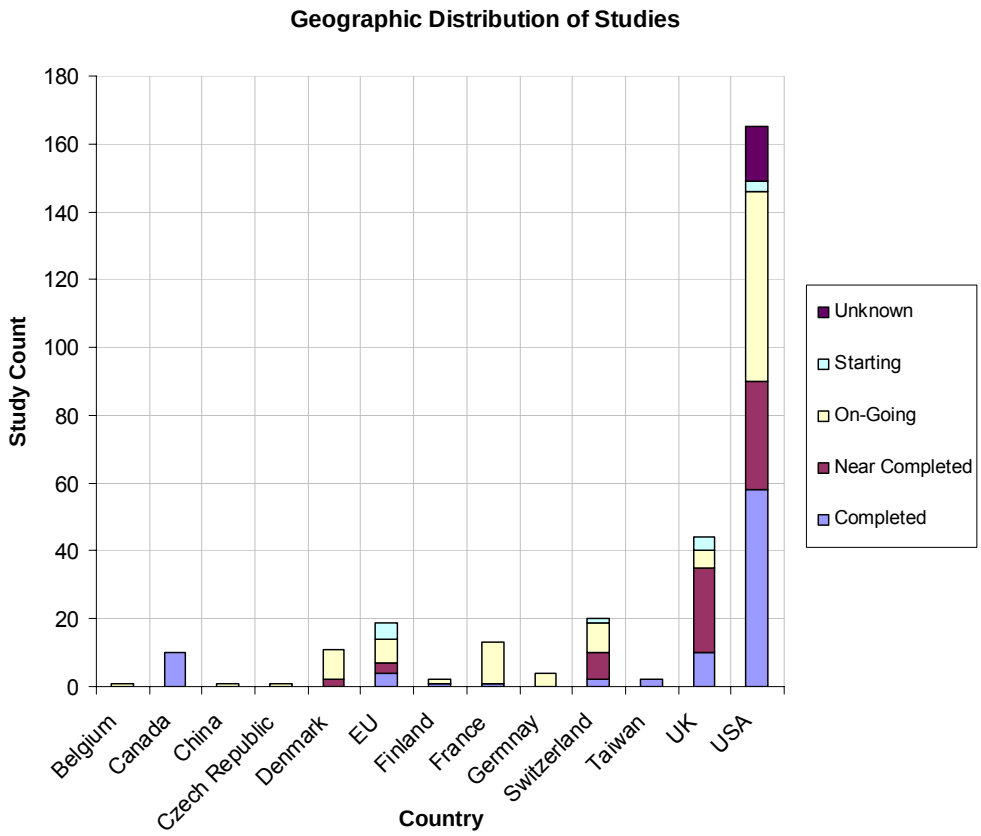

Figure 3.2: Geographic distribution of studies, according to state of progress.

4 ASSESSMENT OF THE RESEARCH OBJECTIVES

4.1 INTRODUCTION

This section of the report contains the synthesis reviews of the studies relevant to each of the Research Objectives. As discussed in the methodology, each of these *synthesis reports* has the same structure. Firstly the RO is described, including any variation or clarification considered appropriate, followed by an overview of the types of studies found to be relevant to the particular RO. This is followed by a description of the key studies that, in the opinion of the reviewer, contributed substantively towards realising the RO. Studies are identified as complete/near completed and ongoing. The studies are identified by their unique study code which can be tracked back to the study list in Appendix 1. The main elements of each study are described, differentiating between those studies which are complete or on the point of completing and those studies which are ongoing. This is followed by a description of the contribution that these studies make towards the RO, an evaluation of the extent to which the RO has been met and an assessment of the remaining gaps required to complete the RO.

4.2 RO02 - METRICS AND ASSOCIATED METHODS FOR THE MEASUREMENT AND CHARACTERISATION OF NANOPARTICLES

The research objective

"To identify the most suitable metrics and associated methods for the measurement and characterisation of nanoparticles."

Overview of the types of studies which have been found to be relevant to this RO

Twenty eight studies were identified as having some relevance to the RO. These broadly addressed both the characterisation of engineered nanomaterials in studies focusing on understanding potential human health and environmental impacts, and on developing and assessing relevant exposure metrics. The studies evaluated ranged from those generating new knowledge that will help identify materials characterisation requirements, to those focussed on developing specific exposure monitoring methods. This broad scope, together with distinct differences between information needs for material characterisation and exposure measurement in many cases, made a comparative assessment of the projects using the weight of evidence rather difficult. Many studies did not align with a systematic development and application of knowledge to measuring relevant characteristics of specific materials and, in most cases, there was little information on which to judge the likely relevance and success of the research.

In assessing the relevance and value of the studies, it was not easy to differentiate between the different information requirements for material characterisation and exposure assessment, and between research that extended general knowledge on characterisation and measurement.

The key studies

Completed or near-completed studies

Seven of the twenty eight listed studies were identified as having been completed. Of these, three studies were assigned a Weight-of-Evidence score of 20 or more: **US264**, **US288** and **US291**. These three studies were funded by and conducted within NIOSH and dealt with the generation and characterisation of airborne nanoscale particles, with

the aim of developing and evaluating occupational exposures to nanoscale aerosols. All three studies address the need to develop and assess new ways of measuring exposure, with an emphasis on investigating approaches to measuring aerosol surface area. This has been identified as a possible alternative exposure metric for nanoscale aerosols. A range of techniques were explored for assessing exposure, including the use of aerosol diffusion charging, electron microscopy and size distribution analysis. The research also explored estimating aerosol surface area through mass and number concentration measurements.

While not conclusive, the research resulted in considerable advances in understanding the possibilities and limitations of alternative measurement techniques. These are reflected in over 20 published papers that draw on the research.

The remaining four studies in this category had Weight-of-Evidence scores ranging from 15 to 19. Three of these represented research funded by and conducted within NIOSH (**US222** and **US303**) and the UK Health and Safety Executive (**UK031**).

The HSE study explored correlations between measuring test aerosols in terms of aerosol mass, surface area and number concentration. The study reported (HSE, 2006) correlations between number, mass and surface area measurements when various aerosol parameters were held constant, but more general relationships were not forthcoming.

The two NIOSH studies addressed associations between aerosol surface area and toxicologically-relevant endpoints. Little information is directly available on the outcomes of the studies (although a number of publications are listed in association with Study **US303**, and both studies contributed to NIOSH publications on nanomaterials in the workplace. Overall, it would appear the research suggested associations between aerosol surface area, biologically relevant activity and toxicity, but that these data were not completely conclusive.

The remaining completed study was a National Science Foundation Advance Fellowship, focusing on the microscopy of nanomaterials (**US248**). As described, the research was not explicitly focussed on addressing health impacts of engineered nanomaterials. Nevertheless, the techniques explored (specifically Electron Energy Loss Spectroscopy) have great significance to understanding health-related characteristics of nanoparticles, if applied appropriately. No information on outcomes from this research was available.

Identified as near to completion is Study **US002**, which is exploring structure-activity relationships for nanoparticles within biological environments, and includes the detailed physical and chemical characterisation of materials under evaluation.

Ongoing studies

Ten studies were identified as ongoing, two of which a Weight-of-Evidence score of 20 or greater, and eight had scores between 15 and 19.

Of the two top-scoring studies, **US086** is a continuation of previous NIOSH research (described above) aimed at developing measurement metrics and instrumentation to evaluate occupational exposures to airborne engineered nanomaterials. It is associated with a number of publications in the area. **US458** is funded by NIOSH and is focussed on evaluating methods for measuring aerosol surface area in the workplace. The work is also associated with a number of publications.

The next-highly rated studies include five European Union-funded research studies. **EU009** ("Nanosafe2") is a broad program of research that addressed multiple aspects of producing and using nanomaterials safely. It includes components of occupational exposure measurement. **EU006** ("Cellnanotox") is addressing correlations between various physicochemical characteristics of nanoparticles, and their potential to have a biological impact on a number of organs within the human body. **EU030** has systematically evaluated the health impact (or potential health impact) of a number of well-characterised nanoparticles. Of relevance to this RO is the comprehensiveness of the physical and chemical characterisation applied to the materials being evaluated, including an array of fourteen different methods designed to assess (amongst other things) material size, shape, surface area and composition. **EU023** ("Nanommune", starting September 2008) is focussed on nanoparticles and their impact on the immune system, but includes a comprehensive physical and chemical characterisation of the materials under investigation. **EU024** ("NanoDevice", expected to start in early 2009) is specifically focussed on developing and evaluating exposure monitoring devices for engineered nanoparticles, and its significance is probably greater than the WoE score suggests. The aim is to develop portable, easy to use devices for measuring airborne nanomaterial exposure in the workplace. Lastly, **UK090** is a UK project funded by the Natural Environment Research Council, exploring the use of oxidative potential as an indicator of nanoparticle toxicity.

Contribution towards the RO

Completed studies

There is insufficient information to evaluate each study in depth in relation to its contribution to RO02. None of the listed studies can be said to be conclusive in providing new approaches or guidelines to characterising and measuring nanomaterials. However, together they contribute to a growing body of knowledge that is informing decisions in government, industry and other organisations such as standards bodies.

Overall, three observations are worth emphasising concerning these studies:

i). They are mainly focussed on addressing the relevance and practicality of using surface area as an exposure metric;
ii). They are limited in scope and conclusiveness;
iii). The most relevant research in this category of studies has been funded and conducted by government organisations established to carry out health and safety research.

Ongoing studies

With one or two exceptions, the most relevant studies to RO02 are either being funded by NIOSH in the US, or the EU in Europe. Beyond a scattering of relevant studies, there is little evidence in this assessment that national general science funding agencies are funding research that will make a significant contribution to the characterisation and measurement of engineered nanomaterials for health purposes. However, this conclusion must be balanced by the likelihood that many studies not specifically focussed on measurement and characterisation or on health impacts—and thus not listed here—will nevertheless add to the body of knowledge that will ultimately inform decisions on how best to measure and characterise nanomaterials when evaluating and monitoring their potential impact.

Extent to which the RO has been met

A number of points are worth highlighting from the studies associated with this research objective:

- Very few of the studies were focussed on systematically developing characterisation or exposure measurement methods for engineered nanomaterials;
- There was very little coherence between the identified studies that would lead to significant advances in risk-related measurement and characterisation methods for different classes of nanomaterials;
- In most cases, there was insufficient information available to judge whether studies would result in credible and relevant new information;
- The predominant materials involved in the studies identified were metal oxides and carbon-based nanomaterials;
- There was an emphasis on measuring occupational exposures. However, very few studies were designed to provide information useful to measuring exposures outside the workplace;
- More specifically, there was a lack of research addressing general measurement methods for characterising nanomaterials and their impact in the environment.

Drawing from the published literature and discussions within the scientific and policy communities over the past few years, scientific and technical questions associated with the characterisation and measurement of engineered nanomaterials include:

- Which physical and chemical characteristics are relevant to the biological behaviour of different nanomaterials?
- How do these characteristics vary over time and within different environments (including biological environments)?
- With what precision and accuracy do these characteristics need to be measured to investigate the potential impacts of different nanomaterials?
- To what extent can existing methods be used to make relevant measurements?
- How can current measurement approaches not normally applied to investigating the potential impact of nanomaterials be applied to research in this area?
- What are the limitations of current characterisation methods, and how can these limitations be overcome?
- Which measurements of nanomaterial characteristics or behaviour best reflect their potential to cause harm?
- How do these metrics vary according to whether materials are being measured outside or inside the body?
- To what extent can existing exposure measurement instruments and methodologies be used to make relevant exposure measurements for engineered nanomaterials?
- What are the limitations of current techniques, and how can these be overcome?

This is not a comprehensive list of research needs, but nevertheless serves to evaluate the extent to which the identified studies for RO02 are likely to address information gaps that need to be filled if clear approaches to engineered nanomaterial characterisation and measurement are to emerge. While it is hard to assess the full scope of many of the listed studies, or the quality of the information that will be generated, it is clear that at best, the current research portfolio will only address a small fraction of the above research needs to any significant degree.

In conclusion, it appears that the EU and research organisations tasked with health and safety research are leading the way in supporting research into health-related nanomaterial characterisation and measurement, and that while valuable research is being conducted, it is barely scratching the surface of what is needed if informed decisions are to be made on appropriate ways to characterise and measure engineered nanomaterials. From this, it would appear that more research is needed, more coordination is needed between research efforts, and greater strategic direction is required to ensure that the information generated has value in making informed decisions.

4.3 RO03 - REFERENCE NANOPARTICLES

The research objective

"To develop standardised, well-characterised reference nanoparticles".

Overview of the types of studies which have been found to be relevant to this RO

Six studies were initially identified for consideration in RO03. A further two studies were considered to have some relevance to RO03 and have been included in the assessment. Sufficient core information was available to classify all studies in terms of their objectives; however, only one study provided evidential information on which to conduct a meaningful assessment. Of the eight studies assessed, one had a Weight-of-Evidence score of 20 or more, five had scores between 12 and 19, and two studies scored less than 10.

The types of study were predominantly associated with the development of candidate reference materials for use with exposure or toxicological analyses. In some cases, candidate materials, characterisation techniques and toxicology analyses were stated but the absence of supporting information on most of the studies precludes verification of progress towards meeting the stated objectives. Despite all of the studies being complete or near-completion, according to their stated timelines, all but one supplied no publications (peer-reviewed or otherwise) or supplied publication citations that were not specifically relevant to the studies considered here.

To supplement the assessment, information regarding on-going activities that are expected to make a contribution towards meeting the objective of RO03 are highlighted. These include reference material development at the US National Institute for Standards and Technology (NIST), the European Union's Institute for Reference Materials and Measurements (IRMM), and related initiatives coordinated by the Organisation for Economic Cooperation and Development (OECD) and International Standards Organisation (ISO).

The key studies

Completed or near-completed studies

Of the specific studies identified, the only study to have provided substantial outputs for assessment and the highest ranked project, was Study **UK073** "Reference materials for engineered nanoparticle toxicology and metrology", which had the objective of providing 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 study, termed REFNANO, took place in the first half of 2007 and is

acknowledged by Defra in the Second UK Government Research Report (Defra, 2007) as a major step forward in the prioritisation of needs for reference materials and measurement methods, including support of nanoparticle toxicology and risk assessment. REFNANO was based on an informed discussion and opinion-gathering activity with representatives from the toxicology, metrology and nanomaterials producer/user communities. A critical aspect was that these communities understand the needs and capabilities of each other. 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 workshops attended by key opinion-leaders in the field, and consultation with other internationally-recognised reference material initiatives. As part of REFNANO's review of toxicology needs, a questionnaire survey of 22 opinion leaders in particle toxicology, who are currently working on nanoparticles, was carried out. There was unanimous agreement that a reference bank of nanoparticle materials was needed. There was also strong agreement that the bank should contain particles selected around 3 main criteria:

i). Industrial Nanomaterials: to select reference nanomaterials on the basis of scale of production and likelihood of exposure;
ii). Hypothesis Driven: to select reference nanomaterials on the basis of how their physicochemical properties are expected to interact with the living system, and that will be useful in answering particular toxicology (and eco-toxicology) questions, e.g. length distribution and its effect on carbon nanotube toxicity;
iii). Distributed Analysis: to select reference nanomaterials in the context of standardised comparative studies.

Based on the discussions and recommendations arising from the two workshops, the REFNANO study developed a series of outputs including:

- a rationale and set of criteria for selection of priority reference/test materials associated with high production volume (HPV) industrial nanomaterials, hypothesis-testing, and distributed analysis;
- a list of seven high priority reference/test materials to meet the needs of toxicology and metrology:
    i. carbon black
    ii. TiO_2
    iii. ZnO
    iv. single-walled and multi-walled carbon nanotubes
    v. polystyrene
    vi. metal & metal oxide
    vii. combustion-derived nanoparticles;
- 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 proposed development schedule for nanoparticle reference materials.

The REFNANO study 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 studies. The project's publicly available final report was peer reviewed by the UK Advisory Committee on Hazardous Substances and the outcomes of the project have been acknowledged in the UK Government's 2nd Nanotechnology Research Report and by organisations influential in reference material development initiatives, including NIST and ISO. The recommendations are consistent with the ongoing discussion of reference materials prioritisation and development by NIST and the OECD's Working Party on Manufactured Nanomaterials (WPMN). The scope of the project was limited to the nomination and justification of several materials, and not able to extend to securing a commitment from those able to implement the development of Reference Materials.

Of the studies scoring between 12 and 19, Study **US453** "Nanoscale Reference Materials for Respiratory Disease Prevention" is part of a wider research program at NIOSH aimed at studying the toxicity of workplace-related aerosols, including those associated with nanotechnology. The purpose of this project is to provide a scientific basis for development of methods to ensure accurate measurement of engineered nanomaterials size and surface area in industrial hygiene samples. It is hypothesised that nanoscale colloidal gold nanospheres can be used as reference materials for nanoparticle size and surface area. The stated project aims are to develop nanoscale reference materials for use in quantifying particle size and particle surface area. Various sizes of electro-statically stabilised gold nanospheres will be generated and particle size characterised using multiple complimentary analytical techniques (microscopy, x-ray diffraction, liquid suspension counter, etc.). It is anticipated that by knowing particle size, it will be possible to characterise particle porosity then particle surface area using complimentary techniques (gas adsorption, microscopy, etc.) and that results from these studies will contribute towards qualifying these gold nanospheres as *respirable masses*. However, no published outputs are available to identify progress with the establishment of gold nanospheres as Reference Materials or data from analysis methods. No detail of the validity of the 'reference' status or whether verification studies will be conducted by other researchers using the developed materials.

Similarly, Study **US264** "Generation and Characterisation of Ultrafine Particles" is also part of the wider NIOSH research program aimed at studying the toxicity of workplace-related aerosols, including those associated with nanotechnology. Methods are being developed to generate and deliver well-characterised particles to exposure systems, enabling particle characteristics responsible for specific toxic responses to be investigated in a systematic manner. The research includes the development of off-line and on-line aerosol and particle characterisation techniques, including methods to measure aerosol surface-area, and methods to characterise the composition and structure of nanometre-diameter particles. Outputs provided by the project leader do not provide evidence of activity towards the establishment of Reference Materials and the study is more suited to RO02.

Study **UK096** "Synthetic polymer nanoparticles: effects of composition and size on uptake, toxicity and interactions with environmental contaminants" is proposed to take an important step towards the provision of a set of well characterised particles by using [unspecified] methods that are in routine use in laboratories to make [unspecified] nanoparticles of three [unspecified] different sizes and three [unspecified] different chemical compositions. The study anticipated to determine the toxicity of these particles to a fungus, an aquatic alga and a freshwater invertebrate, examine whether

the particles are taken up by these organisms and determine how particle size and surface chemistry alter uptake and toxicity. The project also anticipated examining whether the particles increase or decrease the toxic effects of three [unspecified] common pollutants. It was claimed that the results will begin to allow generalisations to be drawn about the likely environmental impacts of nanoparticles and the ways that the physical and chemical characteristics of the particles alter their biological effects. No outputs were available to identify the materials, their properties, progress with their establishment as Reference Materials, analysis methods, or the results of the toxicity testing. No detail of the validity of the 'reference' status or whether verification studies will be conducted by other researchers using the developed materials. The project leader (Dr Alastair Grant, UEA) has stated that the results are exciting, however, as draft publications are currently in preparation, no information was able to be provided within the timeframe of the EMERGNANO review.

Study **US285** "Submicron Particles and Fibers for Toxicological Studies" is aimed at initiating a business venture dedicated to the manufacturing of nanoparticulates for toxicology research. The proposed business venture is stated to be based on a unique set of manufacturing methods to design a wide variety of well-defined nanostructured powders, and a partnership with the Midwest Research Institute has been established to integrate toxicology tests with the nanomanufacturing component. Preliminary toxicology tests on three model systems with real commercial potential, and the design of such nanostructured materials, will serve as a platform to launch a spinoff business venture dedicated to serving the health sciences community and the private sector concerned with the environmental and occupational health impact of new nanotechnology products. The three case studies will include nanoparticle synthesis and preliminary toxicological testing of one metal oxide, one pesticide, and one multiactivity phytochemical. The outcomes are suggested to appeal to a broad clientele for custom synthesis of well-defined and characterised nanomaterials for toxicological evaluation, or for contract research and testing. Whilst a website for LNKChemSolutions exists, no outputs to identify the materials, their properties, progress with their establishment as Reference Materials, analysis methods, or the results of the preliminary toxicity testing are available.

Lastly, two additional European studies are considered to have some relevance to RO03 but scored less than 10 in the Weight-of-Evidence quality assessment due to the absence of any publicly available evidence of productivity. Firstly, the central mission of the Fifth Framework Programme study entitled "A European Virtual Institute on Reference Materials (VI-RM)" (**EUX01**) was a knowledge Network and a facility to encourage the interaction between all stakeholders in the field of Reference Materials. The VI-RM was anticipated to become the meeting place for producers, distributors, users, service providers, and research organisations in the European Union and other countries. The VI-RM was anticipated to coordinate Network Members and facilitate dissemination, exchange of ideas and concepts. The project had the main objective to nurture the concept of a Virtual Institute and to develop into a legally and financially independent organisation. The project concluded in December 2005. At the time of conducting the EMERGNANO review, no evidential information was supplied or could be found on which to assess the project and its contribution towards the RO.

The Sixth Framework Programme project entitled "Nanostrand" (**EUX02**), included a work-package reviewing current nanostandards. The key objective of this work package was to define and assess knowledge of current nanotechnology standardisation developments and trends within National Standards Bodies (NSB), building on the work carried out to date by the European Committee for Standardisation (CEN). The results of the project are anticipated to have an impact on standardisation work and normative research in nanotechnologies. Detailed reports from this project

had still to be published at the time of the EMERGNANO review and it was not possible to appraise the project's findings. A summary report of the project made available provided an indication of the work undertaken. The review of current nanostandards is reported to have consisted of four steps:

i). Collate existing standards data addressing nanotechnologies (EU/international);
ii). Interview other National Standards Bodies in Europe;
iii). Comparing standardisation activities in Europe and other economies outside Europe;
iv). Compilation of developments and trends in nanotechnology standardisation.

The summary report stated that results have been compiled and validated, identifying strengths and weaknesses of standardisation activities in the different European countries in relation to other countries. The different approaches of the NSBs have been compared. The information on development and trends has been segmented by different types of standards (e.g. dimensional, analytical/chemical, performance, health and safety, environment protection). It concluded with key standardisation issues for nanotechnology stakeholders. A report on nanotechnology standards and trends is one of the documentary outputs from the project in the process of being published. The summary document also states that the roadmaps developed in the project have been used so far to steer standardisation programmes in Europe and also as an input for defining priorities for research in the Seventh European Framework Programme. One of the roadmaps focuses on health and safety aspects for standardisation and proposes future strategic activities from 2008-2015. Other outputs are awaited from Study **EUX02**.

Ongoing studies and other activities

Study **UK113** "Model nanoparticles for environmental risk studies" aimed to generate a set of well characterised nanoparticles that show the range of properties that appear to influence (eco)toxicity. The stated focus was on two metallo-nanoparticles, TiO_2 and ZnO, which have a wide range of uses. The available information on the project stated that the goal will be to create [unspecified] metallo-nanoparticles with unique enriched stable isotope ratios. Thus the reference material will not only be well characterised physico-chemically, but also synthesised to have distinct isotopic composition which will make it traceable in both laboratory and environmental experiments. Concentrations in media and in organisms will be quantified using the enriched stable isotope. This combination of tools will allow the assessment of unambiguous relationships between bio-uptake and particle characteristics; and unambiguous relationships between exposure and measures of organism stress. The material will be tested for its reactivity (solubility, surface charge, agglomeration) as a function of pH, ionic strength and the presence of organic matter in model aqueous media (fresh water and seawater). Ecological risk will be assessed under experimental conditions on a marine (Corbula sp.) and a freshwater (Dreissena sp.) bivalve. The project leader (Dr Eva Valsami-Jones, Natural History Museum) indicated that work had only recently started and no published outputs are available to identify progress with the generation of samples of well characterised TiO_2 and ZnO or data from analyses.

The US National Institute of Standards and Technology (NIST) is one of the world's leading organisations advancing measurement science, standards, and technology. In the US, NIST has been called upon to lead the development of materials and analytical standards for nanotechnology environmental, health, and safety research. In January 2008, NIST issued its first reference standards for nanoscale particles (gold spheres nominally 10, 30 and 60 nanometers in diameter) which were developed for the biomedical research community with the National Cancer Institute's Nanotechnology

Characterisation Laboratory (NCL). These new reference materials are three of five nanoparticle samples being used in an ASTM Internationally-sponsored interlaboratory performance-benchmarking study intended to improve the consistency and quality of results across organisations. The comparative study began in January 2008. NIST continues to work in partnership with other national measurement institutes in other countries. In late 2007, NIST signed an agreement with the European Commission's Joint Research Centre Institute for Reference Materials and Measurements (IRMM), which focuses in part on advancing the development and availability of international measurement standards for EHS of engineered nanoscale materials.

The Institute for Reference Materials and Measurements (IRMM) cooperates with international standardisation and metrology bodies and their technical committees to ensure and promote a globally recognised approach for the development and production of reference materials. In response to the need for better characterisation of nanoparticles, IRMM has prepared a new reference material (IRMM-304) for quality control of particle-sizing. The reference material consists of silica nanoparticles with a nominal diameter of 40 nm suspended in an aqueous solution, and can be used for checking the performance of instruments and methods that characterise the particle size distribution of nanoparticles suspended in a liquid medium. It can be used for method development, interlaboratory comparisons and establishing quality control charts. The material has been characterised with dynamic light scattering and disc sedimentation. Three different particle size values were assigned to IRMM-304. The three values correspond to different instrument types (dynamic light scattering and disc sedimentation) and, for the dynamic light scattering method, to different data analysis methods.

The OECD's Working Party on Manufactured Nanomaterials (WPMN) is engaged in studies to further the understanding of the properties and potential risks of nanomaterials including safety testing of a 'representative set' of manufactured nanomaterials. The WPMN identified a representative list of manufactured nanoscale materials for environmental health and safety testing including:

- Fullerenes (C60)
- Single-walled carbon nanotubes (SWCNTs)
- Multi-walled carbon nanotubes (MWCNTs)
- Silver nanoparticles
- Iron nanoparticles
- Carbon black
- Titanium dioxide
- Aluminum oxide
- Cerium oxide
- Zinc oxide
- Silicon dioxide
- Polystyrene
- Dendrimers
- Nanoclays

The WPMN has launched a sponsorship program for testing the representative set of manufactured nanomaterials, with the OECD acting as a clearinghouse for the sponsorship program and assuming responsibility for preparing a guidance manual for sponsors.

The International Standards Organisation Technical Committee (ISO/TC) 229 is working to develop standards for terminology and nomenclature, metrology and

instrumentation including specifications for reference materials. A jointly sponsored international workshop with NIST, OECD and IEC was held in June 2008. The workshop was designed to promote a dialogue among the key players and to capture input and recommendations on relevant matters, including prioritised measurement needs which could be channelled for consideration to the existing technical bodies. One of the workshop's objectives was to identify supporting measures, such as pre-normative and co-normative research and certified reference materials, which are necessary to support the development of documentary standards. Of relevance to the development of reference materials, the workshop identified the following documentary needs for guides/guidance (to include EHS considerations) on:

- Information needed when handling/using nanoparticles;
- Suite of measurement techniques and information the combined data set might provide (which measurement techniques are applicable and what are their limitations);
- Sample preparation for characterisation, including consideration of dispersion and aggregation/agglomeration;
- Sample preparation for toxicology testing;
- Stability considerations relevant to manufactured nanomaterials;
- Application and limitation of surface analysis to nanoparticles;
- Expression of concentration and dosimetry.

Moreover, reference material needs were acknowledged for polydisperse reference materials for instrument/measurement performance. The workshop report highlighted, without specific detail, new efforts in nanotechnology under the Versailles Project on Advanced Materials and Standards (VAMAS), which emphasises collaboration on pre-standards measurement research, inter-comparison of test results, and consolidation of existing views on priorities for standardisation.

The European Committee for Standardisation (CEN) established a technical Committee CEN/TC 352 'Nanotechnologies' at the end of 2005 to develop a set of standards addressing the specifications for reference materials. Much of this work is underway jointly with the aforementioned ISO Technical Committee.

Two further international workshops taking place in late 2008 are expected to make contributions towards the development of reference materials. The first of which, hosted under the NanoImpactNet project (**EU031**) of the Seventh Framework Programme, focuses on strategies to standardise nanomaterials for environmental and ecotoxicological research. The second, hosted by NIST, focuses on enabling standards for nanomaterial characterisation. Outcomes from these workshops were not available for assessment during the timeframe of the EMERGNANO project.

Contribution towards the RO

Standards and, in particular, Reference Materials play a key role in understanding the EHS impacts of engineered nanomaterials. Reference materials for emerging engineered nanoscale materials are needed to provide key information about the characteristics of those materials and their chemical, physical, biological, and other properties that are consistent regardless of how they are applied. They provide researchers with benchmarks to study, monitor and potentially track nanomaterials as they are released into the environment and the workplace, and to assess their potential interactions with human and ecological systems. Reference Materials, along with protocols for their development and use, are needed to support consistency in measurements of critical nanoscale materials. Nanomaterials of known composition,

once routinely available, will contribute across many of the other NRCG Research Objectives.

Equally important are the protocols that accompany reference materials and evidence of international activity towards their development has been highlighted. These provide consistency in interpreting the data obtained using Reference Materials, ensure that they are used in the same way across disciplines and applications, and provide a standard way to use the Reference Materials in different media (e.g. as an aerosol or in a soluble form). Such consistency is vital to regulators, product developers, and researchers alike as this will ultimately ensure published data can be compared in a consistent manner and that there will be a common understanding and interpretation of results.

Hence, at this time, it is apparent that the assessed studies and ongoing activities are contributing productively towards the RO through dialogue to reach a consensus on candidate materials, the rationale for their development, and the required protocols and documentary standards to underpin the standardised use of nanomaterials (be they of formal reference materials status or only test material status) in EHS research. Evidence of any benefit from the limited number of small-scale exploratory or commercially-driven studies reviewed has yet to emerge.

To date, the only substantiated example of effort contributing towards the RO, is the gold nanoparticle reference materials developed by NIST and NCL (RMs 8011, 8012, and 8013). They are being used to evaluate and qualify the methodology and instrument performance related to the physical and dimensional characterisation of nanoscale particles in pre-clinical biomedical research. The gold Reference Materials will also be useful in the development and the valuation of *in vitro* assays designed to assess the biological response (e.g. cytotoxicity, haemolysis) of nanomaterials.

Extent to which the RO has been met

The extent to which the aforementioned key studies contribute toward the development of standardised, well-characterised reference nanoparticles, beyond the justified nomination of candidate materials, is limited. The development is often part of a wider research program. Nevertheless, the nomination of candidate materials is an essential first step and has been taken in the UK *via* the REFNANO project (Study **UK073**) and subsequently by the OECD Working Party on Manufactured Nanomaterials and the US National Nanotechnology Initiative.

The limited progress is perhaps to be expected given the complexity of truly developing a reference material and maintaining its integrity. There is, however, still value in small scale, exploratory studies focussing on key aspects in the development of a reference material. Yet it is evident from the review of known experimental studies, whose objectives are often highly aspirational and generally have yet to be realised, that such studies are being conducted autonomously from the global initiatives in reference material development led by NIST and IRMM amongst others.

There are significant cross-cutting issues in the development of reference materials which influence the extent to which any one study or initiative can reach fruition, including:

- challenges in material considerations;
- experimental methods;
- production (sources, volumes) time scales and cost;

- policy;
- inter-agency cooperation, coordination and inter-laboratory comparisons.

Material considerations and experimental methods, production, time and costs are critical for the design, planning and pre-production of materials. Policy, cooperation, and collaborations are important issues after materials are developed and available for distribution and use.

Overall, progress is being made towards the development of standardised, well-characterised reference nanoparticles, albeit with the developments emerging on a timeframe and scale necessarily longer and smaller than that of other ROs. The on-going initiatives and anticipated project outcomes that are in the final stages of being published are expected to make further progress towards realising the development of reference nanomaterials.

4.4 RO04 - IGNITION AND EXPLOSIVE PROPERTIES

The research objective

To understand the properties of nanoparticles in the context of their ignition and explosion potential, and assess/develop methods for evaluating this.

Overview of the types of studies which have been found to be relevant to this RO

Of the three studies originally identified, two were excluded as not being relevant to the RO. A further two studies and an earlier literature review sponsored by the UK's Health and Safety Laboratory are considered to be of importance to RO04; however the literature review and statements of objectives for the two subsequent studies are all that have been published. To the best of our knowledge, it is apparent that there is a general lack of substantive research activity in the area despite the highlighted need for such research.

The key studies

Completed or near-completed studies

The UK Health and Safety Laboratory (HSL) carried out and published a literature review in 2004 of the knowledge of the fire and explosion risks of metals in the form of nanosized particles. The review concluded that there was very little experimental information to date. Two subsequent HSL-sponsored studies followed this review and were entitled "Explosion Properties of Nanometric Aluminium and Nickel Powder" (**UKX05**) and "Investigation of the Fire and Explosion Properties of Nano-powders" (**UKX04**). The only documentary output sourced was the 2004 literature review. The stated purpose of Study **UKX04** was for 'sharing the costs of testing materials already made by Qinetiq Nanomaterials Ltd so that HSE gets access to the results'. No further information available on open sources was found for this study.

The HSE Research Projects Directory provided more information on the aim of Study **UKX05**, expected to conclude in 2008. The study's aim is to understand the fire and explosion hazards of selected nano powders. Particular areas for investigation include explosion properties; ignition properties; accumulation of electrostatic charge; fire properties; ease with which selected powders can be made to form a cloud; and the suitability of standard test methods. Specific objectives include:

i). Establishing the difference in behaviour of un-oxidised nano powders (protected by an inert environment up to point of release) and oxidised (prolonged exposure to air) nano powders;
ii). Characterising the explosion behaviour of metallic and non metallic nano-powders;
iii). Characterising the ignition behaviour of nano powders in the form of a layer and dispersed cloud;
iv). Characterising the fire behaviour of nano powders as layers, deposits and when packaged/stored;
v). Characterising the electrostatic charging behaviour and electrical resistivity (surface and bulk) of nano powders;
vi). Assessing the dispersion and agglomeration of nano powders and how these influence the fire and explosion issues in general and suitability of standard characterisation tests.

Ongoing studies

NANOSAFE 2: Safe Production and Use of Nanomaterials (**EU009**) was identified as an ongoing study of relevance to this RO. The study's areas of interest include developing an integrated system addressing potential hazards related to nanoparticles, in particular for health and environmental protection. The project seeks to develop detection and characterisation techniques, hazard assessment, safe production processes and applications. This is a high level study involving over 20 industrial, university and research institute partners from across Europe. The study aims to investigate a range of aspects of nanoparticle manufacturing safety and has a strong focus on explosiveness and flammability. A short interim report from February 2008 documents the findings in this area. A new technique has been developed which has yielded useful information such as the flammability of carbon nanotubes being similar to that of carbon black of similar specific surface area; the passivation of nano-aluminium through introducing oxide layers and evidence that agglomerating nanoparticles exhibit reduced explosion violence. The study will also review TiO_2 and a range of metal nanoparticles.

Contribution towards the RO

The scale and specificity of the NANOSAFE 2 programme (**EU009**) and HSL's investigations of the fire and explosive properties of nanopowders (**UKX04**) suggest that these are key studies contributing to the RO. Improved test equipment has been developed and valuable results have already been achieved.

Extent to which the RO has been met

It is apparent from the lack of available information that this RO has been largely overlooked by groups working in the general area. Recently published work in this area is extremely limited in comparison with the vast array of projects addressing nanoparticle toxicology issues. The need for assessment of explosive and flammability properties has been repeatedly highlighted as important (e.g. Knowles, 2006). Quoting from the conclusions of the HSL's 2004 literature review (Pritchard, 2004): 'There is a growing concern over the impact the increased use of nanopowders and other nanomaterials will have on health and safety and the environment. These concerns are almost exclusively centred on the potential toxic effects of nanomaterials. The potential explosion hazards of nanopowders have not been addressed.' However, the response appears to have been somewhat limited. The Strategic Plan for NIOSH Nanotechnology Research: Filling the Knowledge Gaps (NIOSH, 2008), for example, has no studies relating to this theme.

NASA and the United States Air Force run extensive work programs in the area of energetic nanoparticles, mainly focussed on propulsion and weaponry respectively, however the hazard aspects, though implicit, do not appear to be addressed. The reasons why so little information exists in the public domain are not known. Possible explanations include security concerns, commercial sensitivity, the facilities required (few laboratories are equipped and controlled sufficiently) which are limited to Health and Safety laboratories, military and aerospace facilities, mining and weapon companies. Additionally, ignition temperatures often tend to plateau well above the nanoscale for many substances e.g. at 50 µm flour and 40 µm polyethylene, such that the difference in effects between micro and nano particle sizes is effectively irrelevant. Many factors other than particle size (such as moisture content) also affect the explosive nature of particles

The NANOSAFE 2 study (**EU009**) should make a significant contribution to the state-of-the-art investigations of the flammability hazards nanomaterials. The UK's HSL is working on the investigation of the fire and explosion properties of nanopowders. At this time, their work is focussed on the handling of such materials on a small production or laboratory scale and are expected to result in guidance on handling the potential explosion and fire hazard properties. The HSL are also developing further apparatus to test explosion and ignition properties. It would appear that, thus far, the perceived need for research in this area has not been met and that significant knowledge gaps remain.

4.5 RO05 - SOURCES OF NANOPARTICLES

The research objective

The stated research objective is "Further identification of sources of nanoparticles". RO05 sits within the exposure – sources pathways and technologies taskforce areas. RO5 is closely linked to RO06, which is optimisation and development of technologies that enable measurement of occupational and environmental exposure to nanoparticles *via* the air. Quantification is not specifically mentioned in either RO05 or RO06, however clearly it is important. In dealing with the RO we have attempted to identify not just the studies which *identify* sources of nanoparticles, but also those studies which attempt to *quantify* these sources. In considering this question we have taken the view that 'sources' are all of the activities or processes that could give rise to exposure of humans or the environment to nanoparticles. The exposure landscape has been described in the RS/RAEng report (RS/RAEng, 2004), amongst others, and includes, occupational exposure, consumer exposure and exposure from the environment. This is summarised in Figure 4.1 taken from the RS/RAEng report.

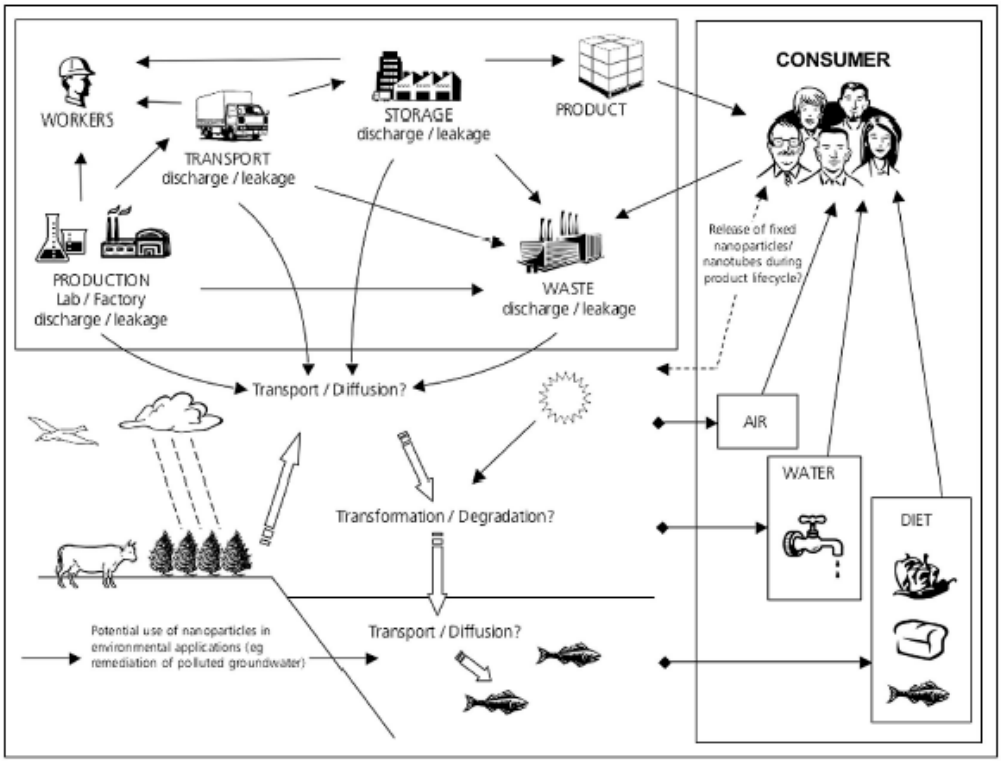

Figure 4.1 Possible exposure to nanoparticles. (Source: Nanosciences and Nanotechnologies: Opportunities and Uncertainties, Royal Society/Royal Academy of Engineering, 2004.)

There are three related questions which together encompass the scope of this RO. These are:

i). What are the sources of nanoparticles which could *potentially* cause exposure to humans or the environment?
ii). What sources *actually* cause exposure?
iii). What are the *characteristics* of these exposures in terms of *intensity*, *duration*, number of *people* exposed, *particle size* and *composition*?

In this review, we have considered the extent to which these questions have been addressed.

Overview of the types of studies which have been found to be relevant to this RO

Given our extended interpretation of this RO, we have identified three types of studies which are able to provide information with which to address the requirements of the RO. These are:

i). reviews and inventories;
ii). modelling studies and;
iii). measurement studies.

Examples of all three have been found as part of the review, either as completed studies or as studies in progress. These studies have addressed a broad range of nanoparticle types including metals and metal oxides, fullerenes and nanotubes. Exposure scenarios which have been addressed in the studies identified include manufacture of nanoparticles, use of nanoparticles in various production activities, use

of nanoparticles in consumer exposure scenarios including cosmetics, food, paint and sources in environmental exposure, including fuel additives.

Thirty one studies were initially identified as being relevant to this RO. On closer inspection six were eliminated as not being relevant to the topic and were not formally reviewed. The total number of studies appraised under RO05 was 25. Fifteen studies exceeding a Weight-of-Evidence score of 17 or greater and have been included in the following appraisal. Of the completed studies, five were identified as being in the reviews and inventory category. This included one study not previously identified as relevant to this RO. Only one completed study was identified with significant modelling activity. Four completed studies were identified as having a level of contribution in the measurement study category.

The key studies

Completed studies

Reviews and inventories

Study **UK005** "Nanoparticles: An occupational hygiene review" was a review activity carried out on behalf of the UK's Health and Safety Executive. A final report is available (Aitken *et al*. 2004). The study was one of the first attempts to define potential exposure scenarios relating to the manufacture and use of nanoparticles. Production processes were described for most of "common" nanoparticle types including, fullerenes, carbon nanotubes, metals and metal oxides and quantum dots. This review identified four main groups of nanoparticle production processes (gas-phase, vapour deposition, colloidal and attrition), all of which may potentially result in exposure by inhalation, dermal or ingestion routes. Of these, only gas-phase processes have the potential to cause exposure to primary nanoparticles by inhalation during the synthesis stage. All processes may give rise to exposure (by inhalation, dermal and ingestion) to agglomerated nanoparticles during recovery, powder handling and product processing. Exposure in downstream manufacturing operations was also identified as plausible.

Study **UKX01** "Manufacture and use of nanomaterials in the UK" followed on from **UK005** and attempted to describe the UK nanoparticle manufacturing industry. A final report is available and there is also a peer-reviewed publication (Aitken *et al.*, 2006). The authors considered that the UK nanoparticle manufacturing industry is at the development stage. Only a handful of companies were identifiable as nanoparticle manufacturers. The main emphasis of nanoparticle manufacturing industry in the UK has so far been the bulk markets in metals and metal oxides, as well as some niche markets such as quantum dots. The review indicated that nanoparticle manufacturing in the UK does not reflect the global emphasis on fullerenes, nanotubes and fibres. Instead, the emphasis is weighted heavily towards the production of nano-metals and metal oxides, with some production of carbon nanotubes and quantum dots (for applications in diagnostics, authentication markers). A large number of university departments, spin-offs, and private companies have developed processes for the manufacture of nanoparticles but may only be producing small experimental quantities for research and development purposes. However, some of these have the potential and capability to scale-up their processes to produce large quantities of nanoparticles.

Study **CH006** is an inventory of manufactured nanoparticles in Swiss industries considering the potential for human exposures. The goal of the study was to evaluate the use of nanoparticles, the currently implemented safety measures, and the number of potentially exposed workers in all types of industry in Switzerland. A targeted telephone survey was conducted among health and safety representatives from 197

Swiss companies. This showed that nanoparticles are already used in many industrial sectors; not only in companies in the apparently new field of nanotechnology, but also in more traditional sectors, such as paints. Forty-three companies declared to use or produce nanoparticles, and 11 imported and traded pre-packaged goods that contain nanoparticles. The following nanoparticles were found to be used in considerable quantities (>1000 kg/year per company): Ag, AlO_x, FeO_x, SiO_2, TiO_2, and ZnO. The median reported quantity of handled nanoparticles was 100 kg/year. The production of cosmetics, food, paints, powders, and the treatment of surfaces used the largest quantities of these nanoparticles.

Study **UK111** is an assessment of current and projected applications of nanotechnology for food contact materials in relation to consumer safety and regulatory implications. Study **UK110** is an assessment of the current and projected applications of nanotechnology in the food sector. These two studies carried out in the UK identified that the currently known and projected applications of nanotechnology for the food sector fall into the following main categories:

- Where food ingredients have been processed or formulated to form nanostructures;
- Where nano-sized, nano-encapsulated or engineered nanoparticle additives have been used in food;
- Where nanomaterials have been incorporated to develop improved, ‘‘active’’, or ‘‘intelligent’’ materials for food packaging;
- Where nanotechnology-based devices and materials have been used (e.g. for nanofiltration, water treatment, nanosensors for food safety and traceability).

Examples of nanostructure formulation include nano- emulsions, surfactant micelles, emulsion bilayers and reverse micelles. For example, low-fat nanostructured mayonnaise, spreads and ice creams claim to be as ‘‘creamy’’ as their full fat alternatives and, hence, offer a healthier option to the consumer. Among the few examples of currently available food additives is the synthetic form of the tomato carotenoid, Lycopene, which has a particle size in the range of 100 nm (BASF’s US Patent US5968251). The main food applications of Lycopene include soft drinks, baking mixtures and blancmanges. Polymer composites incorporating clay nanoparticles are among the first nanocomposites to emerge on the market as improved materials for food packaging. The nanoclay mineral used in these nanocomposites is bentonite, which is a relatively cheap and widely available natural clay derived from volcanic ash/ rocks. Polymer nanocomposites incorporating metal or metal oxide nanoparticles have been developed for antimicrobial ‘active’ packaging, abrasion resistance, UV absorption, and/or strength. The nanomaterials used as UV absorbers (e.g. TiO_2) can prevent UV-degradation in plastics such as polystyrene, polyethylene, and polyvinylchloride. The metal and metal oxide nanomaterials commonly used are Ag, Au, ZnO, SiO_2, TiO_2, Al_2O_3, Fe_3O_4 and Fe_2O_3. Other semi-conductor nanoparticles (e.g. cadmium telluride/ gallium arsenide) have also been used in development of nanocomposites. A number of ‘active’ food contact materials based on the antimicrobial action of nanosilver, have been developed that are claimed to preserve the food materials within by inhibiting the growth of microorganisms. A final report is available for both of these studies and one peer reviewed publication has been identified.

Modelling studies

Study **UK074** considers current and predicted environmental exposure arising from engineered nanomaterials. The objectives of this study were to i) identify the potential releases of engineered NP to the environment; ii) review the fate of NPs in

environmental systems; and iii) to assess the potential current and future environmental exposures. Whilst NPs may be emitted during the manufacturing process, the route of input to the environment will primarily depend on the end use of the NP-containing product. In many of the applications, nanoparticles are in a fixed or bound form, and hence pose minimal risk to the environment. Applications that contain free engineered NP, and/or those that are likely to give rise to a greater likelihood and extent of exposure to the environment (e.g. airborne NP, or those products likely to be disposed of in wastewaters) include cosmetics, paints and coatings, catalysts and lubricants, water treatment and bioremediation products, food and food packaging, human and veterinary medicines and plant protection products. Several products containing NPs that are currently on the UK market were identified. However, due to a lack of published data, the authors were unable to estimate the UK market penetration for these products.

To address the lack of data on usage and environmental fate, a framework of simplistic models and algorithms for estimating concentrations in water, soil and air were developed and applied to a range of NPs. This modelling framework was applied to estimate the likely concentrations of NPs in water and soil for a range of usage scenarios. For the 10% market penetration scenario, which probably overestimates current exposure levels, concentrations of silver, aluminium and fullerene C60 concentrations were predicted to be in the ng/l, whereas, titanium dioxide, zinc oxide, nanolatex and hydroxyapatite are predicted to be in the mg/l range (see Table 4.1, extracted from the report). Predicted concentrations in soil ranged from < 0.01 mg/kg (cerium dioxide) to around 4.3 mg/kg (nanolatex). Predictions were also obtained for concentrations of selected NPs in the air compartment. If, in the future, all of the product types investigated contained engineered NP, then concentrations in water could range from < 1 ng/l (cerium dioxide) to 1 mg/l (nanolatex). This study did not address bio-accumulation.

The exposure data developed in this study provide a benchmark to: i) inform the development of new analytical methodologies for environmental system; ii) inform the design of environmental fate studies; and iii) interpret the significance of existing ecotoxicology data on NPs. A comparison of the results of the exposure estimations with the available ecotoxicological data (Table 4.1) indicates that even the conservative exposure concentrations generated in this study are many orders of magnitude lower than concentrations likely to cause acute effects in invertebrates, fish algae or sub-lethal effects on fish, invertebrates or bacteria.

Whilst this study has identified the potential environmental exposure arising from a range of key NP types, the authors state that the assessment has been limited by the availability of data and knowledge. Work in the future should therefore focus on: i) establishing a detailed knowledge of the content and use of products containing NPs in the UK; ii) developing an understanding of the factors and processes affecting the fate and transport of NPs in the environment; iii) the development and evaluation of more complex exposure assessment models; and iv) the development of a better understanding of the ecotoxicity of NPs under environmentally-relevant exposure situations.

Table 4.1: Summary of exposure and available effects data for selected NPs in the UK environment, assuming 10% market penetration. (Source: Boxall *et al*., 2007)

	Water (µg/l)	Soil (µg/kg)	Air (mg/m^3)	Aggregate size (nm) in water mean and range	Invertebrate EC50 (µg/l)	Fish LC50 (µg/l)	Algae EC50 (µg/l)	Other endpoints
Ag	0.010	0.43	-	-	-	-	-	-
AlO_3	0.0002	0.01	-	-	-	-	-	-
Au	0.14	5.99	-	-	-	-	-	-
CeO_2	<0.0001	<0.01	6×10^{-7}	-	-	-	-	-
fullerenes	0.31	13.2	-	75 (25-500)	> 35000	>>5000	-	effects on invertebrate behaviour at 260 µg/l; bacterial growth effected at 40 µg/l; bacterial phospholipids effected at 10 µg/l
hydroxyapatite	10.1	422	-	-	-	-	-	-
latex	103	4307	-	-	-	-	-	-
organo-silica	0.0005	0.02	-	-	-	-	-	-
SiO_2	0.0007	0.03	-	205 (135-510)	-	-	-	bacterial growth not effected at 500000 µg/l
TiO_2	24.5	1030	7	330 (175-810)	>100,000	>100,000	16,000	effects on invertebrate behaviour at 2000 µg/l; bacterial growth not effected at 100000 µg/l
ZnO	76	3194	-	480 (420 – 640)	-	-	-	90% bacterial growth not effected at 10000 µg/l

Measurement studies

Study **TW002** considers the application of nanotechnology for environmental protection and **TW001** concerns the promotion of a responsible research, development and manufacturing environment for nanotechnology. These are two studies are amongst several being carried out by the same group in Taiwan. In the first study, exposure evaluations of the working environments of two small-scale plasma nanopowder manufacture processes, "Direct-Current Plasma" and "Microwave Plasma", were performed. The average number concentrations for the operating processes were 25255 ± 4705 and 22774 ± 3672 cm^{-3} for direct-current process and microwave process, respectively. The average number concentrations for the non-operating conditions were 14071 ± 995 and 9918 ± 154 cm^{-3} for direct-current process and microwave process, respectively.

In **TW001**, nanoparticle emission of TiO_2 nanopowder coated on different substrates including wood, polymer, and tile, was evaluated in a simulation box and measured with a Scanning Mobility Particle Sizer (SMPS) for the first time. The coating process for the substrate followed the instructions given by the supply company. In the simulation box, UV light, a fan, and a rubber knife were used to simulate the sun light, wind, and human contacting conditions. Among the three selected substrates, tile coated with TiO_2 nanopowder was found to have the highest particle emission (22 cm^{-3} at 55 nm) due to nanopowder separation during the simulation process. The UV light was shown to increase the release of particle below 200 nm from TiO_2 nanopowder coating materials. The results show that, under the conditions of UV lamps, a fan and scraping motion, particle number concentration or average emission rate decreases significantly after 60 and 90 min for TiO_2 on polymer and TiO_2 on wood, respectively. However, the emission rate continued to increase after 2 hours of testing for TiO_2 on tile. It is suggested that nanoparticle emission evaluation is necessary for products with nanopowder coating.

Study **US334** is a TiO_2 nanoparticle exposure study, with the goal of measuring and characterising workplace exposures to fine and ultrafine TiO_2 in both manufacturing and end-user facilities. The specific objectives are threefold: i) characterise airborne TiO_2 exposure metrics by job or process; ii) obtain quantitative estimates of exposure in workers to fine and ultrafine TiO_2 particle sizes by relating the measured exposure metrics to worker exposure; and iii) evaluate a strategy for measuring workplace

exposure to fine and ultrafine TiO_2. A full shift combined with a task based sampling scheme consisting of various real-time and mass based area, and personal aerosol sampling will be employed. In addition, information will be collected on the use of personal protective equipment (PPE) and the types of controls and work practices used to minimize worker exposures to TiO_2. No published data from the study were identified.

In addition to the studies considered in this appraisal, a recent publication by Han *et al*. (2008) is highly relevant. This publication describes the first attempt to measure exposure to carbon nanotubes (CNT) using methods similar to those used to measure asbestos. The study measured exposures in the post production recovery of multi-walled carbon nanotubes (MWCNT) and in a blending activity, part of a composite formulation process. Air samples were taken by drawing air through mixed cellulose ester filters in sampling cassettes (35 mm diameter, 0.8 µm nominal pore size, and 2 inch cowl). Collected samples were counted using a transmission electron microscope (TEM). All objects identified as MWCNT with an aspect ratio greater than 3 were counted. The diameter and length were measured and determined to be 52–56 nm and 1473–1760 nm, respectively. Tests were carried out before and after control measures (essentially isolating the process) were put in place. In mass terms, most of the laboratory MWCNT exposure levels (maximum 0.43 mg m^{-3}) were lower than the current TLV for carbon black (ACGIH 3 mg m^{-3}). However, the number of tubular structures (maximum 194 tubes cc^{-1}) was over (by a factor of almost 1000) the current fibre TLVs (asbestos 0.1 cc^{-1}; glass wool 1 cc^{-1}; rock wool 1 cc^{-1}; refractory ceramic fibre 0.1 cc^{-1}; etc.). The MWCNT lengths that were shorter than 5 µm may differentiate MWCNTs from asbestos and other fibre structures. Health effects of durable shorter fibres remain controversial and the durability of MWCNTs is not clearly known. Other main biological determinants known for fibres, such as aspect ratio, dimension, and deposition, may differentiate MWCNTs from asbestos and other fibre structures.

Ongoing studies

A programme of work has been initiated in Denmark by NRCWE. Started in 2007, it comprises 2 main projects (with a series of associated subprojects):

- Nano-technological materials and products in the plastics industry. (**DK001**; NANOPLAST) Exposure assessment and toxicological properties;
- Nanoparticles in the paint- and lacquer industry. Exposure and toxic properties (**DK008**).

In **DK001**, the aim is to investigate physical, chemical, and toxicological properties of nano-technological materials that will have potentially greater use in the future production of plastic products. The project focuses on polymer nano composites (PNCs) that consist of a polymer matrix containing a uniformly dispersed nano-technological material that can be nanoclay, carbon nanofibres (CNFs), or carbon nanotubes (CNTs). The starting point is to investigate 9 PNCs that are composed from 7 different nano-technological materials (nanoclay, CNFs, CNT) and 3 different polymers. Study **DK001** comprises 3 subprojects:

- Subproject 1. Characterisation of toxicologically relevant physical and chemical properties of nanoclay, carbon nanotubes and carbon nanofibres in pure state and of wear test dust from polymer nano composites;
- Subproject 2. Characterisation of toxicological properties of nano-technological materials for use in plastic products. Specifically carcinogenic effects of nanoclay, carbon nanotubes and carbon nanofibre in pure state and of wear test dust from polymer nano composites;

- Subproject 3. Assessment of the risk of exposure to nano-technological materials in the plastics industry – Exposure scenarios carried out during test productions in the laboratory.

One of the early publications from this study is "Dustiness behaviour of loose and compacted Bentonite and organoclay powders: What is the difference in exposure risk?" (Jensen *et al*., 2009).

The goal of the second study (**DK008**) is to identify and characterise the crucial risks caused by exposure to nanoparticles in the paint- and lacquer industry. The main project will combine knowledge of four research fields and be carried out in close cooperation with the paint- and lacquer industry. The sub-projects in this study are:

- Assessment and exposure risks of nanoparticles and dust generated from nanoparticle-containing paints;
- Characterisation of physical and chemical properties of nanoparticles and dust generated from nanoparticle-containing paints;
- Characterisation of toxic properties of nanoparticles and dust generated from nanoparticle-containing paints;
- Development of model for risk assessment of nanoparticles, for use or expected use in the paint- and lacquer industry.

DK011 is attempting to understand the atmosphere-chemical reactions risk of exposure to chemicals and nanoparticles during use of nanoparticle-based pump and spray products, as well as screening their potential cellular and respiratory effects *in vitro* and *in vivo* at different user scenarios.

Study **EU011** (NANOSH) is investigating inflammatory and genotoxic effects of engineered nanomaterials. This is an EU funded FP7 integrated projects which started in 2007. It has three main parts: i) particle and exposure characterisation; ii) genotoxicity of nanoparticles *in vitro* and *in vivo*; and iii) effects of nanoparticles on microcirculation. In the particle and exposure characterisation part, the objectives are described as:

- to delineate the particle size distribution, dissolution, agglomeration properties, surface area and surface activity of the nanoparticles selected;
- to define exposure levels of selected nanoparticles under laboratory conditions and in workplaces;
- to choose additional nanoparticles based on the initial investigations for exposure studies.

The target is to collect comprehensive exposure data for more than 20 workplaces and to develop appropriate sampling strategies. University laboratories are among the workplaces being studied in the UK. Measurements will be made of mass, number and surface area concentrations and of particle size distribution. Publications have been found relating to the genotoxicity part of this work programme but not as yet for the exposure part.

Study **US176** considers the measurement and control of workplace nanomaterials. According to the published description by NIOSH, this project will provide a fundamental basis for understanding how nanomaterials are released and dispersed into the workplace, demonstrate the way nanomaterials can be monitored, and the way nanomaterial exposure can be controlled. The objective is to conduct detailed nanomaterial workplace evaluations to provide the following:

- A comprehensive workplace characterisation of airborne nanomaterial release and exposure;
- A determination of nanomaterial migration within the workplace;
- A comprehensive qualitative and quantitative (where possible) assessment of engineering controls, ventilation systems, and work practices;
- Recommendations for improvements in engineering controls and work practices where applicable;
- Quantitative assessments of the efficacy of implemented engineering controls and work practices, where applicable, through workplace monitoring.

One of the first case studies published from this programme was an evaluation of exposures in a nanocomposite research laboratory at the University of Dayton Research Institute (UDRI). The UDRI laboratories conduct research, development, and technical service projects on polymeric materials. Activities at the Center include research on plastics, adhesives, elastomers, composites, and engineered nanomaterials. The study team found that measurements made with real-time instruments capable of sizing and determining airborne particle concentrations indicate that most processes did not release substantial quantities of carbon nanofibres when compared to background particle measurements. However, some processes (wet sawing of composite material and the transferring of carbon nanofibres to a mixing vessel) did elevate area airborne particle mass concentrations. Surface sampling indicated that carbon nanofibre material migrated from the laboratory to an adjacent office area, with employee footwear being the most likely means of transport.

Study **US429** involves exposure assessment in tungsten refining and manufacturing. The proposed 3-year study will determine if airborne tungsten oxide fibre concentrations and physicochemical properties vary with production and manufacturing processes in the tungsten industry, and other down-stream industries that consume and incorporate tungsten in their products. The study design is an observational industrial (occupational) exposure assessment of four similarly exposed groups, consisting of 20 workers. The research will identify groups at elevated risk of exposure, document exposure patterns among occupational cohorts, and characterise airborne particle morphology in domestic tungsten production and use among six facilities. No data from this study has been found.

Contribution towards the RO

Completed studies

UK005 was one of the first studies to attempt to systematically identify potential exposure sources. The study identified that there was potential in all of the nanoparticle manufacture processes to have exposure to nanoparticles or agglomerated nanoparticles. The study particularly identified issues of potential exposure during recovery, powder handling and product processing of nanoparticles. The study also identified possibility of exposures occurring both in university research laboratories and in new nanotechnology industry scenarios.

A more extensive study (**UKX01**) was carried out in the UK to identify the types of nanomaterials in use. They found that in the UK at least the emphasis was weighted heavily towards production of metals and metal oxides with some limited production of carbon nanotubes. The focus of this study was also on manufacturing.

CH006 carried out a targeted telephone survey in 197 Swiss companies which identified that nanoparticles are already used in many industrial sectors, including

those such as paints and coatings. Forty-three companies identified that they use or produce nanoparticles, with the most widely used being silver, iron oxide, SiO_2, TiO_2 and ZnO. The medium reported quantity used was 100 kg per year, with several companies using more that 1000 kg per year. The largest quantities of these materials were used in cosmetics, food, paints, powders and surface treatments. Many of the organisations reviewed in this study were users, rather than manufacturers of nanoparticles.

Two studies, **UK111** and **UK110,** looked at potential use of nanoparticles in food and food contact materials. The identified uses of nanomaterials in food included formulation to nano-size emulsions used as food additives, use in food packaging and use of nanotechnology based devices in processing. While the use of nano-structure formulation seems to be increasing, there are relatively few food additives developed in the nanometre size range. One example is the tomato carotenoid lycopene which is used in soft drinks and food processing mixtures. In terms of food packaging, natural clay nano-composites such as bentonite are widely used already. Various use of metal oxides (such as titanium dioxide as a UV absorber) is increasing, as is the use of metal in the metal oxide nanomaterials (such as silver), which is being developed for anti-microbial active packaging.

The aforementioned studies have mapped out a series of potential sources of exposures to nanoparticles in industrial and consumer applications. This landscaping process however is not complete and, as new uses of nanoparticles emerge, it is likely that more scenarios will emerge.

The modelling study **UK074** was an attempt to quantify, through the use of relatively simple models, the potential releases of nanoparticles into the environment. This is perhaps the first attempt for such predictive assessments to be made. The study identified applications that contain three engineered nanoparticles and those that are likely to give rise to exposure to the environment (e.g. airborne nanoparticles, or those products likely to be disposed of in waste waters). These included cosmetics, paints, catalysts, lubricants, water treatment, bio-remediation, food and food packaging, medicines and fuel additives. Estimates produced by the models were in general low, typically ranging from ng/L to mg/L in water and from less than 0.01 mg kg^{-1} to around 4.3 mg kg^{-1} in soil. Predictions were also obtained for concentrations of cerium oxide in air as a fuel additive and titanium dioxide as a consumer application in suntan lotion. Air concentrations of cerium oxide in cities were very low, estimated at 6 x 10^{-7} mg m^{-3}. Local transient concentrations of titanium dioxide in air were estimated at 7 mg m^{-3} for a spray application of suntan lotion. This study is helpful in establishing potential exposure levels for a range of materials and applications. However, the models have not been validated and are relevant only to the scenarios identified. It is nevertheless, a very important study.

The measurement studies identified have contributed some data for various scenarios relevant to both manufacture and use of nanomaterials. The recent work by Han *et al*. (2008) provided quantitative data associated with the manufacture of carbon nanotubes and the use of carbon nanotubes as a component in the manufacture of a composite material. The study demonstrated that there was significant potential for release of nanotubes in to the air and used a method similar to the method for measuring asbestos fibres in air to assess this. High concentrations of CNT in the form of discrete nanotubes (relative to the exposure limit for asbestos fibres) were identified although all of the objects identified were short (less than 2000 nanometres) and would not, therefore, be counted as fibres under normal counting rules. The use of control measures reduced this exposure effectively to zero.

The two studies from Taiwan (**TW001** and **TW002**) looked at the potential release of nanoparticles in to the air during manufacture of metal oxide nanoparticles and the potential release from a surface that had been coated in titanium dioxide. The number of concentrations from the manufacturing process were in the order of 10,000 to 25,000 particles/cm^3, broadly consistent with typical ambient aerosol concentrations in cities or other urban workspaces. No size information was provided. The simulation study demonstrated potential release of very low levels in the order of a few tens of particles/cm^3 during some simulated contact scenarios.

A further titanium dioxide exposure study (**US334)** apparently complete, which has the aim of quantifying titanium dioxide exposures in manufacturing and end use facilities, has been identified. However at this point in time, no data from this study has as yet been identified.

These studies, which identify and quantify the release of nanoparticles either in manufacturing or in end use applications, are necessary, in order to begin to map out and evaluate the potential risks. In terms of completed studies, at this point we only have a handful covering a very small subset of the total scenarios (expressed in terms of materials and applications). So while these are important, they are not at this point definitive.

Ongoing studies

Three large programmes of work have been identified which will contribute significantly to towards the issue of potential sources of nanoparticle release.

Studies **DK001** and **DK008** are looking at potential exposure scenarios in the plastics industry and in the paint and lacquer industry. A range of materials are being considered including nano clays, carbon nanotubes, carbon nanofibres and metal oxides. These are integrated projects which also are looking at toxicological issues. An early outcome from these projects is a paper on dustiness of nanomaterial bentonite. When completed, these projects are likely to make a significant contribution towards understanding the potential release of nanomaterials in these specific applications.

A European study (**EU011**), started in 2007, which has a programme to quantify exposure in a series of occupational scenarios. One focus is exposure in laboratory and research facilities. The target is to collect comprehensive exposure data and develop generic strategies for measurement of exposure. There are no publications from this study relating to exposure available in the public domain at this point.

In the United States, **US176** is a programme of work being carried out by NIOSH which will look at a series of case studies in wide ranging nanomaterial manufacturing and applications. One single study already carried out by this programme in relation to manufacturing/processing of carbon nanofibre has been published. This did indicate elevated mass concentrations for some activities and the presence of surface contamination. Some fibre bundles were identified and, although the number concentrations were not quantified, the impression given is that numbers were low.

Extent to which the RO has been met

The studies completed and those still underway have and will make progress towards meeting the objective set out under RO05. In terms of identifying the sources which could potentially cause exposure to humans and the environment, good work has been done building on that carried out by the Royal Society / Royal Academy of Engineering

to map out the potential exposure landscape. However, each of the pathways represented in Figure 4.1 are complex in themselves. One technique which can be used to track these is that of life cycle analysism, which looks at the (amongst other things) potential for exposure through the complete product life cycle of some product which contains nanoparticles. This systematic approach can be helpful in understanding where exposures are possible. One way to take this forward would be to identify a series of products where nanoparticles are a component and carry out detailed life cycle analysis for each. At this point this has not been done and is a remaining gap to be addressed. Until this structured approach is adopted, information on potential sources is likely to be patchy and incomplete.

In terms of describing sources which actually cause exposure, some progress has been made with some scenarios. Occupational exposures have been detected in synthesis and processing activities. Consumer exposures have been postulated but have not been conclusively demonstrated other than very obvious examples, such as the application of skin care products. With respect to characterising the exposures, many of the studies already identified will make valuable contributions to this issue. It is a complex question, however. Characterisation primarily requires measurement programmes based on either real or simulated exposure scenarios, using equipment or methods which are established and validated. A separate but linked Research Objective (RO06) describes activity towards establishing these methods, many of which are not yet sufficiently good for regular or routine measurement. There are several major measurement programmes now underway and these should be encouraged. It is important to note that the landscape is extremely complex, with many materials products and processes already identified as potentially causing exposure. There is a large programme of work which needs to be carried out in order to quantify and characterise these exposures over the range of important possible exposure scenarios. The studies we have identified in progress, although important, will only cover a small fraction of the potential exposures which are believed to be possible.

4.6 RO06 - EXPOSURE MEASUREMENT (AIR)

The research objective

Research Objective 6 (RO06) is within the scope of NRCG Taskforce 2, exposures – sources, pathways and technologies. The statement of the objective is the *"Optimisation and development of technologies that enable the measurement of occupational and environmental exposure to nanoparticles* via *the air".*

The stated research objective is one which has been identified in large number of review articles (e.g. RS/RAEng, 2004) and refers to the need to develop new approaches to the measurement of both occupational and environmental nanoparticles in air. It should be noted that the objective does not in itself look for more information about what these exposures are, and it is considered that this sits more clearly in RO05. In practice the requirements for occupational exposure measurement are somewhat different to those for environmental exposure measurement. For example, authors such as Maynard and Aitken (2007) have described the need for the development of personal sampling devices for the assessment of occupational exposure. By this we mean small devices which are capable of being worn within the breathing zone of a worker or someone else occupationally exposed. This has implications for the size of the device, the power supply and the cost. In contrast, environmental exposure is typically based on sampling or measurement instruments which are located at a fixed static site. For example, in the UK, the automated urban network (AUN) provides early pollutant concentration from (currently) 96 sites,

collecting information on PM_{10} and PM_5 particulate matter concentrations amongst others. In this case, therefore, the constraints on size cost are much less than would be required for occupational measurements.

RO06 also links to RO02 which has an objective of trying to identify the more suitable metrics and associated methods for measurement and characterisation of nanoparticles. There is therefore broad scope within this objective to deal with approaches and methods which relate to various metrics, including mass concentration, number concentration, surface area concentration, size distribution, composition, particle shape etc. Issues which have been identified in relation to measurement of exposure include:

- Which metric is appropriate to use for which scenario (occupational, environmental) or particle type;
- Discrimination of the NP of interest from any background particles;
- Aggregation of nanoparticles and whether or not it is possible to describe an appropriate maximum size.

A specific issue for high aspect ratio nanomaterials (HARN) such as CNT or metal nanowires is, whether a variant of existing measurement and counting methods used for fibrous aerosols (e.g. asbestos counting methods such as WHO, 1997) can be used in a meaningful way to determine an appropriate measure of exposure.

Overview of the types of studies which have been found to be relevant to this RO

In relation to RO06, twenty-five studies were initially identified as being relevant. Closer analysis indicated that, of these studies, six were outside the scope of the RO, leaving nineteen studies to be assessed in detail. In general, the studies could be divided into three types:

i). Instrument development, the development of new or improved specific instrument or measurement systems;
ii). Optimisation studies, including optimisation/adaptation of specific existing methods, combination of different methods, combination of different metrics to derive alternate indices of exposure and comparison of methods (including both real time measurement instruments and offline analysis methods, for example, image analysis of high aspect ratio nanomaterials using SEM or TEM approaches);
iii). Measurement programmes, broadly field based activities in which different types of measurement systems are used in order to try to characterise the features of a potential nanoparticle release in a workplace or the environment.

Of the 19 studies assessed, fifteen were identified as having a good quality (exceeding a Weight-of-Evidence threshold of 16) and were considered in more detail. These studies included five instrument development projects, six method development projects and three measurement programmes. The final study was sufficiently diverse in its scope as to not fall clearly within any one of these categories. In practice, of course, in all of the categories there was some overlap between the study types. Nevertheless, it is a useful way of discriminating between these types of studies.

Nine of the fifteen studies were either complete or were about to be completed by the end of 2008, whereas six were ongoing with projected end dates of 2009 and beyond. The range of information available was quite variable between the various studies, including those studies which had already been completed. The original information provided or obtained from the project leaders on these studies was supplemented by

additional searches to identify further information, particularly in the public domain through web searches.

The key studies

Completed and near-completed studies

Instrument development projects

Study **US101** concerns the development of an analyser for size and charge characterisation of nanoparticles in research and training. Development of a new laser based instrument to measure simultaneously both particle size and electrostatic charge distributions in real time and on a single particle basis for particles in the size range 10 to 1000 nm in diameter was proposed by a team of researchers of the University of Arkansas at Little Rock (UALR). The instrument will employ a Laser Doppler Velocimeter (LDV) that will analyze the response of particle motion under the excitation of an alternating current electric and acoustic fields synchronously applied at a frequency ranging from 10 to 100 kHz. The diameter and the charge of each particle passing through the LDV sensing volume are determined by measuring the phase lag and the amplitude ratio of the oscillatory particle motion with respect to the applied sinusoidal fields. A high frequency photon correlator and a cooled photomultiplier tube will be used to process signals from the radiation scattered from particles to measure the size and charge distributions at a rate of 1000 particles per second.

Studies **US204** (Elemental Composition of Freshly Nucleated Particles) and **US151** (Chemical Characterisation of Ultrafine Aerosol Particles) have the main objective of developing a method for real-time sampling and analysis of individual airborne nanoparticles in the 5-20 nm diameter range. This size range is much smaller than existing single particle methods for chemical analysis. Nanoparticles will be classified by elemental composition using aerosol mass spectrometry. The aerosol will be drawn directly into a mass spectrometer where individual particles are analysed in real time by laser ablation. To increase the sampling efficiency for particles in the 5-20 nm diameter range, a quadrupole ion guide will be used to focus particles into the ablation laser beam path. Quantitative measurements of elemental composition will be achieved by complete atomisation and ionisation of individual particles using a high energy laser pulse to create a nano-plasma. This method will be used to study a variety of particle formation processes. It is intended that the instrumentation developed will be suitable for field experiments.

US061 is a multi-stakeholder consortium of more than 14 industrial, academic and government organisations formed to sponsor research to further understanding of factors relevant to the assessment and control of occupational exposures to engineered nanoparticles. With particular regard to RO05, this two year research project, led by DuPont scientists, had the aim to help understand: i) how airborne nanoparticles may behave in the workplace; ii) how to monitor and measure occupational exposures to airborne nanoparticles; and iii) how to assess the penetration of engineered nanoparticles through candidate barrier materials for personal protective equipment. One of the 3 identified deliverables was the "Development of a portable aerosol monitor" although no information was provided as to the proposed features of this device.

Method optimisation/development projects

Study **US291** involves the development of fine particle characterisation and monitoring methods. This study, carried out by NIOSH in the US focussed on developing methods

to fully characterise ultrafine aerosols produced in workplaces, attempting to characterise exposure in terms of aerosol number and surface-area, and investigating the surface characteristics of particles through the use of advanced electron microscopy techniques. Combination of the two approaches was intended to provide the tools to enable a greater understanding of toxicologically-relevant particle characteristics to be developed, and allow exposure monitoring against relevant metrics.

Study **US046** involves monitoring and characterising airborne carbon nanotube particles. The proposed research aimed to develop a comprehensive, practical method for sampling, quantification, and characterisation of CNT particles in air. According to the authors, the method will permit classification of sampled particles into three categories: tubes, ropes (bundles of single-walled CNTs bounded by Van der Waals attraction force), and non-tubular particles (soot, metal catalysts, and dust, etc.). The method will also permit calculation of the number concentrations and size distributions for each type, and the shape characters (diameter, length, aspect ratio and curvature) of CNTs. The proposed method used available instrumentation to build an air monitoring system that is capable of sampling and sizing airborne CNT particles in a wide size range by using a 10-stage Micro-Orifice Uniform Deposit Impactor (MOUDI) and an Integrated Diffusion Battery previously developed. The samples of each size fraction will be collected onto silicon-chip substrates and analyzed using Atomic Force Microscopy (AFM). Newly developed software, SIMAGIS® Nanotube Solutions, will be used for AFM image analysis and data processing, which can automatically count nanotubes, nanoropes and particles; and measure the shape characters.

Study **US144** is developing assessment methods for nanoparticles in the workplace. The stated primary objectives of this study were to: i) provide the scientific community and practicing industrial hygienists with verified instruments and methods for accurately assessing airborne concentrations of nanoparticles, and ii) assess the efficacy of respirator use for controlling nanoparticle exposures. The research approach will involve both laboratory and field work. Manufactured nanomaterials covering a range of the types available will be obtained from several sources. The research group will then systematically compare measurements obtained from a variety of sampling instruments, including a novel passive aerosol monitor, with measurements made by transmission electron microscopy (TEM) under controlled laboratory conditions. Field tests will involve the use of the instruments analysed in the lab to quantify and characterise nanoparticle concentrations in workplaces that manufacture or use nanoparticles.

Measurement programmes

US439 - studying the measurement of nanoscale carbonaceous materials - had two specific aims: i) apply multiple methods to characterise carbon nanofiber/nanotube materials in bulk, surface, and air samples (metrics included: particulate carbon; metals; adsorbed organic fraction; particle size, shape, and elemental composition); and ii) generate filter samples of carbonaceous aerosols with known organic and elemental carbon (OC-EC) content and evaluate the suitability of filter sets for quality assurance measurements on nanoscale carbonaceous aerosols.

US331 - examining ultrafine particles in heavy vehicle assembly and components manufacturing plants will evaluate the particle concentrations in a foundry and an engine plant. Aerosol maps will be constructed with data obtained from real-time, aerosol instruments: optical particle counters and condensation particle counters for number concentration; diffusion chargers for surface area concentration; and aerosol photometers for mass concentration. These instruments will allow the investigators to

prepare aerosol maps as a function of particle size [0.01 μm to 5 μm], metric [number, surface area, and mass concentration], and season [winter and summer]. The operations floor plan of the plant will be overlaid onto these maps to identify the source and fate of particle contamination. Collocated with the real-time instruments, filter-based samplers will be used to measure directly respirable, PM1, and PM2.5 mass concentration. The response of the aerosol photometers will be calibrated with these mass measurements. Additional filter samples will be collected near suspected key particle sources; these samples will be analysed chemically. The data from both real-time aerosol instruments and filter-based samplers will be investigated for relationships between particle number, surface area, and mass concentration. In addition, surface area concentration measured with the diffusion chargers will be compared to that estimated from the particle number and mass concentration measuring devices.

Ongoing studies

Instrument development

EU024 – NANODEVICE: Development of Novel Concepts, Methods, and Technologies for the Production of Portable, Easy-to-Use Devices for the Measurement and Analysis of Airborne Engineered Nanoparticles in Workplace Air.

This is major EU Framework study (FP7) involving academic groups, government scientists and instrument manufacturers. It is a highly relevant study aimed exactly at developing new instruments; however the contracts have only just been signed (2008). The programme is due to last 4 years. No outputs are yet available as the project has yet to officially start. The objectives are:

i). To identify relevant physical and chemical properties for specific measurement of engineered airborne NP, and to develop reference materials for NP aerosols;
ii). To develop technologies that enable utilisation of new concepts in miniaturised and field-worthy specific monitors for NP;
iii). To develop methods for calibration and testing of the newly developed concepts, methods and devices in simulated and real life exposure settings.

Method development/optimisation

US433 - Ultrafine TiO_2 Surface and Mass Concentration Analysis - with the purpose of testing the hypothesis that ultrafine titanium dioxide (μTiO_2) surface area can be measured with specificity on heterogeneous particle-laden filter samples using surfactant isotherms and/or fluorescence labelling. This project aims are to: i) develop a model for quantifying bulk ultrafine TiO_2 powder specific surface area using lung surfactant and/or fluorescent labelling; ii) extend the model to evaluate surface area of aerosolized TiO_2 particles collected on filter media in a laboratory chamber; and iii) test the proposed model in a ultrafine TiO_2 primary production workplace.

US427 is determining the diameter distribution of CNT by Raman spectroscopy. The goal of this study is to develop a method to determine the diameter distribution and the dispersion of CNT in various forms: powder, air samples, suspended in aqueous solution, and mixture of CNT and surfactant. Based on these measurements, the investigators will quantify the amount of CNT in a sample. This study seeks to provide a methodology to qualitatively and quantitatively determine a biologically-relevant metric of exposure associated with CNT material. The investigators indicated they would address this issue by using Raman spectroscopy combined with photoluminescence (PL).

US458 - Nanoaerosol Surface Area Measurement Methods. The overall objective of this project is development and evaluation of methods to measure the surface area of airborne nanomaterials with different physicochemical properties over a wide size range of interest. One objective of this project is to investigate the differences between instrument responses to spherical and non-spherical particles, as well as between sub-100 nm and super-100 nm particles. This work may permit extension of the existing theory of diffusion charging and of the instrument to surface area measurements on non-spherical particles. Overall, the complete characterisation of surface area instruments and methods, and their application to determining the toxicity of nanomaterials, will provide a basis for understanding whether surface area is a more appropriate measure than mass for evaluating toxicity.

EU009, the NANOSAFE2 project, is a large EU Framework project (FP6) which has the objective of developing an integrated system addressing potential hazards related to nanoparticles, for health and environmental protection. The project includes developing detection and characterisation techniques, hazard assessment, safe production processes and applications. Specifically in relation to this RO, they plan to optimise the ability of conventional particle counters based on scattering, electrical mobility and impaction to detect industrially manufactured nanoparticle leaks in the air at laboratory scale and fabrication level. The project is also investigating the possibility of capturing and detecting nanoparticles immersed in aqueous solvent with techniques based on specifically binding peptides. The project is due to be completed in 2009.

UK010, HSL NANOCHALLANGE, is a large internal investment programme by the UK's HSL. One of the 3 objectives is efficient sampling and monitoring. This part of the work will develop methods for the collection of airborne engineered nanoparticles in workplaces and their characterisation and discrimination against combustion by-products or ultrafines by transmission electron microscopy (TEM). It will also focus on the evaluation of where biological monitoring might be best applied and on contributions to exposure assessment and studies of the effectiveness of PPE. For the improved collection/characterisation of nanoparticles element of the programme, a start has been made on assessing collection methods (electrostatic and thermal precipitators made or refurbished for use in collecting nanoparticles in the NANOSH (**EU011**) exposure study).

Measurement programme

US176 - The Measurement and Control of Workplace Nanomaterials. The current version of this project was initiated in 2006 to address key research gaps in the NIOSH, CDC-NORA, and NIOSH-NTRC research portfolios on engineered nanomaterials. This project will provide a fundamental basis for understanding how nanomaterials are released and dispersed into the workplace, demonstrate the way nanomaterials can be monitored, and the way nanomaterial exposure can be controlled. Furthermore, this work provides NIOSH with the tools for making recommendations for reducing workers' exposures to nanomaterials.

Contribution towards the RO

Completed and near-completed studies

Method Development

US291 was completed in 2004 and has made a tremendous contribution towards knowledge about methods for assessment of exposure to nanoparticles. The work has resulted in a series of publications although it is not always absolutely certain that the

published work of the lead investigator over that period (Andrew Maynard) relates directly to this project. Nevertheless, a series of references can be identified which cover the scope laid out in the description of the project. These include the following:

- Comparing aerosol surface-area measurements of monodisperse ultrafine silver agglomerates by mobility analysis, transmission electron microscopy and diffusion charging (Ku & Maynard, 2005);
- Observation and measurement of anomalous responses in a differential mobility analyzer caused by ultrafine fibrous carbon aerosols (Ku *et al*., 2007);
- Exposure to carbon nanotube material: aerosol release during the handling of unrefined single-walled carbon nanotube material (Maynard *et al*., 2004).

This programme represents the most coherent and comprehensive portfolio of work to address these issues.

US046 will potentially also make a significant contribution towards the issues relevant to this RO. This project is focussed on the development of a method for quantifying exposure to carbon nanotubes, probably one of the most important, and at the same time challenging, issues in relation to occupational exposure. The approach being investigated in this project is based on adaptations of standard methodology for counting fibrous aerosols in workplace exposure scenarios. The approach suggested is excellent, and comprises an initial size separation of airborne CNT using an impactor and diffusion battery setup followed by image analysis of the collected samples by atomic force microscopy. In principle, this approach could lead to number concentration information for carbon nanotubes, whilst differentiating between tubes, ropes and other non fibrous particles. The successful implementation of this method would provide an invaluable tool in assessing potential occupational exposure to carbon nanotubes. We understand that this project was due to complete in 2008. At this point, however, we have been unable to identify any published outputs from this study and so it is not possible to say the extent to which the proposed approach has been successful.

Instrument Development

US101 is focussed on the development of a specific instrument which may be used to simultaneously measure particle size and electrostatic charge distributions in real time on a single particle basis. While methods for assessing particle size distribution (usually based on electrical charge) are available, this device, in principle, would offer great benefits in terms of the categorisation of aerosols, whether they be in workplace or the environment. Such a device would also have significant possibilities in relation to calibration or comparison with other instruments that use charge as a parameter from which to derive information about particle size. Whether such a device in the form projected would ever find application in the more routine measurement of exposure, either in the environment or an occupational setting, is much less certain at this point.

US204 and **US151** concern the development of aerosol mass spectrometry in which the size and the chemical composition of individual particles are assessed on a real time basis. The purpose of these projects is to extend the existing methods down into the sub 20 nm diameter range. In trying to characterise and differentiate between nanoparticles (engineered nanoparticles) released from a source and those released from the ambient environment, a device of this type could have many useful applications. It is, however, much more likely to be a research tool than a method which can be utilised in routine measurements of exposure.

US061 is a very ambitious study which undertakes to answer many questions in relation to workplace exposure. The study is now complete (end 2007). A specific objective, one of three, is development of a portable aerosol monitor, which if successful, will be an extremely useful outcome. However, although the project is completed, there is no available information that could be found describing this device. Other aspects of the work outwith the scope of this RO have been published more fully. It remains to be seen whether any such device will emerge over the coming months.

US144 is another ambitious project that is intended to provide the community with verified instruments and to look at issues of exposure control in the effectiveness of respirators involving both laboratory and field studies. Although this project is believed to be complete, we have yet to see any significant publication of the outcomes.

Measurement Programmes

US439 is a specific case within the wider NIOSH measurement programme (**US176)**. This particular project involved a measurement programme carried out at a carbon nanofibre plant and looked to correlate relationships between various metrics of aerosol exposure. The work is published as a NIOSH Health Hazard Evaluation Report (NIOSH, 2006) and provides useful experience for other groups seeking to measure in similar circumstances.

US331 is focussed not on engineered nanoparticles, but on incidental particles in the nanoparticle size range arising from foundry activities and engine plants. Despite this, there are potentially useful outcomes, including comparisons between various metrics including mass concentration and particle size. At the current time we are not aware of the published outcomes of these studies which would include relationships between these various metrics. Development of such relationships is clearly important in relation to this topic; however such relationships could not be applied to other particles without further validation. Nonetheless, it will be useful to observe these once they are published.

Ongoing Studies

It is much more difficult to assess the contribution the ongoing studies will make towards the RO. Only a few of these studies have produced anything beyond a statement of their objectives. In this section we will attempt to assess the extent to which the projects will contribute if the objectives are achieved.

Instrument development

The NANODEVICE project (**EU024**) is a highly relevant study aimed exactly at the specific objective of developing new instruments. It is intended to develop technologies that enable utilisation of new concepts in miniaturised and field-worthy specific monitors for NP. The project has a high level of investment (greater that 5 million Euro) and a strong project team involving leading current instrument manufacturers. The type of device(s) being proposed is highly relevant although, at this stage, it is not possible to comment on the range of application or limitations.

Method development/optimisation projects

US433 was expected to start in late 2008. This project is likely to be important in understanding or providing a basis for validation. The project aims to find a way to measure the surface area of the collected sample looking only at the particle type of interest, in this case titanium dioxide, thus discriminating this from background. Such a

technique would certainly be a useful addition to tools available, particularly if this approach could be extended to other NP of interest.

US427 aims to develop a method for dispersion and determination of diameter distribution of carbon nanotubes using Raman Spectroscopy. Based on this, it is intended that this project will provide the basis for quantifying the amount of CNT in a sample. Information on diameter distribution within a sample, coupled with information about the total mass, would provide the basis for estimating the total length of CNT within that sample. However, it would not provide any information on the individual lengths of CNTs. Whether this would be a useful biologically-relevant metric of exposure is not clear.

The overall objective of **US458** is development and evaluation of methods to measure the surface area of airborne nanomaterials with different physicochemical properties over a wide size range of interest. New instruments have recently appeared, based on diffusion charging, which provide a basis for real-time assessment of surface area (e.g. Shin, 2007). However, the interpretation of these instruments, their applicability and their limitations in relation to real workplace NP aerosols are largely unknown. This project aims at the complete characterisation of surface area instruments, methods, and their application and, if successful, will provide an important addition to the tools available.

In the NANOSH project (**EU011**), an outline sampling strategy for workplace exposure monitoring, including contextual information (sampling instruments, where to sample and for how long, etc.), has been agreed using experience gained in NANOSAFE 2 (**EU009**) and in NANOCHALLANGE (**UK010**). All partners have concluded pilot studies. The UK partner (HSL) carried out a monitoring programme in a university's nanotechnology laboratories in February 2007. Initial results show rush hour peaks in traffic in the detection of nanoparticles but no increase over background due to work activities. The difficulties of determining exposure to engineered nanoparticles are very clear. The initial results from the second monitoring campaign carried out in a different university using improved methodology included observations in one clean room where no particles were detected and in another where carbon nanotube agglomerate aerosols were detected. At the time of writing, this represents the only information available concerning the strategy which has been developed or the results obtained. In due course it is expected that, when published, the strategy and approached developed in these studies will have wide application.

Extent to which the RO has been met

Based on the studies already completed, very little progress has been made with this RO. With the notable exception of **US291** (which was completed in 2004 and has largely laid the groundwork for all of the studies discussed here), none of the completed studies have made any contribution of note up to this point despite the declared objectives being highly relevant. In some cases it may be that publications are still in preparation and will emerge over the next year or so. This in itself is disappointing, given that most of the studies are multi-year studies, that many appear to have been complete in 2007, and the urgent need for progress in this area.

One factor of note is that many of these studies have been highly ambitious, and in several cases it is clear that they have been inadequately resourced to deliver on the promised objectives. This may well be the case in other areas for other ROs, and may to some extent reflect on the newness of the area. Whether this is due to overoptimistic claims by the proposers or inadequate funding provided by the funders is not clear. Obviously all parties involved in this process need to be realistic about what can be

achieved and the resources necessary to achieve that. There is some evidence that this is not the case thus far.

The ongoing studies promise much. It is also clear that these are, in general, better resourced than the completed studies and so it might be expected that they will deliver more. If they are all successful in achieving all of their objectives then they could lead to:

- A new personal sampler or samplers for some types of NP;
- Improved measurement strategies, validated for some types of NP;
- A method for measuring the surface area of TiO_2, discriminating from background aerosol;
- A method for estimating total length exposure for CNT;
- Improved understanding on the range of application of real-time surface area measurement methods.

However, even if all of this is achieved there will still be a paucity of information in relation several key issues including:

- A validated method for real time measurement of high aspect ratio nanoparticles (HARN);
- An evaluation of whether fibre counting approaches can be used for HARN;
- A widely applicable method by which NP of interest can be discriminated from background;
- A method for assessment of the agglomeration state for NP aerosols;
- Information about a size selection cut-point for NP.

It is therefore concluded that much work remains to be done.

4.7 RO07 - FATE AND BEHAVIOUR (AIR)

The research objective

The main concerns in relation to safety of nanoparticles originated from existing knowledge on health effects from inhalation exposure to superfine particles, such as combustion-derived particles. In this regard, it is imperative to understand the fate and behaviour of NPs in air, and their aerial dispersal and interactions to enable assessment of potential exposure in the workplace and other scenarios. This research objective aims at developing an understanding of the fate and behaviour of NPs in air.

Overview of the types of studies which have been found to be relevant to this RO

Thirteen studies were sourced for this RO. Of these, two could not be assessed as there was insufficient information in terms of their outputs available. Of the remaining 11 studies, all were assessed using the Weight-of-Evidence framework and are discussed below. Four studies (2 completed or near-completed, and 2 ongoing) scored greater than the threshold of 24 in the WoE appraisal. These studies relate to determination and characterisation of NPs in the workplace, assessment of potential exposure to NPs in the workplace and effectiveness of personal protective equipment. The remaining seven completed, near-completed or on-going studies, whose aims and objectives are very relevant to this review, provided no outcome or experimental results to enable an assessment of their contribution to RO07.

The key studies

Completed or near-completed studies

Of the completed or near-completed studies considered, two are considered to be making a significant contribution (**US159** and **US251**).

Study **US159,** funded by NSF, is aimed at conducting a cross-media assessment of the transport, transformation, and fate of manufactured nanomaterials in atmospheric, aquatic, and terrestrial environments. The experiments focus on carbon NPs (fullerenes, endohedral metallofullerenes, carbon nanotubes). The study measured particle concentration inside and outside a facility where carbonaceous nanomaterials were produced. A high quality journal publication describes the study that measured mass concentrations of fine particles (PM2.5), and their size distribution and photoionisation potential. The measurements were also done for background particle concentration, and inside and outside the fume hood where NPs were produced. The results showed that average PM2.5 and particle number concentrations were not significantly different inside the facility compared to outdoors. The study indicated that the engineering controls at the facility were effective at limiting exposure to NPs. However, the study found considerable differences in particle mass and number concentrations between runs and between days. The results also indicate that physical handling of material results in the aerosolisation of ultrafine particles. The absence of carbonaceous particles outside the fume hood suggests that it is effective at containing NPs. The results of the study provide important information in the context of NP exposure in a workplace scenario.

Study **US251**, funded by NIOSH, aimed at developing partnerships, exposure monitoring instrumentation, operational protocols, and a comprehensive and detailed database of nanoparticles and their properties. The study is linked to an ongoing study (**US176**, highlighted below) and has produced a number of peer-reviewed publications and project reports that are relevant to exposure assessment of airborne NPs. The two projects have undertaken particle characterisation, estimation of possible emissions, routes of exposure, and effectiveness of personal protective equipment. For example, from a study on carbon nanofibers, NIOSH concluded that the potential for release of nano-scale materials does exist during various processes – such as during transfer of the nanofibers to a mixing vessel (probably aggregated material as particle sizes > 500 nm), and the use of wet saw. The report also makes recommendations for controlling the exposure to NPs in different workplace scenarios.

DK011 is attempting to understand the atmosphere-chemical reactions risk of exposure to chemicals and nanoparticles during use of nanoparticle-base pump and spray products as well as screening their potential cellular and respiratory effects *in vitro* and *in vivo* at different user scenarios.

One further near-completed study, **US058** is conducting comparative life cycle analyses of nano- and bulk-materials used in photovoltaic energy generation. However, no outputs were available for appraisal and as a result, the study's contribution to the RO was considered to be low at this time.

Ongoing studies

Of the on-going or starting studies, two are considered to be making a significant contribution towards RO07 (**DK001** and **US176**). Study **DK001** aims to assess exposure and toxicological properties of nano materials and products in the plastics industry. A peer-reviewed publication is available (Jensen *et al*., 2009) that relates to

the measurement of dustiness of nanoclay (Bentonite) and an organically modified nanoclay (Nanofil_5). The study has determined particle size distributions using different particle sizers. The results of the tests have shown intermediate dustiness indices (1,077 - 2,077 mg/kg) for Nanofil_5, Bentonite, and compacted Bentonite, and a high dustiness index for compacted Nanofil_5 (3,487 mg/kg). All powders produced multimodal particle size distributions in the dust cloud with one mode around 300 nm (Bentonite) or 400 nm (Nanofil_5) as well as one (Nanofil_5) or two modes (Bentonite) with peaks between 1 and 2.5 μm. Tests also showed that low-pressure compaction may reduce the risk of particle exposure if powder handling operations involve minimum agitation. The results of the study will be very useful for applications of NPs in packaging materials that have been projected to see a major increase in the coming years.

Study **US176**, funded by NIOSH, is aimed at providing a fundamental basis for understanding how nanomaterials are released and dispersed into the workplace, and to demonstrate the ways for monitoring and control of exposure. Information available so far on exposure assessment of airborne NPs (e.g. carbon nanofibers) suggest that the study has undertaken particle characterisation, possible emissions, routes of exposure, and effectiveness of personal protective equipment. The study also intends to make recommendations for exposure control in workplace scenarios.

For the remaining ongoing studies considered, either only a statement of the objectives or no evidence is available, and hence it has not been possible to fully appraise the contribution of the studies towards the RO. These include **US015** conducting fundamental research of nanoparticle formation in air pollution; **US316** examining nanoparticle processes in the atmospheric environment; **CH019** conducting an analysis of nanomaterial exposure of humans in Switzerland, identifying frequent situations for exposure to today's and possible future use of consumer products on the basis of nanomaterials; **US254** studying experimental and numerical simulations of the fate of airborne nanoparticles from a leak in a manufacturing process to assess worker exposure; **US065** conducting an ultrafine particle intervention study in automotive production plants; and **US147** studying multiscale perspective of carbon nanoparticles in combustion.

Extent to which the RO has been met

The few published articles and reports provide some preliminary information in relation to the determination and characterisation of NPs in the workplace, assessment of potential exposure to NPs in the workplace, and effectiveness of personal protective equipment. Most studies, however, relate to one or two types of NPs and it is unclear whether it will be possible to extrapolate the results to other types. Judging from the aims and objectives of the ongoing studies, it is likely that more relevant scientific evidence to support this RO will emerge in the coming years. The main elements that so far seem to be scarce or missing in relation to the RO include valid analytical tools that can be used in detection and quantification of different NPs in aerial environments in the workplace, and in other emission scenarios. There is also a need for systematic studies on different types of airborne NPs using a range of physicochemical parameters (e.g. size, shape, form, surface area) to generate data on their interactions, fate, behaviour, and dispersal, to allow development of reliable models. It is, nevertheless, reassuring that a few studies carried out so far have indicated the effectiveness of engineering controls and personal protective equipment in preventing or minimising NP exposure in a workplace setting.

4.8 RO08 - EXPOSURE CONTROL DEVICES

The research objective

The formal title of this Research Objective is the development of exposure control devices. This is narrower than could have been applied here. One interpretation of this could be the development of exposure control methods. In a wider definition this would include not just the development of devices such as filtration systems or personal protective equipment, but also management systems, control banding and other activities of that nature. However, in reviewing this RO we have chosen to follow the stated narrower definition of devices.

Important specific questions are as follows:

i). Can nanoparticles pass through respirator filters?
ii). Related to this, can nanoparticles pass through filtration systems used in air cleaning devices?
iii). Can nanoparticles pass through personal protective clothing?
iv). Are workplace engineering control systems (enclosure, ventilation, filtration) effective at reducing or exposure to NP?

An overriding caveat here is that the issue is not just whether nanoparticles can pass through these devices but can be passed round them. Failure of personal protective equipment such as respirators is more likely associated with passage of particles around the device rather than through it.

Overview of the types of studies which have been found to be relevant to this RO

Of the 21 studies originally identified, five were excluded as not being at all relevant to the RO. Eleven studies were of limited relevance, or in the case of completed studies had no assessable output, and scored less than 20 in the Weight-of-Evidence assessment. Five scored 20 or more and were considered to have made or to be making a significant contribution towards the RO and these are appraised in detail below.

Several types of studies could be envisaged as contributing towards this RO. They could include:

- Studies which assess the performance of current devices against the range of nanoparticle challenges;
- Studies which attempt to understand fundamental performance issues relating to these devices through modelling and laboratory investigation;
- Development of new improved devices specifically designed to have enhanced performance in the nanoparticle size range.

Examples of all of these three types of studies have been found and will be discussed below. One further clarification relates to studies carried out in workplaces. In principal, any study which assesses workplace exposures provides an implied evaluation of the control methods which have been used in that workplace. In this sense all such exposure studies could be considered as contributing towards this RO. However, these studies are dealt with in more detail in other ROs, including RO05 and RO06. For clarity, we have not included discussion of these studies within RO08.

The key studies

Completed studies

US216 investigated the penetration of nanoparticles through respirator filter media focussed on particles less than 20 nm. **US083** also investigated penetration of nanoparticles through respirator filter media, with a focus on particles of a few nm. This study took a more mechanistic approach and investigated specifically whether single-fibre filtration theory is valid for engineered nanoparticles, the possible boundaries of the most penetrating particle size range, and the filtration boundaries of nanosized particles in the diffusional capture mechanism range.

A recent publication from the University of Minnesota (Pui *et al.* 2008) reports on the effectiveness of simple in-car filtration systems. The paper, "Recirculating Air Filtration Significantly Reduces Exposure to Airborne Nanoparticles," characterises the reduction of nanoparticle number concentrations when using the recirculation mode of the ventilation system in two types of cars. In simulated heavy-traffic situations, where particle number concentrations can be greater than 50,000 particles/cc for particle diameters less than 50 nanometers (nm), the authors determined that setting the car's air ventilation systems in recirculation mode can decrease the number concentrations to background levels of less than 4000 particles/cc in approximately 3 minutes.

Ongoing studies

US041 is investigating the significance of bypass leakage in filtration systems and, in particular, how this affects exposure to nanometre-scale aerosol particles when recirculation systems are used. Fundamental research to investigate bypass leakage (i.e., aerosols passing around a filter without control) and recirculation (i.e., routing a filtered effluent back into a workspace) is being conducted using experimental and mathematical techniques.

EU028, the NANOSAFE2 project, is a large EU Framework project (FP6) which has the objective of developing an integrated system addressing potential hazards related to nanoparticles, for health and environmental protection. The project includes developing detection and characterisation techniques, hazard assessment, safe production processes and applications. Specifically in relation to this RO, the study is investigating the performance of respirators and protective clothing (gloves and suits) against NP.

US021 is a study developing bench and mist protocols for particulate measurements of protective clothing and ensembles. In order to determine how well ensembles protect wearers, it is necessary to test the entire suit system while it is worn to measure potential leakage through seams, closures, areas of transition to other protective equipment, and any leakage due to movement and activities. The objective of this study is to develop innovative methodology for standardising both bench-scale testing and man-in-simulant test (MIST) procedures for aerosol particle penetration through protective clothing and ensembles.

Contribution towards the RO

Completed studies

Studies **US216** and **US083** have addressed the issue of penetration of nanoparticles through respirators. Filtration theory indicates that commercial respirator filters should be efficient collectors of particles in the nm size range due to capture of particles by diffusion (e.g. Aitken *et al*., 2004). In fact conventional filtration theory suggests that the

most penetrating particle size is likely to be of the order of 300 - 500 nm, which represents a minimum between the mechanisms of diffusion (greater for smaller particles) and impaction (greater for larger particles). For this reason, methods for commercial approval of respirator filters routinely use a challenge aerosol in this size range. However, it has been suggested that this model may break down at particle sizes on a few nm due to a process called thermal rebound (Wang and Casper, 1991).

US216 focussed on developing a methodology by which the penetration of NP through commercial respirator filters or filter materials could be measured and using that to evaluate the performance of commercially available respirators or materials. **US083** took a more fundamental approach, looking at the limitations of single-fibre filter theory.

Relating to **US216**, Rengasamy *et al.* (2008) describe a study investigating the filtration performance of NIOSH-approved N95 and P100 filtering facepiece respirators against six different monodisperse silver aerosol particles in the range of 4-30 nm diameter. They constructed a particle test system which could deliver and measure penetration of monodisperse silver particles. Five models of N95 and two models of P100 filtering facepiece respirators were challenged with monodisperse silver aerosol particles of 4, 8, 12, 16, 20, and 30 nm at 85 L/min flow rate and percentage penetrations were measured. Consistent with single-fibre filtration theory, N95 and P100 respirators challenged with silver monodisperse particles showed a decrease in percentage penetration with a decrease in particle diameter down to 4 nm. Penetrations less than 1 particle/30 min for 4-8 nm particles for one P100 respirator model, and 4-12 nm particles for the other P100 model, were observed. Small, but significant, differences were seen in the results obtained between silver nanoparticle and NaCl particles (the usual test aerosol for commercial testing). The authors state that the filtration data for 4-30 nm monodisperse particles supports previous studies that indicate NIOSH-approved air-purifying respirators provide expected levels of filtration protection against nanoparticles.

Less information was available about **US061** (described under the Enabling studies chapter) and no attributable project report or peer reviewed publication could be identified. From information from the UK's HSE, who were co-sponsors of this project, the test method developed was based on a distribution of particle sizes, rather than discrete monodisperse aerosols. However, these authors also found that N95, N100, and P100 filter media in general perform within their specified filtration efficiency to aerosol nanoparticles diametre < 100 nm.

In **US083**, Shin *et al*. (2008) used a high temperature filtration test system that has been developed to characterise single fibre collection efficiency for monodisperse silver particles over the size range from 3 to 20 nm. A stainless steel screen mesh was used as the test filter to determine whether the classical single fibre efficiency model can be applied to filtration process at elevated temperatures and to determine whether thermal rebound occurs as a result of increasing the thermal speed of the particles. Thermal rebound of particles from a filter surface was predicted to occur at 500K for 3 nm diameter particles based on a theory by Wang and Kasper (1991); however, rebound was not detected in this study.

Pui *et al*. (2008) shows that even relatively crude filtration systems (In car pollen filters) can be highly effective in removing NP.

Ongoing studies

Preliminary results available from the NANOSAFE2 project (**EU028**) are reported in a dissemination report "Efficiency of fibrous filters and personal protective equipments

against nanoaerosols" available from project website (www.nanosafe.org). Few details are provided in this summary level report. Results for HEPA type glass fibre filters challenged by graphite particles show increasing filtration efficiency with decreasing particle size down to 10 nm.

NANOSAFE2 also investigated the performance of skin protective equipment, namely gloves and protective suits. For five types of gloves, nitrile, neoprene, vinyl and two types of latex gloves, test showed significant penetration of 80 nm particles, when tested in a diffusion cell system. 30nm particles showed much lower penetration, although reasons for this were not discussed and there is insufficient information to comment further. The authors recommend that at least two layers of gloves are worn when handling nanoparticles. Tests on suit materials showed that penetration through cotton and paper type suites was substantially greater than though nonwoven type suits (Tyvek, Tychem).

Study **US021** is also investigating the performance of protective suits but the method being developed will test the whole suit, including seals and fastenings, by carrying out a simulated wearing exercise. This will then test the possibility of NP passing around the protective clothing, not just through it. No published data are yet available from this project. Study **US041** is also concerned with leakage around a filtration system, in this case filter units rather than suits, rather than through it. The outcomes of this study will be important in trying to determine the level of leaktightness required in filtration systems.

Extent to which the RO has been met

Given the importance of controlling exposure (no exposure equals no risk) it is disappointing that relatively few studies have been identified which are making a significant contribution towards this RO. This review has only identified a small number (six) of relevant studies. All but one of these studies are being carried out in the US. Most of these studies have investigated the penetration of NP through respirator filters and other (HEPA) filters used in air cleaners. Consistently these studies have found that, as theory predicts, filters are highly efficient collectors of NP and that the efficiency of collection increases as particle size reduces, even down to 4 nm. This seems to form a good basis for understanding that respirators will be effective in removing NP from the air which passes through them. There remains an issue for NP less than 2 nm. Based on the results on the single study which has investigated this, there is still sufficient uncertainty to warrant careful attention to this issue and further investigation. Based on the studies available, we have no information concerning the potential leakage around respirators (face seal leakage) although one ongoing study is addressing this. For Skin Protective Equipment (SPE), such as gloves and suites, evidence of poor performance has been found in one study, based on laboratory tests, and a recommendation to use double gloves has been made. Much more information is required here as to what actually works, in what circumstances and what are the limitations. None of the studies of personal protective equipment has thus far looked at human factors and how these systems can work in a practical sense. Information on this is needed. No studies have been carried out which systematically address the performance of engineering controls which have been implemented in practical settings.

4.9 RO09 - EXPOSURE MEASUREMENT (SOIL AND WATER)

The research objective

Optimisation, development and application of technologies that enable the measurement of exposure to nanoparticles in soil and water.

Overview of the types of studies which have been found to be relevant to this RO

Only one study was identified to be of relevance to this RO, which scored 18 or more in the WoE assessment. There is a great deal of activity in the area. However, possibly due to the generality of the topic, the research knowledge available is dispersed and poorly-focussed.

The key studies

Completed studies

No completed studies were identified in this RO.

Ongoing studies

One on-going study was identified for this RO, study **UK113** 'Model nanoparticles for environmental risk studies'. The abstract describes a method for detecting effect of oxide nanoparticles on marine and freshwater bivalves using a combination of techniques. TiO_2 and ZnO nanoparticles will be synthesised and characterised as reference materials. The project, due to conclude December 2008, should contribute towards the methodology used in environmental impact. Techniques to be used are not described in any substantial detail.

Contribution towards the RO

Ongoing studies

UK113 is a relatively small project based on the effects of TiO_2 and ZnO nanoparticles, but may be more widely applicable if the measurement approach provides information towards a general solution.

Extent to which the RO has been met

It is apparent from the lack of available information that this RO has been largely overlooked by groups working in the general area. A recent SETAC event (September 2008) included in its publicity matter the following: 'While the production of nanomaterials is undergoing exponential growth, their biological effects and environmental fate and behaviour are relatively unknown'. The need for new measurement techniques is clearly important, so it would appear that the need has not yet been met and there remain significant knowledge gaps.

Several groups are working in this area, such as The Brookhaven National Laboratory in the USA, BAUA (Bundesanstalt fur Arbeitschutz und Arbeitsmedzin) in Germany and EMPA in Switzerland. EMPA has undertaken a series of studies looking into the impact of engineered nanoparticles on the environment as a whole (Figure 4.3).

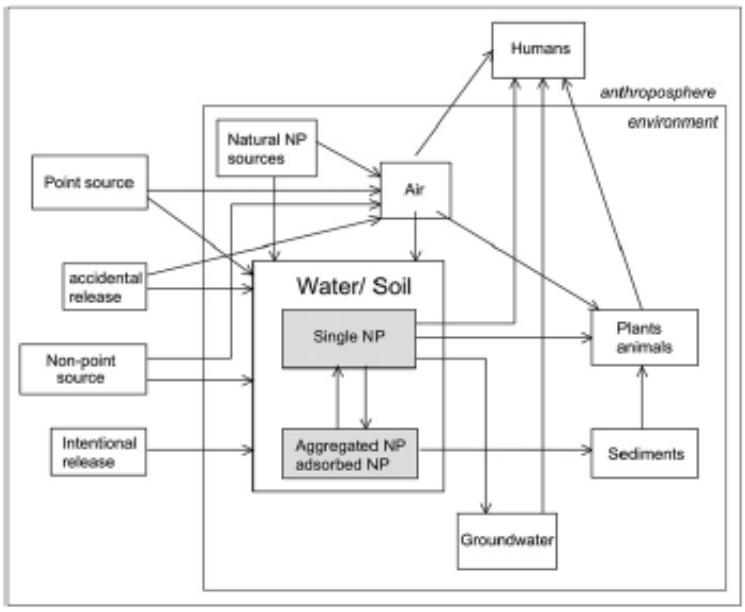

Figure 4.3: Nanoparticle pathways from the anthroposphere into the environment, reactions in the environment and exposure of humans (Source: EMPA).

This approach has included life cycle reviews of the effect of specific nanomaterials such as carbon nanotubes, nanotitania and nanosilver for which flow diagrams on their impact have been constructed (e.g. Figure 4.4). A similar approach has been adopted to study the effects of nanosilver incorporated in antimicrobial products (Blaser *et al*. 2008).

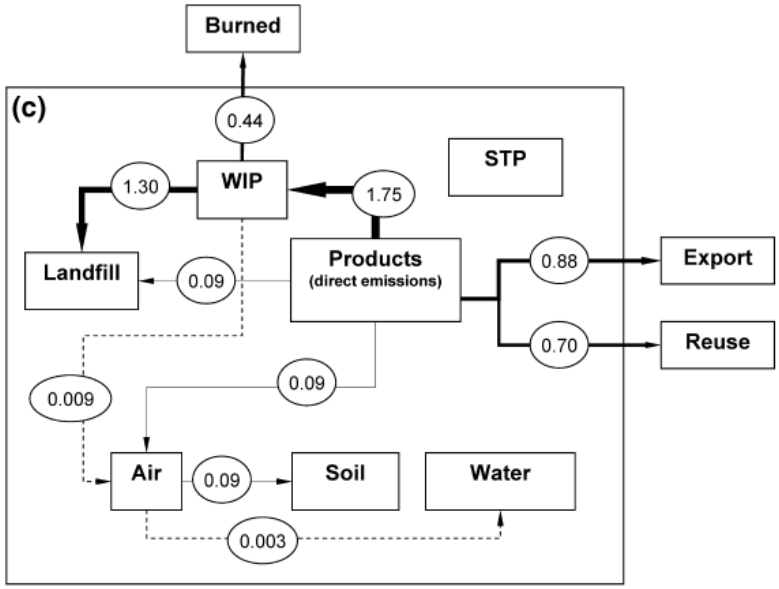

Figure 4.4: Carbon nanotube flows from the products to the different environmental compartments, WIP (waste incineration plant), STP (sewage treatment plant), and landfill (high exposure scenario). All flows are in tonnes/year. The thickness of the arrow is proportional to the amount of material flowing between the compartments. Dashed arrows represent the lowest volume. (Source: EMPA)

The ISO Technical Committee 229, Convener Work Group 3 (Environment, Health and Safety) is also focussed in this area.

Current calls in the area from the EPSRC (Nanometrology and nanotoxicology) and EU Frameworks Programme 7 (Risk assessment of engineered nanoparticles on health and the environment) may contribute resources to this area. For example, two projects entered in the latter call are Long-Term Impact of Engineered Nanoparticles on Environment and Health (nano-LoTIoN) and ENgineered Nanoparticle Impact on Aquatic Environments: Structure, Activity and TOXicology (ENNSATOX), which seek to address the issues of measurement techniques in soil and/or water.

There are likely to be opportunities for characterisation and modelling techniques developed for other fields to undergo transfer into techniques for the analysis of exposures from particles in soil and water.

4.10 RO10 - FATE AND BEHAVIOUR (SOIL AND WATER)

The research objective

This Research Objective aims at developing an understanding of the environmental fate, behaviour and interaction of nanoparticles in soils and water. It is inevitable during manufacture, use and disposal of nanotechnology products that there will be emissions of nanomaterials into the environment. Whilst there is a body of knowledge on dispersal, breakdown, persistence, accumulation etc. of conventional chemicals in the environment, little is known about the potential fate and behaviour of nanomaterials. It is, nevertheless, imperative to understand such mechanisms to assess the current and future environmental exposure to free nanoparticles.

Overview of the types of studies which have been found to be relevant to this RO

Thirty-eight studies relevant to RO10 were initially identified. Three studies were excluded on the basis of being outwith the scope of the RO. Twenty-four studies scored less than 16 in the Weight-of-Evidence. Eleven studies (3 completed, 8 ongoing) scored an overall assessment score of 16 or above and are highlighted in the discussion below.

The studies can be split into two general types:

i). establishment of expert networks to review the state-of-the-art concerning knowledge of environmental fate, behaviour and interactions of nanoparticles in soils and waters as well as development of databases and delivery of workshops and training;
ii). experimental studies to understand the behaviour of nanoparticles in environmental systems.

Experimental studies are exploring a range of nanoparticle types including natural nanomaterials, aged and new materials, fullerenes, carbon nanotubes, silica nanoparticles, metal oxides (titanium oxide, zinc oxide, manganese oxide) and metals (iron, silver). The effects of different particle characteristics, such as size, are also being investigated. Work is being done with unlabelled nanomaterials using a range of detection techniques (FFF-ICP-MS, electron microscopy, DLS) and, in some instances, radiolabelled materials are being employed in the experiments.

The research studies are exploring a range of fate and behaviour processes, including aggregation behaviour, transformation reactions, deposition behaviour, leaching behaviour and uptake into organisms (including soil bacteria, eukaryotes, worms, frogs and aquatic invertebrates) and through food chains. Different environmental systems

are being explored, including surface waters, soils, air, water-sediment systems, water treatment systems and soil/water-organism systems at different levels of complexity (e.g. tightly controlled experiments with laboratory water *versus* real waters; studies with well characterised porous media *versus* studies with real soils and sediments).

The effects of environmental variables such as pH, ionic strength, dissolved organic carbon content and light intensity are being established. In addition, the effects of other anthropogenic substances (e.g. surfactants and detergents), that are likely to occur in the environment alongside nanoparticles, on particle fate and behaviour is being investigated. A number of ongoing studies are looking at how nanoparticles will affect the fate and behaviour of other environmental contaminants, including whether they will affect uptake of these contaminants into organisms.

A number of studies are attempting to develop modelling approaches for the different processes that are being investigated. In the future, it is likely that these models will be invaluable in understanding the impacts of engineered nanoparticles on the environment.

The key studies

A brief account of the high quality evidence is presented below. These studies have investigated detection, characterisation and behaviour of selected manufactured and natural nanoparticles in aquatic systems, interactions of carbon-based NPs with soils and effects on soil microbial communities, the role of nano-scale colloids in particle aggregation and trace metal contaminants, and interaction of environmental contaminants with a range of NP types using modelling and experimental investigations. The outcome of the studies provide important data and information with regard to the fate and behaviour of NPs in the environment.

Completed or near-completed studies

Study **UK095** aimed at investigating the chemistry of NPs under realistic environmental conditions and how this relates to aggregation and sedimentation, which are key processes in environmental transport. The study looks at the detection, characterisation and behaviour of selected manufactured and natural nanoparticles in aquatic systems. The outputs have been published in a number of peer-reviewed articles and in the future should help to better understand the ecotoxicology of nanoparticles.

UK136 aimed at studying C60 fate and transport in natural soils, and developing a numerical simulator to describe the mechanisms. The project is exploring transport and fate of C60 fullerene in saturated and unsaturated soils. Detection of nanoparticles is by DLS and UV. A number of peer-reviewed publications have been produced.

US159 aimed at conducting a cross-media assessment of the transport, transformation, and fate of manufactured nanomaterials in atmospheric, aquatic, and terrestrial environments. The experiments focus on carbon NPs (fullerenes, endohedral metallofullerenes, carbon nanotubes). The focus seems to be on air, with some work on soils, and has therefore also been discussed in RO07.

US259 aimed at providing fundamental information about the impact of carbon NP on water, soil and subsurface ecosystems. The study has explored the interactions of carbon-based NPs with soils and effects on soil microbial communities.

US295 aimed at studying the interactions of an iron-reducing soil bacterium with iron(III) oxide nanoparticles. The study is looking at interactions of natural nanoparticles (although interim report indicates work on engineered NPs) with soil

bacteria. The potential for the nanoparticles to facilitate transport of metal contaminants into organisms is also being investigated.

US243 aimed at investigating the structural dynamics of aggregates of nano-scale colloidal particles and the role of the nano-scale fibrillar biopolymers in inorganic colloid aggregation. The study has explored the role of nano-scale colloids in particle aggregation as well as the interaction of trace metal contaminants with these colloids. A number of journal articles have been produced as well as a final project report.

UK100 aimed at determining the mobility of metal oxide nanoparticles in fresh groundwater systems. The study is looking at the mobility of metal oxide nanoparticles in groundwater but the project is not yet complete and no outputs were available.

US170 is investigating the structural dynamics of aggregates of nano-scale colloidal particles and the role of the nano-scale fibrillar biopolymers in inorganic colloid aggregation. The proposal addresses the role that these structures play in the transport of trace metals and pollutants in natural environmental and engineered systems. The combination of experimental studies encompassing microscopic methods (AFM and EM), synchrotron-based methods (SAXS, XAS) and multi-scale modelling will aid in elucidating the significance of particulate material in aquatic systems. The project is collaborative and multi-disciplinary, combining expertise from chemistry, biology, and modelling to investigate the role of fibrils and macromolecules in inorganic colloid aggregation. Results of this work should help in the understanding the self-cleansing capacity of fresh, estuarine and marine waters through transport and sedimentation. Of special interest is the understanding of the similarities and differences between freshwater and seawater. A number of journal articles have been produced as well as a final project report.

Lastly, **UK059** aimed at quantifying interactions between NPs and wastewater biosolids, and to model their fate with a mechanistic model. The aim of project is to understand the fate of nanoparticles in wastewater system. A range of particle types (metal-oxide, quantum dots, C60 fullerenes, carbon nanotubes) will be studied. Some modelling work is described.

Studies identified but which presented little or no information on which to conduct an appraisal, and scoring 16 or less in the Weight-of-Evidence appraisal, included **US126** assessing the fate of nanomaterials in aquatic systems and the interaction with other contaminants using cell culture assays to assess health risks; **US124** studying lung cells exposed to metals in nanoparticles and combining cell culture models and toxicology assays; and the inter-related studies **US241**, **US242**, **US243**, and **US272** investigating the role of nano-scale colloids in particle aggregation and trace metal contaminants, and interaction of environmental contaminants with a range of NP types (including fullerenes in aquatic systems) using modelling and experimental investigations.

Ongoing studies

US228 aimed at investigating the aggregation and deposition behaviour of CNT in aquatic environments. The study is exploring the behaviour of carbon nanotubes in aquatic systems. A number of journal articles have come out of the work. The effects of a range of particle properties (including functionalisation, size, aggregation state and aqueous solution characteristics) are reported to affect rates of aggregation and deposition.

For many organic chemicals, photodegradation is a significant environmental fate process, and information regarding the rates and products of these reactions are

therefore important in overall risk assessment analysis. The overall objective of study **US003** is to investigate photochemical transformation of buckminsterfullerene (C60) and single wall carbon nanotubes (SWCNT) under conditions of environmental relevance. Due to the strong light absorbance of these materials within the solar spectrum, photochemical transformation in the environment may lead to potentially more water soluble and easily bioaccumulative products. The three sub-objectives are: i) to measure photochemical transformation rates and products of C60 solid films hydrated with aqueous solutions under solar irradiation; ii) to measure solar photochemical transformation of C60 in aqueous humic acid solutions and as clusters in aqueous solution; and iii) to extend these measurements to include the photochemical transformation of single walled carbon nanotubes (SWCNTs) under similar conditions. Natural sunlight (West Lafayette, IN, 40° 26' N, 86° 55' W) and solar-simulated light (λ = 350 ± 50 nm black light phosphor lamps) will be used as the light sources for all photochemical experiments. In most experiments solutions or cluster suspensions of C60 or SWCNTs will be irradiated and the loss rate, product formation rate(s), and spectroscopic and microscopic characteristics of the solutions will be monitored. Initial experiments will examine photodegradation of C60 and SWCNT plated onto test tube walls, hydrated with water containing various naturally occurring substances (i.e., carbonates, humic acids, O_2), with later experiments examining photodegradation of aqueous clusters in the presence of these same substances. Decay and product formation will be followed by HPLC, microscopy (SEM, HRTEM at the Pacific Northwest National Laboratory), and spectroscopic methods. Photochemical experiments with C60 and the more complex SWCNTs in environmentally relevant water-containing solutions, and the identification of reaction products in such experiments, will help assess the potential hazards of these materials when they are released into the aquatic environment.

Ongoing studies identified and worthwhile noting, but which presented little or no information on which to conduct an appraisal and scoring 16 or less in the Weight-of-Evidence appraisal, included:

UK097 - exploring the fate of SiO_2 NPs, arising from use in cosmetics and personal care. The study is nearing completion and no data are yet publicly available. When data are available, they should be helpful in understanding the ecotoxicological implications of SiO_2 nanoparticles in use in consumer products.

US107 - exploring the interactions of colloids with unsaturated porous media. The study involves both experimental and theoretical modelling work.

CH012 - looking at the effects of biopolymers, detergents and biosurfactants on CNT and fullerene dissolution. This data will be valuable in understanding the ecotoxicological implications of these nanoparticles. No data are yet available from the study.

US155 - exploring the interactions of nanoparticles with a range of environmental interfaces (e.g. colloids, atmospheric particulates). It involves both experimental and modelling work. If successful, the developed models should assist in predicting the behaviour of nanoparticles in environmental systems in the future.

US131 - exploring the fate and effects of nanoparticles in aquatic systems. Modelling approaches are being developed.

US123 - exploring the fate and transport of engineered nanoparticles (TiO_2 and Fe) in porous media.

US001- looking at the fate and bioavailability of carbon nanotubes. Work is being done with radiolabelled nanotubes.

US005 - exploring processes of uptake of CdSe and TiO_2 nanoparticles in bacteria and eukaryotes. No output yet available but will involve extensive in situ characterisation.

US056 - exploring the effect of surface coatings on fate, bioavailability and effects of Fe based nanoparticles.

US127 - exploring the fate of nanomaterials in the vadose zone. It combines adsorption/adhesion studies, deposition/dispersion investigations and transport studies.

US181 - exploring the fate and transformation of C60 Nanoparticles in Water Treatment Processes.

US188 - looking at the fate of Fe nanoparticles in the environment.

US154 - exploring the transport and microbial impacts of a range of nanoparticles (fullerenes, TiO_2, ZnO, Fe(0)).

US271 - exploring the aggregation of fullerenes in aquatic systems. Study combines experimental work as well as modelling.

US309 - exploring the uptake and effects of a range of nanoparticles into a terrestrial food chain (worms, frogs). Effects of particle characteristics on bioavailability and routes of uptake are being assessed.

EU022 - synthesising and characterising nanoparticles and considering human toxicological and ecotoxicological effects. The study will look at bioaccumulation/bioavailabiliy and explore effects at biomarker through to population level.

US133 - exploring the fate of a range of metal based nanoparticles in aquatic systems, as well as uptake into daphnids and Hyallela.

US158 - attempting to understand the physical and chemical properties of manganese oxide nanoparticles.

Contribution towards the RO

It is clear from the assessment of available evidence that a significant number of projects are now looking at those factors and processes that might affect the fate and behaviour of a wide range of nanoparticles in soils and waters. These studies are likely to provide experimental data and modelling approaches that support the RO. However, the majority of these projects are ongoing and detailed outputs are only available for a few projects. We are therefore some way from meeting the RO. There also seems to be significant overlap between many of the ongoing projects so it might be more appropriate to work in a more co-ordinated manner in order to make the best use of the financial resources available.

The main elements that so far seem to be scarce or missing in relation to the RO include valid analytical tools that can be used in detection and quantification of different NPs in complex environmental matrices. There is also a need for systematic studies on different types of NPs using a range of physicochemical parameters (e.g. size, shape, form, surface area) and environmental variables (e.g. pH, ionic concentration) to generate data on fate and behaviour of NPs to allow development of reliable models. There is also a need for assessment of human and environmental exposure in a full lifecycle analysis approach to identify critical stages that can lead to exposure to free NPs and that can be targeted for risk management.

Extent to which the RO has been met

The majority of projects are ongoing and detailed outputs are only available for a few projects. We are therefore some way from meeting the RO. There also seems to be significant overlap between many of the ongoing projects so it might be more appropriate to work in a more co-ordinated manner in order to make the best use of the financial resources available.

4.11 RO11 - TOXICOKINETICS

The research objective

RO11 - Research to establish a clear understanding of the adsorption of nanoparticles *via* the lung, skin and gut and their distribution in the body (i.e. toxicokinetics), identifying potential target organs/tissues for toxicity assessment.

Overview of the types of studies which have been found to be relevant to this RO

Forty-four studies were sourced for RO11. Out of these studies, 18 could not be assessed as there was insufficient information in terms of their outputs available; most of these came with a title only or an inadequate statement of objectives. Of the remaining 26 studies assessed using the Weight-of-Evidence framework, fourteen scored 12 or above and twelve studies scored less than 12. These are discussed below. The types of study were predominantly *in vivo* and *in vitro* studies, with a couple of modelling studies.

Despite studies being completed, as regards their stated timelines, the vast majority supplied no peer-reviewed publications or supplied publications that were not relevant to the projects considered here.

The key studies

Completed or near-completed studies

In studies **US193** and **US048** the same workers developed an animal/cell exposure system for welding fume NP generation and published a number of studies documenting its use. They have studied inflammatory effects in the lungs and different types of fume and have reviewed the literature on the effects of inhaled metals on the brain. Judging from the research published so far on the lungs and the progress report, this group will shortly be publishing findings pertaining to translocation, neurological and cardiovascular effects in the peer reviewed literature.

US115 has addressed the potential effects of manufactured nanomaterial aerosol on human health, investigating and comparing these to ultrafine carbonaceous particles typically found in the environment from combustion processes. The researchers have repeated the studies of Oberdorster *et al*., and shown that NP TiO_2 causes inflammation following a modest exposure. However, they did not use larger TiO_2 particles for comparison.

US156 has several outputs that are important and which appear in peer-reviewed journals. Research findings suggest that: i) mainly iron mediates the pro-inflammatory effects of CNT *in vitro via* oxidative stress, induced due to Fenton chemistry; ii) instilled CNT produce severe fibrosis and inflammation; iii) dispersion of CNT makes them more inflammogenic in rat lungs; and iv) the inflammatory effects of quantum dots.

US249 evaluated several types of nanoparticles for toxicity in the conducting airways and alveolar region of the lung, and investigated their mechanisms of toxicity by comparing the effects of pulmonary exposure to fine versus ultrafine metal oxide particles. The data obtained will contribute to the development of a toxicological database necessary for hazard identification and to the NIOSH Nanotechnology and Health and Safety Research Program. The project will provide data to NIOSH, OSHA, and EPA for nanoparticle exposure risk assessment and prevention. This project has extensive aims in understanding nanoparticle toxicology. To date, one peer reviewed paper has described a dispersion medium for nanoparticles that uses albumen and phosphatidyl choline, biochemicals found in the lungs.

UK030 examined a range of common bulk nanoparticle types for their ability to cause oxidative stress and genotoxicity. This study in fact comprises two integrated projects, both funded by the Colt Foundation, in which a panel of bulk NP are assessed for their intrinsic free radical activity and their toxicity. Toxicity was assessed *in vitro* and *in vivo* and attempts made to relate the toxicity to the intrinsic free radical activity. There was no simple relationship between the two variables. Whilst the most free radically active particle nickel oxide was inflammogenic, only one of three aluminium oxide NP types, which had no free radical activity, was also inflammogenic. The *in vitro* test of haemolysis did predict the inflammogenicity better than the intrinsic free radical activity. A paper was submitted to Environmental Health Perspectives in July 2008.

EU030, a soon to be completed study, has set out to investigate the possible adverse health effects from exposure to NP and will aid in developing novel methods for NP risk assessment, specifically new toxicological methods for emerging NP evaluation; new information to help regulate environmental exposure to NP.

US095 sought to characterise the mechanisms which can translate into systems study and address the human health risks associated with exposure to nanomaterials that are of specific interest to Air Force, such as nanosize toxicity effect of aluminium and silver nanoparticles. The specific aims include: i) characterisation of nanomaterials before, during and after biological interaction (these experiments are designed to understand the physicochemical nature of these particles and their impact on producing toxicity); ii) evaluate nanomaterial toxicity using *in vitro* cell models, such as phenotype under concern of exposure: macrophage, liver, PC12 cells, T-cell (Jurkat cell); and iii) analyse toxicogenomics of mammalian cells exposed to NPs. The proposed biochemical and genetic endpoints were expected to provide a comprehensive view of how NPs are toxic to different cell types (phenotypes) and the molecular mechanisms involved. The assays proposed represent vital biological functions and were expected to provide a general sense of the impact of NP on cells. Using quantitative measures of cell viability, mitochondrial function and redox status, NP were classified as to whether they are of high, intermediate or low toxicity when compared to a control. Generation of reactive oxygen species, reduced glutathione and mitochondrial membrane potential were measured to determine if oxidative stress is the mechanism of toxicity. The degree of apoptosis, cell proliferation and DNA synthesis were determined by standard techniques. The investigators hoped to gain insight into the mechanisms underlying the inflammatory/immune responses to NP. Previous studies by the investigators revealed that one mechanism by which NP are toxic to mammalian cells is, in part, through increased production of reactive oxygen species, which results in oxidative stress, and eventually apoptosis. The physicochemical aspects of NP will likely determine their interactions with cells with respect to their uptake, translocation, interference with cellular functions, and deposition within cellular substructures. The multiple cellular functional assays used in this study present a comprehensive array of data which allow the identification of how size, composition, and other characteristics of NP might affect cellular uptake and

toxicity. The investigators acknowledged the challenges with standardisation of NP exposure when conducting experiments at the cellular level and proposed to address the issue of exposure, including homogeneous nanoparticle suspension and agglomeration, as well as NP turbidity in solution, and their impact on toxicity assessment.

UK012 has completed commissioned work by Defra in which the authors set out a risk based approach for research, in terms of the identification of hazard and a structured approach to determining likely exposure to the identified hazard, i.e. nanoparticles and nanotubes.

Ongoing studies

Of the ongoing studies scoring greater than 12 in the Weight-of-Evidence appraisal, study **US428** is an extensive and ambitious study that has four aims: i) evaluate nanoparticle chemical composition (bulk and surface) before, during and after inhalation exposure studies; ii) determine the impact of nanoparticle physical morphology (agglomeration size, agglomeration state and nanoparticle shape) on nanoparticle toxicity; iii) determine if pulmonary clearance is impaired by inhaled nanoparticles and if impaired clearance increases the risk of pulmonary infection; and iv) compare lung inflammation produced by co-exposure of nanoparticles with other inflammatory substances and relative to the nanoparticles alone (common aerosols present in the indoor and outdoor environments including endotoxins and sulphate aerosols [e.g. ammonium sulphate]). Peer reviewed studies have shown that:

i). Mice exposed acutely to 0.77 or 7.22 mg m^{-3} nanoparticles demonstrated minimal lung toxicity or inflammation. Mice exposed sub-acutely (8.88 mg m^{-3}) and necropsied immediately, 1 or 2 weeks post exposure had higher counts of total cells and alveolar macrophages in the brochoalveolar lavage (BAL) fluid compared to sentinels. However, mice recovered by 3rd week post exposure. Other indicators were negative;

ii). Using murine models size effects of inflammatory response in instillation and acute inhalation exposures of TiO_2 nanoparticles with manufacturers' average particles sizes of 5 and 21 nm were investigated. The properties of the primary nanoparticles, aerosol and instillation solution for both sized nanoparticles were evaluated. Mice were acutely exposed in a whole-body exposure chamber or through nasal instillation and toxicity was assessed by enumeration of total and differential cells, determination of total protein, LDH activity and inflammatory cytokines in BAL fluid. Lungs were also evaluated for histopathological changes. Results show the larger TiO_2 nanoparticles were found to be moderately, but significantly, more toxic. The nanoparticles had different agglomeration states which may be a factor as important as the surface and physical characteristics of the primary nanoparticles in determining toxicity.

Study **US072**, a NIOSH project, is an important one for risk assessment. For human health the most important aspect is the extension of the lung dosimetry model for NP. In this project, a current rat lung dosimetry model will be extended to include biologically relevant paths for the transport of nanoparticles to the blood and other organs, as well as excretion. Published data will be used to calibrate the model, and data from new studies at NIOSH and elsewhere will be used to validate the model. The model will be extrapolated to humans using human data where available. Interspecies extrapolation will be applied to extrapolate, from the rat model, the parameter values not available in humans. The lung dosimetry models will be used in conjunction with rodent dose-response data to estimate risk of adverse health effects in workers exposed to NP.

Study **DE005,** on the basis of limited information to evaluate at this stage, is a large-scale study focusing on the possible impact of nanomaterials on humans at the work place or consumers. Thirteen companies, universities and research institutes are contributing their expertise to this partnership. They work together to develop generally accepted measuring and test methods that can be utilised to analyse the safety issues relating to nanomaterials. The study partners also intend to create new nanoparticles and use model systems to analyse their effects on human health.

Study **UK071** offers an outline scoping study to determine whether high aspect ratio nanoparticles (HARN) should raise the same concerns as do asbestos fibres. This study has been influential in setting the research agenda for CNT in the UK.

DK001 aims to investigate physical, chemical, and toxicological properties of nanomaterials that will obtain massive use in the future production of plastic products. The project focuses on polymer nano composites (PNCs), but does not specifically address the issue of toxicokinetics.

Of the ongoing studies scoring less than 12 in the Weight-of-Evidence appraisal, but worthy of acknowledgement, **DE003** will set out to assess the toxicology of functionalised CNTs, namely Polyether-Etherketone and Polyurethane composite materials. Specifically, the authors will study cytotoxicity of the material but give very little insight as to how this will be carried out and in which models. **DK008** may help to address the issue of toxicokinetics with respect to NPs generated following sanding of paint and lacquer. However, no information for assessment has been provided. **BE001** comprises a multidisciplinary research team including chemists, physicists, biologists, physicians and pharmacists and has five years to develop representative *in vitro* models using cell cultures mimicking tissues exposed to nanomaterials (carbon nanotubes, silicon and titanium carbides). These *in vitro* tissues models include skin, lung and intestine, which constitute the main potential routes of contact with nanoparticles. At the same time, *in vivo* studies on nanoparticle effects will be made in order to validate the *in vitro* models. The objective of Study **DK012** is to establish and validate toxicological test systems in order to perform an integrated hazard characterisation of free nanoparticles of silver, ZrO_2, SiO_2 and TiO_2 using a tiered approach.

US320 is an ambitious study which aims at understanding robust large scale manufacturing of nanoparticles and their toxicology, but has had no output so far. Briefly, the proposed study will develop the fundamental understanding needed to manufacture well controlled and characterized elemental, carbon-based nanoparticles. Mechanisms of particle-cell interactions will be evaluated and potential adverse/beneficial effects will be determined using a tiered testing approach utilising both *in vitro* and *in vivo* (rat) models.

Contribution towards the RO

This is a broad RO – 'Research to establish a clear understanding of the adsorption of nanoparticles *via* the lung, skin and gut and their distribution in the body (i.e. toxicokinetics), identifying potential target organs/tissues for toxicity assessment'.

The studies address a number of the aspects of the RO i.e. adsorption of nanoparticles *via*:

i). The lung – this most certainly encompasses the vast majority of the studies included in this RO (**EU030**, **UK012**, **US193**, **US048**, **US156**);
ii). The skin – very few if any studies (**DE004**);

iii). The gut – very few if any studies (**DE004**).

Extent to which the RO has been met

Overall, the RO is being partially met in terms of the global research identified here. Key gaps/ weaknesses are:

i). This review of ongoing studies has failed to demonstrate that there is any comprehensive attempt to gain the toxicokinetic (particokinetic) data required to reach the aims of Hazard identification and Derivation of plausible dosimetry;
ii). There is no study that sets out with the aim of simply assessing the accumulation of particles in a wide variety of organs after inhalation exposure to a range of different NP;
iii). A potential structure activity relationship that governs penetration at each different portal of entry is not being sought;
iv). No systematic studies on the potential of different kinds of nanoparticles to get into the blood, the lymph or the brain;
v). No specific studies on whether carbon nanotubes behave like asbestos, in terms of whether they translocate to the pleural mesothelium and length-relatedness of this effect;
vi). No studies specifically addressing the interstitialisation of inhaled nanoparticles and the consequences in terms of fibrosis etc.;
vii). Quantum dots have been shown to pass through epidermis to the dermis, albeit in small amounts, raising issues of susceptibility – a recent study by Mortensen *et al*. (2008), has described work where nude mice which were UV irradiated demonstrated a deeper penetration of quantum dots into the skin;
viii). Quantitative estimation of translocation to brain has only been seriously addressed in one US laboratory, using a limited range of nanoparticles types.

4.12 RO12 - TRANSLOCATION AND CELLULAR TOXICITY

The research objective

Research to establish a clear understanding of inter- and intra-cellular transport and localisation of NPs and their cellular toxicity.

Overview of the types of studies which have been found to be relevant to this RO

Forty-eight studies were sourced for this RO. Out of these studies, nine could not be assessed as there was insufficient information in terms of their outputs available; most of these came with a title only or an inadequate statement of objectives. Of the remaining thirty-nine studies fully assessed, fifteen scored 11 or above and are described below. Twenty-four scored less than 11, of which 6 are also mentioned below.

The types of study were predominantly *in vivo* and *in vitro* studies, with a couple of modelling studies.

Despite studies being completed, as regards their stated timelines, the majority supplied no peer-reviewed publications or supplied publications that were not relevant to the projects considered here.

The key studies

Completed or near-completed studies

US120 has used both *in vitro* and *in vivo* approaches to provide dose-, size- and composition-related toxicological information about nanoparticles and will also explore their translocation to extrapulmonary tissues. The study will primarily use nanoparticles composed of iron oxide (Fe_2O_3, 3-25 nm) but, for specific experiments, bulk composition and surface coatings will be varied. For *in vitro* studies, they will use human alveolar epithelial and umbilical vein endothelial cells and monitor their uptake of nanoparticles as well as inflammation- and oxidative stress-related responses using a range of doses. For *in vivo* studies, rats will receive intratracheal instillation and intravenous injection exposures. Endpoints related to lung inflammation, inflammatory cell activation, and oxidative stress, as well as those indicating vascular endothelial injury and acute phase responses, will be assessed. Comparisons will be made with ultrafine (20 nm) TiO_2 particles. Tissues from exposed rats (lung, liver, and olfactory bulb) will also be examined for the presence of the particles following intratracheal and intranasal instillations and intravenous injection exposures.

CA011 focuses on quantum dots and sets out to determine their biodistribution and toxicity. The project will synthesize five different sizes and three different shapes of quantum dot nanostructures and systematically evaluate their fate, metabolism, and clearance in macrophages.

CA006 has developed fluorescently tagged (RGD peptide) rosette nanotubes (RN), which are different than the commonly used single-walled carbon nanotubes. The RGD peptide plays important role in the function of neutrophils and the study will examine how RGD-RN interact with the cells *in vitro* and *in vivo*. These studies will clarify fundamental processes of interaction of RN with the cells with an aim of using RN as smart weapons for precise delivery of drugs to treat diseases such as pneumonia.

CA007 is targeted at better understanding the interaction between peptide molecules (and potentially proteins) and bulk-produced nanomaterials, such as carbon-based nanotubes and fullerene-based materials.

CA001 and **CA009** both address the role of quantum dots and will quantify the interactions of Quantum dots with cell membranes, cellular proteins, DNA, and whole cells, with the goal of identifying the exact conditions under which the particles can damage cells. This should allow for the design of simple tests for researchers to use that will predict the cellular toxicity of nano-sized materials based upon physical properties such as size and fluorescence. This work will also determine whether specific classes of molecules, including DNA molecules, make Quantum dots more reactive and hence more toxic, which will allow for recommendations for handling and disposing of these particles in biological experiments.

US263 has four main objectives: i) to compile reproducible gene expression profiles of primary human lung cells exposed to single-walled carbon nanotubes (SWCNT), ii) to apply different tiers of computational analysis to determine if toxicity is present; iii) to compare across biological tissues with previous data from human skin cells (obtained with NSF SGER award); and iv) to lay the foundation for a "systems biology" approach for predictive toxicity by merging traditional risk assessment methods with new toxicogenomics computational methods.

Ongoing studies

UK017 addresses the potential route of uptake of nanoparticles by cells and their impacts on antigen processing. Some work has been carried out on the uptake of quantum dots into cells and the effect of the surface on cell uptake and toxicity. The mechanisms of toxicity of zinc nanoparticles and the effects of different types of quantum dots has been studied, but no data has yet been made available on the antigen processing.

US125 also focuses on the membrane and aims to identify the cell internalisation process by characterising the dynamic behaviour of between individual NP and membrane lipids at the encounter site, and the involved subcellular structures. These workers also intend to determine the involvement of selected membrane receptors in the attachment and internalisation of manufactured NP with specific properties, by exploring molecular interactions between the receptors and ligand-coated or naked particles.

US411 uses a highly physicochemical approach to understanding the interaction of membranes and NP; it is difficult to tie this to toxicology but there may be relevance and future advances are anticipated.

CH016 takes a similar approach and examines interactions between lung cells after exposure to combustion–derived and manufactured NP. However, this is a study designed to deliver NP payload to tumour cells and so is highly specific and not immediately relevant to NP toxicity arising from exposure to environmental NP. No data for this project is available yet.

EU006 is another study that seeks to relate physicochemical properties to NP toxicity, but little data is provided. A similar approach is taken in **EU030** where new toxicological approaches are used to produce a tool for hazard ranking of novel particle-producing technologies.

EU008 will connect nanoparticle properties in physiological solution to mechanism of uptake into and transport in cells, with a further view to the final cellular location of nanoparticles with the intra- and inter-cellular processes disrupted. **EU003** examines modes of interaction between NP and cells for a range of NP.

UK026 investigates the uptake of nanoparticles by hepatocytes and Kupffer cells, the impact on cell function and potential for excretion into bile. This study is predicated on the argument that NP may reach the blood *via* the respiratory tract, gastrointestinal tract or skin and then be removed from the circulation by the Kupffer cells of the liver and hence may result in exposure to hepatocytes. Moreover, medical NP might also be directly injected into the circulation. No copies of papers are provided but there are a number in preparation.

US023 is a clinical study of patients with artificial joints which release metal and metal particles by wear. This is a unique exposure situation which probably has little relevance to other portals for entry. In addition, all the particles are fairly unusual and unlikely to be encountered in any other scenario. None of this research is likely to help establish a clear understanding of inter- and intra-cellular transport and localisation of nanoparticles and their cellular toxicity. It may do so, but the objectives are not sufficiently detailed to know what the aims in this regard are. This appears to be largely a clinical study and so may never allow for the correct models to understand these objectives.

US129 - This study may help to understand inter- and intra-cellular transport and localisation of nanoparticles and their cellular toxicity. This proposed research will directly compare the biological disposition, persistence, and toxicity of two commercial nanoscale and non-nanoscale metal oxide classes and will determine the impact of particle class, particle size, and surface area on NPs that are of significant commercial relevance for nanotechnology (SiO_2 and Al_2O_3). The primary objectives will be executed after repeated inhalation studies (F344 rats, 6 hours/day, 5 days/week for 6 weeks to nanoscale- and micron-size materials of the same composition). These are to: i) determine biological disposition (translocation/elimination/persistence) by measurement of residual metal oxide in plasma and six target organs (lung, brain, liver, kidney, spleen, intestine); and ii) determine local (lung/respiratory tract) and systemic toxicity by measurements of sensitive biochemical markers of inflammation and oxidative stress, in addition to histopathological analysis. For both i) and ii), measurements will be made immediately post exposure, 4 weeks post exposure, and 17 weeks post exposure to complement previous studies in their laboratory on micron-size SiO_2.

US313 - This study may help to understand inter- and intra-cellular transport and localisation of nanoparticles and their cellular toxicity.

US422 is from a group which has published a number of studies that are highly physicochemical regarding membrane NP interactions - it is difficult to tie this to toxicology but there may be relevance. Future studies are looked forward to.

US211 - This study may yield important information on genes involved in cellular toxicity of NP but, by using a yeast indicator cell, there will need to be a number of validation steps to gain relevance to human responses. A commercially-available library of approximately 4,800 yeast deletant mutants will be systematically screened for sensitivity to fullerene and to fullerol, under controlled conditions in which the physical-chemical properties of these particles will be manipulated through formulation of the solution chemistry to mimic a range of potential environmental conditions. Particle size, shape, surface charge, specific surface area, and the surface functionality of these materials will be measured and correlated with particle toxicity. Genes missing in mutants found to exhibit sensitivity will likely encode functions that normally provide protection. Uptake of nanomaterial will be assessed by indirect immunofluorescence.

Extent to which the RO has been met

Overall, the RO is being partially met in terms of the global research identified here.

Clearly many types of nanoparticles are taken up by cells – especially *in vitro* cell culture conditions. This has been partially addressed for a limited number of materials. Key gaps/ weaknesses are:

i). There are clearly generic issues such as fluorescent tagging of nanoparticles, which may change their activity;
ii). Lots of studies are utilising quantum dots due to their visibility, but these are not typical nanoparticles;
iii). Addressing the issue of translocation within cells – are different nanoparticles preferentially taken up in the mitochondria or nucleus resulting in concentrated areas of oxidative stress and potential respiratory burst disruption?
iv). What are the structural correlates determining protein particle interactions and transport of important proteins within a cell – detrimental changes in protein

folding and packaging? Fibrillation of proteins, which is especially pertinent in the brain;

v). Different target cell specific toxicity i.e. brain cells, liver cells (canniculae formation), if nanoparticles become blood born – importance of platelet aggregation and activation as a risk factor in cardiovascular disease;

vi). Issues of addressing the specific aspects of toxicity, which may well be subtle i.e. primary and secondary toxicity for cells e.g. with respect to the cardiovascular system, there may well be direct particle exposure in the blood or alternatively blood effects due to local inflammation in the lungs;

vii). Carbon nanotubes – differential responses with long versus short nanotubes in relation to the exquisite sensitivity of the mesothelium to fibrous particles. There is also the issue that carbon nanotubes have the potential to act like nanoparticles, causing a florid fibrotic effect in the lung – potential for identifying the role of fibroblasts.

4.13 RO13 - TOXICITY MECHANISMS (OXIDATIVE STRESS, INFLAMMATION AND GENOTOXICITY)

The research objective

RO13 - Research to establish a clear understanding of whether oxidative stress, inflammatory effects and genotoxicity apply to nanoparticles.

Overview of the types of studies which have been found to be relevant to this RO

Forty-one studies were sourced for this RO. Out of these studies, five could not be assessed as there was insufficient information in terms of their outputs available; most of these came with a title only or an inadequate statement of objectives. Of the remaining thirty-six studies fully assessed, thirteen scored 18 or above and are described below. Twenty-three scored less than 18, of which 12 are also mentioned below.

The types of study were predominantly *in vivo* and *in vitro* studies with a couple of modelling studies. Despite studies being completed, as regards their stated timelines, the majority supplied no peer-reviewed publications or supplied publications that were not relevant to the projects considered here.

The key studies

Completed studies

UK030 is a study that focuses on a range of metal oxide nanoparticles and tests the hypothesis that the intrinsic free radical or oxidative activity of the panel is related to their ability to cause inflammation. The study measured both free radical activity using electron paramagnetic resonance and a dye that is fluorescent under oxidising conditions, to investigate free radical generation. A range of other simple endpoints of toxicity, cell death and haemolysis were also tested and all of these were related to the ability to cause lung inflammation when injected into rat lungs at equal surface area dose. The results showed that there was a poor relationship between intrinsic oxidative stressing activity and ability to cause inflammation. That is, very high free radical generation particles did cause inflammation but for moderate free radical generating particles there was no relationship. One particle, an alumina, was inflammatory but had no detectable free radical or oxidant activity. This key study suggests that there are other mechanisms by which NP generate inflammation, other than *via* oxidative stress

directly from the particle. However, it should be noted that particles with little intrinsic ability to generate free radicals in a chemical system may still have ability to cause oxidative stress inside cells.

UK051 examined a very specific aspect of nanoparticle toxicity, namely whether High Aspect Ratio Nanoparticles (HARN) might act like asbestos in having length-dependent toxicity to the mesothelium. CNT were used as the most common HARN currently in production and shown to have length–dependent inflammogenic effects following direct exposure of the peritoneal mesothelium in mice by intraperitoneal injection. Long CNT (longer than approximately 20 μm) were highly inflammogenic and also caused granulomas to form rapidly on the diaphragm, a site where long fibres accumulate due to the clearance mechanisms that operate in the peritoneal cavity. Short CNT (less than approximately 5 μm) had no such effects, despite being injected at the same mass dose. Inflammation is considered to play an important role in asbestos pathogenicity and this study suggests that CNT could have the same effects. The events involved in inflammatory cell activation and oxidative stress generation are complex and intercellular interactions are likely to be important in amplifying inflammation.

Oxidative stress was identified as a mechanism of toxicity in dysfunction of the peripheral vessels following inhalation of NP TiO_2 in **US096**.

US120 studied low-toxicity platinum nanoparticles, in comparison with other metal NPs, and defined some of the surface characteristics that affected cellular uptake. The particles were taken up by cells following *in vitro* exposures, but did not induce changes in cell oxidative stress or viability. This correlated with the low, inherent oxidative capacity of the particles (i.e. DCF oxidation). This suggests a good correlation between acellular oxidation assay and inflammogenicity, for low oxidative particles at least. The study recommends that acellular oxidation could be used as one in a set of assays to screen the toxic potential of nanomaterials of unknown activity.

UK025 has examined a number of common nanoparticles including TiO_2 and carbon black, but also quantum dots. These studies have demonstrated that oxidative stress is involved in the pro-inflammatory effects of nanoparticles in macrophages and that one action of oxidative stress is to activate inflammation. **UK025** has addressed a number of aims associated with the ability of a range of commercially relevant particles and nanoparticles to generate ROS leading to oxidative stress in macrophages. The study has demonstrated the ability of NP such as latex, TiO_2 and carbon black to cause oxidative stress and to activate NF-κβ and AP-1, leading to cytokine gene expression. Ongoing studies on a range of nanoparticles are examining their ability to activate other oxidative stress signalling pathways, including the transcription factor Nrf-2, and the expression of antioxidant defences such as HO-1.

UK090 brought together nanotoxicologists and particle experts from around the globe and addressed the very issue as to whether oxidative stress was a sufficiently common mechanism for the action of nanoparticles and that it might be adopted as an exposure metric, given that methods could be developed to assess the oxidative potential of airborne particles in the environment. Thus, oxidative potential would serve as an adjunct to mass or particle number metrics, as an exposure metric that might be closer to the effective dose that drives pathological change and so might improve risk management. The overall conclusion was that this was a plausible aim in the medium term and that there should be an effort to develop real-time tests of airborne particle oxidative potential that might be used in the general environment and in NP workplaces, to assess this parameter of the airborne dust.

UK071 was a scoping study intended to determine whether high aspect ratio nanoparticles (HARN) should raise the same concerns as do asbestos fibres. It concluded that there should be concern and that more research is warranted, especially on exposure.

US093 specifically addressed the NP oxidative stress hypothesis in the skin. This study especially concentrates on whether transition metals drive such effects when NP penetrate into the skin. One peer review publication shows that, after 18 h of exposure of human keratinocytes to SWCNT, oxidative stress and cellular toxicity were indicated by formation of free radicals, accumulation of peroxidation products, antioxidant depletion, and loss of cell viability. Exposure to SWCNT also resulted in ultrastructural and morphological changes in cultured skin cells.

The completed studies in **CH001** provide interesting and potentially useful models for studying such interaction *in vitro*. These 3-D cell systems use epithelial cells, endothelial cells, fibroblasts and dendritic cells and so may provide improved models for studying cellular/molecular interactions. These systems are in use for this purpose in **CH002,** to determine the role of radicals and the expression of cytokines as a measure of inflammatory potential.

US095 has not yet produced any publications but the aims suggest that the output of this studies should be important. The study aimed to investigate nanotoxicity using aluminium and silver nanoparticles and multiple *in vitro* model systems - rat liver cells, alveolar rat macrophages, human Jurkat T cells, neuroendocrine cells, keratinocytes and germline stem cells. Endpoints include cell viability, mitochondrial function and redox status. NP will be classified as to whether they are of high, intermediate or low toxicity when compared to a control. Generation of reactive oxygen species, reduced glutathione and mitochondrial membrane potential were to be measured to determine if oxidative stress is the mechanism of toxicity.

In **US122** inhalation exposures were carried out with types of carbon nanotubes (single-walled, bundled and multiwalled) of known size and composition. Ultrafine titanium dioxide (TiO_2) and ultrafine carbon black (CB) were also to be aerosolised to compare their potential adverse effects on the lungs. Cellular, biochemical and histological assays were carried out in the respiratory tract to determine effects of nanoparticle exposure. These measures included indicators of oxidative stress, inflammation, cell injury and repair, and metabolic change. The role of metal contaminants, present as catalysts used in the manufacturing process, were studied using nanotube preparations with these trace metals/contaminants removed. No published output from this study is available as yet.

US222 focussed on three classes of manufactured nanoparticles: catalysts (aluminosilicates), titanium, and carbon. For the catalysts and titanium samples, nanoparticles (<100 nm) and micron-sized particles of similar bulk composition were studied. For carbon, carbon black and single-walled carbon nanotubes were utilised. Nanoparticles of aluminosilicates and titanium were synthesised, whereas the other particles were to be obtained from commercial sources. Characterisation involved electron microscopy, surface area, surface and bulk composition. Reactivity of the particles with regard to acidity, reaction with antioxidants simulating the lung lining fluid, coordination of iron, and Fenton chemistry were carried out using spectroscopic methods. *In vitro* oxidative stress and inflammatory responses upon phagocytosis of the particles by macrophages and pulmonary epithelial cells were to form the toxicological/biological end points of the study. No published output is available as yet.

US337 focussed on model carbon nanofibers and nanotubes synthesised by non-catalytic templating routes from high-purity liquid-phase precursors. This approach allows control of size and shape, and CNT so-produced are free of transition metal impurities. Metal doping (spiking) and surface oxidation of these pure nanocarbons were then to be carried out to assess directly the effects of metals and hydrophilicity. A panel of fibrous and tubular nanocarbons were to be synthesised, post-processed, and characterised, then assessed for the following toxicologic endpoints (over a range of doses): (i) phagocytosis; (ii) cell toxicity; (iii) induction of proinflammatory gene expression; and (iv) genotoxicity. Toxicity of the CNT will be compared to asbestos fibres and titanium dioxide nanoparticles. No published output is available yet.

Ongoing studies

EU011 is a multidisciplinary study that examines a range of NP towards risk assessment. NPs will be characterised and used to determine exposures in workplaces and assess pro-inflammatory, genotoxic and microcirculatory effects.

UK093 is a pilot study to determine whether a structure-activity model can be constructed for NP toxicity. A panel of 12 NP types are being characterised and subjected to a battery of pro-inflammatory and toxic effects. The data will be mined to determine whether structure and toxic activity can be modelled.

US055 is a highly productive programme that has developed a paradigm for oxidative stress from NP in eliciting a graded response in target cells. This dose-dependent hierarchical response ranges from induction of antioxidant defence (low level NP-derived oxidative stress), to inflammatory gene expression (moderate NP-derived oxidative stress), to apoptosis (high level NP-derived oxidative stress). This paradigm has been tested for a number of NP and agreement found. This group has also examined the origins of intracellular oxidative stress and have identified the mitochondria as likely targets for NP in inducing oxidative stress in dosed cells. This model had been developed using a range of NP including fullerenes, CNT, TiO_2, carbon black. The same group have a new study that examines TiO_2, ZnO and Cerium oxides (CeO_2/Ce_2O_3) , chosen based on high volume of production. They will assess their potential for airborne spread and ability to induce airway inflammation through the generation of reactive oxygen species (ROS). The group will particularly address how a variation in the physicochemical characteristics of the engineered NP influences their toxicity at portal-of-entry cellular targets in the lung. They will also determine whether the predictive hierarchical oxidative stress model is operative with this panel of NP.

US048 has shown that oxidative stress and inflammation are important mechanisms in the action of welding fume NP. The endpoints arise as a result of metals in the welding fume.

EU019 specifically addresses the role of oxidative stress in the adverse effects of NP on the brain and CNS. The role of particle-derived oxidative stress in aiding penetration to the brain, enhancing protein fibrillation and causing oxidative stress, will be studied. The project has not yet begun, however, and so no data are yet available.

EU004 is specifically directed towards elucidating the relationships between physicochemical properties and reactive oxygen species induced by nanoparticles and their inflammogenicity and toxicity in human epithelial cells. Therefore it is highly relevant to the RO but there are no data or publications or reports as yet.

US129 is determining the impact of particle size and surface area on NPs that are of significant commercial relevance for nanotechnology, such as SiO_2 and Al_2O_3. Amongst

a number of endpoints that are to be examined following aerosol exposure, are local (lung/respiratory tract) and systemic toxicity by measurements of sensitive biochemical markers of inflammation and oxidative stress, in addition to histopathological analysis. Little further information is available and no published output is available.

US163 is an ongoing study which aims include establishing the extent to which Single Walled Carbon Nanotubes (SWCNT) alone are pro-inflammatory to lung cells and tissue, characterisation of the role of iron in these effects using genetically manipulated cells and animals, as well as antioxidant interventions. The study will also determine the potential for SWCNT and microbial stimuli to interact synergistically to promote macrophage activation, oxidative stress, and lung inflammation. Finally, the study should reveal the extent to which SWCNT are effective in inducing apoptosis and whether apoptotic cells exert their macrophage-dependent anti-inflammatory potential during *in vitro* and *in vivo* SWCNT exposure. No data is available as yet.

US320 has a special feature, in that the group are generating their own NP in a reactor for their studies. Particle-cell interactions will be evaluated initially and potential adverse/beneficial effects will be determined using a tiered testing approach. At first, *in vitro* studies will be performed using specific target cells, such as lung epithelial, vascular endothelial, and neurons, to evaluate the oxidative stress-inducing potential of the engineered nanoparticles. This will be followed by *in vivo* studies in rats with delivery of the particles to the circulation and to the lung, followed by evaluation of potential adverse pulmonary and systemic effects. No published output is available as yet.

The ongoing study **US326** tests the hypothesis that physicochemical properties of NP determine their interactions with cells with respect to their uptake, translocation, interference with cellular functions and disposition within cellular substructures and tissues. The ultimate goal is to develop and test computational models that can forecast a nanostructure-induced cellular response. In other words, assessing whether there is a structure-activity relationship for NP. Not much detail is given, only objectives, but it is likely that oxidative stress and inflammatory markers will be measured as 'activities' since this group have been developing this hypothesis.

How studies contribute toward the RO

'Research to establish a clear understanding of whether oxidative stress, inflammatory effects and genotoxicity apply to nanoparticles' - this is a broad RO. It would be surprising indeed (to this reviewer at least) if oxidative stress, inflammation and genotoxicity were not found to be involved in the pathogenic action of NP, since these processes underlie the pathogenic effects of all other harmful particles so far studied (asbestos , quartz, etc.). They also underlie the toxicity of any number of chemical toxins. It is also true to say that, since these form the dominant hypothesis for mainstream nanotoxicology, these are the endpoints that are being sought, so it will be no surprise if they are found (you cannot find what you do not look for!). However, the choice of oxidative stress, inflammation and genotoxicity are logical. More interesting and of importance to nanotoxicology is the relationship between NP structure and these endpoints i.e. the structure function relationship. The predictable heterogeneity in the oxidative stressing and inflammatory effects of NP is a most important aspect of defining the role of oxidative stress and inflammation and could allow predictive toxicology of NP. Subsequently many studies directly contribute to the question of whether oxidative stress and inflammation are involved in the action of NP. However, given the nature of toxicology, whereby doses are increased mostly *in vitro*, until an effect is reached, the relevance of such findings for plausible exposures is questionable. Few studies contribute to the issue of genotoxicity of NP.

Extent to which the RO has been met

The RO has been met, but with considerable number of caveats:

i). Some NP are used a lot (TiO_2, Carbon black and CNT) whilst some are used much less (alumina, ZnO, q- dots, CeO_2 etc) and so the generic nature of the findings are questionable;
ii). There can be little doubt that there will be heterogeneity in the action of NP in causing oxidative stress and inflammation – therefore we are on a case-by–case assessment unless some structure function rules can be derived;
iii). Several studies purport to address the structure activity relationship fully, through oxidative stress and inflammation as the activities allied to various structures being measured in a panel of NP (**US337**, **US326**, **US438**, **EU004**, and **UK093**);
iv). Other studies carry out more limited structure activity studies e.g. fixing on limited activities such as role of iron, oxidative stress biopersistence or shape (**US163**, **US122**, **US222**, **US048**, **UK051**, **UK025**, and **DK009**);
v). *In vivo* studies are at a premium (**US055**, **US438**, **US120**, **US129**, **US163**, and **US320**) and, where there are *in vivo* studies, they are mostly confined to a small subset of NP for proof-of-concept type studies;
vi). There are few studies addressing genotoxicity – only **US337** (nanotubes) **DK009**, and **UK030** - and so there is a gap here;
vii). Few studies are likely exposing to NP at or near plausible exposures and then assessing oxidative stress, inflammation and genotoxicity;
viii). No studies with susceptible models are described, except for synergism between CNT and microbial inflammogens (**US163**) *in vitro*;
ix). There are few studies addressing the oxidative stress hypothesis for cardiovascular (CV) effects (**US096**) or the brain (**EU019**), so there is a need for *in vivo* exposures to NP followed by measurement of oxidative stress in the vessel wall and in other CV targets or in the brain;
x). The focus on oxidative stress and inflammation can be seen as logical, but it does stifle other thinking. For example, in one study (**UK093**) an alumina with no oxidative stressing activity turned out to be inflammogenic. Therefore, there are definitely other mechanisms for inflammation, in addition to oxidative stress.

4.14 RO14 - DEPOSITION, DISTRIBUTION, TOXICITY, PATHOGENICITY AND TRANSLOCATION POTENTIAL AND PATHWAYS FOR NANOPARTICLES IN THE AIRWAYS AND LUNG AND THEIR POTENTIAL IMPACTS ON THE CARDIOVASCULAR SYSTEM AND BRAIN

The research objective

RO14 - Research to establish a clear understanding of the deposition, distribution, toxicity, pathogenicity and translocation potential and pathways for nanoparticles in the airways and lung and their potential impacts on the cardiovascular system and brain.

Overview of the types of studies which have been found to be relevant to this RO

Fifty-three studies were sourced for this RO. Out of these studies, nine could not be assessed as there was insufficient information in terms of their outputs available; most of these came with a title only or an inadequate statement of objectives. Of the remaining forty-four studies fully assessed, sixteen scored 13 or above and are described below. Twenty-three scored less than 18, of which twelve are also

mentioned below. The types of study were predominantly *in vivo* and *in vitro* studies, with a couple of modelling studies. Some of the studies included did not address the RO and were excluded. Despite studies being completed, as regards their stated timelines, the majority supplied no peer-reviewed publications or supplied publications that were not relevant to the projects considered here.

The key studies

Completed or near-completed studies

In studies **US193** and **US048** (an ongoing study discussed later), the same workers developed an animal/cell exposure system for welding fume NP generation. These workers have constructed the exposure system for welding fume exposures and published a number of studies documenting its use. They have studied inflammatory effects in the lungs and different types of fume and have reviewed the literature on the effects of inhaled metals on the brain. Judging from the research published so far on the lungs and the progress report, this group will be shortly publishing findings pertaining to translocation and neurological and cardiovascular effects in the peer reviewed literature.

Study **US060** specifically addressed transport of NP from the nose, *via* the olfactory nerves to the brain. The study uses nasal epithelium explants, nasal instillations in mice and inhalation studies. In addition, data derived from these studies were to be used by Chemical Industry Institute of Toxicology scientists who have developed dosimetry models that describe nasal and lung deposition of particles, as well as other models developed to describe the olfactory transport of inhaled materials by laboratory animals.

Study **US156** aimed to identify where in the lungs inhaled nanomaterials might deposit, the health risks that might arise from such deposition, and to what extent the nanomaterials might translocate to other organs of the body after depositing in the lungs. Although completed, the data in peer reviewed journals so far has been restricted to carbon nanotube studies and their local effects on the lungs, such as acute inflammation with early onset yet progressive fibrosis and granulomas. More publications on the systemic effects are to be anticipated in future publications.

Study **US306** was also part of the NIOSH programme and specifically addressed CNT and their effects in the lungs. The unusually pro-fibrotic effects of CNT were demonstrated and *in vitro* studies underlined the importance of iron as a mediator of free radical generation. Study **US029** was also a NIOSH study on the cardiovascular effects of lung deposited CNT. ApoE mice exposed to CNT by four injections showed increased atherogenesis and evidence of mitochondrial DNA damage and oxidative stress in the aorta. This evidence showing an effect of a NP on the vessel wall and atherosclerosis following pulmonary deposition is potentially important.

In study **US262** there has been an attempt to investigate toxicokinetics of NP. In an interim report the workers show that gold NP reach the brain from the nose after nasal instillation and by inhalation. Smaller particles accumulate in the brain to a greater extent than larger NP. For quantum dots, coating with polyethyleneglycol (PEG) altered the translocation to the brain, with more than twice the amount of PEG coated quantum dots found in the brain compared to PEG-amine coated quantum dots. Gold NP instilled into the lungs, bypassing the nose, only showed up in the brain in very miniscule amounts (*via* the blood brain barrier). Intravenous injection showed that liver is the major organ of uptake; spleen and bone marrow also displayed a significant uptake of the rat serum albumin-coated gold particles as determined by neutron

activation analysis (NAA). PEG coating enhanced the retention in the blood compartment and lowered accumulation of nanogold in the liver. ICPMS analysis of brain tissue following intravenous injection showed that - unlike administration into the lower respiratory tract, following which PEGylated nanogold was not found in the brain- there was significant brain accumulation of these particles at 1 and 24 hours post-exposure. All translocation was small in quantity.

Study **US303** was focussed on the surface area metric and aimed to expose cells in culture and rats by inhalation to equal surface area doses of nanoparticles and large particles. The overall aim of these studies was to determine if particle surface area was a more appropriate metric for exposure than particle mass. **US122** involved short-term exposure to a range of well characterised nanoparticle types delivered to rats by inhalation. The investigators will seek oxidative stress and inflammatory endpoints, as well as repair and toxicity endpoints in the lungs. They will attempt to relate the endpoints to metal contamination and size of the NP types used.

Study **US437** evaluated the potential neurotoxic effects of exposure to MWCNT and silica in a murine model. In an interim report they describe that exposure of mice by pharyngeal aspiration to MWCNT or silica elicited neuro-inflammation in discrete brain areas. Specifically, the expression of CCL2, TNFα and IL6 increased (2 to 10-fold; $P < 0.05$) in the olfactory bulb, hippocampus and frontal cortex, within 24 hours following exposure. This inflammatory outburst was a consequence of microglial activation, in response to perturbation of the neuronal microenvironment and function.

Study **US068** comprised pulmonary toxicology studies in rats to evaluate the acute lung toxicity of intratracheally instilled pigment-grade TiO_2 particles (rutile-type particle size = approximately 300 nm) versus nanoscale TiO_2 rods (anatase = 200 nm x 35 nm) or nanoscale TiO_2 dots (anatase = approximately 10 nm) compared with a positive control particle type, quartz. Exposures to nanoscale TiO_2 rods or nanoscale TiO_2 dots produced transient inflammation and cell injury effects at 24 hours post-exposure and were not different from the pulmonary effects of larger sized TiO_2 particle exposures. Contrary to previous studies, the results suggest that nanoscale particle types are not necessarily more cytotoxic or inflammogenic to the lung than larger sized particles of similar composition, but emphasises the importance of surface reactivity as a factor that contributes to particle toxicity.

In Study **CH020** a panel of nanoparticles (gold, carbon black, quantum-dots and fullerenes) were subjected to comprehensive characterisation and then tested in a range of tests including toxicokinetics, effects on platelets and on liver cells. The aim was to relate NP characteristics to the various endpoints. Gold NP were found to persist in the blood to a greater extent if they were smaller and the charged quantum dots were more active in causing platelet aggregation than uncharged quantum dots. Although completed, there has been no peer review publication of the data in journals as yet or a bringing together of the structure activity information, but this will be done.

In Study **US034** the objective was to use NP and nanotubes bought from major suppliers to extend existing models of particle deposition in the respiratory tract of rats and humans to cover the range of size for nanoparticles. Semi-empirical models of penetration from the upper respiratory tract (URT) were to be used to describe regional deposition fraction in the URT. The approach included verification of the model with experimental data obtained in casts of both human and rat upper respiratory tract, as well as *in vivo* studies of respiratory tract deposition. This kind of information on the deposition characteristics of NP is potentially important for risk assessment.

Ongoing studies

Study **US048** seeks to evaluate the translocation of welding fume metals from the lungs to other organ systems, including central nervous system and cardiovascular system. In addition, the potential neurotoxic effects in animals after inhalation of welding fume are being evaluated.

The central hypothesis of Study **US452** is that inflammatory mechanisms govern the systemic micro vascular dysfunction that follows ultrafine PM exposure, and the severity of this dysfunction is augmented in clinically relevant populations. Short term exposures to high airborne mass concentrations of fine and ultrafine TiO_2 were carried out. There was no lung inflammation with any treatment. However there was disruption of endothelium-dependent vasodialation with fine TiO_2 exposures but a greater effect with the ultrafine TiO_2. Previous studies have suggested that PMN that are marginated in the blood vessels may be responsible for the dysfunction in the endothelium.

Study **US079** should provide important data on the relationship between NP agglomeration and deposition characteristics.

Study **US220** addressed the important issue of the long-term cardiovascular impact of NP inhalation. Nickel, titanium, and carbon nanoparticles will be produced using a spark generator with pure electrodes of nickel, titanium, and carbon. ApoE (-/-) mice will be exposed to filtered air or various concentrations of nanoparticles for 6 hours per day, 5 days per week, for up to 6 months. The development and progression of atherosclerosis will be assessed using non-invasive ultrasound biomicroscopy (UBM) as well as serial morphometric measurements. Serial endpoints such as vasoconstriction response, macrophage infiltration, NOS expression level (both inducible and endothelial isoforms), ROS, and 3-nitrotyrosine production will also be investigated as the major mechanisms involved in vascular dysfunction leading to nanoparticle enhanced atherosclerosis. An interim report shows that nickel NP have been used and inhaled nickel NPs can induce significant acute and chronic pulmonary inflammation, systemic inflammation and, in the long term, accelerated and exacerbated atherosclerosis.

In Study **US249** TiO_2 nanowires and nanospheres will be synthesised and characterised. Their ability to generate ROS and cause cellular oxidative stress will be assessed. The differential toxicity of TiO_2 nanospheres and nanowires on the pulmonary and central nervous systems will also be assessed after pulmonary deposition *via* pharyngeal aspiration in mice.

In study **US422** mice will be exposed to multi-walled carbon nanotubes by pharyngeal aspiration. Up to 56 days post-exposure toxicity to the lung and brain will be assessed. To evaluate lung toxicity, bronchoalveolar lavage studies will be conducted to determine the inflammatory profile. To evaluate neurotoxicity, the extravasation of immunoglobulin G into the brain and expression of endothelial cell and gap junction proteins, as indicators of blood-brain barrier integrity will be assessed, along with neuro-inflammatory and oxidative stress responses.

Study **DE003** has provided little detail but intends to focus on the cytotoxicity of carbon nanofibres and carbon nanotubes. It will involve comprehensive physicochemical characterisation of the materials and will make it possible to reference the cytotoxicity investigated in terms of standardised material properties. Several simulation tools will also be adapted to investigate the uptake and distribution pathways of carbon nanomaterials in human organisms, which will facilitate the prediction of the dose-response.

Study **DE005** did not provide sufficient information for full evaluation but is a large-scale study aimed at understanding mechanisms, including genotoxicity and oxidative stress.

Study **BE001** aims to evaluate the potential toxicity of CNT and NP. New relevant tissue models of reconstituted skin, respiratory epithelium and intestinal epithelium will be developed and validated to assess the toxicity related to nanoparticles, using histology, cytotoxicity and genotoxicity tests. Acute and subchronic toxicity studies will be carried out on animal models using different exposure pathways, such as the skin, the respiratory tract and the intestinal tract.

Study **EU019** is predicated on the assumption that NP can reach the brain and addresses the likely results of such transfer. In particular, the study addresses the particle parameters that control breaching of the blood-brain barrier (BBB) and the consequences for amyloid fibril burden, oxidative stress and neurotoxicity.

Study **EU023** addresses procurement, synthesis, and physico-chemical characterisation of representative categories of nanomaterials, followed by assessment of adverse immunological effects. A panel of *in vitro* and *in vivo* model systems will be used, as well as state-of-the-art transcriptomics and lipidomics protocols.

Study **EU028**, amongst a variety of approaches, will develop new equipment for detecting NP in air and, of special relevance, a miniaturised translocation test system.

Study **UK089** focuses on the behaviour of nanoparticles following inhalation and possible transition into the blood stream. Multiwalled carbon nanotubes and titanium dioxide nanoparticles will be investigated as to their interactions with synthetic lung lining liquid and blood plasma. The effect of size, shape and structure, and how they affect their behaviour in solution, specifically their solubility, surface area, surface charge, surface roughness and tendency for agglomeration, will be tested in the two media.

DK009 aims to study the mechanism of cardiovascular, hepatic and vascular effects of NP in light of the inflammatory and oxidative stress hypotheses.

Two studies, **US432** and **US435**, will assess the *in vitro* genotoxicity of carbon nanotubes, using different endpoints. **CH016** is a potentially valuable study using co-cultures to mimic the lung. This can be used for studying cellular interactions after NP exposure and so may have utility for assessing translocation pathways. **EU030** aims to characterise a panel of NP and subject them to a battery of toxicity tests as well as limited toxicokinetics. The overall aim is to relate structural correlates to toxicity and toxicokinetics.

How studies contribute toward the RO

The overall extent to which key studies contribute toward the RO are described below. This is a broad RO – 'Research to establish a clear understanding of the deposition, distribution, toxicity, pathogenicity and translocation potential and pathways for particles in the airways and lung and their potential impacts on the cardiovascular system and brain.'

The following studies address a number of the aspects of RO14:

i). Deposition - this is rather poorly addressed, being the focus of only three studies (**US034**, **US079**, and **US156**);
ii). Distribution and translocation - several studies directly address the issue of translocation and redistribution from portal of entry (**DK009**, **CA008**, **EU030**, **US029**, **US048**, **US060**, **US129** and **US220**, **US262** and **US422**);
iii). Pathogenicity - there are a number of studies that superficially address mechanism but this is better covered in other RO; however genotoxicity is addressed in **US432** and **US435**;
iv). Cardiovascular system - few studies (**DK009**, **US048**, **US452**, **US096**, and **US220**) address the cardiovascular (CV) system as a target, despite the PM10 literature suggesting that this is likely to be a major target for manufactured NP;
v). Neurological studies - this is addressed in a few studies (**US048**, **US060**, **US437**, **US129**, **US168**, **US262**, **US320**, and **US422**).

In terms of the particle used, there is a huge emphasis on carbon nanotubes (especially the NIOSH studies) and on titanium dioxide. There are a very few with silica or alumina, some quantum dots and some gold NP. Few studies generate their own NP – **US220** is a key exception.

Extent to which the RO has been met

Overall the RO is being partially met in terms of the global research identified here.

Key gaps/ weaknesses are:

i). Use of a narrow range of particle types - e.g. lots of CNT studies - no mention of, for example, CeO_2;
ii). Piecemeal studies on translocation to brain or CV but no studies on mass balance toxicokinetics;
iii). Virtually no *in vivo* dosimetry studies marrying up toxicokinetics with studies of response i.e. no information on dose versus response, which is important;
iv). Few studies on the CV impact – a few studies on blood but the real impact of particles is likely to be on atherosclerosis – only one study seriously addressing this issue;
v). Few studies addressing the issue of deposition and effects on clearance;
vi). Despite numerous CNT studies, no studies addressing the asbestos/HARN paradigm e.g. translocation to the pleura, role of HARN length or biopersistence etc.;
vii). Few studies on brain transfer for common NP;
viii). The important issue of the origin of any CV effects i.e. blood transfer of NP versus indirect effects of pulmonary inflammation- is not directly addressed in any study.

4.15 RO15 - TOXICITY (SKIN)

The research objective

From the available information on nanomaterials used in the 800 currently available consumer products listed in the Woodrow Wilson inventory, the largest number of currently available nanotechnology products is in the health and fitness sector, which includes cosmetics and personal care products. This is followed by other applications including paints and coatings, electronics, food and food packaging. A major concern,

therefore, in relation to consumer safety of nanotechnology products is the potential for exposure through the dermal route. On the other hand, skin is designed to prevent entry of pathogens and particulate materials into the body. It is, therefore, imperative to understand whether different types of NPs can penetrate the skin, and whether the dermal uptake can lead to any toxic effects. The Research Objective for this area relates to the current use of nanoparticles in consumer products and considers whether there is a need to further our understanding of dermal uptake, penetration and toxicity in the skin.

Overview of the types of studies which have been found to be relevant to this RO

Eight studies were identified as relevant to RO15. Four scored 18 or more and were considered to have made or be making a significant contribution towards the RO. The remaining four studies presented little relevant evidence and scored 10 or less in the Weight-of-Evidence assessment.

The studies range from the study of interactions and effects of NPs and welding fumes on skin, to potential consumer safety implications arising from application of nanotechnologies for food and food packaging.

The key studies

Completed studies

Of the four highest scoring studies in the Weight-of-Evidence appraisal, **US139** and **US093** relate to evaluation of the interactions and effects of NPs on skin.

The focus of **US139** was to assess the nature of interactions between NPs and the skin, including dermal absorption, cutaneous toxicity as well as the ability to distribute to the skin after systemic exposure. The studies used NPs of different sizes, shapes and compositions to study the absorption, distribution and toxicity to the skin. The project studied the interaction between different NPs (fullerenes, derivatised fullerenes, carbon nanotubes, quantum dots) and the skin, using well-characterised *in vitro* skin models. Consideration of the final project report indicates that the studies have been well conducted, and therefore results provide very useful information in relation to safety of the four types of NPs in relation to skin.

US093 investigated dermal effects of NPs and whether toxicity is dependent on their penetration to skin, induction of oxidative stress, and content of transition metals. The study used a combination of inflammatory response with metal oxide particles, or iron-containing SWCNT to investigate the potential synergistic enhancement of damage to cells and tissue. *In vitro* studies using unpurified and purified SWCNTs showed the potential artefacts that might arise due to contaminants. A reduction in cytotoxicity of (unpurified) SWCNTs in human keratinocytes was observed on removal of iron catalyst particles. Studies also showed that *in vitro* exposure of epidermal cells to nanosized TiO_2 generated hydroxyl radicals, and activated AP–1 through phosphorylation of MAP kinase signalling pathways.

Two further high scoring studies - **UK110** and **UK111** - reviewed literature to assess implication of the current and projected applications of nanotechnology in food and food packaging in relation to consumer safety and regulatory controls. The increased ability of nanosized particles *via* the gastrointenstinal tract has been discussed in relation to potential effects on consumer health. The studies have also highlighted potential regulatory inadequacies and gaps in knowledge that need further research.

UK110 assessed the implications of nanotechnology applications for food ingredients and additives in relation to consumer safety and regulatory controls. A peer-reviewed article (Chaudhry *et al*., 2008) highlights the state-of-the-art and the potential consumer safety and regulatory implications that might arise from the application of nanotechnologies for food ingredients and additives. The study highlighted a number of potential consumer safety and regulatory issues arising from application of nanotechnologies to food additives and supplements.

UK111 reviewed the implications of nanotechnology application for food contact materials in relation to consumer safety and regulatory controls. Similarly, a peer-reviewed article has been published highlighting state-of-the-art and the potential consumer safety and regulatory implications that might arise from nanotechnology applications for food contact materials. Migration of NPs from two nanotechnology-derived food contact materials has been determined and no migration (nano-clay-PET bottles) to insignificantly low migration (nano-silver-polypropylene containers) was found.

Ongoing studies

Study **US193** has the stated aim of developing well-characterised inhalable welding fumes to investigate pulmonary, immune, and dermal exposure in rats. The project aims to develop a system for generation and characterisation of inhalable welding fumes, and investigate pulmonary, immune, and dermal reactions to exposure in rats. However, results from this study are not yet available.

Study **EU007** is investigating the link between effects and physicochemical properties, and the potential role of NPs to carry environmental contaminants. However, results on the outcome of the study are not available.

Study **BE001** aims to carry out risk assessment of nanoparticles on human health using *in vitro* and *in vivo* models. The project involves several research teams who aim to carry out characterisation of NPs, determining physicochemical properties, and studying their interaction with biological systems through *in vitro* and *in vivo* studies. The outcome of the project is expected to be helpful in informing risk assessment strategies for NPs.

Lastly, study **US057** relates to impact of physiochemical properties on skin absorption of manufactured nanomaterials. The project aims to investigate the relationship between structural aspects (e.g. particle size, surface charge, hydrophobicity and solvent effects) and permeability of NPs, to develop a quantitative model for use in risk assessment.

Extent to which the RO has been met

The assessment has shown that the scientific evidence available to address RO15 is currently limited. There are only a very few completed studies that provide some relevant information to the RO, but they represent only initial attempts to investigate the potential interactions and effects of NPs on the skin, and through ingestion (gastrointestinal) route. A few ongoing studies aim to develop well-characterised inhalable welding fumes for dermal exposure studies, and to investigate the link between effects and physicochemical properties of NPs, and the potential role of NPs to carry environmental contaminants. However, detailed results of the ongoing studies are not yet available, and it is possible that they will yield more evidence in the future.

There is therefore still a need for systematic studies to develop the basic understanding of dermal uptake, penetration and toxicity of well-characterised NPs in the skin.

Research also needs to address relevant NPs that are used, or are likely to be used, in dermal products, or those that may come in contact with skin during the whole lifecycle of materials/ products. It is also clear from the two studies on the use of nanomaterials in food and food packaging that very little is known in regard to potential exposure, uptake, toxico- dynamics/ kinetics of NPs through the ingestion route. There is a need for research to address this aspect, and also whether data on uptake, penetration and bioavailability of NPs from one exposure route (e.g. inhalation) can be extrapolated to another routes (e.g. dermal or gastrointestinal routes).

4.16 RO16 - TESTING STRATEGIES

The research objective

The research objective for RO16 is 'Human Health Hazard and Risk Assessment'. To reach this objective, data on the toxicity (hazard) of nanoparticles are required. For risk assessment, data on NP physico-chemical characteristics and exposure (occupational, environmental, consumer) are also needed.

Overview of the types of studies which have been found to be relevant to this RO

Eighteen studies were identified as relevant to the RO. Seventeen scored 14 or more in the Weight-of-Evidence assessment and were considered to have made or be making a contribution towards the RO. The remaining one study is of potential value but presented no information upon which to conduct an appraisal.

The three types of studies relevant to this RO are toxicology (including toxico-kinetics), hazard identification i.e. particle characterisation, exposure and risk assessment studies.

All the toxicology studies reviewed here have an element of particle characterisation. They are, in general, studies using *in vitro* models of human or animal cell lines. Some have included animal experiments. There is no study involving humans identified in this review. Another data gap is the lack of data on occupational, environmental and consumer exposure.

The key studies

Completed or near-completed studies

Eight completed or nearly completed studies have been identified. The studies can be organised into two categories:

i). scientific studies which generate relevant data on exposure, hazard and risk of nanoparticles;
ii). review and synthesis studies aimed at reviewing state-of-the-art knowledge and disseminating it.

In this respect, **EU012**, **US183**, **US263**, **EU030** belong to the former category while **UK075**, **US318**, **DK002** and **UK057** belong to the latter.

EU012 contributes new data on the aerosolisation of platinum nanoparticles for toxicology research. The objective is to investigate physical changes which NP aerosols undergo after release into the workplace environment under realistic scenarios. This information is essential to understand the characteristics of NPs when

they reach a human receptor after transport over a distance from a NP. Knowledge obtained from the study was used to develop recommendations to EC regarding:

- Test aerosols for nanotoxicology studies;
- Testing of filters and protective equipment in the workplace;
- Research priorities.

This project has generated important data for aerosol physicists as well as inhalation toxicologists to design their experiments and interpret their data.

US183 examines the cellular and molecular mechanisms of toxicity of nanoparticles. The methodology available is beyond the state of the art and is considered promising. However, there is little data currently available on this project.

DK002 is a review of existing data for dissemination and risk management. Specifically, its objectives are: i) to identify and assess the potential risks of nanotechnologies; ii) explore and further develop current decision-making tools for dealing with complex and uncertain emerging risks; and iii) provide recommendations on how to govern nanotechnologies within a precautionary framework focussed at protecting human health and the environment without hindering innovation.

EU030 is a small project to investigate the hazard and exposure of 5 engineered NP (Gold, Fullerenes, Quantum Dots, Single Wall Carbon Nanotubes and Carbon Black). The target systems considered are: Pulmonary, Hepatic, Cardio-Vascular. The investigators used both *in vitro* and *in vivo* models (healthy and susceptible individuals). The hazard data were combined with exposure information to derive a risk index for these NP. The methodology and findings of this project were used as background for many FP7 projects. This project is therefore instrumental in driving the shape of future nanotoxicology research.

US263 - This study investigates the gene expression profiling of Single-Walled Carbon Nanotubes as a unique safety assessment approach. However, there is no publication to date.

UK075 - This study is a review for an Assessment of Regulatory Testing Strategies and Methods for Characterising the Ecotoxicological Hazards of Nanomaterials. A report is available from the Defra Science and Research Projects website and is relevant to the RO, but does not address the need for beyond-the-state-of-the-art tests needed for regulations.

US318 - This study identifies approaches for regulating Environmental Impacts of Nanomaterials. It is relevant to the RO but it falls short in contributing relevant tools for risk assessment of NP.

UK057 reviews and evaluates different approaches on risk assessment for their relevance to nanoparticles issues. This project is likely to make a very important contribution to future research direction, both in terms of science and regulation. This project is part of the current initiative of the UK government to assess the state of the art in exposure, hazard and risk of NP.

Ongoing studies

Ten studies were identified as either ongoing, soon to commence or were assigned an unknown status (due to the absence of publicly available information) but were considered important to acknowledge. All of these studies are scientific studies which

generate relevant data on exposure, hazard and risk of nanoparticles. One study scoring less than 14 in the Weight-of-Evidence appraisal was **FR008**, an ongoing study of potential value but which presented only a title that could be appraised.

US426 is a study generating relevant data on chemical, structural and superstructural determinants of nanocarbon toxicity for risk assessment.

EU003 is an FP6 study which has the potential of developing *in vitro* tests but no useful data appear yet.

DE001 is the very comprehensive German research initiative. It covers all aspects of the nanoparticles issues – Exposure, Hazard and Risk. For toxicology, it includes both *in vitro* and *in vivo* studies. Exposure data will be supplied by the German industry. This is an ongoing project and some data have emerged.

US253 is a study sponsored by Dupont. Some data are available but does not go far enough in assessing alternative tests. However, future data may be more promising.

EU025 is a small project from the Health Thematics to develop alternative toxicology tests for nanoparticles used in medical diagnosis. Both **EU023** and **EU025** follow the same approach and include a WP on risk assessment.

EU023 is a FP7 project in the final stage of negotiation. This project looks specifically at the immune system. Of interest, there is a WP in this project specialising in functionalising nanoparticles, without the adverse properties identified in the corresponding toxicology WP.

EU022 is a FP7 study which is in the final stage of negotiation. It is potentially useful and will be relevant to the RO when data begin to appear.

DK012 is a potentially relevant study but no data is available.

US436 is a NIOSH sponsored study which is potentially relevant to the RO but no data are available so far.

Contribution towards the RO

Completed studies

The completed studies have raised awareness about the complexity in various issues regarding engineered nanoparticles, and have demonstrated an approach for combining exposure and hazard to evaluate the NP risk to human health. This approach is currently being adopted by two ongoing studies: **EU023** and **EU025**. There were many reviews on different aspects of the nano issues in order to assess the state-of-the-art, such as for risk assessment. The general impression is that the complete studies have laid the ground work to identify useful approach and knowledge gaps in science and regulation.

Ongoing studies

The ongoing studies are much more focussed in terms of their deliverables. The infrastructure for dissemination and communication is also well laid out. For (eco)toxicology, there are promising studies (**EU023** and **EU025**) which are likely to deliver interesting data for risk assessment.

Extent to which the RO has been met

It is clear that we are still some way from meeting the RO. However, it is also evident that research has become much more focussed in meeting the RO. There are still many knowledge gaps. Most important are the data on workplace, environmental and consumer exposure. For Hazard assessment, data begin to emerge, demonstrating the translocation of nanoparticles to various secondary body organs beyond the portal of entry. This implies that a hazard assessment must be based on the most sensitive target organ. Finally, the current hazard information is from *in vitro* and (few) animal studies. These data must be used to extrapolate to humans situations where the risk assessment is ultimately needed.

4.17 RO17 - UPTAKE, TOXICITY AND EFFECTS OF NANOPARTICLES ON GROUNDWATER AND SOIL MICROORGANISMS, ANIMALS AND PLANTS, ESPECIALLY IN THE CONTEXT OF REMEDIATION

The research objective

Research to establish the uptake, toxicity and effects of nanoparticles on groundwater and soil microorganisms, animals and plants, especially in the context of remediation.

Overview of the types of studies which have been found to be relevant to this RO

Initially fort-five studies were identified as being potentially related to RO17. Two were excluded on the basis of being outwith the scope of the RO. Of the remaining forty-three, studies included terrestrial and aquatic organisms, with microorganisms, invertebrates and vertebrates from both terrestrial and aquatic group environments. The NP studied included metals, which were limited to silver and iron, while metal oxides included TiO_2, CeO_2, FeO, Fe_2O_3 and ZnO. Carbon particles included simple carbon nanoparticles, C60 and nanotubes. Organic materials were also included in the form of polymers, with a number of studies not specifying the nanoparticles studied. Only one study clearly addressed bioremediation (**US191**), but this was focussed on aquatic species rather than a groundwater or soil dwelling organism. The study investigating FeO might also be relevant to bioremediation, but this is not stated explicitly within the project information. All studies addressed toxicity to some extent, and several addressed uptake (six studies). No studies focussed specifically on groundwater species. On inspection of the studies assigned initially to RO17, nineteen were found to be within the scope of the RO: **UK096**, **UK098**, **UK103**, **UK104**, **UK105**, **UK106**, **UK107**, **UK108**, **UK109**, **US005**, **US056**, **US118**, **US128**, **US154**, **US191**, **US225**, **US227**, **US259**, and **US309**. A number were reallocated to RO18, although there is a degree of overlap between RO17 and RO18. Ten studies scoring 11 or more in the Weight-of-Evidence appraisal were considered to be most relevant to RO17 (**UK096**, **UK106**, **UK107**, **US118**, **US128**, **US154**, **US191**, **US225**, **US259**, and **US309**). These studies, and the key studies having with relevance to both RO17 and RO18 also scoring 11 or more in the WoE assessment, are described below.

The key studies

Completed or near-completed studies

Study **US191** investigated the toxicity of nanoparticle iron used in remediation to standard EPA species (*Daphnia magna* and fathead minnow). The project uses standard endpoints (e.g. *Daphnia* immobilisation) allowing comparison of relative

toxicity with other environmental contaminants for regulatory purposes. In addition, the project is supplemented with micro-array analysis of gene expression for the exposed fathead minnow, which has now contributed to a database to compare the non-lethal effects of environmental contaminants. The study found no significant indication of toxic effects of nano iron in either the fish (5 day exposure to reasonably high concentration) or *Daphnia* (48h LC50 55ppm, which is equivalent to bulk Fe). However, the results present a partial picture, as the study only used two species and a 2000 gene microarray. The study has not been published but forms the basis of a report.

Study **US118** investigated a range of quantum dots conjugated to biological molecules, their potential stability in aquatic and soil environments and their potential toxicity to a range of microorganisms. This study provides some evidence that nucleobase-conjugated CdSe quantum dots were actively taken up by soil and aquatic bacteria leading to toxicity as indicated by reduced doubling time. There have not, however, been any reports or publications identified that can be used to assess the output of this study.

Study **US259** focusses on the effects of carbon nanoparticles on microbial processes. The authors suggest that there may be a shift in the structure of soil microbial populations in systems exposed to these NP. The project team propose to draw information from the ratio of key fatty acids taken from the phospholipid fatty acids fraction (PLFA) and relate it to a background status of the soil microbial populations. They will also use genetic approaches, (e.g. density gradient gel electrophoresis (DGGE) with both bacterial and fungal primers), as well as enzyme assays for dehydrogenase, urease, cellubiase; and respiration and trapping of CO_2 to estimate aerobic activity in the presence of the carbon NP. No outputs for this project have been provided.

Study **US154**, which assesses the microbial impacts of nanoparticles, investigates the hypothesis that nanomaterials that generate reactive oxygen species or free radicals will hinder heterotrophic and phytosynthetic activities. This project aimed to investigate both fullerenes and metallic nanoparticles (e.g., TiO_2, ZnO and Fe0) in terms of their transport and microbial impacts. The study includes particle characterisation (size, shape, functionality, reactivity, aggregation, deposition potential, and bioavailability) and then uses this information to screen nanomaterials of varying sizes and properties for bactericidal activity. The study also aims to discern bacterial physiologic characteristics that might result in resistance or susceptibility to catalytic nanomaterials, to evaluate the potential for fullerene biotransformation by bacteria and fungi, and to assess the impact of nanomaterial releases on microbial diversity and community structure. No abstracts, publications or reports have been identified.

Study **UK106** aims to investigate the impact of manufactured nanomaterials on groundwater microbial communities and the subsequent recovery of these communities. The study proposed is cross-disciplinary (environmental microbiologists and material scientists) and uses a systematic approach to assessing nanomaterial toxicity to groundwater microbial communities. Again this study will synthesise and characterise their own nanomaterials before exposure to laboratory microcosms for 4 months in freshly collected groundwater contaminated with the pesticide isoproturon and the environmental/industrial contaminant trichloroethene. Impacts will be determined in terms of relative abundance of microbial populations (genetic fingerprinting), changes in the communities' ability to biotransform groundwater contaminants (metabolic profiling) and shifts in the functional diversity of the community. No abstracts, publications or reports are identified.

UK102 aims to investigate the effects of C60 fullerenes and carbon nanotubes on marine mussels. In a previous study this group demonstrated that haemocytes of blue mussels can take up C60 *via* endocytosis or phagocytosis leading to cytotoxicity. The results showed increase in protein carbonyls and lipofuscin at both 0.1 and 1 mg/L for C60, but only at the 1 mg/L level for CNT. This indicates oxidative damage with toxic induction one order of magnitude less for the CNT. Lysosomal stability in digestive gland cells only revealed a significant decrease for 1 mg/L C60 and no effects for CNT. Feeding tests on mussels did not show any significant toxic response, although feeding rate was stimulated at 1 mg/L CNT. This study will investigate interactions of the nanoparticles with algae, mussel growth, cytotoxicity to hepatopancreatic digestive cells and histopathology of exposed tissues associated with ROS induced oxidative stress, although the abstract does not stipulate what this might entail. The authors suggest that their studies will be used to predict the consequences of environmental release of nanomaterials including damage to health, ecological risk and possible food chain risks for humans. The study has generated one submitted publication.

UK101 is a one year pilot study investigating the consequences of exposure of fish to nanoparticles by ingestion. The study objectives include measurement of the responses to carbon nanotubes and manufactured titanium dioxide nanoparticles in terms of histopathology, a range of health indices, and a number of toxicological and biochemical endpoints. Results of this work presented at recent conferences suggest that the ingestion of these particles induced an oxidative stress in the CNS and liver, as well as aggressive behaviour. Therefore, this study is possibly more appropriate for RO18 than RO17.

UK096 investigates the effects of synthetic polymer nanoparticle composition and size on uptake, toxicity and interactions with environmental contaminants. This study aims to use nanoparticles made within their own laboratory facility and systematically relates the characteristics to their biological effects in fungi, aquatic algae and a freshwater invertebrate. Endpoints include examination of how characteristics of size and surface chemistry influence uptake and toxicity. In addition, the study aims to examine interactions with pollutants. No abstracts, publications or results are identified.

US225 focuses on the response of aquatic and terrestrial microorganisms to carbon-based manufactured nanoparticles. The main aim of this project is to investigate the effect of carbon-based nanomaterials on the ecology and toxicity of aquatic and soil microorganisms. This work is conducted by addressing four objectives: i) solubility studies: behaviour in natural materials, redistributions, and stability; ii) enviro-toxicology: protocol development, aquatic bacterial response, chemical and metabolites, oestrogenicity; iii) effects: microbial biomass structure and function, impacts; and iv) mass balance and fungal utilisation. No outputs were identified from this study.

UK107 addresses the environmental effects of nanoparticles using a bacterial bioassay with cultures of cyanobacteria. The aim of the study is to test the hypothesis that the biological membrane is critical in the mechanisms of toxicity of the nanoparticles.

US128 investigates the toxicity of carbon-based one-dimensional nanomaterials on aquatic organisms that inhabit sediment-water interfaces and aims to identify factors controlling the toxicity to sediment-dwelling organisms. Carbon nanotubes, nanofibers, and silicon carbide nanowires often contain heavy metals (e.g., Fe, Co, Ni, Cu, and Cr), mostly introduced as catalysts for their manufacturing. This project assesses the interaction between heavy metals and carbon-based nanomaterials by studying the effects of exposures on a range of organisms inhabiting the water-sediment interface.

Ongoing studies

For the project **DK007** only the statement of objectives is available, which indicates that the study will develop methods for evaluation of the acute, chronic and genetic effects of NPs in aquatic organisms, the links between these effects and physicochemical properties of NPs, and whether they can act as carriers of contaminants. The study aims to include development of methods to assess the acute, chronic and genetic effects of nanoparticles, including assessment of the relative importance of the physical-chemical properties of nanoparticles in inducing these effects. The study also aims to investigate the role of nanoparticles as carriers of contaminants. Although publications are indicated, it is unclear if they are a direct result of this project or of a group of projects.

UK091 investigates uptake of nanoparticles by ingestion in aquatic invertebrates, fish and human *in vitro* models. The main aim of the study is to assess and compare the potential for different species, including humans, to take up nanoparticles from the environment or food sources following ingestion, and to assess the subsequent potential mechanism of toxicity for a variety of particle types. In all of the models studied, the results indicate relatively low toxic effects of CeO_2, but the potential of silver nanoparticles to cause harm to aquatic invertebrates at relatively low concentrations. The studies will make a very useful contribution to the knowledge needed to assess the risk of the NPs. A number of abstracts have been presented at international meetings, but no publications have been generated at this time.

Project **CH003**, involves quantitative risk assessment of nanoparticles in the environment, exposure modelling and ecotoxicological considerations. A peer-reviewed publication is available for this study. The study uses a life-cycle perspective to model the expected concentrations of NPs from their release into the environment. The NPs modelled were silver, TiO_2 and CNTs. The study also compares the predicted environmental concentrations (PEC) with the predicted no effect concentrations (PNEC) derived from the literature, to estimate the possible risk. The PEC values for nano-TiO_2 in water have been estimated to be close to or higher than the PNEC, whereas much lower for CNT and silver. The study will provide a basis for quantitative risk assessment of the NPs in the environment.

Study **US197** uses genomic markers to investigate the innate immune response of rainbow trout to nanoparticles that differ in physicochemical characteristics. The innate immune response includes markers of macrophage activity and function at sub-lethal particle concentrations. This study investigates the hypothesis that nanomaterials of dissimilar chemical composition will stimulate different patterns of trout macrophage gene expression, and nanomaterials of similar chemical characteristics (e.g. charge, shape, functional group) may be grouped with respect to their bioactivity, expressed as a particular gene response pattern. The idea is that such techniques and approaches can be used in the future for risk assessment purposes. This project has only generated abstracts.

Study **US133** investigates a number of hypotheses: i) that metal-containing manufactured nanomaterials pose an exposure and toxicological risk to aquatic organisms, either *via* the nanoparticle itself or *via* liberation of dissolved metal ions - this will be investigated using a number of different water compositions; ii) that exposure in the water column will be a function of dissolution rate and aggregation rate of the nanoparticle – this will be investigated using *Daphnia magna*; and iii) that exposure to benthic organisms may occur, after aggregation and settling of nanoparticles, through ingestion of sediment using *Hyalella azteca* organisms. The

study uses CdSe and CdSe/SnS quantum dots, with ZnS and ZnO nanoparticle for comparison. No study outputs have been identified that detail the results of this project.

US102 investigates the types of colloidal particles that might carry radionuclides to groundwater. This will include assessment of the physicochemical characteristics, such as charge and morphology, that can be used to predict transport through soils and sediments. This project has not yet published abstracts, publications or reports.

US104 focuses on sesquioxides, which are common products of chemical weathering and important constituents of most soils. Currently it is not fully known how the size and concentration of oxides and the composition of soil organic matter affect destabilisation of carbon from the oxide surfaces and how the oxides are distributed in the soil system. This project focuses on the assessment of threshold oxide concentrations beyond which organic-matter-oxide associations become irreversible. The project also aims to establish the influence of changing soil moisture conditions (due to global climate change scenarios) on carbon stabilisation and transport of nano-sized oxides, and to develop a relationship between reactivity, aggregation and transport of nanoparticles in the soil system. Again, due to the early nature of this study, no outputs were available to assess.

US309 focuses on the interactions between nanoparticle size and chemical composition for 5 and 100 nm copper, silver, and gold nanoparticles in determining bioavailability, tissue distribution and toxicity in a simulated terrestrial food chain, consisting of soil, earthworms (*Eisenia fetida*), and bullfrogs (*Rana catesbeiana*). Genomic and microspectroscopic techniques will be employed to determine the tissue distribution and cellular effects of the nanoparticles.

UK086 uses stickleback fish to investigate C60, but not remediation nanoparticles. The study objectives include toxicity, but not uptake. **CH009** studies green algae *Chlorella vulgaris* treated with carbon black (combustion byproduct) and carbon nanotubes (nanoparticle), but not remediation particles. The objectives indicate interactions will be investigated, however these are not clearly described.

Contribution towards the RO

Progress has been made in this area through the results and developments of recent studies. Nevertheless, it is still a field where more focus is required, given that most studies have concentrated predominantly on pelagic aquatic systems. In addition, the environmental effects of nanomaterials used in remediation have so far not been well studied, with progress being slowly made in this area.

Extent to which the RO has been met

Although the studies considered in this appraisal are relevant to ecotoxicology, environmental studies, fate, risk assessment using environmental species, it is not clear, in general, how well they fit within the scope of RO17 since very few studies actually focus on remediation particles. A few studies do assess impacts on microbial and sedimentary systems and these have been included. It is clear from this assessment that more work is required in this area.

Many of the studies initially categorised into RO17 were in fact aquatic species e.g. fish, several aquatic invertebrates and some primary producers, rather than those that might live in groundwater, although of course there are actually very few organisms that do live in groundwaters.

Most of the studies conducted focus on uptake and/or toxicity of nanoparticles, with only very few specifically investigating remediation nanoparticles. Some interesting data is emerging relating to the uptake and toxicity of nanoparticles to a range of invertebrates and microorganisms, but relatively little for plants. Most of these studies often provide details of the physicochemical characteristics of nanoparticles that drive uptake and toxicity, which can then potentially be applied to other types of nanoparticles such as those used in remediation. Almost no groundwater or soil exposure scenarios are studied and so there is much work to be done with organisms in such models.

4.18 RO18 - MECHANISMS OF TOXICITY, TOXICOKINETICS AND *IN VIVO* EFFECTS OF NANOPARTICLES TO KEY ECOLOGICAL GROUPS

The research objective

Research to establish the mechanisms of toxicity, toxicokinetics and *in vivo* effects of nanoparticles to key ecological groups (including invertebrates, vertebrates (e.g. fish) and plants). A key aspect of such work should be the facilitating of knowledge transfer from human toxicological studies to inform ecotoxicology.

Overview of the types of studies which have been found to be relevant to this RO

A total twenty-six studies were initially identified to be of relevance to this RO. Three studies were excluded on the basis of being outwith the scope of the RO. One further study could not be assessed as there was insufficient information available. Of the remaining twenty-two studies assessed under the Weight-of-Evidence appraisal, fifteen scored 11 or above and are discussed below. In these studies, a range of nanomaterials and test systems have been considered with a key focus on aquatic species, and on metal and carbon nanoparticles.

The key studies

Completed or near-completed studies

US090 examined the biophysical interactions between single-walled carbon nanotubes (SWCNTs) and naturally occurring lysophospholipids, and their subsequent toxicity in cells and in whole animals (e.g. *Daphnia magna*). A range of results has been generated by this project and five publications were produced. Data indicate biomodification of coated nanotubes takes place mediated by an aquatic crustacean. These interactions are important in effecting alteration of physical-chemical properties of carbon nanotubes.

Study **US092** focussed on the toxicity of a range on NM on aquatic species. Specifically it concentrated on the following points: i) how do filter-feeding organisms such as bivalve molluscs; and zooplankton (e.g. *Daphnia magna*) regulate the intake and distribution of NP and ii) how are fish impacted as they are exposed to these nanoparticles *via* the gills? The goal was to explore uptake and distribution of nanoparticles, as well as oxidative stress endpoints and gene expression changes. Results from this project and other work from the lead researcher have generated a range of refereed publications detailing the specific and general effects of studied nanoparticles.

Study **US126** is a wide ranging project that investigates the fate, transport, transformation and toxicity of manufactured nanomaterials, specifically in drinking

water. The toxicity assessments involved *in vitro* studies with human cell lines in order to assess the impact of exposure to drinking water *via* ingestion. At this time there is only an abstract available to assess this project, although a list of outputs is included. The project description suggests that nine different nanomaterials (metal oxides [TiO_2, iron(III) oxide, ZnO, nickel oxide, and silica], functionalised quantum dots and hematite NPs), which have been extensively characterised within the project, are investigated, and their interaction with pollutants such as anions, metals and organic molecules, interactions with virus particles and coagulation of particles. Although the study indicates that quantum dots behaved differently, metal oxides and hematite aggregated readily, indicating that most metal oxide NPs would aggregate under drinking water treatment scenarios. The study also used Caco2 BBe (human intestinal cells), and found that NPs flattened down the microvilli, allowed passage of 1% to ~10% of the particles, and led to >25% decrease in TEER (Trans-Epithelial Electrical Resistance) relative to controls, but only at very high NP concentrations. This project has led to six publications and presentations so far with four other currently planned or already submitted.

Study **US259** focuses on the effects of carbon nanoparticles on microbial processes. The authors suggest that there may be a shift in the structure of soil microbial populations in systems exposed to these NP. The project team proposed to draw information from the ratio of key fatty acids taken from the phospholipid fatty acids fraction (PLFA) and to relate it to a background status of the soil microbial populations. They also used genetic approaches, e.g., density gradient gel electrophoresis (DGGE) with both bacterial and fungal primers, as well as enzyme assays for dehydrogenase, urease, cellubiase; and respiration and trapping of CO_2 to estimate aerobic activity in the presence of the carbon NP. It is unclear if any publications have been generated from this project given that very little information is actually included in the material submitted.

FR001 focussed on the physico-chemical and biological interaction between three bacterial species (*Ralstonia*, *Syncechocystis*, *E. coli*) and five nanoparticles (Fe_2O_3, TiO_2, CeO_2, ZnO and fullerenes). From a biological point of view, the cytotoxicity, genotoxicity and nanoparticle influence on the three bacteria's metabolism was assessed by survival tests, proteomic analysis and genomic analysis. The key aim of the project was to examine any relationship between the properties of the nanoparticles and their biological impact. No information regarding outputs is included in the material submitted by the authors.

US119 aims to assess the factors controlling the fate and effect of single-walled carbon nanotubes (SWCNTs) and their synthetic byproducts in estuarine seawater, sediment, and sediment-ingesting organisms. Interactions of SWCNTs and by-products on the disposition of model organic contaminants in estuarine sediments, and their toxicity and bioavailability to estuarine invertebrates is also a key component of this project.

Ongoing studies

UK027 investigates the uptake and subsequent toxicity of a range of nanoparticles on aquatic and terrestrial invertebrates. The study has used *Daphnia magna* treated with fluorescent polystyrene beads to assess uptake and translocation, as well as TiO_2, carbon black and silver nanoparticles to assess uptake and toxicity (acute and chronic) at a range of concentrations. The study has used standard protocols, supplemented with modifications to make them more relevant to nanoparticles, as well as the development of new techniques to assess biochemical effects (e.g. oxidative stress). This study has generated abstracts, reports and has contributed data to chapters and submitted publications.

UK086 is a multidisciplinary pilot study that aims to investigate both genomic (organ-specific changes in gene expression) and oxidation-related biological responses in stickleback fish exposed to a range of fullerenes that vary in terms of physicochemical characteristics. The authors indicate that genomics was chosen as it is an "open" technology which does not have the “disadvantage” of hypothesis directed research which can focus on one parameter or process. This project has generated abstracts so far, but no reports or publications at this time.

UK091 investigates the uptake of nanoparticles by ingestion in aquatic invertebrates, fish and human *in vitro* models. The main aim of the study is to assess and compare the potential for different species, including humans, to take up nanoparticles from the environment or food sources following ingestion, and to assess the subsequent potential mechanism of toxicity for a variety of particle types. The results indicate reduced toxic effects of CeO_2, but the potential of silver nanoparticles to cause harm to aquatic invertebrates at relatively low concentrations. The observed effects were mirrored by tests on aquatic vertebrates and cell systems. The studies will make a very useful contribution to the knowledge needed to assess the risk of the NPs. A number of abstracts have been presented at international meetings, but no publications have been generated at this time.

UK101 assesses the dietary toxicity of nanoparticles on fish. During the project the responses of the fish to a 4-week dietary exposure to two major types of nanoparticles, carbon nanotubes and manufactured titanium dioxide nanoparticles, are studied. Measurements include histopathology, a range of health indices, detailed toxicological and biochemical investigations of the fish tissues, and especially gut function. No publications are listed but several presentations at Conferences have taken place.

UK102 aims to investigate the effects of C60 fullerenes and carbon nanotubes on marine mussels. In a previous study this group demonstrated that haemocytes of blue mussels can take up C60 *via* endocytosis or phagocytosis leading to cytotoxicity. The results showed increase in protein carbonyls and lipofuscin at both 0.1 and 1 mg/L for C60, but only at the 1 mg/L level for CNT. This indicates oxidative damage with toxic induction one order of magnitude less for the CNT. Lysosomal stability in digestive gland cells only revealed a significant decrease for 1 mg/L C60 and no effects for CNT. Feeding tests on mussels did not show any significant toxic response, although feeding rate was stimulated at 1 mg/L CNT. This study will investigate interactions of the nanoparticles with algae, mussel growth, cytotoxicity to hepatopancreatic digestive cells and histopathology of exposed tissues associated with ROS induced oxidative stress, although the abstract does not stipulate what this might entail. The authors suggest that their studies will be used to predict the consequences of environmental release of nanomaterials, including damage to health, ecological risk and possible food chain risks for humans. The study has generated one submitted publication.

UK106 aims to investigate the impact of manufactured nanomaterials on groundwater microbial communities and the subsequent recovery of these communities. The study proposed is cross-disciplinary (environmental microbiologists and material scientists) and uses a systematic approach to assessing nanomaterial toxicity to groundwater microbial communities. Again this study will synthesise and characterise their own nanomaterials before exposure to laboratory microcosms for 4 months in freshly collected groundwater contaminated with the pesticide isoproturon and the environmental/industrial contaminant trichloroethene. Impacts will be determined in terms of relative abundance of microbial populations (genetic fingerprinting), changes in the communities' ability to biotransform groundwater contaminants (metabolic

profiling) and shifts in the functional diversity of the community. No abstracts, publications or reports have been identified.

US111 focuses on the development of an *in vivo* system for the rapid assessment of the toxicity of nanomaterials at multiple levels of biological organisation (i.e. molecular, cellular, systems, organismal). The project uses the test organism zebrafish and aims to identify structural properties of nanomaterials that lead to adverse biological consequences. Five refereed publications were identified.

Study **US112** included the objectives to investigate the characteristics of aqueous C60 aggregates, the impact of dissolved organic material (humic and fulvic acids) on behaviour of these aggregates, and to evaluate in fish (zebrafish and channel catfish) the bioavailability and toxicity of C60 (aqueous and dietary). The project assesses changes in gene expression, histopathology, and bioaccumulation of C60 in tissues. Experiments will include chronic exposures of zebrafish from embryos to adult life stages, to allow assessment of survival, growth, biomarker gene expression in specific tissues, and histopathology. The biomarker genes identified in zebrafish will also be investigated in the channel catfish using RT-PCR, in addition to bioaccumulation of C60 among tissues and histopathology. There are currently no abstracts, publications or reports identified for this project.

US128 investigates the toxicity of carbon-based one-dimensional nanomaterials on aquatic organisms that inhabit sediment-water interfaces and aims to identify factors controlling the toxicity to sediment-dwelling organisms. Carbon nanotubes, nanofibers, and silicon carbide nanowires often contain heavy metals (e.g., Fe, Co, Ni, Cu, and Cr), mostly introduced as catalysts for their manufacturing. This project assesses the interaction between heavy metals and carbon-based nanomaterials by studying the effects of exposures on a range of organisms inhabiting the water-sediment interface.

Contribution towards the RO

Of the completed or near-completed studies, **US090** uses whole organisms and therefore investigates toxicity *in vivo*. In addition it also looks at interactions with biomolecules. **US092** also studies uptake and effects *in vivo* on a range of aquatic invertebrates. **US126** focus on drinking water and cell line studies and so is perhaps less relevant within the scope of RO18. **US259** focuses on the effects of carbon NP on microbial processes. It is unclear if there is transfer of knowledge between different systems included in this project. Similarly, **FR001** focussed on microbial studies. These studies are important to assess changes in an important system, microbes; however, the relevance within this RO18 may be limited. Similarly **US119** focussed on the study of a wide range of invertebrate organisms and microbes, and the study is relevant in the area of ecotoxicology and environmental fate and effects. However, the knowledge transfer aspect is less clear.

Of the ongoing studies, **UK027** uses biochemical assays (e.g. total antioxidant capacity, glutathione content) to assess mechanisms of toxicity. Toxicokinetics includes assessment of effects of nanoparticles on *Daphnia magna* on a daily basis for up to 21 days. The species used is an aquatic invertebrate and therefore covers the use of *in vivo* models in key ecological groups. The biochemical assays chosen for this study have been influenced by our current understanding of the effects of nanoparticles in human/mammalian and rodent models. Although the focus is important from an ecotoxicological point of view, it is unclear to what extent human-based studies have helped with the design and approach. Similarly the same can be said for study **UK086**, which focuses on organ-specific changes in gene expression in fish exposed to NP, although some oxidation-related biological responses are also studied. **UK091** focuses

on a range of environmental and human model systems and so addresses the focus of RO18 perfectly. The focus of study **UK101** is the assessment of the dietary toxicity of nanoparticles on fish and so fits well within this RO. **UK102** focuses on effects of specific NP on marine mussels and is relevant to this RO. Study **UK106** investigates the impact of NM on groundwater microbial communities and the subsequent recovery of these communities, and so the relevance to RO18 is a bit more limited. **US111** focuses on the development of an *in vivo* system for the rapid assessment of the toxicity of nanomaterials at multiple levels of biological organisation (i.e. molecular, cellular, systems, organismal) and so can be considered very relevant within the scope of RO18. **US112** assesses the effects of specific NM on a range of endpoints in two fish species and fits well within the scope of RO18.

Extent to which the RO has been met

The studies assessed under RO18 have made a useful inroad in the areas listed within this RO in terms of improving the understanding of kinetics of nanoparticle uptake in invertebrate and vertebrate models and relating this to toxicity. In addition, there are now a few studies focussing on microbial organisms and these provide information on effect assessment at individual level but also at community level, although this may not be so relevant within this RO. The number of studies only covers a limited range of species and material types. There is some effort to relate study design and interpretation to human toxicology of nanoparticles, but there is much more to be done. While significant inroads have been initiated, there is still much work to be done on all aspects of RO18.

4.19 RO19 - DEFINE ENDPOINTS TO BE MEASURED IN ECOTOXICOLOGICAL STUDIES AND ASSESS HOW FIT FOR PURPOSE CURRENT STANDARD TESTS FOR PERSISTENCE, BIOACCUMULATION AND TOXICITY ARE WHEN CONSIDERING NANOPARTICLES

The research objective

Define endpoints to be measured in ecotoxicological studies and assess how fit for purpose current standard tests for persistence, bioaccumulation and toxicity are when considering nanoparticles. This should lead to the defining of a suite of standard PBT protocols for use in environmental hazard assessment.

Overview of the types of studies which have been found to be relevant to this RO

In total 12 studies were identified to be of relevance to RO19. All studies were assessed under the Weight-of-Evidence appraisal. Nine studies scored 11 or above and are discussed below. In these studies, a range of nanomaterials and test systems were used. As for RO17 and RO18, the key focus was on aquatic species and on metal and carbon nanoparticles.

The key studies

Completed or near-completed studies

Study **FR001** focussed on the physico-chemical and biological interaction between three bacterial species (*Ralstonia*, *Syncechocystis*, *E. coli*) and five nanoparticles (Fe_2O_3, TiO_2, CeO_2, ZnO and fullerenes). From a biological point of view, the cytotoxicity, genotoxicity and nanoparticle influence on the three bacteria's metabolism was assessed by survival tests, proteomic analysis and genomic analysis. The key

aim of the project was to establish a relationship between the properties of the nanoparticles and their biological impact. No information regarding outputs is included in the material submitted by the authors.

Study **US119** aims to assess the factors controlling the fate and effect of single-walled carbon nanotubes (SWCNTs) and their synthetic byproducts in estuarine seawater, sediment, and sediment-ingesting organisms. Interactions of SWCNTs and byproducts on the disposition of model organic contaminants in estuarine sediments, and their toxicity and bioavailability to estuarine invertebrates is also a key component of this project.

Ongoing studies

For study **DK007**, only the statement of objectives is available, which indicates that the study will develop methods for evaluation of the acute, chronic and genetic effects of NPs in aquatic organisms, the links between these effects and physicochemical properties of NPs, and whether they can act as carriers of contaminants. The study aims to include development of methods to assess the acute, chronic and genetic effects of nanoparticles, including assessment of the relative importance of the physical-chemical properties of nanoparticles in inducing these effects. The study also aims to investigate the role of nanoparticles as carriers of contaminants. Although publications are indicated it is unclear if they are a direct result of this project or of a group of projects.

Study **EU003** focuses on in vitro tests of interaction of engineered nanoparticles with cells, including the identification of the modes of NP-cell interaction using laboratory-developed cellular models on the field investigations. No publications are listed.

Study **US108** will study the safety of specific nanomaterials when used as antifoulants. It will focus on CNT and will assess a variety of endpoints on bivalve molluscs, including genetic endpoints as well as a variety of cellular endpoints. Live whole organism assays will include oysters, barnacles, *Daphnia magna* and fat head minnow fry. No publications are listed.

US110 evaluates the potential risks of bioaccumulation of manufactured nanomaterials in aquatic organisms. The objectives of this project are: i) to develop suitable manufactured nanomaterial bioaccumulation testing procedures to assure data accuracy and precision, test replication, and the comparative value of test results; ii) to evaluate how the forms of these manufactured nanomaterials affect the potential bioavailability and bioconcentration factor (BCF) in phytoplankton; iii) to determine the potential biomagnification of manufactured nanomaterials in zooplankton; and iv) to determine the potential biomagnification of manufactured nanomaterials in fish. The bioconcentration, bioaccumulation, and biomagnification of manufactured nanomaterials will be evaluated in a simulated food chain and aquatic organisms, consisting of algae, daphnia, and zebrafish. No publications are listed.

US111 focuses on the development of an *in vivo* system for the rapid assessment of the toxicity of nanomaterials at multiple levels of biological organisation (i.e. molecular, cellular, systems, organismal). The project uses the test organism zebrafish and aims to identify structural properties of nanomaterials that lead to adverse biological consequences. Five refereed publications were identified listed.

US112 studies the characteristics of aqueous C60 aggregates, the impact of dissolved organic material on behaviour of these aggregates, and the bioavailability and toxicity of C60 (both aqueous C60 aggregates and dietary C60) in fish by assessing changes

in gene expression, histopathology, and bioaccumulation of C60 in tissues. Zebrafish (*Danio rerio*) and channel catfish (*Ictalurus punctatus*) are the species investigated in this research. No publications are identified.

US121 aims to assess bioavailability and trophic transfer of NP in a simplified model food chain. The overall objectives of the project are to: i) evaluate the bioavailability and toxicity of manufactured nanoparticles (ZnO) as a function of particle size to the model soil bacteria, *Burkholderia cepacia*, and the model detritivore *C. elegans* as referenced against aqueous Zn^{2+}; ii) evaluate the ability of manufactured ZnO nanoparticles to be transferred from one trophic level to the next as assessed in the simple food chain consisting of pre-exposed *B. cepacia* and *C. elegans*; and iii) evaluate the synergistic or antagonistic effects of manufactured ZnO nanoparticles on the toxicity of Cu^{2+} to *B. cepacia* and *C. elegans*. No publications are listed.

Contribution towards the RO

Of the completed studies, **FR001** and **US119** address the areas covered by this RO to a point, but the scope is necessarily incomplete given that the coverage is limited to specific species and endpoints.

Of the ongoing studies, **US108** has a number of relevant objectives within this RO. It includes the validation of a new protocol/model of cellular mediated calcification in bivalve molluscs. This new model will be used along existing standard species such as *Daphnia magna* and fathead minnow. These models will be used to develop a safe antifouling strategy for marine surfaces using single walled nanotubes. **US110** will develop testing procedures suitable for the assessment of nanomaterial bioaccumulation and evaluation of how different nanomaterials affect the potential bioavailability and bioconcentration factor (BCF) in phytoplankton. This will be followed by an assessment of the potential biomagnification of nanomaterials in zooplankton and fish. **DK007** focuses on the development of methods for the evaluation of the acute, chronic and genetic effects of NPs in aquatic organisms, the links between these effects and physicochemical properties of NPs, and whether they can act as carriers of contaminants. In this context it fits well within RO19. **EU003** focuses on *in vitro* tests of interaction of engineered nanoparticles with cells, including the identification of the modes of NP-cell interaction using laboratory-developed cellular models on the field investigations. Studies **US111**, **US112** and **US121** fit well within the scope of RO19.

Extent to which the RO has been met

Projects listed use current standard species as well as new models to assess nanomaterial toxicity. While they use standard species it is not clear whether standard protocols are used and assessed for their suitability. **US110** is the only study addressing bioaccumulation and bioconcentration of nanoparticles.

In conclusion, there are some studies completed and some ongoing. These have begun to address RO19, but there is still much work to be done using a range of nanoparticles and a range of species types to develop standard protocols and to assess persistence, bioaccumulation and toxicity of nanoparticles.

5 PROGRESS AND GAPS

5.1 INTRODUCTION

In this section the outputs from the previous chapter have been summarised to distil out the key issues, progress and gaps in relation to each of the ROs. These have been presented in a tabular format to highlight the important elements. More detail can be found in the previous chapter. These summaries have been arranged in the order presented for each task force area.

5.2 METROLOGY, CHARACTERISATION, STANDARDISATION AND REFERENCE MATERIALS

RO 02 - To identify the most suitable metrics and associated methods for the measurement and characterisation of nanoparticles
Relevant issues • Which physical and chemical characteristics are relevant to the biological behaviour of different nanomaterials? • How do these characteristics vary over time and within different environments (including biological environments)? • With what precision and accuracy do these characteristics need to be measured to investigate the potential impacts of different nanomaterials? • To what extent can existing methods be used to make relevant measurements? • How can current measurement approaches not normally applied to investigating the potential impact of nanomaterials be applied to research in this area? • What are the limitations of current characterisation methods, and how can these limitations be overcome? • Which measurements of nanomaterial characteristics or behaviour best reflect their potential to cause harm? • How do these metrics vary according to whether materials are being measured outside or inside the body? • To what extent can existing exposure measurement instruments and methodologies be used to make relevant exposure measurements for engineered nanomaterials? • What are the limitations of current techniques, and how can these be overcome?
Progress • None of the listed studies can be said to be conclusive in providing new approaches or guidelines to characterising and measuring nanomaterials. However, together they contribute to a growing body of knowledge; • Studies are mainly focussed on addressing the relevance and practicality of using surface area as an exposure metric; • Studies are limited in scope and conclusiveness.
Gaps • Very few of the projects were focussed on systematically developing characterisation or exposure measurement methods for engineered nanomaterials; • There was very little coherence between the identified projects that would lead to significant advances in risk-related measurement and characterisation methods for different classes of nanomaterials; • In most cases, there was insufficient information available to judge whether projects would result in credible and relevant new information; • The predominant materials involved in the projects identified were metal oxides and carbon-based nanomaterials; • There some emphasis on measuring occupational exposures. However, very few projects were designed to provide information useful to measuring exposures outside the workplace; • More specifically, there was a lack of research addressing general measurement methods for characterising nanomaterials and their impact in the environment.

RO 03 - To develop standardised, well-characterised reference nanoparticles
Relevant issues • Very limited number of studies addressing this issue; • Studies were predominantly associated with the development of candidate reference materials for use with exposure or toxicological analyses; • Much of the activity thus far has been on identifying candidate materials – typically with a workshop type approach; • Equally important are the protocols that accompany reference materials, for their storage and use; • In some cases, candidate materials, characterisation techniques and toxicology analyses were stated, but the absence of supporting information on most of the projects precludes verification of progress towards meeting the stated objectives.
Progress • Progress in understanding of reference materials, test materials and how they may be used is developing; • Reasonable level of agreement about the likely candidate materials; • Some commercial materials beginning to emerge, being produced by organisations such as NIST and IRMM.
Gaps • Very few reference nanomaterials are available at this stage; • Little evidence that issues such as storage, distribution are being addressed in the public domain; • No robust process in place by which these materials will be delivered in time.

RO 04 - To understand the properties of nanoparticles in the context of their ignition and explosion potential, and assess/develop methods for evaluating this
Relevant issues • Relevant types of studies would include adaptation of existing methods for assessing ignition and explosion potential for smaller quantities relevant to NP, extending models down into the NP range and collecting data for a range of NP types.
Progress • Little progress has been made, only two studies having been identified. Commercial and military aspects of research on explosive properties of nanoparticles are, however, much more widely reported.
Gaps • It is apparent from the lack of available information that this RO has been largely overlooked by groups working in the general area. Work in this area is very limited in comparison with the vast array of projects addressing nanoparticle toxicology issues. The need for assessment of explosive and flammability properties has been repeatedly highlighted as important (Pritchard, 2004; Knowles, 2006); however the response appears to have been somewhat limited.

RO 09 - Optimisation, development and application of technologies that enable the measurement of exposure to nanoparticles in soil and water
Relevant Issues • This has been interpreted as including analytical techniques and studies on the behaviour of nanoparticles in natural materials.
Progress • Generally little progress has been reported, however approaches considering life cycle reviews of specific nanoparticles may prove useful in determining the extent of exposure in defined environmental areas.
Gaps • It is apparent from the lack of available information that this RO has been largely overlooked by groups working in the general area. The need for new measurement techniques is clearly important, so it would appear that the need has not been met and there are significant knowledge gaps.

5.3 EXPOSURES – SOURCES, PATHWAYS, AND TECHNOLOGIES

RO 05 - Further identification of sources of nanoparticles
Relevant issues • What are the sources of nanoparticles which could potentially cause exposure to humans or the environment? • What sources actually cause exposure? • What are the characteristics of these exposures in terms of intensity, duration, number of people exposed, particle size and composition?
Progress • In terms of identifying the sources, good work has been done which builds on work done by the Royal Society to map out the potential exposure landscape primarily through review and inventory projects; • In terms of describing sources which actually cause exposure, some progress has been made with some scenarios. Occupational exposures have been identified in both synthesis and processing activities; • Studies already identified will make valuable contributions towards characterising exposures. Programmes are underway and these should be encouraged.
Gaps • There are many complex exposure pathways and only a very few have been considered. • Life cycle assessment approach is missing e.g. identify a series of products where nanoparticles are a component and carry out detailed life cycle analysis for each; • Consumer exposures have been postulated but have not been conclusively demonstrated, other than very obvious examples such as the application of skin care products; • Characterisation primarily requires measurement programmes either on real or simulated exposure scenarios using equipment or methods which are established and validated (dealt with under RO6); • The landscape is extremely complex with many materials, products and processes already identified as potentially causing exposure. There is a large programme of work which needs to be carried out in order to quantify and characterise these exposures over the range of important possible exposure scenarios. Studies identified will only cover a small fraction of the potential exposures which are believed to be possible.

RO 06 - Optimisation and development of technologies that enable the measurement of occupational and environmental exposure to nanoparticles *via* the air

Relevant issues

- Which metric is appropriate to use for which scenario (occupational, environmental) or particle type?
- Discrimination of the NP of interest from any background particles;
- Aggregation of nanoparticles and whether or not it is possible to describe an appropriate maximum size;
- For high aspect ratio nanomaterials (HARN) such as CNT or metal nanowires, can variants of existing measurement and counting methods used for fibrous aerosols be used in a meaningful way to determine an appropriate measure of exposure?
- Relevant studies include *instrument development* e.g. development of a new personal sampler, *optimisation studies* including optimisation/adaptation of specific existing methods, combination of different methods, combination of different metrics derive alternate indices of exposure and comparison of methods, including both of real time measurement instruments or offline analysis methods (e.g. image analysis of high aspect ratio nanomaterials using SEM or TEM approaches), *Measurement programmes,* broadly field based activities in which different types of measurement systems are used in order to try to characterise the features of a potential nanoparticle release in a workplace or the environment.

Progress

- Very limited progress in completed or near completed studies. Many studies seem overambitious in their aims;
- New or ongoing studies promise a great deal including:
    - A new personal sampler or samplers for some types of NP;
    - Improved measurement strategies, validated for some types of NP;
    - A method for measuring the surface area of TiO_2, discriminating from background aerosol;
    - A method for estimating total length exposure for CNT;
    - Improved understanding on the range of application of real-time surface area measurement methods.

Gaps

- Even if all of the studies deliver what they promise, many significant gaps will remain. These include:
    - A validated method for real time measurement of HARN;
    - An evaluation of whether fibre counting approaches can be used for HARN;
    - A widely applicable method by which NP of interest can be discriminated from background;
    - A method for assessment of the agglomeration state for NP aerosols;
    - Information about a size selection cut-point for NP.

RO 07 - Fate and Behaviour of Nanoparticles in Air
Relevant issues • Studies could deal with the release of nanoparticles into the air and their subsequent behaviour (e.g. transport, mixing, agglomeration, deposition); • Significant overlaps with RO5, RO6 and RO8.
Progress • The few published articles and reports provide some preliminary information in relation to the determination and characterisation of NPs in the workplace, assessment of potential exposure to NPs in the workplace, and effectiveness of personal protective equipment; • From the aims and objectives of the ongoing studies, it is likely that more relevant scientific evidence to support this RO will emerge in the coming years. It is, nevertheless, reassuring that a few studies carried out so far have indicated the effectiveness of engineering controls and personal protective equipment in preventing or minimising NP exposure in a workplace setting.
Gaps • Studies dealing with transport, mixing agglomeration and deposition were generally not found; • Studies which are available focus primarily on the occupational setting i.e. indoor air; • Most studies, however, relate to one or two types of NPs and it is unclear whether it will be possible to extrapolate the results to other types; • The main elements that so far seem to be scarce or missing in relation to the RO include valid analytical tools that can be used in detection and quantification of different NPs in aerial environments in the workplace, and in other emission scenarios; • There is also a need for systematic studies on different types of air-borne NPs using a range of physicochemical parameters (e.g. size, shape, form, surface area) to generate data on their interactions, fate, behaviour, and dispersal to allow development of reliable models.

RO 08 - Development of exposure control devices
Relevant issues • Can nanoparticles pass through respirator filters? • Can nanoparticles pass around respirator face-pieces (face seal leakage)? • Related to this, can nanoparticles pass through filtration systems used in air cleaning devices? • Can nanoparticles pass through personal protective clothing? • Are workplace engineering control systems (enclosure, ventilation, filtration) effective at reducing or exposure to NP?
Progress • Relatively few studies have been identified which are making a significant contribution towards this RO. All but one of these studies are being carried out in the US. Most of these studies have investigated the penetration of NP through respirator filters and other (HEPA) filters used in air cleaners; • Consistently these studies have found that, as theory predicts, filters are highly efficient collectors of NP and that the efficiency of collection increases as particle size reduces, even down to 4 nm; • For Skin Protective Equipment (SPE), such as gloves and suits, evidence of poor performance has been found in one study, based on laboratory tests, and a recommendation to use double gloves has been made.
Gaps • No information concerning the potential leakage around respirators (face seal leakage), although one ongoing study is addressing this; • For SPE, much more information is required here as to what actually works, in what circumstances and what the limitations are; • None of the studies of personal protective equipment have thus far looked at human factors and how these systems can work in a practical sense. Information on this is needed; • No studies have been carried out which systematically address the performance of engineering controls which have been implemented in practical settings; • This RO could have been extended to the development of exposure control *methods* including the development management systems, control banding and other activities of that type.

RO 10 - Research to understand the environmental fate, behaviour and interaction of nanoparticles in soils and water

Relevant issues

- The research studies are exploring a range of fate and behaviour processes, including aggregation behaviour, transformation reactions, deposition behaviour, leaching behaviour and uptake into different organisms (including soil bacteria, eukaryotes, worms, frogs, aquatic invertebrates) and through food chains;
- Different environmental systems are being explored, including surface waters, soils, air, water-sediment systems, water treatment systems and soil/water-organism systems at different levels of complexity (e.g. tightly controlled experiments with laboratory water *vs* real waters; studies with well characterised porous media *vs* studies with real soils and sediments);
- The effects of environmental variables such as pH, ionic strength, dissolved organic carbon content and light intensity are being investigated;
- In addition, the effects of other anthropogenic substances (e.g. surfactants and detergents), that are likely to occur in the environment alongside nanoparticles, is being investigated;
- Studies are also attempting to develop modelling approaches for the different processes that are being investigated.

Progress

- Significant numbers of projects are now looking at those factors and processes that might affect the fate and behaviour of a wide range of nanoparticles in soils and waters. These studies are likely to provide experimental data and modelling approaches that support the RO.

Gaps

The main elements that so far seem to be scarce or missing in relation to the RO include:

- Valid analytical tools that can be used in detection and quantification of different NPs in complex environmental matrices;
- Systematic studies on different types of NPs using a range of physicochemical parameters (e.g. size, shape, form, surface area) and environmental variables (e.g. pH, ionic concentration) to generate data on fate and behaviour of NPs to allow development of reliable models;
- Assessment of human and environmental exposure in a full lifecycle analysis approach to identify critical stages that can lead to exposure to free NPs and can be targeted for risk management.

5.4 HUMAN HEALTH HAZARD AND RISK ASSESSMENT

RO 11 - Research to establish a clear understanding of the adsorption of nanoparticles *via* the lung, skin and gut and their distribution in the body (i.e. toxicokinetics), identifying potential target organs/tissues for toxicity assessment

Relevant issues

- The types of study were predominantly *in vivo* and *in vitro* studies with a couple of modelling studies.

Progress

- For a limited number of nanoparticles, iridium and carbon, in a very few laboratories, there has been good toxicokinetics following inhalation; these have not related particle characteristics to behaviour, except for limited studies with two different sizes of iridium;
- There have been limited studies tracking the fate of NP of different sizes of gold injected into the blood; the relevance of such studies to lung exposure is not known since passage through the lungs might change the particle surface coating, leading to different toxicokinetics compared to those arising following direct injection into the blood;
- There are piecemeal studies using instillation of suspensions of particles (e.g., on the fate of TiO_2 nanoparticles instilled into the nose of rats and particokinetics after instillation); these can be criticised on the basis of the non-physiological basis of the exposure;
- Very limited studies therefore suggest that some NP can gain access to the blood and the brain after inhalation exposure; the general relevance of this to all NP, structure-relatedness of these findings or the relevance to man (these are only shown in animals), are not yet known.

Gaps

- This review of ongoing studies has failed to demonstrate that there is any comprehensive attempt to gain the toxicokinetic (particokinetic) data required to reach the aims of hazard identification and derivation of plausible dosimetry;
- There is no study that sets out with the aim of simply assessing the accumulation of particles in a wide variety of organs after inhalation exposure to a range of different NP;
- A potential structure activity relationship that governs penetration at each different portal of entry is not being sought;
- No systematic studies on the potential of different kinds of nanoparticles to get into the blood, the lymph or the brain;
- No specific studies on whether carbon nanotubes behave like asbestos, in terms of whether they translocate to the pleural mesothelium and length-relatedness of this effect;
- No studies specifically addressing the interstitialisation of inhaled nanoparticles and the consequences in terms of fibrosis etc;
- Quantum dots have been shown to pass through epidermis to the dermis, albeit in small amounts, raising issues of susceptibility – a recent study by Oberdorster *et al*., has described work where nude mice which were UV irradiated demonstrated a deeper penetration of Quantum dots into the skin;
- Quantitative estimation of translocation to brain is only being seriously addressed in one US laboratory using a limited range of nanoparticles types.

RO 12 - Research to establish a clear understanding of inter and intracellular transport and localisation of NPs and their cellular toxicity
Relevant issues • The types of study were predominantly *in vivo* and *in vitro* studies with a couple of modelling studies.
Progress • Advances in 'between cells and tissue' transport are described in RO11 above; • As regards intercellular transport, there have been studies using a limited number of NP, demonstrating that some nanoparticles can move between different cellular compartments and can cross membranes by free diffusion; • Subsequently, nanoparticles have been described as locating to the mitochondria, nucleus and being found free in the cytoplasm, unlike larger particles; this has clear implications for 'compartment-specific' toxicity.
Gaps • No straightforward study on the intracellular location of a range of different NP with time after exposure; • No studies directly addressing whether nanoparticles are preferentially taken up in the mitochondria or nucleus resulting in concentrated areas of oxidative stress and potential respiratory burst disruption; • No systematic study of structural correlates determining protein particle interactions and transport of important proteins within a cell such as detrimental changes in protein folding and packaging; • No specific studies addressing key generic issues such as whether fluorescent tagging of nanoparticles changes their location in the cell. Lots of studies are utilising quantum dots due to their visibility, but these are not typical nanoparticles; • No addressing of different target cell specific toxicity i.e. brain cells, liver cells (canaliculae formation); • No specific studies addressing carbon nanotubes and the sub-cellular targets of the length-dependent toxicity seen with carbon nanotubes , echoing asbestos (Poland). Translocation from blood into atherosclerotic plaques and their interactions with components of the plaque.

RO13 - Research to establish a clear understanding of whether oxidative stress, inflammatory effects and genotoxicity apply to nanoparticles

Relevant issues

- It would be surprising if oxidative stress, inflammation and genotoxicity were not found to be involved in the pathogenic action of NP, since these processes underlie the pathogenic effects of all other harmful particles so far studied (asbestos, quartz, etc.). They also underlie the toxicity of any number of chemical toxins. It is also true to say that these form the dominant hypothesis for mainstream nanotoxicology.

Progress

- The RO has been met to some extent and many studies are assuming the oxidative stress/inflammation hypothesis;
- More interesting and of importance to nanotoxicology is the relationship between NP structure and these endpoints i.e. the structure function relationship. The predictable heterogeneity in the oxidative stressing and inflammatory effects of NP is a most important aspect of defining the role of oxidative stress and inflammation and could allow predictive toxicology of NP. Subsequently many studies directly contribute to the question of whether oxidative stress and inflammation are involved in the action of NP. However, given the nature of toxicology, whereby doses are increased mostly *in vitro*, until an effect is reached, the relevance of such findings for plausible exposures is questionable. Few studies contribute to the issue of genotoxicity of NP.

Gaps

- Some NP are used a lot (TiO_2, Carbon black and CNT) whilst some are used much less (alumina, ZnO, quantum dots, CeO_2 etc.) and so the generic nature of the findings are questionable;
- Some limited attempts at structure activity relationships (SAR) - there can be little doubt that there will be heterogeneity in the action of NP in causing oxidative stress and inflammation – therefore we are on case-by–case assessment unless some structure function rules can be derived;
- Several studies purport to address the structure activity relationship fully through oxidative stress and inflammation as the activities allied to various structures being measured in a panel of NP;
- Other studies carry out more limited structure activity studies e.g. fixing on limited activities such as role of iron, oxidative stress biopersistence or shape;
- *In vivo* studies are at a premium and where there are *in vivo* studies they are mostly confined to a small subset of NP for proof-of-concept type studies;
- There are few studies addressing genotoxicity – only three were identified;
- Few studies are exposing subjects to NP at or near plausible exposures and then assessing oxidative stress, inflammation and genotoxicity;
- No studies with susceptible models are described except for synergism between CNT and microbial inflammogens *in vitro*, despite the fact that the only data available that may tell us about the effects of (combustion-derived) nanoparticles suggest that a susceptible background of airways inflammation or CV disease is where the main effects of manufactured NP will be found;
- There are few studies addressing the oxidative stress hypothesis for CV effects or the brain so there is a need for *in vivo* exposures to NP then measurement oxidative stress in the vessel wall and in other CV targets or in the brain;
- The focus on oxidative stress and inflammation can be seen as logical but it does stifle other thinking. For example, in one study an alumina with no oxidative stressing activity turned out to be inflammogenic. So there are definitely other mechanisms for inflammation than oxidative stress.

RO14 - Research to establish a clear understanding of the deposition, distribution, toxicity, pathogenicity and translocation potential and pathways for nanoparticles in the airways and lung and their potential impacts on the cardiovascular system and brain
Relevant issues • A broad RO. Toxicokinetic study types include deposition, distribution and translocation.
Progress • Deposition is rather poorly addressed, being the focus of only three studies; • Distribution and translocation - several studies directly address the issue of translocation and redistribution from portal of entry; • Pathogenicity - there are a number of studies that superficially address mechanism but this is better covered in other RO; however genotoxicity is addressed in two studies; • Cardiovascular (CV) system - few studies (5) address the CV system as a target, despite the PM10 literature suggesting that this is likely to be a major target for manufactured NP; • Neurological studies - this is addressed in a few studies.
Gaps • Use of a narrow range of particle types - e.g. lots of CNT studies - no mention of e.g. CeO_2; • Piecemeal studies on translocation to brain or CV, but no studies on mass balance toxicokinetics; • Virtually no *in vivo* dosimetry studies marrying up toxicokinetics with studies of response i.e. no information on dose versus response, which is important; • Few studies on the CV impact – a few studies on blood but the real impact of particles is likely to be on atherosclerosis – only one study seriously addressing this issue; • Few studies addressing the issue of deposition and effects on clearance; • Despite numerous CNT studies, no studies addressing the asbestos/HARN paradigm (e.g. translocation to the pleura, role of HARN length or biopersistence etc); • Few studies on brain transfer for common NP; • The important issue of the origin of any CV effects i.e. blood transfer of NP versus indirect effects of pulmonary inflammation- is not directly addressed in any study.

RO15 - Given the current use of nanoparticles in consumer products there is a need to further our understanding of dermal uptake, penetration and toxicity in the skin
Relevant issues • As a result of the consumer products emphasis, it was considered appropriate to expand the scope to take into account ingestion exposure due to the possible presence of NP in food and food contact materials.
Progress • There are only a very few completed studies that provide some relevant information to the RO, but they represent only initial attempts to investigate the potential interactions and effects of NPs on the skin, and through ingestion (gastrointestinal) route; • A few ongoing studies aim to develop well-characterised inhalable welding fumes for dermal exposure studies, and to investigate the link between effects and physicochemical properties of NPs, and the potential role of NPs to carry environmental contaminants. However, detailed results from these studies are not yet available.
Gaps • There is a need for systematic studies to develop the basic understanding of dermal uptake, penetration and toxicity of well-characterised NPs in the skin; • Research also needs to address relevant NPs that are used, or are likely to be used, in dermal products, or those that may come in contact with skin during the whole lifecycle of materials/ products. It is also clear from the two studies on the use of nanomaterials in food and food packaging that very little is known in regard to potential exposure, uptake, toxico- dynamics/ kinetics of NPs through the ingestion route. There is a need for research to address this aspect, and also to assess whether data on uptake, penetration and bioavailability of NPs from one exposure route (e.g. inhalation) can be extrapolated to another routes (e.g. dermal or gastrointestinal routes).

RO16 – Human Health Hazard and Risk Assessment
Relevant Issues • A strategy for assessing the potential hazard of NP, involving both *in vitro* and *in vivo* experiments.
Progress • There are only few completed studies with NP. However, the test protocols remain to be validated; • Ongoing and new studies claim to address the issue and are likely to generate useful data for a future testing strategy.
Gaps • The gaps for a useful testing strategy are: (i) lack of validation of testing protocols; (ii) concordance between *in vitro* and *in vivo* and relevance to human situations. Point (ii) is especially important as it contributes to the 3R (reduction, replacement, refine) principle.

5.5 ENVIRONMENTAL HAZARD AND RISK ASSESSMENT

RO17 - Research to establish the uptake, toxicity and effects of nanoparticles on groundwater and soil microorganisms, animals and plants, especially in the context of remediation
Relevant Issues • Initially, studies included in this RO encompassed microorganisms, invertebrates and vertebrates from both terrestrial and aquatic groups environments; • Studies on fate and modelling were also included within this RO; • Although RO17 mostly included studies that are not specific to remediation, these studies focussed on uptake by, and toxicity of, nanomaterials to microorganisms, animals and plants. Many of these were aquatic rather than terrestrial species, thus not fully fitting within this RO and have been considered under RO18. After discussion at the workshop it was decided that RO17 addresses a very specific objective that is in fact a sub-objective of RO18, therefore ensuring that the need for studies to address specifically remediation nanoparticles is explicit; • Many of the studies initially categorised into RO17 were in fact aquatic species e.g. fish, several aquatic invertebrates and some primary producers, rather than those that might live in groundwater, although of course there are actually very few organisms that do live in groundwaters (which has a specific definition and is distinct from surface waters).
Progress • There is some interesting data emerging relating to the uptake and toxicity of nanoparticles to a range of invertebrates and microorganisms, but relatively little for plants. Most of these studies often provide details of the physicochemical characteristics of nanoparticles that drive uptake and toxicity, which can then potentially be applied to other types of nanoparticles such as those used in remediation.
Gaps • Most of the studies conducted focus on uptake and/or toxicity of nanoparticles, with only very few specifically investigating remediation nanoparticles. There is therefore much work to be done with these particles; • Of the species studied most studies used animals, followed by microorganisms. Only one used plants; • Almost no groundwater or soil exposure scenarios are studied and so there is much work to be done with species in such models.

RO18 - Research to establish the mechanisms of toxicity, toxicokinetics and *in vivo* effects of nanoparticles to key ecological groups (including invertebrates, vertebrates (e.g. fish) and plants). A key aspect of such work should be the facilitating of knowledge transfer from human toxicological studies to inform ecotoxicology
Relevant Issues • A range of nanomaterials and test systems were studied. The focus was on aquatic species and on metal and carbon nanoparticles; • Facilitation of knowledge transfer from human toxicological studies to inform ecotoxicology (and vice versa) is also important.
Progress • The studies assessed have made a useful inroad in the areas listed within this RO in terms of improving the understanding of kinetics of nanoparticle uptake in invertebrate and vertebrate models and relating this to toxicity; • In addition, there are now a few studies focussing on microbial organisms and these provide information on effect assessment at individual level and also at community level, although this may not be so relevant within this RO.
Gaps • The studies only cover a limited range of species and material types; • There is some effort to relate study design and interpretation to human toxicology of nanoparticles, but there is much more to be done.

RO19 - Define endpoints to be measured in ecotoxicological studies and assess how fit for purpose current standard tests for persistence, bioaccumulation and toxicity are when considering nanoparticles. This should lead to the defining of a suite of standard PBT protocols for use in environmental hazard assessment
Relevant Issues • A range of nanomaterials and test systems were studied. As for RO17 and RO18, the focus was on aquatic species and on metal and carbon nanoparticles.
Progress • Projects listed use current standard species as well as new models to assess nanomaterial toxicity. While they use standard species it is not clear whether standards protocols are used and assessed for their suitability; • Only one project is addressing bioaccumulation and bioconcentration of nanoparticles.
Gaps • A great deal of work needs to be done, using a range of nanoparticles and a range of species types, to develop standard protocols and to assess persistence, bioaccumulation and toxicity of nanoparticles.

6 ACTIVITIES EXTERNAL TO THE SCOPE OF THE RESEARCH OBJECTIVES

Although they are broad in their scope, the ROs do not describe complete coverage of all the important issues and questions relevant to the health, safety and environmental risks arising from exposure to nanoparticles. In reviewing the studies, more than 50 were identified which were relevant to these issues but did not fit within the scope of any of the ROs. These included:

- Enabling activities;
- Life cycle analysis;
- Effectiveness of risk assessment approaches.

These studies were not formally reviewed according to the WoE appraisal as it was not possible to identify appropriate generic criteria by which they could be assessed. The most important of those in each category are described below.

6.1 ENABLING ACTIVITIES

Most of the studies fell into the category of enabling activities. These activities, which include observatories, networking activities and capability building, have played an important role in not only aiding realisation of the benefits nanotechnologies can offer *via* its many applications, but also in bringing together scientists to share an understanding of the effects of nanomaterials and nanoparticles on human health and the environment, maintaining public engagement and raising awareness of the need to develop nanotechnologies in a responsible manner in order to ensure its future. The clear added value of these observatories cannot, therefore, be ignored under the scope of this review.

6.1.1 UKX03 - SAFENANO

SAFENANO is an initiative focussed solely on provision of independent, impartial advice concerning the potential risks to the environment and human health from nanoparticles. It was established in 2006 by the IOM and funded by the UK Technology Strategy Board (TSB) and Scottish Enterprise.

SAFENANO's aims include provision of strategic advice on nanotechnology safety, health and environmental issues to UK Government and relevant stakeholder groups, co-ordination of UK industry around these issues, lowering the risk of Small and Medium sized Enterprise entry into the commercialisation of nanotechnologies, and assisting in the risk assessment of existing products containing nanoparticulates.

SAFENANO achieves its objectives primarily *via* a web-based information service (www.safenano.org), focussed on interpreting and transferring the emerging scientific evidence about the potential risks to health and the environment arising from the development and use of nanotechnology. SAFENANO's online presence has been active since August 2007. The SAFENANO website is updated on a daily basis with news covering scientific, industry, governmental, regulatory and societal developments under the umbrella of nanotechnology health and safety. In addition, SAFENANO distributes a weekly bulletin and quarterly newsletter, and staff are involved in a variety of nanotechnology EHS related projects worldwide.

EU021 - ObservatoryNANO

ObservatoryNANO is a European Observatory on Nanotechnologies, led from within the UK and funded through the European Commission's Framework 7 program (FP7). Launched in 2008, the observatory's mission is 'to present reliable, complete and responsible science-based and economic expert analysis across technology sectors, establish dialogue with decision makers and others regarding the benefits and opportunities, balanced against barriers and risks, and allow decision makers to take action to ensure that scientific and technological developments are realized as socio-economic benefits.'

In order to meet these aims, the Observatory is to analyse 10 key technology sectors within nanotechnology, and produce comprehensive and regularly updated technology reports. Areas to be analysed within each technology sector include scientific and technological trends, economic realities and expectations, ethical and societal issues, regulation, standardisation and legislative issues, and potential impacts on health, environment and safety.

The Observatory's main output is *via* its website (www.observatory-nano.eu). In addition, a quarterly newsletter is circulated to provide updates on the latest reports available, and workshops and symposia are planned. ObservatoryNANO's first symposium is to be held in March 2009, and its first full reports are due in spring 2009. Until this time, it will not be possible to judge fully the Observatory's realistic value.

EU031 - NanoImpactNet

NanoImpactNet is a multidisciplinary European network of over 27 different institutions, working on the health and environmental impact of nanomaterials. This project is led from within Switzerland and is funded for 4 years through the European Commission's FP7 program. Active since early 2008, the network's principal focus is on two-way communication, to ensure efficient dissemination of information to stakeholders and the European Commission, while at the same time obtaining input from the stakeholders about their needs and concerns. As such, its primary aim is to 'create a scientific basis to ensure the safe and responsible development of engineered nanoparticles and nanotechnology-based materials and products, and will support the definition of regulatory measures and implementation of legislation in Europe.'

To meet this goal, NanoImpactNet provides a platform for a range of workshops and integrative conferences; and will publish reports, information packages, best practice documents and a protocol and nomenclature database. To date, the project's main output has been *via* its regular newsletters and website (www.nanoimpactnet.eu), which is regularly updated and includes information from members across the EU on their latest research amongst other content. The network has held three workshops to date and the reports of these workshops are currently in preparation.

NanoImpactNet provides a valuable resource for monitoring developments from network members as they emerge. The ultimate output of the project is to centralise protocols, consensus reports, data compilations and other outputs into a European databank on nanomaterials risk and safety assessment, and to provide a generalised framework to interpret the findings from all of the ongoing and future studies on nanomaterial impacts on health and the environment in a policy-relevant way. However, as the network is still relatively new, not enough output has been generated to date to evaluate whether the project will fully meet the goals outlined.

NLX01 - KIR Nano

KIR nano is a Dutch nanotechnology risk observatory run from within the Netherlands' National Institute for Public Health and the Environment (RIVM). It is a partnership between RIVM, and the Dutch Ministries of Housing, Spatial Planning and the Environment (VROM), Health, Welfare and Sport (VWS) and Social Affairs and Employment (SZW). Its main objective is to serve as an information hub for all nanotechnology risk related matters, supporting decision makers both within Dutch government and on a wider scale with other stakeholders in the field.

KIR nano aims to co-operate and exchange information with research organisations both within the Netherlands and on an international level, in order to identify potential risks from nanotechnology development, both to humans and the environment. On a national level, its staff act as a liaison point for Dutch Government, providing advice and answering questions on the field as requested, authoring regular reports into aspects of nanotechnology risk and contributing to meetings and workshops on the risks of nanotechnology. On an international level, KIR Nano personnel are involved in a number of EC Framework projects, as well as advisory bodies including the European Food Safety Authority (EFSA) and the EC's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR).

FRX01 - Observatory for Micro and Nano Technologies (OMNT)

The Observatory for Micro and Nano Technologies (OMNT) is a joint venture between the French technological research organisation (CEA) and national scientific research centre (CNRS), creating a network of over 200 experts, to monitor scientific breakthroughs and emerging trends in micro and nanotechnologies across multiple sectors. Its goal is to advise and inform government ministries, research institutes, industry, and the wider nanoscience community.

One of the 10 key themes covered within this observatory is the effect of nanoparticles on health. Within this theme, the OMNT aims to monitor developments across multiple fields. These include: toxicology and ecotoxicology; exposure detection and monitoring; metrology, characterisation and property of nanoparticles; risk assessment; worker and consumer protection; regulation and standardisation; life cycle of nanoparticles, their future in the environment and analysing sources of exposure.

The OMNT's main output is *via* a series of publications, including a bi-monthly 'Nanodigest' and series of synthesis reports; these are however available only on a commercial basis. In addition, the OMNT hosts an annual seminar and has a website translated into both French and English (www.omnt.fr).

USX01 - International Council on Nanotechnology (ICON)

Based within Rice University, the International Council on Nanotechnology (ICON) was founded in 2004 *via* industry funding, as an extension of the US National Science Foundation Center for Biological and Environmental Nanotechnology. Its mission is 'to develop and communicate information regarding potential environmental and health risks of nanotechnology, thereby fostering risk reduction while maximizing societal benefit.'

ICON has several working groups, which focus on risk governance, best practices, EHS knowledge base expansion and communication. Its main outputs are *via* its website (http://icon.rice.edu), which includes a virtual journal and an electronic knowledge base for accessing peer-reviewed nanotechnology publications.

Communication activities include regular forums, events and projects, through which it promotes effective nanotechnology stewardship using risk assessment research and communication.

US323 – International Research Needs Assessment is one noteworthy project covered within the umbrella of ICON. The project, which completed in 2007 and reported in May 2008, was funded by the US National Science Foundation. Its aim was to develop a document assessing current scientific understanding of nanomaterial hazards for different classes of nanomaterials and identifying critical research needs and timeframes when results are needed by industry, governments, academia and the public. The project attempts to set the stage for future efforts to develop standard protocols for assessing the environmental and health impacts of nanomaterials. The project was conducted *via* two multidisciplinary meetings, one focussed on nanomaterials and characterisation, and the second focussed on toxicology. Its final output was a report written in collaboration with the Project on Emerging Nanotechnologies entitled *International Assessment of Nanotechnology Environment, Health and Safety Research Needs* (available from http://icon.rice.edu/index.cfm).

USX02 - Project on Emerging Nanotechnologies

The Project on Emerging Nanotechnologies (PEN) is a US-based initiative established in April 2005 as a partnership between the Woodrow Wilson International Center for Scholars and the Pew Charitable Trusts.

Its aim is to ensure that as nanotechnologies advance, possible risks are minimised, public and consumer engagement remains strong and the potential benefits of new technologies are realised. It achieves this goal through informing debate and creating an active public and policy dialogue, whilst maintaining a neutral position.

PEN's main outputs are online *via* its website (www.nanotechproject.org), through a regular series of events (of which it hosts various online and physical seminars, workshops and discussions), and a series of themed publications. In addition, PEN hosts several nano inventories, of which one is dedicated to tracking current research underway in the field of nanotechnology EHS.

UKX03 - HSE Horizon Scanning

Horizon Scanning is a program by which the UK Health and Safety Executive (HSE) aims to identify or predict (and thus prepare for) emerging risks in the workplace, and assess their potential impact on the distribution of resources, existing priorities and delivery for work related health and safety. Nanotechnology was first raised as an issue within this process in 2004, and falls under the science and technology branch of the activity.

HSE's goal for nanotechnology horizon scanning is to monitor the latest scientific research into exposure to and potential health effects from nanomaterials, with a particular emphasis on the occupational setting. The main output of this program is HSE's 'Helpdesk NanoAlert', which provides an overview of study results published in peer-reviewed scientific papers. This alert service is generally published twice a year, although this can vary.

EU014 - NANOTOX

NANOTOX was a support action funded under the European Commission's Framework 6 program. The global aim of this activity was to provide investigative support for the elucidation of the toxicological impact of nanoparticles on human health and the environment. This was to be achieved *via* targeted review activities examining properties, use, toxicology and ecotoxicology of nanoparticles, the findings from which would be used to develop guidelines and recommendations for use by policy makers and regulators.

NANOTOX was part of a wider cluster project, IMPART-NANOTOX. However, NANOTOX ended before completion and thus did not achieve its original deliverables, ultimately making it of little impact. IMPART continued to a second phase after the dissolution of NANOTOX, and is considered separately under **EU002**.

EU002 – IMPART

IMPART is a Swiss-led Coordination Action, which commenced in February 2005 and was funded under the European Commission's Framework 6 Program. Its primary aim is to prevent knowledge of the health and environmental implications of nanoparticles from lagging behind the technological advances. In order to meet this objective, IMPART aims to foster communication links between numbers of regional, national and international initiatives to reduce duplication of effort, pool expertise and facilitate co-operation between networks.

IMPART was part of a wider cluster project, IMPART-NANOTOX. IMPART is now the only remaining active partner after early closure of the NANOTOX project (see **EU014**). IMPART's activities to date have included a series of workshops and training sessions, and construction of a database which contains workshop proceedings, reports and a listing of key players in the area of nanotechnology health and safety research. The project was due to complete in August 2008, final reports are therefore imminent.

US061 – NOSH Consortium

The Nanoparticle Occupational Safety and Health (NOSH) Consortium was an ambitious multi-stakeholder consortium established in 2005 and led by DuPont. Formed from over 14 industry, academic and government organisations, its aim was to obtain further understanding of factors relevant to control of exposures to engineered nanoparticles over its 2 year span. In particular, emphasis was placed on deliverables to further understanding of: i) how airborne nanoparticles may behave in the workplace; ii) how to monitor and measure occupational exposures to airborne nanoparticles; and iii) how to assess the penetration of engineered nanoparticles through candidate barrier materials for personal protective equipment. A summary of deliverables and a report on findings was published by HSE in December 2007 (HSE, 2007). The consortium achieved some of their aims; for example test methods to measure filtration efficiency of commercially available filter media to aerosol nanoparticle exposure were developed that can distinguish filtration efficiencies as a function of particle size. However, delivery on others (e.g. design and manufacture of a new personal sampler) still remains outstanding.

EU020 - ENRHES

The 'Engineered Nanoparticle - Review of Health and Environmental Safety' project, funded under the European Commission's FP7 program, commenced in September 2008 and is anticipated to review data on health and environmental safety of four

classes of engineered nanoparticles: fullerenes, carbon nanotubes (CNTs), metals, and metal oxides, with the aim of informing priorities for future research, risk management actions and regulation.

Other important enabling activities for which less information was obtained include **US063** - Nanotechnology Characterization Laboratory (NIST); **DK005** – Evaluation and control of occupational health risks from nanoparticles; **US067** - Nanotechnology Information Dissemination (NIOSH); **UK014** Engineered nanoparticles in the natural aquatic environment; **US237** - Developing a Web-Based Nano-Information Library (NIOSH) and **CN003** Health and Safety Impacts of Nanotechnology.

6.2 LIFE CYCLE ANALYSIS

Life cycle analysis, as a holistic and comprehensive impact assessment tool, is being used increasingly in nanotechnology to evaluate how a product or material affects ecosystems and human health, considering life stages from pre-production, service through to end-of-life and waste disposal / recycling. Two studies have been identified which are focussing on life cycle approaches. However, neither has yet provided a significant contribution.

US058 - Comparative Life Cycle Analysis of Nano and Bulk-materials in Photovoltaic Energy Generation. In this study, the information derived from the LCA of bulk material based PV will be extrapolated to the processes used for their nanomaterial equivalents. For each of the life stages of PV (i.e., material production, cell/module manufacture, installation, operation/maintenance, recycling and disposal), resource utilisation, process efficiencies, extra controls/steps, conversion efficiencies, recyclability and the environmental fate of the micro and the nonmaterial alternatives will be investigated. In this way, data and relationships will be built that will enable the quantification of the environmental effects of nanomaterials from existing micromaterial life-cycle inventory data. The study was due to conclude in 2008.

US138 - A Life Cycle Analysis Approach for Evaluating Future Nanotechnology Applications. However, limited information is available for this project and it is not clear if this project is specifically addressing risk issues.

6.3 EFFECTIVENESS OF RISK ASSESSMENT APPROACHES

Improving the integration of hazard and exposure data into risk assessment frameworks is an important development for safety regulation and may assist in the identification of priorities for further EHS research. Much of the work described in this project will be used to try to assess, and ultimately manage risk. There are a large number of models and frameworks which have in the past been and continue to be used to assess potential risks of chemicals or other substances. The question of how these may be used with NP and what the limitations, if any, would be, is an appropriate question to consider but has not been identified in an RO. Nevertheless, there are some ongoing studies which are considering this issue. We have identified two below.

A study developing risk assessment tools, technically outwith the objectives of RO16, is **DK004** - Framework Development for Adaptive Environmental Risk Assessment and Uncertainty Analysis for Nanomaterials. The study aims to: i) develop and apply uncertainty analysis tools for environmental risk assessment of nanomaterials in order to identify and document critical areas of uncertainty; ii) develop a framework for adaptive environmental risk assessment for nanomaterials in various forms; and iii) evaluate nano-based environmental technologies with respect to environmental risks

and the applicability of the developed frameworks on these technologies. The study is very promising but has just begun and is due to complete in 2010.

US282 - NIRT: Evaluating Oversight Models for Active Nanostructures and Nanosystems: Learning from Past Technologies in a Societal Context. No additional information is available for this study.

6.4 ISSUES NOT CURRENTLY BEING INVESTIGATED

In addition, there are a number of topics which are considered potentially important by the project team but currently do not seem to be being investigated to any extent. Three are highlighted below.

The first is development of improved, evidence based, guidance on the control of exposure. While there is generic guidance available, specific guidance for control of NP is absent. Guidance which has been developed (e.g. BSI 2007, ISO 2008), although helpful, is not evidence based. While some of the studies identified will undoubtedly contribute to this, none of the studies identified have it as a specific objective. Study types which would be helpful here would include studies to investigate the effectiveness of exposure control management approaches, such as control banding, and studies to develop and evaluate exposure models.

A second largely un-researched area is ingestion as a route of exposure. It is widely reported that nanoparticles are available as food supplements (nanosilver is perhaps the best known example) and that other nanoparticles are incorporated into food. Information about which nanoparticles and in what quantities is not available. Nor have we been able to identify any research which is considering the possibility that ingested nanoparticles are able to cross the gut wall, although this has been widely speculated. Given the potential for this route to expose very large numbers of individuals and the importance of food in relation to public concerns about safety, the lack of activity in this area is surprising.

Finally, we have been unable to identify studies which are integrating the synthesis of new materials or the development of new products directly with risk assessment of the materials or processes in any meaningful way. Even though at this stage, a great deal of work needs to be done to validate that the risk assessment processes for nanoparticles, it would not be premature to begin to incorporate them into development projects. An argument could be made to suggest that they should be incorporated into all nanomaterials development projects.

7 RISK ASSESSMENT

The objective of the risk assessment appraisal was to assess whether there is sufficient information provided in the ROs to determine whether there is a risk associated with manufactured nanomaterials or whether the precautionary principle should be invoked with identified nanomaterials. It should be noted, however, that we have only considered the information and expert opinions within the ROs and not taken into account the wider body of literature that may exist for some of the longer established nanomaterials.

Firstly in this chapter, the Precautionary Principle and its applicability to potential health and environmental risks for application to the manufacture, use and disposal of nanomaterials is considered. Consideration is then given to the synthesis reports generated from the ROs in general, and then finally the overall impact and concerns related to individual nanomaterials.

7.1 PRECAUTIONARY PRINCIPLE

The Precautionary Principle has been endorsed internationally on many occasions. At the Rio Conference on the Environment and Development in 1992, world leaders agreed on the principles of the Precautionary Principle, stated in the following terms: *'In order to protect the environment, the precautionary approach shall be widely applied by States according to their capability. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation" (Rio Declaration Principle 15).*

In 2000, the European Commission adopted a Communication on the use of the Precautionary Principle providing a general framework for its use in EU policy, and to avoid unwarranted adoption of the precautionary principle as a disguised form of caution (Commission of the European Communities, 2000). The framework to be followed should be that:

- if a preliminary scientific evaluation shows that there are reasonable grounds for concern that a particular activity might lead to damaging effects on the environment, or on human, animal or plant health, which would be inconsistent with the protection normally afforded to these within the European Community, the Precautionary Principle is triggered;
- if the Precautionary Principle is triggered, decision-makers then have to determine what action is necessary, taking into account the potential consequences of taking no action, the uncertainties inherent in the scientific evaluation, and consulting interested parties on the possible ways of managing the risk. The adopted measures should be proportionate to the level of risk and to the desired level of protection. They should be provisional in nature pending the availability of more reliable scientific data;
- action is then undertaken to obtain further information enabling a more objective assessment of the risk. The measures taken to manage the risk should be maintained so long as the scientific information remains inconclusive and the risk unacceptable.

Within this, when action is deemed necessary, then the measures taken should (amongst other things) be *proportional* to the chosen level of protection, *non-discriminatory* in their application, *consistent* with other measures previously taken, based on *examination of benefits and costs* of both the action and inaction,

continuously reviewed taking into account new scientific data, and *assign responsibility* for producing the scientific evidence required to move away from the precautionary response to a more robust risk assessment. Furthermore, the implementation of the precautionary principle should be initiated with a scientific evaluation (as complete as possible), including the evaluation of and degree of uncertainty, ensuring that the decision-makers are aware of the degree of uncertainty attached to the results. The process itself should be transparent and involve all stakeholders in the issue, and the scientific evaluation should be ongoing until it is possible to reasonably determine the risks associated with the substance. It may also be necessary to place the burden of proof onto the manufacturer or importer and assign them the responsibility for producing the scientific evidence necessary for a comprehensive evaluation.

The precautionary principle is, however, only relevant when there is a potential risk, even if this risk cannot be fully quantified or demonstrated due to the insufficiency of the scientific data. It cannot be used to justify the adoption of unsupported decisions. Factors identified by the Commission include the identification of potentially negative effects without further data, a scientific evaluation of the potential adverse effects, and scientific uncertainty in risk assessment. In each case, a scientific examination of the available data must occur and the conclusions drawn must be supported by evidence.

Within this context, we believe that the precautionary principle is directly applicable to emerging nanotechnologies. However, this report can only indicate the opinion of the authors as to whether the precautionary principle should be considered in these cases and cannot be taken as the decision of the regulatory bodies. This report does not indicate what measures should be taken if the precautionary principle is invoked. It is noted that in the UK, the COSHH Regulations 2002 provides an existing framework for protection against hazardous materials in the workplace that will also account for novel substances which might represent an equivalent risk to existing hazardous substances. In addition, the HSE are able to produce specific precautionary guidance where appropriate and, as an example, they are producing specific guidance on potential exposure to carbon nanotubes based of recent experimental findings.

7.2 CONSIDERATION OF RESEARCH OUTPUTS

RO02 – Identify the most suitable metrics and associated methods for measurement and characterisation of engineered nanomaterials

The research projects identified under this RO mainly considered metal oxide and carbon-based nanomaterials. The identified projects did not provide much detail or information to satisfy the RO and those that did, had an emphasis on occupational exposure measurement.

Research projects considered the generation and characterisation of airborne (nanoparticles) NP to:

- Address the need to develop and assess new methods of exposure measurement;
- Measure aerosol surface area (SA) (by aerosol diffusion charging, electron microscopy, size distribution analysis, estimation of aerosol surface area by mass and number concentration measurements);
- Identify a correlation between number, mass and surface area, but no other relationships were identified;
- An association (although slight) between aerosol SA, biologically-relevant activity and toxicity, but these studies were not conclusive.

Whilst no study showed a new approach or developed guidelines to characterise and measure engineered NP due to limited scope and conclusiveness, the identified studies mainly considered the relevance and practicality of SA as an exposure metric. The most relevant and applicable studies were government-funded and will add to the body of knowledge surrounding this area.

We note that there are international activities to harmonise best practice in nanomaterial characterisation. A good example is the activities of the OECD Working Party on Manufactured Nanomaterials and their Lists of Manufactured Materials and Endpoints for Phase One of the Testing Programme (details are available on the OECD webiste). A series of papers on the "Safety of Manufactured Nanomaterials" have been published by an international committee (over 100 experts) under the auspices of the OECD and thus will have international agreement and status. It is proposed that a comprehensive series of physicochemical aspects be defined for the following list of representative nanomaterials:

- Fullerenes (C60)
- Single-walled Carbon Nanotubes
- Multi- walled Carbon Nanotubes
- Silver nanoparticles
- Iron nanoparticles
- Carbon black
- Titanium dioxide
- Aluminium oxide
- Cerium oxide
- Zinc oxide
- Silicon oxide
- Polystyrene
- Dendrimers
- Nanoclays

This is also relevant to RO03.

RO03 – Develop standardised, well-characterised reference nanoparticles

The identified studies in this RO were either ongoing (Nanostrand; NIST; ISO/TC29), had no associated publications (Synthetic polymer nanoparticles; model nanoparticles for environmental risk studies; nanoscale reference materials for respiratory disease prevention; generation and characterisation of ultrafine particles; submicron particles and fibres for toxicological studies; European Virtual Institute on Reference Materials), or were only used to identify nanoparticles that have been highlighted as reference nanoparticles by interested parties (REFNANO; OECD Working Party on Manufactured Nanomaterials and the US National Nanotechnology Initiative). One project has developed reference gold nanoparticles (spheres with diameters of 10, 30, and 60 nm) however there is no mention of these being (or intending to be) independently verified (NIST). Another project has manufactured reference silica nanoparticles (nominal diameter of 40 nm) in aqueous solution for the calibration of particle size analysis techniques; however, three different particle size values were assigned and thought to be due to different instruments and data analysis methods.

The projects manufacturing reference nanoparticles have not been reported to have independently validated the few reference nanoparticles manufactured and the intensive characterisation of already available commercial nanoparticles has not been

reported. The lack of consolidated data on commercially-available nanoparticles and the lack of ongoing research that will consider this area is a concern and suggests that later projects are using poorly characterised nanoparticles without reference to a known material. This increases the uncertainty in the results that have been collected and the conclusions drawn from this data.

RO04 – Understand the properties of nanoparticles in the context of their ignition and explosion potential, and assess/develop methods for evaluating this

Whilst only one relevant research project was identified as being relevant to this RO (NANOSAFE 2: safe production and use of nanomaterials), it had a strong focus on explosiveness and flammability. This project is ongoing but has already identified a number of outcomes, including the flammability of carbon nanotubes (CNT) being similar to that of carbon black of similar specific surface area; the passivation (reduced reactivity) of nano-aluminium through introducing oxide layers; and evidence that agglomerating nanoparticles exhibit reduced explosion violence. While this project and the recently started HSL project will provide further useful information as they progress, the available data pool is likely to remain limited.

RO05 – Further identification of sources of nanoparticles

A number of studies were identified for inclusion under this RO and the reviewer also included a number of studies that were not identified by the data collection in Activity 3. In general, the studies considered the routes of human exposure (inhalation, dermal and ingestion) of nanoparticles during manufacture of nanomaterials.

Occupational exposure during the manufacture of nanomaterials was considered in the UK which identified both industry and universities as sectors for nanomaterial production concentrating mainly on metals, metal oxides and quantum dots. Whilst the universities only produced a small amount of material, it was suggested that it would be easy to scale up these processes. A separate study considered the use of manufactured nanomaterials and the occupational exposure in downstream products in industrial sectors in Switzerland such as cosmetics, food, paints, powders and surface treatments (which was the largest sector). This study suggested that specific nanoparticles (Ag, Al-O_x, Fe-O_x, SiO_2, TiO_2, ZnO) are already used in significant quantities (i.e. > 1000 kg/year/company), with a median reported quantity of handled nanoparticles of 100 kg/year. The use of nanoparticles in food was further categorised as originating from processed food ingredients (nanoemulsions), engineered nanoparticle additives, packaging containing nanoparticles (“nanocomposites”, polymers containing clay nanoparticles), and from devices used during food manufacture (filtration or sensors).

The measurement of exposure has concentrated on exposure during manufacture of nanoparticles using established measurement guidelines. The materials that have been measured include TiO_2 and CNT. Emission exposure of TiO_2 after coating onto wood, polymer or title substrates was shown to vary with the substrate and also over time, with a significant reduction in emission after 60 minutes for both the wood and polymer substrates which was affected by the presence of UV light. This suggests that the conditions must be taken into consideration when estimating nanoparticle exposure from substrates. Multi-walled CNT (MWCNT; aspect ratio greater than 3, majority shorter than 5 µm therefore not strictly fibres) were measured with similar techniques used in the monitoring of occupational asbestos exposure and showed that aerosol fibres were generated during manufacture, but with suitable control mechanisms this exposure was reduced to virtually zero. A case study of CNT exposure has also been done, but no results have been recorded by the reviewer.

The potential exposure of environments to nanoparticles have been modelled in simple scenarios; however these are limited by the lack of data surrounding the content and use of products, the factors and processes in the fate and transport of nanoparticles, and the understanding of the ecotoxicity of nanoparticles. Therefore further development and evaluation of such models is needed.

Ongoing studies consider the dustiness of nanoclays, the inflammatory and genotoxicity of engineered nanoparticles (which will help to define exposure levels and the effect of control mechanisms), and the measurement and control of workplace nanomaterials (CNT case study).

In general, occupational exposure in synthesis and processing activities has previously been identified and is currently under further investigation, however the consumer exposure, whilst suggested, has not been demonstrated (except for in obvious cases such as skincare applications). This may be an issue when considering life cycle analysis methods of monitoring and estimating exposure.

RO06 – Optimisation and development of technologies that enable the measurement of occupational and environmental exposure to nanoparticles *via* air

This RO considered studies which aimed to detect and measure nanoparticles in air, however one ongoing project developing an aqueous detection method was included. The completed and ongoing studies were considered to have made little or no contribution to the RO statement, with the exception of one (completed in 2004).

The main concerns arising from the projects identified in this RO were that the techniques would only be appropriate for spherical nanoparticles, would need to be validated, would be able to separate engineered nanoparticles from incidental or environmental nanoparticles, and may not be applicable for different materials.

RO07 – Understanding the fate and behaviour of nanoparticles in air

The identified studies in this RO provide evidence that there is potential for release of aerosol nanoparticles (or airborne nanoparticles) from various processes, especially during their manufacture and physical handling (**US251**, **US176**, **US159**, **DK001**). This has been shown to be effectively eliminated with local exhaust ventilation and current manufacturing control methods which had a similar particle count to that of the outside environment (**US159**, PM2.5 only measured). However, this was shown to vary according to run and day.

Whilst physical handling was shown to increase particle release of fibres (**US159**) and nanoclays (**DK001**), the risk of exposure was also shown to reduce with low pressure compaction (**DK001**) but not shown to vary with particle modification (**DK001**).

In summary, the current laboratory safety methods in place may be considered to be sufficient for reducing the exposure of manufacturing personnel to nanoparticles. However, the reviewer does emphasise that the studies only relate to one type of nanoparticle and it is not clear whether it will be possible to extrapolate these results to other types of nanoparticles.

RO08 – Development of exposure control devices

A limited number of studies have been identified for this RO which have mainly considered exposure to airborne nanomaterials. These have been investigated for their passage through respirator filters and other (HEPA) filters used in air cleaners. The collection of airborne nanoparticles, and therefore estimation of exposure, has been shown to be sufficient in filters.

Filtering facepiece respirators (recommended by NIOSH) were shown to be effective filters and showed a decrease in percentage penetration with a decrease in particle diameter (30 nm to 4 nm diameter); however there were small significant differences between the particle penetration of silver nanoparticles compared to those of the normal test substance, NaCl (**US216**). This raises the question as to whether the aerosol behaviour of a nanoparticle can be considered to be standard or whether it changes. Whilst in general the effectiveness of the filtration devices was expected, the separate process of thermal rebound may affect the passage of particle sizes of a few nm (where particles may literally bounce through a filter enabling passage) was investigated using 3 nm diameter silver nanoparticles where this phenomenon did not occur (**US083**).

Dermal exposure to nanoparticles has been considered in light of the performance of SPE (gloves and suits). In these cases, there have been some evidence of poor performance of SPE and a recommendation of double gloving has been made. Separately, the study showed that increased penetration occurred through cotton and paper-type suits when compared to non-woven type suits (**EU028**).

The studies have not considered the impact of human factors (e.g. the effect of face-seal leakage with exposure) and this area will need further consideration.

RO09 – Optimisation, development and applications of technologies that enable the measurement of exposure to nanoparticles in soil and water.

Two ongoing projects were identified from Activity 2 as being relevant to this RO. It has been identified that the test methods used in this area are not suitable and that new measurement techniques are required. There are significant knowledge gaps.

RO10 – Research to understand the environmental fate, behaviour and interaction of nanoparticles in soils and water.

This RO has identified both expert networks reviewing current knowledge and experimental studies considering the behaviour of nanoparticles.

The reviewer states that some of the studies will be very useful, but has failed to elaborate further.

RO11 – Research to establish a clear understanding of the absorption of nanoparticles *via* the lung, skin and gut and their distribution in the body (i.e. toxicokinetics), indentifying potential target organs/tissues for toxicity assessment.

The vast majority of studies identified by Activity 2 for this RO considered *in vitro* studies with exposure scenarios that would not be typical in industrial or consumer exposure. Within these, instillation or airborne-like exposure mechanisms to lungs were used for the majority of studies. Only a few studies considered effects to skin and gut. There were a large number of studies that have yet to publish results.

In general, there is a possibility of inflammation in the lung after exposure to TiO_2 nanoparticles of different sizes (**US115**, **US428**) and that this is thought to increase with particle size (**US428**; limitations were different agglomeration states), quantum dots have been shown to be inflammatory (**US156**) and instilled CNT produce fibrosis and inflammatory response (**US156**) *in vivo* which is more pronounced if the fibres are dispersed. Iron was found to mediate the pro-inflammatory effects of CNT *in vitro* (**US156**).

The inhalation of TiO_2 nanoparticles (2-5 nm diameter) causes only minimal lung toxicity and inflammation in mice after acute exposure (whole body exposure, 4 hours, 0.77 and 7.22 mg/m^3). Sub-acute exposure (4 hours/10 days) resulted in a higher total cell count and increase in alveolar macrophages in BAL fluids (**US115**). Exposure of male C57Bl/6 mice (inhalation, 4 hrs/day, 10 days) to TiO_2, Cu and Fe nanoparticles (8.9, 3.7 and 3.6 mg/m^3, respectively, median airborne particle size diameters of 88, 188 and 199 nm, respectively) caused pulmonary inflammation, with Cu nanoparticles causing the most inflammation and TiO_2 nanoparticles the least, and some resolution within 3 weeks of exposure (**US115**; **US428**).

The reviewer identified some important knowledge gaps that are not covered under the identified studies, including structure-activity relationships to determine penetration; the affect of material on distribution and translocation; the interstitialisation of inhaled nanoparticles; potential of nanoparticles to penetrate the dermis; and the potential of nanoparticles to translocate to the brain.

RO12 – Research to establish a clear understanding of inter- and intra-cellular transport and localisation of nanoparticles and their cellular toxicity.

The studies identified in Activity 2 that fall into this RO consider the cellular toxicity of quantum dots, single walled CNT (SWCNT), TiO_2, Fe_2O_3, SiO_2, ZnO and Al_2O_3 concentrating on epithelial cells, umbilical cells, human lung cells, hepatocytes, Kupffer cells as well as protein, DNA and cell membrane interactions. In general, the studies partially met the RO with knowledge gaps identified, however little or no published information is currently available to draw any conclusions.

RO13 – Establish a clear understanding of whether oxidative stress, inflammatory effects and genotoxicity apply to nanoparticles.

Nanotoxicologists and particle experts have suggested that oxidative stress is a common mechanism for the action of nanoparticles and may be adopted as an exposure metric for the oxidative potential of airborne particles in the environment in the medium term (**UK090**).

TiO_2, carbon black and quantum dots have all provoked oxidative-stress mediated inflammatory responses in macrophages (**UK025**). The relationship between free radical generation and inflammation is unclear as medium free radical generation does not correlate with the inflammatory response, whereas nanoparticles that cause high free radical generation have a related inflammatory response (**UK030**). Latex, carbon black and TiO_2 have also been demonstrated to cause oxidative stress and activation of NF-κB and AP-1 resulting in cytokine gene expression (**UK025**). Oxidative stress has also been identified as a mechanism of toxicity in the dysfunction of peripheral vessels of rats following the inhalation of TIO_2 nanoparticles (**US096**), which was dose-dependent and arose following exposures of 2 to 12 hours at airborne mass concentrations of 1.5 to 10 mg/m^3 (deposited mass doses of 7 to 150 μg).

The administration of silver nanoparticles (35 nm) caused greater cytotoxicity in C3A cells (human liver cell line) with LD_{50} 50 µg/ml compared to 330 µg/ml when exposed to larger particles (0.6 - 1.6 µm diameter) (**UK091**). No cytotoxicity with CeO_2 nanoparticles in C3A cells was observed.

Platinum nanoparticles, along with other metal nanoparticles, were taken up by Human Umbilical Vein Endothelial Cells (HUVEC) following *in vitro* exposure but did not induce changes in cell oxidative stress of viability (**US120**).

High aspect ratio nanomaterials (HARN) fibres were shown to have some similar effects to asbestos fibres. Long HARN CNT (20 µm length) were shown to be highly inflammogenic following direct exposure of the peritoneal mesothelium in mice by IP injection and caused granulomas to form rapidly on the diaphragm. Importantly, short CNT (<5 µm) did not provoke similar effects (**UK051**).

Exposure of human keratinocytes to SWCNT for 18 hours caused oxidative stress and cellular toxicity, as indicated by the formation of free radicals, accumulation of peroxidation products, antioxidant depletion, and loss of cell viability as well as ultra-structural and morphological changes in cultured skin cells (**US093**).

Whilst the focus on oxidative stress and inflammation is logical, the reviewer emphasises that other mechanisms of toxicity are possible, e.g. alumina nanoparticles that had no oxidative stressing activity were inflammogenic (**UK093**). Therefore, there are mechanisms for inflammation other than oxidative stress that may be important and that need to be explored. These may be nanomaterial-specific rather than generic.

RO14 – Research to establish a clear understanding of the deposition, distribution, toxicity, pathogenicity, and translocation potential and pathways for nanoparticles in the airways and lung and their potential impacts on the cardiovascular system and brain.

In general, the opinion of the reviewer was that a number of the RO aspects have been met to varying degrees:

i). Deposition - partially addressed in **US034**, **US079**, and **US156**;
ii). Distribution and translocation - directly addressed in **DK009**, **CA008**, **EU030**, **US029**, **US048**, **US060**, **US129** and **US220**, **US262** and **US422**;
iii). Pathogenicity - superficially addressed mechanism of toxicity, but this is better covered in other ROs; genotoxicity is addressed in **US432** and **US435**;
iv). Cardiovascular system - Few studies (**DK009**, **US048**, **US452**, **US096**, **US220**) address the cardiovascular system as a target, despite the PM10 literature suggesting that this is likely to be a major target for manufactured NP;
v). Neurological studies - addressed by studies **US048**, **US060**, **US437**, **US129**, **US168**, **US262**, **US320**, and **US422**.

In terms of the particles studied, there is an emphasis on CNTs and TiO_2 but studies also covered quantum dots, nickel, alumina, silica, and gold NP

Gold nanoparticles were found to persist in blood to a greater extent if they were smaller and uncharged (**CH020**). PEG-coated gold nanoparticles were shown to be present in the brain after installation in the nose, and smaller particles were more likely to accumulate in the brain. When instilled into the lungs, and bypassing the nose, the nanoparticles only showed up in the brain in very small amounts, suggesting that the blood brain barrier prevents movement into the brain from the blood. When rats were injected with RSA-coated gold nanoparticles, the particles were shown to be mainly

taken up in the liver, but also the spleen and bone marrow. However, PEG-coating enhanced the retention of the nanoparticles in the blood compartment (decreasing the amount in the liver) (**US262**).

TiO_2 shapes were investigated (rods, particles and dots) and when instilled intratracheally, produced transient inflammation and cell damage which did not differ between shapes or sizes, suggesting that type of particle does not necessarily alter cytotoxic or inflammatory effects on lung but that surface reactivity should still be taken into account (**US068**). Short-term exposure to high airborne mass concentrations (concentration range unstated) of fine and ultrafine TiO_2 did not produce any lung inflammation but did cause disruption of endothelium-dependent vasodilation with fine TiO_2, which was thought to be due to the margination of polymorphonuclear leukocytes (PMNs) in the blood vessels (**US452**).

Quantum dots were shown to be more active in causing platelet aggregation when coated compared to uncoated (**CH020**), and that coating with PEG altered the translocation into the brain.

CNT inhaled into lungs (**US156**) showed pro-fibrotic effects and iron mediated free radical generation. Apo E mice injected with CNT (4 injections, site not specified) showed increased atherogenesis, mitochondrial DNA damage, and oxidative stress to the aorta (**US306**). Pharyngeal aspiration and exposure to MWCNT elicited neuroinflammation in discreet brain areas. There was increased expression of CCL_2, TNFα, and IL6 in the olfactory bulb, hippocampus, and frontal cortex. The inflammatory outburst was thought to be due to microglial activation in response to the perturbation of neuronal microenvironment and function (**US437**).

Inhaled nickel nanoparticles were shown to induce acute and chronic pulmonary inflammation and systemic inflammation. Long-term exposure also resulted in accelerated and exacerbated atherosclerosis in Apo E mice exposed for 6 hours/day for 5 days/week, up to 6 months in total (**US220**).

Welding fume nanoparticles are also investigated in studies identified under this RO, but no further data was available.

RO15 – Given the current use of nanoparticles in consumer products there is a need to further our understanding of dermal uptake, penetration and toxicity in the skin.

The expert opinion suggests that the evidence available to address whether there is a need to further understanding of dermal uptake, penetration and toxicity in the skin due to consumer products is very patchy. Only 3 studies provide initial attempts to investigate the potential interactions and effects of engineered nanomaterials on skin, or to develop well-characterised inhalable welding fumes for dermal exposure studies (detailed results of these studies are not available), and these do not reflect the commercially-available nanomaterials in consumer products. We are aware that there have been previous studies to examine skin penetration from cosmetic-grade TiO_2.

One study did suggest that SWCNT was cytotoxic in cultured human keratinocytes; however this was believed to be due to iron catalyst impurities, as the transcription factor AP-1 was not activated to such an extent when the system was exposed to purified SWCNTs (**US093**). This further emphasises the need for careful characterisation and exposure conditions. The same study did, however, show that nanosized TiO_2 generated hydroxyl radicals in epithelial cells and activated AP-1 through phosphorylation of MAP kinase signalling pathways.

RO16 – To develop testing strategies for human health hazard assessment and assess how fit for purpose current test methods are as applied to nanoparticles.

The identified studies in this RO have raised awareness about the complexity of various issues regarding engineered nanoparticles. The general impression of the reviewer is that the completed studies have laid the ground work to identify useful approaches and identified knowledge gaps in science and regulation, whilst the ongoing studies are much more focussed and attempt to address the knowledge gaps as well as the dissemination and communication between disciplines.

The main knowledge gaps are related to workplace, environmental and consumer exposure. Hazard assessment studies have demonstrated the translocation of nanoparticles to various secondary body organs beyond the portal of entry, implying that a hazard assessment must be based on the most sensitive target organ. However, this information is mainly from *in vitro* studies and there are few animal studies, making the extrapolation to humans more uncertain. This emphasises the need for a better knowledge of the toxicokinetics of nanoparticles and fits in well with the need for this aspect to be considered under the new EU REACH regulations.

RO17 – Research to establish the uptake, toxicity and effects of nanoparticles on groundwater and soil microorganisms, animals and plants; especially in the context of remediation.

In general, most of the studies that were initially identified within this RO focussed on the uptake and toxicity of nanomaterials on aquatic species. The title of the RO suggested a focus on studies on groundwater and soil systems which addressed especially remediation. There were, however, few identified studies that considered toxicity in the context of remediation, plants, or groundwater and soil microorganisms.

From the identified studies, there were data relating to a number of specific nanomaterials, but there was not clear information regarding physicochemical characterisation, either because it was not carried out or it was not detailed specifically in the information provided. This affects the usefulness of the effects data provided.

Functionalised quantum dots were shown to behave differently when compared to other studied nanomaterials (**US126**). In this study metal oxides tended to aggregate readily when exposed to the different drinking water treatment scenarios. A range of nucleobase-conjugated CdSe quantum dots (size and conjugation not reported) were also shown to be actively taken up by soil and aquatic bacteria and reducing the doubling time of the bacteria, a sign of toxicity (**US118**).

Nano-iron (form not mentioned; used in remediation) was shown to have no significant indication of toxic effects in *Daphnia* and the fathead minnow, although the tests were not all-inclusive since only two species were studied, and only 2000 genes were printed on the DNA array (**US191**). Iron oxide was shown, along with other metal oxides, to aggregate readily in drinking water (**US126**).

RO18 – Research to establish the mechanisms of toxicity, toxicokinetics and *in vivo* effects of nanoparticles to key ecological groups (including invertebrates, vertebrates (e.g. fish) and plants) – facilitating of knowledge transfer from human toxicological studies to inform ecotoxicology.

The identified studies in this RO have not provided any significant information as to the mechanisms of toxicity and toxicokinetics in ecological groups and studies in plants

appear to be generally absent (with the exception of one study using algae). The absence of plant data is a significant and a severe knowledge gap which must be addressed.

In general, the identified studies consider carbon-based and metal nanoparticles, with Zebrafish and Daphnia being the most common test species. Studies have been included that address the impact of nanoparticles on microbial organisms, but these have not yet been completed. However, data has been generated showing cytotoxicity in mussles after uptake of C_{60} *via* endocytosis or phagocytosis and CNT showing an effect at high concentration (mass but not number concentration). Whilst this data is significant, suggesting that carbon-based nanoparticles may have an ecotoxicological impact, the mass concentration administration leads to some confusion and emphasises the need for a standardised method of determining concentration.

TiO_2 was shown to aggregate readily in drinking water (**US126**). In separate modelling studies, TiO_2 was shown to have a predicted environmental concentration (PEC) equal to or greater than the predicted no effect concentration (PNEC; PEC= 0.7 – 16µg/L and PNEC < 1 µg/L, respectively) which is of considerable concern (**CH003**; PNEC determined for algae and Daphnia; no further details available). This was not the case with silver nanoparticles or CNT. This is however a modelled result and, whilst based on identified studies for the country in consideration (Switzerland), there is a large degree of uncertainty included within the model.

The administration of 0.1 mg/L silver nanoparticles (35 nm) caused greater mortality in *D. magna* when compared to particles of 0.6 - 1.6 µm diameter (60% and 15%, respectively (**UK091**) and inhibited growth at 0.1 mg/L compared to 1 mg/L bulk material. CeO_2 nanoparticles had a relatively low toxic effect compared to silver nanoparticles and showed a dose-dependant growth inhibition at 0.01 and 10 mg/L, which was also seen with the bulk material (0.01 mg/L), as well as a reduction in moulting frequency at 10 mg/L compared to controls (**UK091**).

C60 was shown to be more toxic than CNT, decreasing lysosomal stability in marine mussel digestive gland cells at 1 mg/L concentrations whilst the same concentration of CNT had no effects. An increase in protein carbonyls and lipofuscin at both 0.1 and 1 mg/L was noted with C60 but only at 1 mg/L for CNT. 1 mg/L CNT stimulated feeding in mussels, but there was no effect with C60.

RO19 – Define endpoints to be measured in ecotoxicological studies and assess how fit for purpose current standard tests for persistence, bioaccumulation and toxicity are when considering nanoparticles – lead to the defining of a suite of standard PBT protocols for use in environmental hazard assessment.

Activity 2 identified two completed and twelve ongoing studies of interest to this RO. The majority of studies use standard species (but have not stated whether they use standard methods) to determine ecotoxicological effects of nanoparticles. The nanoparticles studied have been identified as C_{60}, ZnO, Fe_2O_3, TiO_2, CeO_2, CNT, and fullerenes (specifically identified). Whilst only one project (**US110**) considers bioaccumulation and bioconcentration of nanoparticles, other studies (**US121** and **US112**) consider the bioavailability and tropic transfer in a simple food chain model and the bioaccumulation in tissues of nanoparticles, respectively. The information on the ecotoxicological effects of nanoparticles and whether current standard tests are appropriate is limited and will need to be explored more thoroughly before a confident conclusion can be drawn.

7.3 ASSESSMENT OF USE, EXPOSURE AND TOXICITY

Metal nanoparticles (including alumina) are passivated by the introduction of a naturally-occurring oxide layer (RO04) and the agglomeration of nanoparticles reduces their explosive violence (RO04). The aerosol behaviour of a nanoparticle may be affected by the chemical components (RO08).

Physical handling increases the release of fibres and nanoclays. This can be reduced with compaction (nanoclays) but not with particle modification (RO07; only shown with one type of nanoparticle). However, personal protection equipment (PPE), such as local exhaust ventilation and protective suits, has been shown to be effective for preventing exposure to nanoparticles (although the suit material must not be woven). Protective gloves were shown to be less effective, but were suitable if doubled up (RO08).

Certain high aspect ratio (HARN) fibres have been shown to have some similar effects to asbestos fibres in lung tissue (RO14).

Titanium dioxide (TiO_2) particles
Use and exposure - Used in significant quantities in industry (RO05), emission exposure varies with coated substrate (RO05).
Mechanisms of toxicity - There is a possibility of inflammation in the lung after exposure to TiO_2 nanoparticles of different sizes and this increased with particle size, dose based on surface area (RO11). Research is currently ongoing into the cellular toxicity (RO11), but no results are currently available. TiO_2 has been shown to provoke oxidative stress mediated inflammatory responses in macrophages (RO13), and has been demonstrated to cause oxidative stress, as well as activated NF-κB and AP-1 resulting in cytokine gene expression (RO13). The mechanism of toxicity in peripheral vessel dysfunction after inhalation is *via* oxidative stress (RO13).
Inhalation toxicity – Intratracheally-instilled rods, particles and dots produced transient inflammation and cell damage which did not vary with particle size or shape (RO14), however the route of exposure was not considered environmentally relevant. Short-term exposures to high airborne mass concentration of fine particles cause disruption of endothelium-dependent vasodilation. This was thought to be due to polymorphonuclear cells marginated in the blood vessels (RO14).
Dermal toxicity - Nanosized particles were shown to generate hydroxyl radicals in epithelial cells and activated AP-1 through the phosphorylation of MAP kinase signalling pathways (RO15).
Environmental toxicity – TiO_2 aggregates readily in drinking water (RO17), and the predicted environmental concentration (PEC) is equal to or greater than the predicted no effect concentration (PNEC; no toxic endpoint stated), resulting therefore in a potential scenario that requires further consideration.

Quantum dots
Use and exposure - Used in industry and academia within the UK (RO05).
Mechanisms of toxicity – Quantum dots have been shown to cause more platelet aggregation when coated with PEG compared to uncoated (RO14) and that coating changes the translocation of the particles into the brain (PEG coated quantum dots found in higher concentrations than amine-PEG coated quantum dots; RO14).
Inhalation toxicity – no information supplied in the RO.
Dermal toxicity – no information supplied in the RO.
Environmental toxicity – Functionalised quantum dots did not aggregate readily in drinking water compared to metal oxides (RO17). Nucleobase-conjugated quantum dots (CdSe) were actively taken up by soil and aquatic bacteria and had a toxic effect reducing doubling (RO17).

Carbon nanotubes (CNT) including single walled CNT (SWCNT) multiwalled CNT (MWCNT) – long and short NT

Use and exposure - Used in industry and academia within the UK (RO05). CNTs have similar flammability as compared to carbon black (RO05). Current measuring techniques may only be appropriate for spherical nanoparticles rather than rod or plate-shaped nanoparticles (RO06). Local exhaust ventilation exposure control mechanisms have been shown to be highly effective at reducing exposure (RO05). A case study of CNT exposure has been done, but no results were discussed in the RO (RO05). Some inflammatory effects of CNTs have been attributed to contamination of the test system with iron catalysts (RO14), so the results and methodologies must be carefully considered.

Mechanisms of toxicity – currently under investigation for cellular toxicity (RO12). Mice injected with CNT (location not stated) showed increased atherogenesis, mitochondrial DNA damage, oxidative stress to aorta (RO14). CNT is cytotoxic in muscles after uptake at high mass concentration (RO18).

Inhalation, instillation and intraperitoneal toxicity – CNT inhaled into lungs showed pro-fibrotic effects and iron-mediated free radical generation (RO14). Pharyngeal aspiration and exposure to MWCNT elicited neuroinflammation in discreet brain areas thought to be due to microglial activation (RO14). Long HARN CNT (20 µm length) were shown to be highly inflammogenic following direct exposure of the peritoneal mesothelium in mice by IP injection and caused granulomas to form rapidly on the diaphragm whereas short CNT (< 5 µm) did not provoke similar effects (RO14).

Dermal toxicity – Exposure of human keratinocytes to SWCNT caused oxidative stress and cellular toxicity, as well as ultra-structural and morphological changes in cultured skin cells (RO15).

Environmental toxicity – currently under investigation for ecotoxicological effects (RO19), but the PEC/PNEC ratio has been calculated to be below 1 and is therefore of lesser concern than other nanoparticles (RO17). C_{60} nanoparticles have been shown to be more toxic than CNTs (RO18).

Iron oxide (Fe_xO_x)

Use and exposure - Used in significant quantities in industry (RO05). Used in industry and academia within the UK (RO05).

Mechanisms of toxicity – currently under investigation for cellular toxicity (RO12).

Inhalation toxicity – no information supplied in the RO.

Dermal toxicity – no information supplied in the RO.

Environmental toxicity – currently under investigation for ecotoxicological effects (RO19).

Cerium oxide (CeO_2)

Use and exposure – Identified use as a diesel fuel additive, hence provides the possibility of widespread environmental exposure. However, modelling of exposure indicates very low levels (RO5, **UK074**).

Mechanisms of toxicity – no information supplied in the RO.

Inhalation toxicity – no information supplied in the RO.

Dermal toxicity – no information supplied in the RO.

Environmental toxicity – CeO_2 has been shown to have a reduced toxic effect on aquatic invertebrates when compared with bulk particles and with other nanoparticles (RO18). Further work is currently taking place in the area of ecotoxicology (RO19).

Zinc oxide (ZnO)

Use and exposure - Used in significant quantities in industry (RO05). Used in industry and academia within the UK (RO05).

Mechanisms of toxicity – currently under investigation for cellular toxicity (RO12).

Inhalation toxicity – no information supplied in the RO.
Dermal toxicity – no information supplied in the RO.
Environmental toxicity – currently under investigation for ecotoxicological effects (RO19).

Carbon black (CB)
Use and exposure – Extensively used in the rubber (tyre and rubber-goods) and printing industries, Flammable (RO05).
Mechanisms of toxicity – CB has been shown to provoke oxidative stress-mediated inflammatory responses in macrophages (RO13), and has been demonstrated to cause oxidative stress, as well as activated NF-κB and AP-1 resulting in cytokine gene expression (RO13).
Inhalation toxicity – no information supplied in the RO but we are aware of a wealth of previously published investigations.
Dermal toxicity – no information supplied in the RO.
Environmental toxicity – no information supplied in the RO.

Gold nanoparticles (Au)
Use and exposure – Reference gold nanoparticles (10, 30 and 60 nm in diameter) are being developed for use in experimental tests (RO03).
Mechanisms of toxicity – no information supplied in the RO.
Inhalation toxicity – Gold nanoparticles were found to persist in blood to a greater extent if they were smaller and uncharged (RO14). PEG-coated gold nanoparticles were shown to be present in the brain after installation in the nose, and that smaller particles were more likely to accumulate in the brain, but when installed into the lungs a very small amount showed up in the brain, suggesting that the blood brain barrier prevents movement into the brain from the blood (RO14). RSA-coated gold nanoparticles were taken up in the liver, spleen and bone marrow of rats after injection but PEG-coating enhanced the retention of the nanoparticles in the blood compartment decreasing the amount in the liver (RO14).
Dermal toxicity – no information supplied in the RO.
Environmental toxicity – no information supplied in the RO.

Silver nanoparticles (Ag)
Use and exposure - Used in significant quantities in industry (RO05). Thermal rebound was shown not to occur with filtration devices (RO08).
Mechanisms of toxicity – no information supplied in the RO.
Inhalation toxicity – Penetration of HEPA filters by silver nanoparticles differed significantly (4 – 30 nm diameter, no indication whether a greater or fewer number of particles penetrated the filter) when compared to normal test compound (NaCl) suggesting that the aerosol behaviour differs for different nanoparticles (RO08).
Dermal toxicity – no information supplied in the RO.
Environmental toxicity – The PEC/PNEC ratio was less than 1 (RO17) but silver nanoparticles (no size stated) were shown to have harmful effects on aquatic invertebrates at low concentrations (RO17).

Silica nanoparticles (SiO_2)
Use and exposure - Used in significant quantities in industry (RO05). Reference SiO_2 nanoparticles are currently being manufactured for use in calibrating particle size analysers (40 nm in aqueous solution) (RO03).
Mechanisms of toxicity – currently under investigation for cellular toxicity (RO12).
Inhalation toxicity – whilst mentioned as being studied for inhalation toxicity, no further information was provided (RO14). We are aware that previous studies are available.
Dermal toxicity – no information supplied in the RO.
Environmental toxicity – no information supplied in the RO.

Aluminium oxide (AlO_x)
Use and exposure - Used in significant quantities in industry (RO05). Alumina nanoparticles are passivated by the introduction of a naturally occurring oxide layer (RO04).
Mechanisms of toxicity – currently under investigation for cellular toxicity (RO12). Alumina nanoparticles that had no oxidative stress-related activity have been shown to be inflammogenic suggesting another method of toxicity (RO13).
Inhalation toxicity – whilst mentioned as being studied for inhalation toxicity, no further information was provided (RO14).
Dermal toxicity – no information supplied in the RO.
Environmental toxicity – no information supplied in the RO.

Nickel (Ni)
Use and exposure – no information supplied in the RO.
Mechanisms of toxicity – no information supplied in the RO.
Inhalation toxicity - Inhaled nickel nanoparticles were shown to induce acute and chronic pulmonary inflammation and systemic inflammation with long term exposure resulting in accelerated and exacerbated atherosclerosis (RO14).
Dermal toxicity – no information supplied in the RO.
Environmental toxicity – no information supplied in the RO.

Nanoclays
Use and exposure – Ongoing studies into the dustiness of nanoclays (RO05). Physical handling has been shown to increase the release of nanoclays but is reduced with low pressure compaction (RO07). Present in food packaging as complexes with polymers (RO05).
Mechanisms of toxicity – no information supplied in the RO.
Inhalation toxicity – no information supplied in the RO.
Dermal toxicity – no information supplied in the RO.
Environmental toxicity – no information supplied in the RO.

7.4 CONSIDERATION OF THE APPLICATION OF THE PRECAUTIONARY PRINCIPLE

Titanium dioxide
Titanium dioxide nanoparticles are used in significant amounts (greater than 1000 kg/company/year). They are potentially inflammatory, and have been shown to induce oxidative stress in cell culture which is mediated by the generation of free radicals. They have the potential to be environmentally detrimental as their estimated environmental concentration has been calculated to be greater than their no effect concentration, however the nanoparticle size and type was not further defined, the toxic effect not stated, and the data were based on a modelling study. This must be further investigated and steps taken to ensure the environmental safety of titanium dioxide nanoparticles. The ROs have not identified a sufficient body of evidence to make a risk assessment feasible for this nanomaterial, however there is some evidence to suggest that titanium dioxide nanoparticles may be present in the environment at concentrations greater than the predicted no effect concentration.

Iron oxide
Whilst produced in significant amounts, there was no evidence presented in the ROs to suggest that iron oxide nanoparticles are detrimental or beneficial to human health or the environment, therefore a risk assessment was not possible.

Quantum dots
Quantum dots have been shown to cause platelet aggregation, however there is no information on other toxicological properties. Quantum dots do not readily aggregate in drinking water. Limited exposure as experimental use and normally held in solution or a solid matrix. Quantum dots are a general name given to a range of compounds (including cadmium telluride and cadmium selenide) therefore it will be necessary to consider each chemical compound separately. The ROs have not identified a sufficient body of evidence to make a risk assessment feasible for this group of nanomaterials.

Cerium oxide
Although suggested to have relatively low aquatic invertebrate toxicity, the ecotoxicological properties of cerium oxide nanoparticles is currently under investigation. Data suggests that CeO_2 nanoparticles are not cytotoxic *in vitro* (human hepatic cell line), however this may be cell specific and will require further investigation. However, the ROs have not identified a sufficient body of evidence to make a risk assessment feasible for this nanomaterial.

Zinc oxide
Whilst produced in significant amounts, there is no evidence to suggest that zinc oxide nanoparticles are detrimental or beneficial to human health; or the environment. However, the toxicological effects and ecotoxicological effects of zinc oxide nanoparticles are currently being investigated and the ROs have not identified a sufficient body of evidence to make a risk assessment feasible for this nanomaterial.

Carbon black
Carbon black particles are flammable and have been shown to produce oxidative stress mediated inflammatory responses in cell culture. The ROs have not identified a sufficient body of evidence to make a risk assessment feasible for this nanomaterial.

Carbon black consists of near-spherical colloidal primary particles which exist outside the production chamber as aggregates consisting of a number of fused primary particles. These aggregates may consist of a few or hundreds of particles, or the particles can be bound together by van der Waals forces in more loosely associated agglomerates (IARC; 1996). The average primary particle diameters in several commercially produced carbon blacks range from 10 to 500 nm, while the average aggregate diameters range from 80 to 810 nm (DFG, 1999, IARC, 1996). The particles are likely to be contaminated by chemicals including polycyclic aromatic hydrocarbons (PAHs), elemental sulphur (DFG, 1999; IARC, 1996; McCunney *et al*., 2001). Industrial exposure to carbon black particles has been shown to vary with occupation. Carbon black has been thoroughly reviewed by the International Agency for Research on Cancer (IARC, 1996) who concluded that there was "*inadequate evidence in humans for the carcinogenicity of carbon black…sufficient evidence in experimental animals for the carcinogenicity of carbon black* [and its extracts]". It has been classified as "*possibility carcinogenic to humans (Group 2B*)". The risk associated with carbon black particles has previously been characterised.

Nickel
Inhaled nickel nanoparticles have been shown to cause pulmonary inflammation (acute exposure) and systemic inflammation (chronic exposure) which resulted in atherosclerosis in mice models. However, the ROs have not identified a sufficient body of evidence to make a risk assessment feasible for this nanomaterial.

Silicon oxide
Whilst produced in significant quantities, there is no evidence to suggest that amorphous silica nanoparticles are detrimental or beneficial to human health or the environment. Ongoing studies are considering their toxicological behaviour. The ROs have not identified a sufficient body of evidence to make a risk assessment feasible for this nanomaterial.

The inhalation of crystalline silica causes irritation and inflammation in the lungs, with chronic exposure causing the condition silicosis, characterised by unique histological nodules and fibrotic scarring of the lung (Green and Vallvathan, 1996). Animal studies have provided lowest observed adverse effect levels (LOAEL) ranging from 1.0 mg/m^3 (24 month study) to 2 mg/m^3 (6 month study) but it is noted that inhaled silica is much more toxic to humans than rodents (the reported human LOAEL is 0.02 to 0.05 mg/m^3) with the main mechanism of damage being cytokine release and apoptosis produced as a result of receptor-mediated signalling (Hamilton *et al*., 2008). The risk associated with silica particles has previously been characterised.

Aluminium oxide
Whilst produced in significant quantities, there is no evidence to suggest that aluminium oxide nanoparticles are detrimental or beneficial to human health or the environment. However they have been shown to be inflammogenic in cell culture but did not cause oxidative stress. The ROs have not identified a sufficient body of evidence to make a risk assessment feasible for this nanomaterial.

Carbon nanotubes
Carbon nanotubes are increasingly used in industry and research. It has been shown that PPE is effective if used correctly (gloves need to be doubled up). Whilst the cellular toxicity of carbon nanotubes is currently under investigation, they have been shown to cause mitochondrial DNA damage, oxidative stress to the aorta and to be cytotoxic in muscles at high mass concentrations. After installation into the lungs, they have been shown to be pro-fibrotic with some similar effects to asbestos fibres. The inflammatory response has been shown to be *via* iron-mediated free radical generation. CNT have also been shown to cause oxidative stress in keratinocytes and cultured skin cells. The ecotoxicological effect of CNTs is currently under investigation.

The potential of CNTs to cause oxidative stress and cytotoxicity in cell culture, along with the pro-fibrotic response after installation of CNTs into the lungs suggests that caution should be used when handling CNTs. There is currently no indication as to whether CNTs can be released from matrices in products in which they are currently found. Whilst the ROs have not identified a sufficient body of evidence to make a risk assessment feasible for this nanomaterial, there is sufficient evidence to suggest that CNT may be harmful to human health and therefore the use of the precautionary principle should be considered in this case. As noted, we are aware that the UK HSE is in the process of developing specific guidelines for the control of exposure to CNTs.

Nanoclays
Whilst produced and used in food packaging, there is no evidence to suggest that nanoclays are detrimental or beneficial to human health or the environment. The ROs have not identified a sufficient body of evidence to make a risk assessment feasible for this nanomaterial.

Silver
The RO reports present indicative evidence of the harm of silver nanoparticles at low concentrations on aquatic invertebrates, which suggest that the environmental release

of silver nanoparticles will be detrimental for the environment and that any industry/institute using silver nanoparticles should consider taking the necessary steps to reduce or eliminate the potential exposure of the environment to these nanoparticles. The ROs have not identified a sufficient body of evidence to make a risk assessment feasible for this nanomaterial; however there is sufficient evidence to suggest that silver nanoparticles may be harmful to the environment and therefore the use of the precautionary principle should be considered in this case.

Gold

Gold nanoparticles will translocate into the brain after installation in the nose, but this does not occur if the particles are installed into the lungs. As small particles are more likely to travel further into the respiratory system before being removed, it is likely that the exposure to humans will be through the lungs. After injection, coated gold nanoparticles were found in the liver, spleen and bone marrow of rats, although a different coating enhanced the retention of the nanoparticles in the blood compartment. Whilst this suggests that gold nanoparticles translocate within the body, there is no suggestion of toxicity in mammals or in the environment. The ROs have not identified a sufficient body of evidence to make a risk assessment feasible for this nanomaterial.

7.5 SUMMARY

This report draws upon the data identified from the previous activities. Whilst the authors acknowledge that other published studies are available for a number of these NP described above, these do not fall under the remit of this project and have not been considered in the opinions stated above. The authors are aware that some background data has not been included in the RO reports (including reports of ecotoxicological effects of silver and toxicological effects of carbon black nanoparticles). However, the ROs have not identified a sufficient body of evidence to make a risk assessment feasible for any of the NPs described.

The precautionary principle requires that there is a preliminary scientific evaluation showing reasonable grounds for concern that the nanomaterial might lead to damaging effects on the environment, or on human, animal or plant health. For the majority of the identified nanomaterials, the body of evidence identified was not sufficient to suggest that the nanomaterials will cause harm and therefore the precautionary principle should not be considered in these cases. However, three different nanomaterials have been identified that give rise to sufficient concern from the results presented within the RO reports. There is evidence that carbon nanotubes may have an adverse effect on human health; and that silver nanoparticles and titanium dioxide nanoparticles are detrimental to the environment. In these specific cases further investigation as to the need to invoke the precautionary principle is required, taking into consideration all available data.

Further information may be available in the wider literature that was not considered as part of this project. The authors acknowledge that the presented emerging data on nanomaterials described within this project suggest that there is uncertainty, particularly surrounding the ecotoxicological effects of nanomaterials, and that further data should be collected in this area. Whilst certain areas of investigation are extensively covered, for example the pulmonary effects of nanoparticles, other areas require more attention, including the environmental fate of nanomaterials in water and the dermal or ingestion absorption of nanomaterials.

In conclusion, whilst the precautionary principle can be applied to selected nanomaterials as a result of the data investigated within this project, there is an

identified need for further information (which may already be present within the wider literature). Therefore the data presented here must indicate caution over the use of nanomaterials and, if considered alongside the wider literature, there may still be a case for the application of the precautionary principle to certain manufactured nanomaterials.

8 DISCUSSION

8.1 LIMITATIONS OF THE STUDY

The remit undertaken in the EMERGNANO study was an extremely challenging one. It was to identify (recently completed and current) research activities on a worldwide basis, assess the quality and relevance of that activity, to map that against the 18 research objectives published by Defra, and to consider whether, within that emerging data, there was sufficient evidence to justify implementation of the precautionary principle. The plan was to complete the study within a timescale of six months. The constraints of the project, in relation to the timescale for delivery, the resources available and that the focus was on active, rather that published work means that that we cannot be certain to have captured all of these studies, or that we have captured all of the information available on these studies. Nevertheless, the authors consider that EMERGNANO has been successful in identifying and appraising the overwhelming majority of the current work worldwide.

In preparing a proposal to address the remit of this project, we considered, but ultimately rejected, a conventional literature review to identify relevant studies. It was considered likely that many studies ongoing would not yet have information in the literature and so would not be identified by this route. In addition, for those studies which were identifiable, it was not always possible to relate this back to the funding source. Given that an objective here was to evaluate the usefulness and contribution of these planned strategic investments in research, this would not be optimal. Finally the volume of studies now appearing in the literature, particularly in relation to toxicology issues, meant that a review which attempted to cover all dimensions of the risk issue simply could not be done within the allocated timescale or resources. One consequence of this strategy is that important contributions or papers arising from studies which were not funded at a national or strategic level, such as a single PhD study at a university, would not be identified in this approach. For this reason, it was requested that individual reviewers used their own knowledge of the particular area to identify significant and important pieces of work which were otherwise not picked up by our search strategy.

The first limitation concerns study identification. We were fortunate in undertaking this study that we had access to the existing database of research activities held by the Woodrow Wilson Project on Emerging Nanotechnologies. Indeed without access to this database, it is doubtful if the study could have been completed at all. However, the Wilson Center database is a comprehensive, but not exhaustive, catalogue of international nanotechnology risk-related research and, while it provided an essential starting point for this project, it left gaps in the knowledge-base that required further exploration. Our strategy to add to this list was: i) to identify other reviews of existing projects, such as that produced by the European Commission (EC, 2008); ii) identification of resources held on “national” websites or major research programmes in nanotechnology which we were aware of or which were identified through web based searching strategies; and iii) by direct communication with our extensive network of contacts and collaborators. Where possible this was followed up by direct contact with identified persons with responsibilities for the programmes which we found. Whilst this approach could not guarantee identification of all studies, and we cannot exclude that important studies have been missed, we consider that the studies identified represent the overwhelming majority of active or recently active studies which have been funded at a national or strategic level. We consider that the study list identified represents the most comprehensive list available at this point in time.

Having identified the studies, it was necessary to gather together the output from these studies, both published and unpublished. Again, a multiple strategy was adopted here which involved searching for publicly available resources relating to the study and contacting the identified study leader directly by email. Direct contact with study leaders was chosen in preference to going through national contact points since it was considered that this approach would lead to a greater response rate. In practice the response from study leaders was variable. Less than half of those contacted were willing or able to provide additional information. Given the intensity of activity in this area and the demands on researchers, this is perhaps not surprising. However this must also be considered a limitation to the study. Incomplete information about a specific study limits the extent to which the quality and relevance of the study can be assessed.

Finally, the studies identified differed widely in terms of their relevance, quality and state of completion. To try to assess in a coherent way, a quantitative weight of evidence (WoE) approach was developed and applied in an attempt to introduce more rigor into the review process. In retrospect, we consider this approach to have been necessary but ambitious. Whilst all of the reviewers were involved in the development of approach, feedback from the reviewers was mixed in relation to its viability and overall usefulness. A longer study would have provided the opportunity for this approach to be further developed, evaluated and finally validated before being used throughout such an important study. In practice the evaluation step was truncated and was limited to only two of the reviewers. Overall, the applicability of the approach was compromised by the lack of information available for many of the studies. In some cases reviewers had little more than a study title to go on in completing their evaluation. In these cases the quality of the review cannot be as strong as in situations where more complete information is available. Nevertheless, we consider that the quantitative WoE approach was entirely necessary in order to impose some structure to this review process. As such, we would consider that any future review on this scale should use a weight of evidence approach. What we have provided is a basis on which a more robust approach could be developed.

8.2 QUANTITATIVE OUTPUTS

The quantitative outputs reported earlier may be considered in two parts. Firstly, based on the data gathered, an evaluation has been made regarding the distribution, in terms of both numbers of studies and total value of studies, on issues including:

- Distribution by Task Force Area of work (metrology, exposures, human health and environmental hazard);
- Distribution by relevant RO;
- Distribution by geographic area.

The data on study numbers is more robust then the data on value of the studies. For many studies no information of funding was obtained. For other studies it was not possible to validate whether figures already held were accurate. Finally, we attempted to convert all of the funding to a single currency, so as to facilitate comparison. However, this activity has coincided with unprecedented fluctuations in the world currency markets, with widespread changes on a day to day basis. For this reason, any comparisons based on value of research must be considered indicative only.

There are clear discrepancies between the number of studies (and value) in each of the Task Force Areas. Most of the studies are in the Human Health area (171), followed by exposures (107), environment (77) and characterisation (40).

Given the importance of minimising exposure, particularly of recognising that there are many uncertainties in hazard assessment, this difference is striking. There may be several reasons for this. One is that, at elementary level, the question "how toxic is this material?" is asked much more frequently and apparently with more concern than "which people are exposed to this material?". Second is that broadly toxicity is an intrinsic property of the material whereas exposure is entirely dependant on the scenario i.e. conditions of use and process. The intrinsic nature of toxicity is however somewhat illusory. There are many ways to answer the question of toxicity and an appropriate response to the toxicity question relates to what is the route of exposure, what is the nature of the material to which people are exposed, to which organ is toxicity being investigated, what is the health end point. So, there are many sub-questions to the toxicity question. Thirdly, toxicity questions may be addressed (albeit not completely) with relatively limited resources i.e. these can be carried out in a laboratory, often with staff at a PhD level. In contrast, exposure studies typically need some kind of field work, and access to production or use facilities where nanomaterials are being used. The above are not intended as a complete analysis of the situation, clearly, but are rather suggestions as to why this imbalance may occur.

It is also useful to comment on the relative weight given to the balance between Human Health and Environment. There are some clear regional variations. On a world-wide basis, toxicology studies largely outweigh eco-toxicology studies, by more than two to one. In the UK, however, there seems to be an almost even balance between the number of toxicology studies and the number of eco-toxicology studies. For example, to the best of our knowledge, in the EU framework project there are no ecotoxicological studies funded up to this point in time. It is not clear why this should be, although certainly the emphasis on eco-toxicology within the RO's must be considered to be a factor.

The difference between the numbers of studies in each RO is even more striking with wide disparities even within a Task Force Area Force; for example, in the Metrology TFA, RO02 had 28 identified studies, whereas RO09 had only 1. These disparities may not be problematic and indeed may be appropriate.

8.3 RESEARCH OBJECTIVES - PROGRESS AND GAPS

Progress and gaps are described in detail in Chapter 4 and are summarised for each RO in Chapter 5. These are critical outputs for EMERGNANO and the detail provided there will not be replicated here. Instead the reader is directed to these key sections of the report. In this section, we will discuss the wider, more general issues which we have drawn from our examination of these studies and the information which has been available to us.

For a large number of studies there was almost no information available within the public domain. It would be inappropriate to point to the specific studies for which this was the case but, even for some studies which had apparently completed, it was not possible to find information in the public domain, nor was it possible to get any further information from the project leader. Clearly, for this worldwide effort into understanding the potential risks associated with nanomaterials, it is critical that studies which have been carried out are published, preferably within the peer reviewed literature. A study with no publicly available outputs makes no real contribution to wider public knowledge. We accept that in making this statement some of the studies do in fact have published work which we have been unable to identify trough our search strategy. Funding

organisations should promote and foster publication of the work which they fund and should require (and resource) adequate dissemination of the research outputs.

A second issue relates to what could be described as unrealistic aims of many of the studies which have been funded. Examination of some of these studies has identified very ambitious objectives but with clearly inadequate resources allocated by which these objectives can be addressed. This issue needs to be considered both by researchers, who should be realistic in what they claim to be able to achieve within a programme of work, and by programme funders, who should not expect answers to all questions within modest resources. To some extent this may be due to the emerging nature of the field. It appears that the funding of more recent studies is more proportionate to the promised deliverables.

It was noted that there was substantial overlap between many of the ROs. In the Metrology area, there are four quite distinct ROs, but some overlap with ROs in other areas. RO02, in particular the "associated methods for measurement and characterisation of NP" overlaps substantially with RO06 and RO07. Within the Exposures, ROs 5, 6, and 7 are highly related and contain many of the same studies. For the Human Health TFA, there is substantial overlap between ROs 10, 11, 12, 14. This may be quite appropriate from a scientific perspective but actually made the analysis quite challenging, both in study allocation and in the review activity where individual studies had to be reassessed several times (or assessed by different assessors) from (slightly) different perspectives.

In TFA1, "Metrology, characterisation, standardisation and reference materials", the reviewers found that none of the studies could be said to be conclusive in providing new approaches or guidelines to characterising and measuring nanomaterials. Nor was the selection and/or development of exposure metric or metrics addressed well. Studies were focussed on addressing mainly the relevance and practicality of using surface area, which, whilst important, is unlikely to be relevant for all NP. Progress has been made in identifying candidate materials which may be used to develop characterised reference nanoparticles for toxicology. Candidate lists, along with minimum characterisation specifications have been developed and some commercial reference materials are beginning to emerge, but there is little evidence that issues such as storage, distribution and protocols for use are being addressed. Only two studies were identified as addressing potential risk of explosion of NP and only one study was identified as addressing the issue of measurement of exposure to nanoparticles in soil and water. Overall, the specific objectives within this RO appear to have been very sparsely addressed.

In TFA2, "Exposures, sources, pathways and technologies", some work has been done on establishing inventories of nanoparticle use and application, and on trying to map out some of the potential exposure pathways. However, there are many complex exposure pathways and only a very few have been considered. Little is known about nanoparticles in relation to consumer exposure and work in relation to nanoparticles in food seems to be entirely missing. Use of a life cycle assessment approach is missing. Little progress has yet been made in relation to development of measurement technologies for nanoparticles in air. Although there is some evidence that ongoing studies may produce some devices, such as personal samplers, and approaches for some types of nanoparticles, major questions remain. These include discrimination between NP and the background particles, and the evaluation of whether fibre counting methods can be applied to high aspect ratio nanoparticles. It now seems clear that filtration systems will be effective against nanoparticles and several studies have found improving collecting efficiency as particle size decreases. Studies have not, thus far, specifically addressed the performance of engineering controls as they are

implemented in practical settings. Issues of leakage round filtration systems and the effectiveness of skin protective equipment are also under-researched.

In TFA3, "Human health hazard and risk assessment", there is an absence of studies aiming to describe the accumulation of particles in a variety of organs after inhalation. There are no specific studies on whether carbon nanotubes and other high aspect ratio nanoparticles behave like asbestos in terms or translocation to the pleural mesothelium. In general, there is no attempt to try to identify potential structure activity relationships that govern penetration at any of the important boundaries. Many studies are addressing the issue of oxidative stress – inflammation. Several studies are also attempting to address structural activity relationships in relation to this but, as yet, little progress has been made. There are few *in vivo* studies being carried out, making a comparison between *in vitro* and *in vivo* data problematic. Genotoxicity is only being addressed in a few studies. Few studies are exposing nanoparticles at or near plausible exposure levels and also few studies addressing genotoxicity. Dermal uptake is not being addressed to any great extent and, as yet, toxicological testing strategies have not evolved to any level of agreement.

In TFA4, "Environmental hazard and risk assessment", studies have improved the understanding of kinetics of nanoparticle uptake in invertebrate and vertebrate models and have related this to toxicity. In addition, there are now a few studies focussing on microbial organisms and these provide information on effect assessment at individual level but also at community level. However, studies only cover a limited range of species and material types. There is some effort to relate study design and interpretation to human toxicology of nanoparticles, but there is much more to be done. Only one project is addressing bioaccumulation and bio-concentration of nanoparticles.

We have identified a very large body of work which is either completed or already underway. It is clear from the reviewers' synthesis reports that at present, based on the evidence we have been able to collect regarding these studies, progress has been disappointing. Whilst many studies are undoubtedly contributing in an incremental way to the advancement of knowledge, few of the key questions have been resolved.

8.4 STUDIES AND ISSUES WHICH ARE EXTERNAL TO THE RESEARCH OBJECTIVES

Although they are broad in their scope, ROs do not describe complete coverage of the important issues and questions relevant to the health, safety and environmental risks arising from exposure to nanoparticles. In reviewing the studies underway, more than 50 studies were identified which were relevant to these issues but did not fit within the scope of any of the ROs. These included:

- Enabling activities
- Life cycle analysis
- Effectiveness of risk assessment approaches

These studies were not formally reviewed according to the WoE appraisal as it was not possible to identify appropriate generic criteria by which they could be assessed.

An important category is the enabling activities. A total of 19 of these have been identified. These activities, which include observatories, networking activities and capability building, have played an important role in not only aiding realisation of the benefits nanotechnologies can offer *via* its many applications, but also in bringing together scientists to share understanding of the effects of nanomaterials and NP on

human health and the environment, maintaining public engagement and raising awareness of the need to develop nanotechnologies in a responsible manner in order to ensure its future.

Two studies have been identified which are focussing on life cycle approaches. However, neither has yet provided a significant contribution.

Two studies have been identified which are focussing on development and evaluation of risk assessment approaches. Again, at this point, neither has yet provided a significant contribution.

In addition, there are a number of potentially important topics which currently do not seem to be being investigated to any extent. Three are highlighted below.

The first is development of improved, evidence based, guidance on the control of exposure. While there is generic guidance available, specific evidence-based guidance for control of NP is absent. While some of the studies identified will undoubtedly contribute to this, none of the studies identified have it as a specific objective. Study types which would be helpful here would include studies to investigate the effectiveness of exposure control management approaches, such as control banding, and studies to develop and evaluate exposure models.

A second largely un-researched area is ingestion as a route of exposure. It is widely reported that nanoparticles are available as food supplements (nanosilver is perhaps the best known example) and that other nanoparticles are incorporated into food. Information about which nanoparticles and in what quantities is not available. Nor have we been able to identify any research which is considering the possibility that ingested nanoparticles are able to cross the gut wall, although this has been widely speculated.

Finally, we have been unable to identify studies which are integrating the synthesis of new materials or the development of new products directly with risk assessment of the materials or processes in any meaningful way. Even though at this stage, a great deal of work needs to be done to validate the risk assessment processes for nanoparticles, it would not be premature to begin to incorporate them into development projects.

8.5 RISK ASSESSMENT AND THE PRECAUTIONARY PRINCIPLE

In this study, we were asked to consider whether there is sufficient information to provide a risk assessment for identified nanomaterials or, if not possible, whether there is sufficient information identified to invoke the precautionary principle for one or more of the identified nanomaterials using the information collected during this report.

We therefore considered the information and data generated from the RO assessment activities to form an opinion as to whether there is sufficient information to identify and quantify risks to human health and/or the environment from manufactured nanomaterials that may lead to a requirement for implementation of the precautionary principle. The report is therefore limited by the data collected and presented in the RO reports.

The framework followed was consistent with the Communication on the use of the Precautionary Principle providing a general framework for its use in EU policy, and to avoid unwarranted adoption of the precautionary principle as a disguised form of caution (Commission of the European Communities, 2000). Namely, if a preliminary scientific evaluation shows that there are reasonable grounds for concern that a

particular activity might lead to damaging effects on the environment, or on human, animal or plant health, which would be inconsistent with the protection normally afforded to these within the European Community, the Precautionary Principle is triggered.

Information relevant to the following materials was collated and assessed:

- Titanium dioxide (TiO_2) particles
- Quantum dots
- Carbon nanotubes (CNT) including single walled CNT (SWCNT) multiwalled CNT (MWCNT) – long and short NT
- Iron oxide (Fe_xO_x)
- Cerium oxide (CeO_2)
- Zinc oxide (ZnO)
- Carbon black (CB)
- Gold nanoparticles (Au)
- Silver nanoparticles (Ag)
- Silica nanoparticles (SiO_2)
- Aluminium oxide (AlO_x)
- Nickel (Ni)
- Nanoclays

This list is broadly comparable to the list of substances identified by OECD as their list of representative nanomaterials. Materials on the OECD list not assessed (as there was no specific evidence) were Fullerenes (C60), Polystyrene and Dendrimers

The authors are aware that a proportion of background data has not been included in the RO reports (including, for example, reports of ecotoxicological effects of silver and toxicological effects of carbon black nanoparticles). Identified information on nanomaterial exposure was limited. However, based on the information presented we have not identified a sufficient body of evidence in any case to make a risk assessment feasible for these nanomaterials.

The precautionary principle requires that there is a preliminary scientific evaluation showing reasonable grounds for concern that the nanomaterial might lead to damaging effects on the environment, or on human, animal or plant health. For the majority of the identified nanomaterials, the body of evidence identified was not sufficient to suggest that the nanomaterials will cause harm and therefore the precautionary principle should not be considered in these cases. However, three different nanomaterials have been identified that give rise to sufficient concern from the results presented within the RO reports. There is evidence that carbon nanotubes may have an adverse effect on human health; and that silver nanoparticles and titanium dioxide nanoparticles are detrimental to the environment and, in these specific cases, further investigation as to the need to invoke the precautionary principle is required, taking into consideration all available data.

8.6 LESSONS FOR FUTURE

The EMERGNANO project provides many lessons for the future at both national and international level. In a general sense, funding should be more strategically developed and more focussed on the specific aspects and gaps which have been identified in this and other projects. From a UK perspective it is clear that not all of the gaps can possibly be covered given the likely limitations to funding. However, for the 18 ROs which have been considered, little progress has been made. The implication for this is

that future funding should be much more targeted, much more focussed and much more strategic if the UK wishes to achieve these stated research objectives.

Given the difficulties noted, it would be appropriate to provide more focus on work which enables effective risk assessment and management. For example, this could include, for a single class of NP within a group of applications (e.g. metal oxides in sunscreens) work to assess potential exposures throughout the lifecycle (production, use, disposal), the toxicity within the formulations used and evaluation of the risk management arrangements being applied. The current arrangement in which the research objectives are arranged broadly according to research disciplines does not facilitate multi-disciplinary work of this type. Consideration should be given as to whether this represents the best model for assessing and realising future priorities.

The lack of information available in the public domain from many studies is disappointing. Future research should have as a requirement the need to publish the work, preferably in a peer-reviewed form and to have a commitment (and resource provided) to share the outcome of that work with the wider research community. This could include, for example, active participation in review activities such as EMERGNANO. There is also a requirement for both researchers and funding organisations to be more realistic in the scope of projects relative to the resources and timescale requested. Work in this area is resource intensive and should be recognised and funded as such.

Greater emphasis should also be placed on work which is integrated with existing studies and expertise. The reviews suggest that single stand-alone studies until now do not seem to have contributed significantly to the knowledge base in this area.

All of this implies a much more strategic joined-up funding approach. It is clear that within the UK the multiple routes to funding have yet to deliver this strategic level approach. The same is also true internationally.

The EMERGNANO project has provided a clear benchmark against which progress may be judged both nationally and internationally. This is highly relevant to future UK activity in this area and towards setting and assessing performance against future priorities.

This project has collected and synthesised a wealth of information, and provides a unique snapshot of worldwide research activity. It is recommended that at some future point (12-18 months) the exercise is repeated to determine how much progress has been made.

In the meantime there are a number of activities which we consider would be relevant, but lie outwith the scope of the current project. These include:

- further development of the database which underlies the EMERGNANO, to enhance the quality of data within and to make the database itself more robust and to some extent, more public;
- to publish the report in the form of a peer-reviewed paper, recognising that the challenge of condensing such a wealth of information into such a format is substantial;
- further, more detailed, analysis of the quantitative data collected specifically in relation to national trends, delivery dates and the work currently underway.

9 CONCLUSIONS

EMERGNANO has been a challenging piece of work. Broadly it has been an attempt to identify and evaluate worldwide activity in nanotechnology risk research, specifically those programmes which have been funded at a national or strategic level.

The timescale and resource issues in particular, placed certain constraints on how the work could be carried out, and how robust the outcomes of the work are. We consider that what has been achieved has been close to the specified remit, but cannot exclude that some important studies have been overlooked. However, with this caveat, we feel able to draw conclusions regarding the nature of the work, its relevance and remaining gaps.

Since 2004, there has been an enormous undertaking of activity in this area. Work has been funded by both national government activity and in research programmes within each country and at an international level. We identified 158 studies which had been completed in this period or were about to complete within the next few months, and 119 studies which were scheduled for completion in 2009 and beyond.

We found a widespread imbalance in the work being carried out (in terms of numbers of studies) between the four main thematic areas and between the eighteen research objectives. The largest number of studies were in the Human Health area, followed by the Exposures area. Numbers in both the Environment and Characterisation areas were substantially lower. The distribution by RO was even more striking. RO14 had 44 studies identified as relevant, whereas RO9 had only one study.

There are large regional variations in the type of study funded in different countries or economic areas, perhaps reflecting different national priorities or capabilities.

A disappointing aspect was that we were unable to identify significant output from many of the studies involved in the programme, including studies which had already been completed. We accept, in relation to this, that we have not captured all of the information available on these studies and it is quite likely that there is some information that we have not been able to identify by the various routes through which we attempted to do so.

Our assessment of the available research outputs in the context of the NRCG research objectives, has revealed that some important contributions have been made. These include:

- In terms of characterisation and reference materials, progress has been made in identifying candidate materials which may be used to develop characterised reference nanoparticles for toxicology. Candidate lists, along with minimum characterisation specifications, have been developed and some commercial reference materials are beginning to emerge;
- In terms of exposure assessment and control, it is now clear that filters, such as those used in respiratory protective equipment and in air cleaning systems, are highly effective in removing nanoparticles from the air;
- In toxicology, the lack of mass balance toxicokinetics for any nanoparticle and the patchy nature of the published toxicokinetic data is a severe impediment to identifying extra-pulmonary hazards. This feeds through to problems in utilising plausible doses, for example, in an *in vitro* study with liver cells or blood components. The use of only a very limited number of particle types and sizes makes it impossible to know whether all NP act the same as regards

toxicokinetics, or whether there will be a structure activity relationship that highlights certain sizes and surface chemistries as factors enhancing or limiting potential of any NP to translocate or be toxic, as seems likely;

- In ecotoxicology, studies have improved the understanding of kinetics of nanoparticle uptake in invertebrate and vertebrate models and have related this to toxicity. In addition, there are now a few studies focussing on microbial organisms and these provide information on effect assessment at individual level and also at community level.

In projects which are just starting or have just started, there is some evidence to support the view that the work in these projects will deliver much more in terms of output than the projects which are currently just closing. However, it remains to be seen whether these new projects will in practice deliver their stated objectives. It would be appropriate to review these projects at some future time point, perhaps in 12-18 months.

It is clear nonetheless, that the major gaps in the knowledge base still remain. This was the overall view of the Royal Society report in 2004 (RS/RAEng, 2004), and it remains the view of this review. The major gaps have been identified in Chapter 5 and will not be reproduced in detail here. It is nonetheless the case that, in all of the major thematic areas (characterisation, exposure, toxicology and ecotoxicology), and all of the specific ROs, there is a substantial work remaining to be done. We conclude that the programme of research activity has yet to deliver step changes in the knowledge base on these issues.

In assessing quality and completeness for the purpose of carrying out a risk assessment, we did not identify a sufficient body of evidence in any case to make a risk assessment feasible.

However, from the results presented within the RO reports, three different nanomaterials have been identified that give rise to sufficient concern. There is evidence that carbon nanotubes may have an adverse effect on human health; and that silver nanoparticles and titanium dioxide nanoparticles are detrimental to the environment. In these specific cases, further investigation as to the need to invoke the precautionary principle is required, taking into consideration all available data.

The EMERGNANO project has been a unique attempt to identify and assess worldwide progress in relation to nanotechnology risk issues. On an international basis, we have identified and assessed 673 projects for which there has been a wide disparity in the information available, across four major thematic areas. We have also mapped these projects against the eighteen research objectives set in the UK by the NRGC/DEFRA. This has been achieved over a period of six months. We consider that what has been achieved, has been successful in identifying the overwhelming majority of important studies and having these studies assessed as to their output and relevance by some of the leading researchers currently working in this area. We have, as part of the project, achieved a comprehensive listing of projects and produced detailed comments and assessment on the outputs of those considered to be most relevant. It is our view that EMERGNANO (this report and the accompanying data collected) represents the best available picture of current strategic research. As such, EMERGNANO presents an excellent basis for assessing progress of these and other studies in the future.

10 REFERENCES

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10.2 SELECTED WEB LINKS

SAFENANO: www.safenano.org

ObservatoryNANO: http://www.observatory-nano.eu/

KIRnano: http://www.rivm.nl/rvs/075_nanotechnologie/KIR_nano/

NanoImpactNet: www.nanoimpactnet.eu

HSE Horizon Scanning: http://www.hse.gov.uk/horizons/nanotech.htm

WWICS Project on Emerging Nanotechnologies: http://www.nanotechproject.org/

International Council On Nanotechnology: http://www.icon.rice.edu/index.cfm

OMNT: http://www.omnt.fr/index.php?lang=eng&page=accueil

IMPART: http://www.impart-nanotox.org/impart_summary.html

NANOTOX: http://www.impart-nanotox.org/nanotox_summary.html

NOSH: http://www.hse.gov.uk/horizons/nanotech/consortiumsummary.pdf

ENRHES: http://nmi.jrc.ec.europa.eu/project/ENRHES.htm

APPENDIX 1 - LIST OF STUDIES

Study Key	State Of Progress	Country	Study Title	Funding Source/s	Budget (£)	Assessed under Research Objective/s
BE001	On-Going	Belgium	Risk assessment of nanoparticules on human health using in vitro and in vivo models	FP5/Walloon Region of Belgium	7953818	11,14,15
CA001	Completed	Canada	Interactions between semiconductor nanoparticles and biomembranes and DNA	NSERC	49691	12
CA003	Completed	Canada	Understanding Transport and Association of Nanoparticles in Biological Systems	NSERC	49691	12
CA004	Completed	Canada	Interactions Between Nanoscale Materials and Blood	NSERC	49691	11,14
CA006	Completed	Canada	Mechanisms of cellular interactions of functionalized rosette nanotubes	NSERC	49691	12
CA007	Completed	Canada	Recognition and Physicochemical Characterisation of Nanomaterial-Peptide Interactions	NSERC	49691	12
CA008	Completed	Canada	Dynamics, distribution and photochemistry of quantum dots in blood vessels	NSERC	49691	14
CA009	Completed	Canada	Understanding the light-induced cytotoxicity of quantum dots: a cellular, phototophysical and analytical mechanistic approach	NSERC	49691	12
CA010	Completed	Canada	Fate of nanoparticles in mammalian cells: effect of composition, shape, size and surface charge	NSERC	49691	12
CA011	Completed	Canada	Effect of a nanostructure's size and shape on uptake, degradation, & clearance in primary macrophages	NSERC	49691	12,13
CA012	Completed	Canada	Nanoparticles in Phospholipid Membrane Environments	NSERC	49691	12
CH001	Near Completed	Switzerland	Development of a particle exposure system to investigate the inflammation and toxicity potential of nanoparticles in an epithelial airway barrier model	Doerenkamp-Zbinden Foundation, FFVFF Foundation	28845	13
CH002	On-Going	Switzerland	Protein - carbon nanotubes interaction, uptake and the influence on oxidative stress and inflammation as key factors in nanoparticles - cell interaction		191371	13
CH003	On-Going	Switzerland	Quantitative risk assessment of nanoparticles in the environment: Exposure modelling and ecotoxicological considerations	EMPA	146659	17
CH005	Near Completed	Switzerland	Centre for toxicology and fine dust research. Electron microscope tomography for the study of the distribution of nano-tissue and cells	Schenkung von Dr. Alfred Bretscher	1201741	02
CH006	Near Completed	Switzerland	Nanoinventory: manufactured nanoparticles in Swiss industries and the potential for human exposures	Multiple	144223	05
CH007	Near Completed	Switzerland	Particle-lung interaction: mechanisms and effects on lung cell function	Swiss National Science Foundation	124989	14
CH009	Starting	Switzerland	Ecotoxicology of Nanoparticles: Biota-Nanoparticle-Pollutant Interactions in aqueous systems - Comparison of Black Carbon and Carbon Nanotubes	Swiss National Science Foundation	76696	17,18
CH010	On-Going	Switzerland	How to assess the adequacy of safety measures for manufactured nanoparticles	Financed by the participating institutes		05,08
CH011	On-Going	Switzerland	Use of nanoparticles in industry: safety aspects	Other	86530	05
CH012	On-Going	Switzerland	Solubilisation of carbon nanotubes and fullerenes in natural waters under environmental conditions	Swiss National Science Foundation	68291	10
CH013	Completed	Switzerland	Nanorisk: Safety and Risks of Carbon Nanotubes	CTI (Swiss Innovation Promotion Agency), BAG (Federal Office of Public Health), BAFU (Federal Office for the environment), EMPA (Materials science and technology research institution)	267248	11
CH014	Near Completed	Switzerland	Behaviour of ultrafine Particles in tissue and cells of the lung –Importance for our health	Swiss Federal Office for Environment	28845	14
CH015	Near Completed	Switzerland	Health effects of manufactured nanoparticles_ molecular and cellular biology and toxicology	DFG (SPP 1313)	95446	12,13
CH016	On-Going	Switzerland	Interplay of lung cells and their cellular responses upon exposure to combustion-generated ultrafine particles and manufactured nanoparticles	Swiss National Science Foundation	94470	12,14
CH017	Near Completed	Switzerland	Comparison of the effect of asbestos fibres and Carbon-Nanotubes	Swiss Federal Office for Environment	36055	11
CH019	On-Going	Switzerland	Analysis of nanomaterials exposure on humans in Switzerland – Identification of frequent situations for exposure situations with today's and possible future use of consumer products on the basis of nanomaterials	BAG (Federal Office of Public Health)	86542	05,07
CH020	Completed	Switzerland	NeuroCNTox - Neurotoxicity of Carbon Nanotubes	EMPA (Materials science and technology research institution)	113945	14
CH021	Near Completed	Switzerland	Interaction of ultrafine particles with the internal surface of the lung	Bangerter-Stiftung	72109	14
CH023	On-Going	Switzerland	In vitro reactivity of fine and ultrafine particles	French AFSSET and Institut universitaire romand de	169416	06
CH024	On-Going	Switzerland	Fate of hydrophilic nanoparticles in biological environment	Swiss National Science Foundation	86707	10
CN001	On-Going	CHINA	Health and Safety Impacts of Nanotechnology: Exploring Solutions	Ministry of Science and Technology, China		11

Study Key	State Of Progress	Country	Study Title	Funding Source/s	Budget (£)	Assessed under Research Objective/s
CZ001	On-Going	Czech Republic	Study of transport of inhaled nano-sized particles (Ag, Pb, Cd) and their allocation in organs			05,11
DE001	On-Going	Germany	Identification and assessment of the effects of engineered nanoparticles on human and environmental health	BMBF	874920	16,19
DE003	On-Going	Germany	The TRACER-Project: Toxicological Assessment and Functionalisation of Carbon Nanotubes	BMBF	1272611	05,11,14
DE004	On-Going	Germany	Toxicological assessment and functionalisation of carbon nanotubes	BMBF and industrial project partner	1193073	11
DE005	On-Going	Germany	NANOCARE: Development of Inhalation Toxicity Model for Testing of Nanomaterials	BMBF	4056447	11,13,14
DK001	On-Going	Denmark	NANOPLAST: Nano-technological materials and products in the plastics industry: Exposure assessment and toxicological properties	Danish Working Environment Research Fund	540860	05,07,11
DK002	Near Completed	Denmark	Risk analysis and governance of nanomaterials	Technical University of Denmark	190892	16
DK003	On-Going	Denmark	Biopolymer Nanocomposite Films for use in Food Packaging Applications	Government	839942	05
DK006	On-Going	Denmark	Translocation of Nanoparticles and Ultrafine Particles across Tissue Barriers in Mice.	Danish Ministry of Interior and Health, Research Centre for Environmental Health's Fund, and The Danish Medical Research Council	238615	12
DK007	On-Going	Denmark	Environmental Effects of Engineered Nanoparticles	Technical University of Denmark	190892	17,19
DK008	On-Going	Denmark	NanoKem: Nanoparticles in the paint- and lacquer industry. Exposure and toxic properties.	The Danish Working Environment Research Fund and National Research Centre for the Working Environment (DK)	1069062	05,11,12
DK009	On-Going	Denmark	Cardiovascular and Genotoxic Effects of Nanoparticles	University of Copenhagen (DK) and the Research Centre for Environment and Health (Ministry for Interior and Health)	267248	13,14
DK010	On-Going	Denmark	Engineered Nanoparticles and Development of Airway Allergy	National Research Centre for the Working Environment	198845	13
DK011	On-Going	Denmark	Characterisation and toxicological evaluation of nanoparticles from liquid-based nanofilm products	Nanocover Scandinavia A/S	288452	05,07,11,14
DK012	On-Going	Denmark	SUNANO - Risk assessment of free nanoparticles	The Danish Strategic Research Council, Programme Commission on Nanoscience, Biotechnology and IT (NABIIT)	846286	11,16
DK013	Near Completed	Denmark	Air Pollution in a Life Time Health Perspective	DRA	2665596	02,11
EU003	On-Going	EU	Development of an integrated platform for nanoparticle analysis to verify their possible toxicity and the eco-toxicity	DG Research	2221688	12,16,19
EU004	Near Completed	EU	Nano-particle characterization and toxicity	DG Research	143275	13
EU006	On-Going	EU	Cellnanotox: cellular interaction and toxicology with engineered nanoparticles	DG Research	2067993	02,11,12
EU007	Completed	EU	Quality of skin as a barrier to ultra-fine particles	DG Research	873324	15
EU008	On-Going	EU	Nanointeract: development of a platform and toolkit for understanding interactions between nanoparticles and the living world	DG Research	2624760	12
EU009	On-Going	EU	Nanosafe 2: safe production and use of nanomaterials	DG Research	5567543	02,04,06
EU011	On-Going	EU	Nanosh: inflammatory and genotoxic effects of engineered nanomaterials	DG Research	1908916	05,13
EU012	Completed	EU	Nanotransport: the behaviour of aerosols released to ambient air from nanoparticle manufacturing - a pre-normative study	DG Research	357922	08,16
EU015	Completed	EU	MAAPHRI: Multidisciplinary Approach to Airborne Pollutant Health Related Issues: Modelization with Combustion Engine Exhausts	EU	1307441	11,14
EU017	Completed	EU	NANOSAFE: Risk Assessment in Production and use of Nanoparticles with Development of Preventive Measures and Practice Codes	EU	256739	05,08,
EU019	Starting	EU	NeuroNano	FP7		13,14
EU022	Starting	EU	The reactivity and toxicity of engineered nanoparticles: Risks to the environment and human health	FP7		10,11,12,13, 14,16,17,18, 19
EU023	Starting	EU	Comprehensive assessment of hazardous effects of engineered nanomaterials on the immune system	FP7		02,11,12,13, 14,16
EU024	Starting	EU	Nanodevice: Novel concepts, methods and technologies for the production of portable, easy to use devices for the measurement and analysis of airborne engineered nanoparticles in workplace air	FP7		02,06

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EU025	Starting	EU	NANOTEST: Development of methodology for alternative testing strategies for the assessment of the toxicological profile of nanoparticles used in medical diagnostics	FP7		16
EU028	On-Going	EU	Safe Production and Use of Nanomaterials	FP6	5567543	02,04,05,06, 08,14
EU030	Near Completed	EU	PARTICLE RISK: Risk Assessment for Particle Exposure	FP6	635968	02,11,12,14, 16
EUX01	On-Going	EU	VI-RM - A European Virtual Institute on Reference Materials	EC		03
EUX02	Near Completed	EU	Nanostrand - Standardization related to Research and Development for Nanotechnologies	EC		03
FI002	Completed	Finland	Inflammatory and genotoxic effects of engineered nanoparticles	Finnish Work Environment Fund, Finnish Institute of Occupational Health	214753	12
FI003	On-Going	Finland	Nanohealth	Finnish academy of sciences	621989	12
FR001	Completed	France	Analyse multi-échelle des interactions physico-chimiques et biologiques entre des nanoparticules manufacturées et des bactéries	ANR, CEA, CNRS	340423	18,19
FR002	On-Going	France	Impact sur cellules rénales des nanoparticules manufacturées : Etude In Vitro des effets cellulaires et moléculaires après exposition aiguë et chronique Impact on renal cells by engineered nanoparticles. in vitro study on cellular and molecular effect	AFSSET		11,12
FR003	On-Going	France	Devenir des nanoparticules minérales manufacturées dans les milieux aquatiques et les sols Institut de Physique du Globe de Paris Fate of engineered mineral nanoparticles in aquatic and soil environment. Institute of global physics (PARIS)	AFSSET		10,17
FR004	On-Going	France	NANOP : niveaux, déterminants et variabilités des nanoparticules dans l'environnement intérieur Level, determinant and variability of nanoparticles in the internal environment	AFSSET		11,12
FR005	On-Going	France	Evaluation in vitro de la réactivité des particules fines et ultrafines In vitro evaluation of the reactivity of fine and ultrafine particles	AFSSET		12,13
FR006	On-Going	France	Toxicité respiratoire des nanotubes de carbone: Université Caholique de Louvain Respiratory toxicity of carbon nanotubes: Catholics University of Louvain	AFSSET		14
FR007	On-Going	France	Signatures toxicologiques de nanoobjets manufacturés sur des cellules humaines après inhalation ou ingestion Toxicological signs of engineered nanoobjects in human cells after inhalation and ingestion	ANR		12,13,14
FR008	On-Going	France	Tests parallélisés sur puce à cellule de cytotoxicité aigue de nanoparticules à morphologie contrôlée Parallel tests on cellular chips of acute cytotoxicity of nanoparticles of controlled morphologies.	ANR		13,16
FR009	On-Going	France	Effets des nanotubes de carbone sur l'appareil respiratoire. Rôle de leurs caractéristiques physico-chimiques Effects of carbon nanotubes on the respiratory tracts. The role of physico-chemical characteristics.	ANR		11
FR010	On-Going	France	Evaluation de l'Influence de la Nature des Nanotubes de Carbone sur la Santé Humaine et l'Environnement Evaluation of the influence of the nature of carbon nanotubes on human health and the environment.	ANR		14,18
FR011	On-Going	France	Synthèse, détection et toxicologie de nanoparticules métalliques (Au, Ag, Pt). Synthesis, detection and toxicology of metallic nanoparticles	ANR		02,11
FR012	On-Going	France	Toxicologie des Nanoparticules : Influence de la taille, de la composition chimique et de la réactivité de surface sur leurs effets pulmonaires et rénaux Toxicology of nanoparticles: Influence of size, chemical composition and surface reactivity	ANR		11,14
FR013	On-Going	France	Caractérisation in situ de la surface des aérosols fins et ultrafins Characterisation in situ of the fine and ultrafine aerosols.	ANR		02
TW001	Completed	TAIWAN	Promoting responsible R&D and manufacturing environment of nanotechnology	Taiwan EPA	69071	05,08
TW002	Completed	TAIWAN	Study in Applying Nanotechnology for Environmental Protection	Government	117846	05
UK005	Completed	UK	Nanoparticles: An occupational hygiene review	Government	15000	05
UK010	On-Going	UK	Nanochallenge	HSL/HSE	400000	06

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UK012	Completed	UK	A scoping study to identify hazard data needs for addressing the risks presented by nanoparticles and nanotubes	DEFRA	34500	11,13
UK017	Near Completed	UK	The potential routes of nanoparticle uptake by cells and their impacts on antigen processing	Napier University	45000	12
UK025	Completed	UK	Cellular and molecular responses to oxidative stress	Colt Foundation	150000	13
UK026	Near Completed	UK	Effects of nanoparticles on liver cells	EU	150000	12
UK027	Near Completed	UK	Ecotoxicology of nanoparticles	DEFRA/CSL	48000	18
UK030	Near Completed	UK	Mechanisms of nanoparticle and nanotube-induced pulmonary toxicity	Colt Foundation	176267	02,11,12,13
UK031	Completed	UK	The Assessment of Different Metrics of the Concentration of Nano (Ultrafine) Particles in Existing and New Industries	HSE	97073	02
UK047	Completed	UK	Blood and cardiovascular effects of nanoparticles	EU	76000	14
UK051	Near Completed	UK	Nanotube toxicity (PhD studentship University of Edinburgh Medical Faculty)	University of Edinburgh Medical Faculty	50000	13
UK057	Near Completed	UK	Evaluation of Risk Assessment Approaches for Manufactured Nanomaterials	DEFRA	51765	16
UK071	Near Completed	UK	An outline scoping study to determine whether high aspect ratio nanopartcles (HARN) should raise the same concerns as do asbestos fibres	DEFRA	57349	11,13
UK073	Near Completed	UK	Reference materials for engineered nanoparticle toxicology and metrology	DEFRA	124795	03
UK074	Completed	UK	Current and Predicted Environmental Exposure arising from Engineered Nanomaterials	Government	47731	05
UK075	Completed	UK	An Assessment of Regulatory Testing Strategies and Methods for Characterising the Ecotoxicological Hazards of Nanomaterials	DEFRA	24560	16
UK086	Near Completed	UK	Genomic and oxidation-related biological responses in fish exposed to fullerenes of different physicochemical characteristics	NERC	28828	17,18
UK088	Near Completed	UK	Nanotoxicology of Fine PM: The Role of Surfactant and Collectins in Short-Term Health Effects of PM Air Pollution	NERC	122070	12
UK089	On-Going	UK	Hazards of nanoparticles to the environment and human health	NERC	118300	14
UK090	On-Going	UK	Determinants of Oxidative Potential, A Health-Based Metric to Assess Particulate Matter Toxicity	NERC	62126	13
UK091	On-Going	UK	Assessing human exposure, uptake and toxicity of nanoparticles from contaminated environments	NERC	103327	17,18
UK092	Near Completed	UK	An exploratory study investigating the physiochemical characteristics of ambient air particles responsible for the dysregulation of pulmonary genes	NERC	114452	14
UK093	On-Going	UK	A proof of concept study for a structure activity model for the toxicity of nanoparticles	NERC	48273	13
UK094	Near Completed	UK	Visualisation of Nanoparticles in the Environment	NERC	19668	04
UK095	Near Completed	UK	Understanding the fate and behaviour of manufactured nanoparticles in natural waters	NERC	48327	10
UK096	Near Completed	UK	Synthetic polymer nanoparticles: effects of composition and size on uptake, toxicity and interactions with environmental contaminants.	NERC	61991	03,17
UK097	Near Completed	UK	Pharmaceutical and cosmetic silica nanoparticles: towards an understanding of their structure, fate and behaviour in aquatic systems	NERC	63880	10,17,18
UK098	Near Completed	UK	Nanoparticle immunotoxicity using an environmental sentinel as a model	NERC	38994	17,18
UK100	Near Completed	UK	Manufactured Nanoparticle Migration in Groundwaters	NERC	57982	10
UK101	Near Completed	UK	Dietary Exposure to Nanoparticles in Fish: A Pilot Study.	NERC	55384	17,18
UK102	Near Completed	UK	Effects of C-60 fullerenes and carbon nanotubes on marine mussels.	NERC	19850	17,18
UK103	Near Completed	UK	Nanoscale zerovalent iron (nZVI) impact on soil microbial communities	NERC	64682	17,18
UK104	Starting	UK	Interaction of Nanoparticles with Microbial Populations during Particle Transport	NERC	48316	17,18
UK105	Starting	UK	Impact of manufactured nanoparticles on the catabolic capabilities and phenotypic structure of soil microbial communities	NERC	56857	17,18
UK106	Near Completed	UK	Impact and recovery of groundwater microbial communities exposed to manufactured nanomaterials (MNM).	NERC	53435	17,18
UK107	Near Completed	UK	Biomembrane interactions in the toxicology of nanoparticles to microorganisms	NERC	20016	17,18
UK108	Starting	UK	An investigation into the effects of nanoparticles on the bacterial diversity of freshwater and coastal marine sediments	NERC	37997	17
UK109	Starting	UK	A study of the effects of silver surface chemistry on bactericidal properties of silver nanoparticles.	NERC	20167	17
UK110	Completed	UK	Assessment of the current and projected applications of nanotechnology in the food sector	FSA		05,11,15

Study Key	State Of Progress	Country	Study Title	Funding Source/s	Budget (£)	Assessed under Research Objective/s
UK111	Near Completed	UK	Assessment of current and projected applications of nanotechnology for food contact materials in relation to consumer safety and regulatory implications.	FSA	68500	05,15
UK113	Near Completed	UK	Model nanoparticles for environmental risk studies	NERC	56564	03,08,17
UKX01	Completed	UK	A scoping study into the manufacture and use of nanomaterials in the UK	DEFRA	39329	05
UKX04	Near Completed	UK	HSL - Fire and Explosion Properties of Nanopowders	HSE	305621	04
UKX05	Completed	UK	Explosion Properties of Nanometric Aluminium Powder and Nickel Powder	HSE	10000	04
US001	On-Going	USA	Carbon Nanotubes: Environmental Dispersion States, Transport, Fate, and Bioavailability	EPA	184958	10,19
US002	Near Completed	USA	Structure-function Relationships in Engineered Nanomaterial Toxicity	EPA	186507	02,12
US003	On-Going	USA	Photochemical Fate of Manufactured Carbon Nanomaterials in the Aquatic Environment	EPA	99421	10
US005	On-Going	USA	Bioavailability and Fates of CdSe and TiO_2 Nanoparticles in Eukaryotes and Bacteria	EPA	198934	10,12,17
US015	Completed	USA	CRAEMS: Fundamental Studies of Nanoparticle Formation in Air Pollution	NSF	611002	07
US021	On-Going	USA	Development of Bench and Mist Protocols for Particulate Measurements of Protective Clothing and Ensembles	NIOSH		08
US023	On-Going	USA	Systemic Implications of Total Joint Replacement	NIH	2874289	12
US029	Completed	USA	Role of CNTs in Cardiovascular Inflammation & COPD Related Diseases	NIOSH	447617	14
US030	Completed	USA	Review of Best Practices for Nanotechnology Safety	ICON	27354	08
US034	Completed	USA	Mechanistic Dosimetry Models of Nanomaterial Deposition in the Respiratory Tract	EPA	186507	14
US035	Unknown	USA	Direct Reading Instrument Metrology	NIOSH		06
US038	On-Going	USA	Respirator Testing and Certification	NIOSH		08
US041	On-Going	USA	Bypass Leakage and Recirculation of Workplace Aerosols	NIOSH		08
US045	On-Going	USA	Nanoparticle Disruption of Cell Function	NIH	182461	12
US046	Near Completed	USA	Monitoring and Characterizing Airborne Carbon Nanotube Particles	NIOSH	198941	06
US047	Completed	USA	Cytotoxicity of Nanoparticles	NSF	45756	12
US048	On-Going	USA	Neurotoxicity after Pulmonary Exposure to Welding Fumes Containing Manganese	NIOSH		06,11,13,14
US055	On-Going	USA	Development of methods and models for nanoparticle toxicity screening: Applications	NIH	179209	13
US056	On-Going	USA	The Effect of Surface Coatings on the Environmental and Microbial Fate of Nano iron and Fe oxide Nanoparticles	EPA	198941	10,17
US057	On-Going	USA	Impact of Physiochemical Properties on Skin Absorption of Manufactured Nanomaterials	EPA	194772	11,15
US058	Near Completed	USA	Comparative Life Cycle Analysis of Nano – and Bulk-materials in Photovoltaic Energy Generation	EPA	99470	07
US059	On-Going	USA	Biological Fate & Electron Microscopy Detection of Nanoparticles During Wastewater Treatment	EPA	198443	10,17
US060	Completed	USA	Olfactory transport of inhaled nanoparticles	American Chemistry Council	188994	11,14
US065	On-Going	USA	An Ultrafine Particle Intervention Study in Automotive Production Plants	NIOSH		05,07,08,14
US066	On-Going	USA	NIR Absorbing Nanoparticles For Cancer Therapy	NIH	227674	11
US068	Unknown	USA	Pulmonary Toxicity Screening Studies with Nano vs. Fine-Sized Particles in Rats.	DuPont		14
US072	On-Going	USA	Nanoparticles: Dosimetry and Risk Assessment	NIOSH		11
US079	On-Going	USA	Lung Deposition of Highly Agglomerated Nanoparticles	NSF	198941	14
US083	Completed	USA	Filter Efficiency of Typical Respirator Filters for Nanoscale Particles	NIOSH		08
US086	On-Going	USA	Generation and Characterization of Occupationally Relevant Airborne Nanoparticles	NIOSH		02
US088	On-Going	USA	The chemical and physical nature of particulate matter affecting air, water, and soil quality.	USDA		07,10
US090	Completed	USA	SGER: Aquatic Nanotoxicology of Nanomaterials and Their Biomolecular Derivatives	NSF	14921	18
US091	Completed	USA	New Instruments for Real-Time, High-Resolution Characterization of Nanoparticles in the Environment	NSF	52719	06
US092	Completed	USA	Fullerene, carbon nanotube, and reactive nano iron particle toxicity in aquatic species.	Lonestar Nanotechnology fund		18
US093	Completed	USA	Dermal Effects of Nanoparticles	NIOSH	348147	13,15
US095	Completed	USA	Biological Interactions of Nanomaterials	DOD	149206	11,12,13
US096	Completed	USA	Systematic Microvascular Dysfunction Effects of Ultrafine Versus Fine Particles	NIOSH	298411	13,14
US098	On-Going	USA	Near-Infrared Fluorescence Nanoparticles for Targeted O*	NIH	1439640	11

Study Key	State Of Progress	Country	Study Title	Funding Source/s	Budget (£)	Assessed under Research Objective/s
US099	Near Completed	USA	Nanoparticles for efficient delivery to solid tumors	NIH	165660	12
US101	Near Completed	USA	IMR: Development of an Analyzer for Size and Charge Characterization of Nanoparticles in Research and Training	NSF	124849	06,08
US102	On-Going	USA	Sorption and availability of metals and radionuclides in soils	USDA		17
US104	On-Going	USA	Reactivity, aggregation and transport of nanocrystalline sesquioxides in the soil system	USDA	61409	17
US107	Near Completed	USA	Colloid interfacial reactions in open microchannel representing unsaturated soil capillaries	USDA	47746	10
US108	On-Going	USA	Cellular and materials-based studies of marine invertebrates to advance biomineralization, antifouling and nanotechnology fields	USDA		19
US110	On-Going	USA	Methodology Development for Manufactured Nanomaterial Bioaccumulation Test	EPA	198825	19
US111	On-Going	USA	A Rapid In Vivo System for Determining Toxicity of Manufactured Nanomaterials	EPA	198941	18,19
US112	On-Going	USA	Ecotoxicology of Underivatized Fullerenes (C60) in Fish	EPA	197353	17,18,19
US113	On-Going	USA	Effects of Ingested Nanoparticles on Gene Regulation in the Colon	EPA	99470	12,13
US114	Completed	USA	Absorption and Release of Contaminants onto Engineered Nanoparticles	EPA	166015	10
US115	Completed	USA	Impacts of Manufactured Nanomaterials on Human Health and the Environment - A Focus on Nanoparticulate Aerosol and Atmospherically Processed Nanoparticulate Aerosol	EPA	166613	11
US118	Completed	USA	Transformations of Biologically-Conjugated CdSe Quantum Dots Released Into Water and Biofilms	EPA	165170	17
US119	Completed	USA	Chemical and Biological Behaviour of Carbon Nanotubes in Estuarine Sedimentary Systems	EPA	166489	17,18,19
US120	Completed	USA	Iron Oxide Nanoparticle-Induced Oxidative Stress and Inflammation	EPA	166613	12,13
US121	Near Completed	USA	The Bioavailability, Toxicity, and Trophic Transfer of Manufactured ZnO Nanoparticles: A View from the Bottom	EPA	180877	19
US122	Completed	USA	Health Effects of Inhaled Nanomaterials	EPA	166612	13,14
US123	On-Going	USA	Agglomeration, Retention, and Transport Behaviour of Manufactured Nanoparticles in Variably-Saturated Porous Media	EPA	198461	10
US124	Completed	USA	Responses of Lung Cells to Metals in Manufactured Nanoparticles	EPA	165597	10,11
US125	Near Completed	USA	Internalization and Fate of Individual Manufactured Nanomaterial within Living Cells	EPA	99470	12
US126	Completed	USA	The Fate, Transport, Transformation and Toxicity of Manufactured Nanomaterials in Drinking Water	EPA	226295	10,17,18
US127	Near Completed	USA	Hysteretic Accumulation and Release of Nanomaterials in the Vadose Zone	EPA	186507	10
US128	On-Going	USA	Aquatic Toxicity of Carbon-Based Nanomaterials at Sediment-Water Interfaces	EPA	198695	17,18
US129	Near Completed	USA	Chemical Fate, Biopersistance, and Toxicology of Inhaled Metal Oxide Nanoscale Materials	EPA	186507	11,12,13,14
US130	Near Completed	USA	Effects of Nanomaterials on Human Blood Coagulation	EPA	186507	14
US131	Near Completed	USA	Assessing the Environmental Impacts of Nanotechnology on Organisms and Ecosystems	EPA	186507	10,17,18
US132	Near Completed	USA	Acute and Developmental Toxicity of Metal Oxide Nanoparticles to Fish and Frogs	EPA	186507	17
US133	On-Going	USA	Nanoparticle Stability in Natural Waters and its Implication for Metal Toxicity to Water Column and Benthic Organisms	EPA		10,17
US134	On-Going	USA	Nanoparticle Toxicity in Zebrafish	EPA	198094	17
US135	Near Completed	USA	Evaluating the Impacts of Nanomanufacturing via Thermodynamic and Life Cycle Analysis	Government	186507	05
US136	Near Completed	USA	Fate and Transport of Carbon Nanomaterials in Unsaturated and Saturated Soils	EPA	196603	10
US137	Completed	USA	Implications of Nanomaterials Manufacture and Use: Development of a Methodology for Screening Sustainability	Government	49606	05
US139	Completed	USA	Evaluated Nanoparticle Interactions with Skin	EPA	163615	15
US140	On-Going	USA	Aquatic Toxicity of Waste Stream Nanoparticles	EPA	198855	17
US144	Near Completed	USA	Assessment Methods for Nanoparticles in the Workplace	NIOSH	198941	06,08
US147	On-Going	USA	Carbon Nanoparticles in Combustion: A Multiscale Perspective	NSF	119365	07
US151	Completed	USA	Chemical Characterization of Ultrafine Aerosol Particles	NSF	180539	06
US154	Near Completed	USA	Microbial Impacts of Engineered Nanoparticles	EPA	186507	10,17
US155	Near Completed	USA	NIRT: Nanoparticle-Environment Interfaces: Interactions in Natural Systems	NSF	745903	10

Study Key	State Of Progress	Country	Study Title	Funding Source/s	Budget (£)	Assessed under Research Objective/s
US156	Completed	USA	Pulmonary Deposition and Translocation of Nanomaterials	NIOSH	447617	11,14
US158	On-Going	USA	Investigating the Surface Structure and Reactivity of Bulk and Nanosized Manganese Oxides	NSF	163913	10
US159	Near Completed	USA	Nanotox: Cross-Media Environmental Transport, Transformation, and Fate of Manufactured Carbonaceous Nanomaterials	NSF	174073	07,10
US163	On-Going	USA	Lung Oxidative Stress/Inflammation by Carbon Nanotubes	NIOSH	746028	13
US168	Near Completed	USA	Nanoparticles for siRNA delivery to mammalian neurons	NIH	248739	14
US170	Completed	USA	The Role of Nano-Scale Colloids in Particle Aggregation and Trace Metal Scavenging in Aquatic Systems	NSF	258760	10
US176	On-Going	USA	The Measurement and Control of Workplace Nanomaterials	NIOSH		05,06,07,08
US179	Near Completed	USA	CNS Gene Delivery and Imaging in brain Tumor Therapy	NIH	3299013	13
US181	Near Completed	USA	Fate and Transformation of C60 Nanoparticles in Water Treatment Processes	EPA	186507	10
US182	Near Completed	USA	NIRT: Investigating Nano-carbon Particles in the Atmosphere: Formation and Transformation	Government	917176	05
US183	Completed	USA	Reverse Engineering Cellular Pathways from Human Cells Exposed to Nanomaterials-Development of Novel Risk Assessment Methods	NSF	99470	16
US188	Near Completed	USA	NIRT: Nanoparticle Fe as a Reactive Constituent in Air, Water, and Soil	NSF	696293	10
US190	Unknown	USA	Development of Computer-Aided Face Fit Evaluation Methods	NIOSH		08
US191	Completed	USA	Rapid Environmental Impact Screening for Engineered Nanomaterials: A Case Study Using Microarray Technology	Project on Emerging Nanotechnologies	14921	17
US192	On-Going	USA	Performance Test of High APF Respirators	NIOSH		08
US193	Completed	USA	Pulmonary, Immune, and Dermal Effects of Welding Fumes	NIOSH		11,14,15
US197	On-Going	USA	Innate Immune Response of an Aquatic Vertebrate Model to Manufactured Nanoparticles Assessed Using Genomic Markers	EPA	198349	17
US204	Completed	USA	Elemental Composition of Freshly Nucleated Particles	EPA	193967	06
US211	On-Going	USA	Genomics-based Determination of Nanoparticle Toxicity: Structure-function Analysis	EPA	99467	02,12
US215	Completed	USA	Pulmonary Toxicity of Diesel Exhaust Particles	NIOSH		13
US216	Completed	USA	Penetration of Nanoparticles Through Respirator Filter Media	NIOSH	248676	08
US220	On-Going	USA	Long Term Cardiovascular Effects of Inhaled Nanoparticles	NIH	176975	14
US222	Completed	USA	Role of Surface Chemistry in the Toxicological Properties of Manufactured Nanoparticles	NIOSH	198941	02,13
US225	Near Completed	USA	NIRT: Response of aquatic and terrestrial microorganisms to carbon-based manufactured nanoparticles.	NSF	795763	17
US227	Completed	USA	Short-Term Chronic Toxicity of Photocatalytic Nanoparticles to Bacteria, Algae, and Zooplankton	EPA	166554	17
US228	On-Going	USA	Aggregation and Deposition Behaviour of Carbon Nanotubes in Aquatic Environments	NSF	198941	10
US229	Completed	USA	From Nanoparticles to Novel Protective Garments	NIOSH	49735	08
US238	Completed	USA	NIRT: Nanoscale Processes in the Environment: Nanobiogeochemistry of Microbe/Mineral Interactions	NSF	497352	17
US241	Completed	USA	The Role of Nano-Scale Colloids in Particle Aggregation and Trace Metal Scavenging in Aquatic Systems	NSF	159275	10
US242	Completed	USA	The Role of Nano-Scale Colloids in Particle Aggregation and Trace Metal Scavenging in Aquatic Systems	NSF	120966	10
US243	Completed	USA	The Role of Nano-Scale Colloids in Particle Aggregation and Trace Metal Scavenging in Aquatic Systems	NSF	136602	10
US244	Near Completed	USA	Nanotox: Biochemical, Molecular and Cellular Responses of Zebrafish Exposed to Metallic Nanoparticles	NSF	174073	17,18
US247	On-Going	USA	NIRT: Design of Biocompatible Nanoparticles for Probing Living Cellular Functions and Their Potential Environmental Impacts	NSF	658370	12
US248	Completed	USA	ADVANCE Fellow: Microscopy of Nanomaterials	NSF	223228	02
US249	Near Completed	USA	Pulmonary Effects of Exposure to Various Nanoparticles	NIOSH		11,14
US250	Completed	USA	Portable Monitors for Airborne Metals at Mining Sites	NIOSH		06
US251	Completed	USA	Nanoparticle in the Workplace	NIOSH	198941	05,06,07,08
US253	Unknown	USA	Development of Alternative In Vitro Methods to Assess Pulmonary Toxicity of Inhaled Fine and Nano-sized Particles	DuPont		16
US254	On-Going	USA	Experimental and Numerical Simulation of the Fate of Airborne Nanoparticles from a Leak in a Manufacturing Process to Assess Worker Exposure	NSF	198941	07

Study Key	State Of Progress	Country	Study Title	Funding Source/s	Budget (£)	Assessed under Research Objective/s
US259	Completed	USA	Repercussion of Carbon Based Manufactured Nanoparticles on Microbial Processes in Environmental Systems	EPA	166613	10,17,18
US262	Completed	USA	Size Dependent Neuronal Translocation of Nanoparticles	NSF	99469	14
US263	Completed	USA	Nanotox: Gene Expression Profiling of Single-Walled Carbon Nanotubes: A Unique Safety Assessment Approach	NSF	149206	12,16
US264	Completed	USA	Generation and Characterization of Ultrafine Particles	NIOSH		02,03
US271	Near Completed	USA	Collaborative Research: Fullerene Aggregation in Aquatic Systems	NSF	115548	10
US272	Completed	USA	Collaborative Research: Fullerene Aggregation in Aquatic Systems	NSF	58066	10
US273	Completed	USA	NIOSH Current Intelligence Bulletin: Welders and Parkinsonism	NIOSH		08
US274	Near Completed	USA	NIOSH Current Intelligence Bulletin: Evaluation of Health Hazard and Recommendations on Occupational Exposure to Titanium Dioxide	NIOSH		11,14
US285	Completed	USA	Submicron Particles and Fibers for Toxicological Studies	NIH	167999	03
US286	On-Going	USA	NIRT: Micropatterned Nanotopography Chips for Probing the Cellular Basis of Biocompatibility and Toxicity	NSF	917387	13
US288	Completed	USA	Physical Characteristics of Ultrafine Particles	NIOSH		02
US291	Completed	USA	Development of fine particle characterization and monitoring methods	NIOSH		02,06
US293	On-Going	USA	Characterization and Communication of Chemical Hazards	NIOSH		06
US294	On-Going	USA	Emerging Issues for Occupational Respiratory Disease	Government		05
US295	Near Completed	USA	Environmental Biogeochemistry and Nanoscience: Applications to Toxic Metal Transport in the Environment	NSF	59682	10
US296	On-Going	USA	Longitudinal Surveillance/Beryllium Disease Prevention	NIOSH		02
US303	Completed	USA	Particle Surface Area As a Dose Metric	NIOSH	497352	02,14
US306	Completed	USA	Pulmonary Toxicity of Carbon Nanotube Particles	NIOSH	447617	14
US307	On-Going	USA	Ultrafine Aerosols from Diesel-Powered Equipment	NIOSH		08
US309	On-Going	USA	The Fate and Effects of Nanosized Metal Particles along a Simulated Terrestrial Food Chain Investigated Using Genomic and Microscopic Techniques	EPA		10,17
US313	Near Completed	USA	Multifunctional Nanoparticles for Intracellular Delivery	NIH	673985	12
US316	Near Completed	USA	NIRT: Nanoscale Processes in the Environment: Atmospheric Nanoparticles	NSF	830221	07
US318	Completed	USA	Identifying and Regulating Environmental Impacts of Nanomaterials	NSF	64656	16
US320	On-Going	USA	NIRT: Understanding Robust Large Scale Manufacturing of Nanoparticles and Their Toxicology	NSF	696293	02,11,12,13, 14
US326	On-Going	USA	Multidisciplinary University Research Initiative: Effects of Nanoscale Materials on Biological Systems: Relationship between Physiochemical Properties and Toxicological Properties	DOD	2735437	12,13
US331	Completed	USA	Ultrafine Particles in Heavy Vehicle Assembly and Components Manufacturing Plants	UAW, International Truck and Engine Corporation	54709	05,06
US334	Completed	USA	Titanium Dioxide (TiO_2) Nanoparticle Exposure Study	Government	198941	05
US337	Completed	USA	Physical and Chemical Determinants of Nanofiber/Nanotube Toxicity	EPA	166613	13
US339	Completed	USA	NER: Fullerene-Microbe Interactions: Implications for Disinfection and Risk Assessment	NSF	74603	17
US411	On-Going	USA	The Interaction of Polycationic Organic Polymers with Biological Membranes	NIH	553393	12
US421	On-Going	USA	Modulation of Qdot nanoparticle toxicity by glutathione in GCL transgenic mice	NIEHS		12
US422	Unknown	USA	Investigations of Multi-Walled Carbon Nanotube Toxicity	NIOSH		12,14
US425	Starting	USA	Project 5: Nanotechnology-Based Environmental Sensing	NIEHS		6
US426	On-Going	USA	Chemical, structural and superstructural determinants of nanocarbon toxicity	NIEHS		16
US427	Unknown	USA	Determination of diameter distribution of carbon nanotubes by Raman Spectroscopy	NIOSH		02,06
US428	Starting	USA	An Integrated Approach Toward Understanding the Tox of Inhaled Nanomaterials	NIOSH; NIEHS; RFA; ES	596823	11,14
US429	Unknown	USA	Exposure Assessment in Tungsten Refining and Manufacturing	Government		05
US430	Unknown	USA	Specific biomarkers for unusual toxicity of nanomaterials	NIOSH		14
US432	Unknown	USA	Potential Aneuploidy Following Exposure to Carbon Nanotubes	NIOSH		14
US433	Unknown	USA	Ultrafine TiO_2 Surface and Mass Concentration Analysis	NIOSH		02,06
US434	On-Going	USA	Personal Exposure to Engineered Nanoparticles	NIOSH Career Development Grant	159582	06
US435	Unknown	USA	Mutagenicity assessment of carbonaceous nanomaterials	NIOSH		14

Study Key	State Of Progress	Country	Study Title	Funding Source/s	Budget (£)	Assessed under Research Objective/s
US436	Unknown	USA	Cell-based assessment for iron nanoparticles induced health effects	NIOSH		13,16
US437	Unknown	USA	Occupational exposures and potential neurological risks	NIOSH		14
US438	Starting	USA	Nano-Biological Interactions and Toxicity of Engineered Metal Oxide Particles	NIEHS		13
US439	Unknown	USA	Measurement of Nanoscale Carbonaceous Materials	NIOSH		02,06
US452	On-Going	USA	Remote Microvascular Dysfunction after particulate matter exposure	NIEHS		14
US453	Unknown	USA	Nanoscale Reference Materials for Respiratory Disease Prevention	NIOSH		03
US458	Unknown	USA	Nanoaerosol Surface Area Measurement Methods	NIOSH		02,06

APPENDIX 2 - WEIGHT-OF-EVIDENCE FRAMEWORKS

	Metrology, Characterisation, Standardisation & Reference Materials ***(RO 2, 3, 4 & 9)***		***Exposures - Sources, Pathways & Technologies*** ***(RO 5, 6, 7, 8 & 10)***		***Human Health Hazard & Risk Assessment*** ***(RO 11, 12, 13,14, 15 & 16)***		***Environmental Hazard & Risk Assessment*** ***(RO 17, 18 & 19)***	
What type of research is being conducted?	**4**	Generic / multiple proven (or validated) metric / RM / method	**10**	Generic validated model	**5**	Human, with controls or Meta-analysis	**6**	Multi-organism mesocosm type / food chain / mulitgeneration with full appropriate controls
	3	Single / specific proven (or validated) metric / RM / method	**9**	Specific validated model or Quantitative data for a generic scenarios, with the inclusion of contextual information	**4**	Human, without controls or *In vivo*, with controls	**5**	Organism with full appropriate controls, short & long-term tests
	2	Generic / multiple unproven (or unvalidated) metric / RM / method	**8**	Quantitative data for generic scenarios, without inclusion of contextual information	**3**	*In vivo*, without controls or In vitro, with controls or Model with supporting data	**4**	Organism with full appropriate controls, short-term tests
	1	Single / specific unproven (or unvalidated) metric / RM / method	**7**	Quantitative data for a specific scenario, with the inclusion of contextual information	**2**	In vitro, without controls or Model, without supporting data	**3**	*In vitro* with full appropriate controls or Model with supporting data
			6	Quantitative data for a specific scenario, without inclusion of contextual information	**1**	Case report / Expert Opinion	**2**	Experiments without proper controls or Model, without supporting data
			5	Generic unvalidated model			**1**	Case report / Expert Opinion
			4	Specific unvalidated model				
			3	Identification of scenarios				
			2	Plan describing a process to address needs				
			1	Identification of needs only				

	Metrology, Characterisation, Standardisation & Reference Materials ***(RO 2, 3, 4 & 9)***	***Exposures - Sources, Pathways & Technologies*** ***(RO 5, 6, 7, 8 & 10)***	***Human Health Hazard & Risk Assessment*** ***(RO 11, 12, 13,14, 15 & 16)***	***Environmental Hazard & Risk Assessment*** ***(RO 17, 18 & 19)***
For RO 2,3,4 & 9 only, what standing does the research have?	**4** Recommended metric / RM / method **3** Implementation process reported **2** Implementation plan reported **1** Needs identification reported	n/a	n/a	n/a

	Metrology, Characterisation, Standardisation & Reference Materials ***(RO 2, 3, 4 & 9)***	***Exposures - Sources, Pathways & Technologies*** ***(RO 5, 6, 7, 8 & 10)***	***Human Health Hazard & Risk Assessment*** ***(RO 11, 12, 13,14, 15 & 16)***	***Environmental Hazard & Risk Assessment*** ***(RO 17, 18 & 19)***
What is the study's level of output?	**7** Systematic review **6** Review **5** Journal publication **4** Final report **3** Interim report **2** Abstract **1** Statement of Objectives only			

	Metrology, Characterisation, Standardisation & Reference Materials ***(RO 2, 3, 4 & 9)***	***Exposures - Sources, Pathways & Technologies*** ***(RO 5, 6, 7, 8 & 10)***	***Human Health Hazard & Risk Assessment*** ***(RO 11, 12, 13,14, 15 & 16)***	***Environmental Hazard & Risk Assessment*** ***(RO 17, 18 & 19)***
What test material / analyte characterisation was done?	**4** Determined & reported, with in situ characterisation **3** Determined & reported - characterised as supplied **2** Reported - as per label **1** Inferred from data presented **0** None			

	Metrology, Characterisation, Standardisation & Reference Materials ***(RO 2, 3, 4 & 9)***	***Exposures - Sources, Pathways & Technologies*** ***(RO 5, 6, 7, 8 & 10)***	***Human Health Hazard & Risk Assessment*** ***(RO 11, 12, 13,14, 15 & 16)***	***Environmental Hazard & Risk Assessment*** ***(RO 17, 18 & 19)***
What level of peer review was employed?	**3** Independent			
	2 Sponsor			
	1 Internal			
	0 None / Unknown			

	Metrology, Characterisation, Standardisation & Reference Materials	***Exposures - Sources, Pathways & Technologies***	***Human Health Hazard & Risk Assessment***	***Environmental Hazard & Risk Assessment***
How specific & relevant to the RO is the study?	**3** High specificity & High relevance			
	2 Low specificity but High relevance			
	1 High specificity but Low relevance			
	0 Low specificity & Low relevance			

	Metrology, Characterisation, Standardisation & Reference Materials ***(RO 2, 3, 4 & 9)***	***Exposures - Sources, Pathways & Technologies*** ***(RO 5, 6, 7, 8 & 10)***	***Human Health Hazard & Risk Assessment*** ***(RO 11, 12, 13,14, 15 & 16)***	***Environmental Hazard & Risk Assessment*** ***(RO 17, 18 & 19)***
How reliable is the study?	**3** Reliable, without condition			
	2 Reliable, but with condition			
	1 Not reliable			
	0 Not assignable			

APPENDIX 3 - WEIGHT-OF-EVIDENCE SCORE DISTRIBUTIONS

Figures A3.1 to A3.18 provide an overview of scores awarded to studies by assessors within each Research Objective. The threshold over which studies were considered to be of the most value scientifically is shown (where applicable) as a dashed grey line.

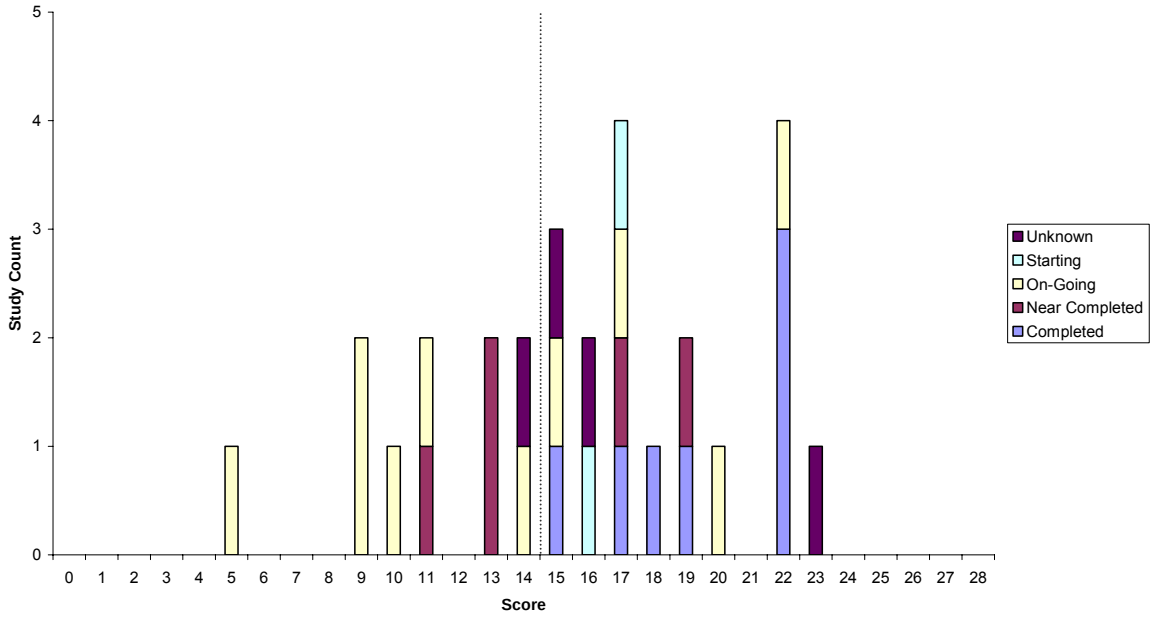

Figure A3.1: Research Objective 2

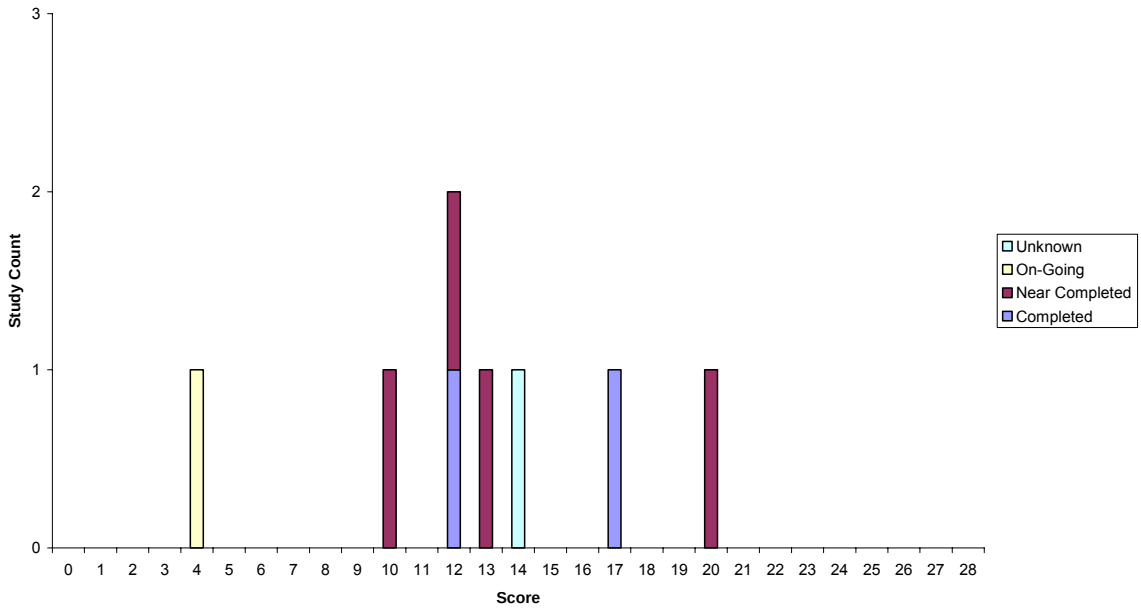

Figure A3.2: Research Objective 3

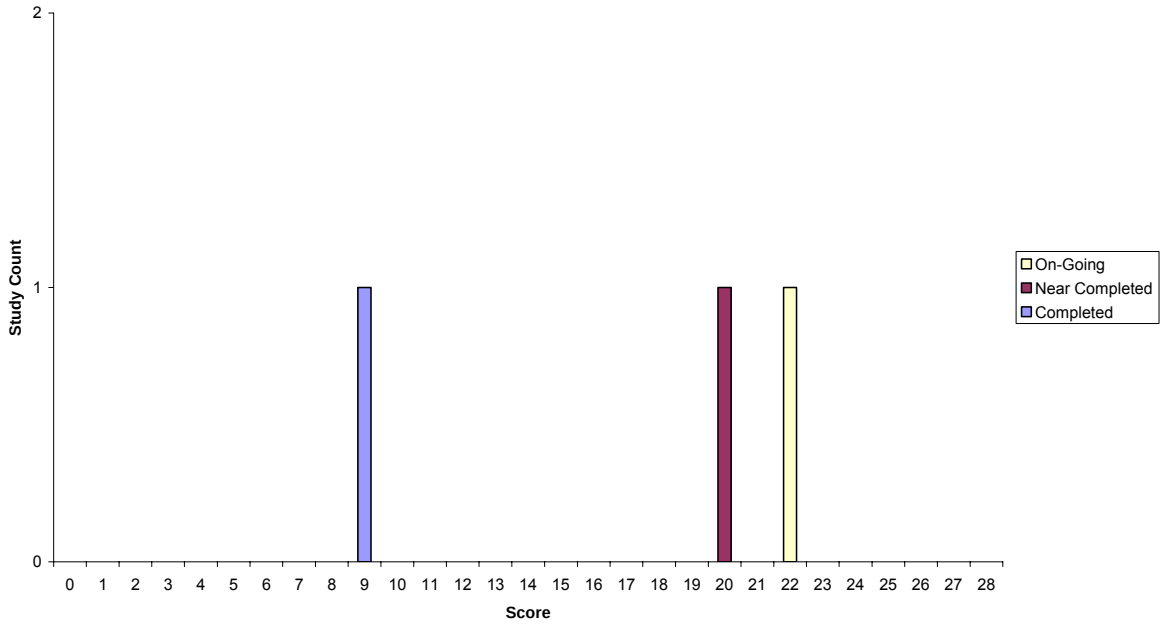

Figure A3.3: Research Objective 4

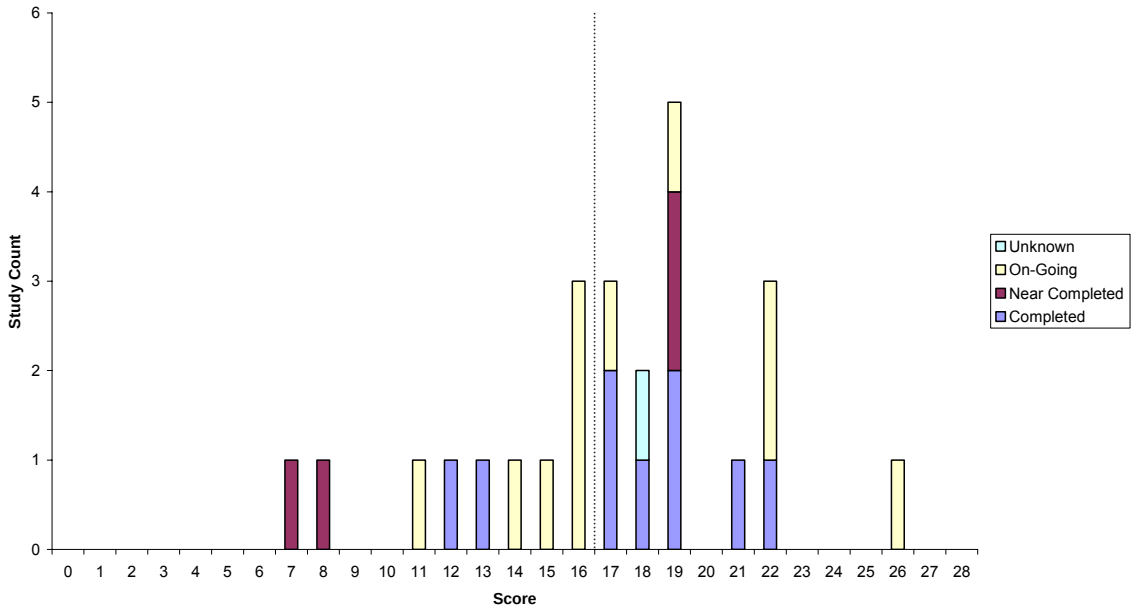

Figure A3.4: Research Objective 5

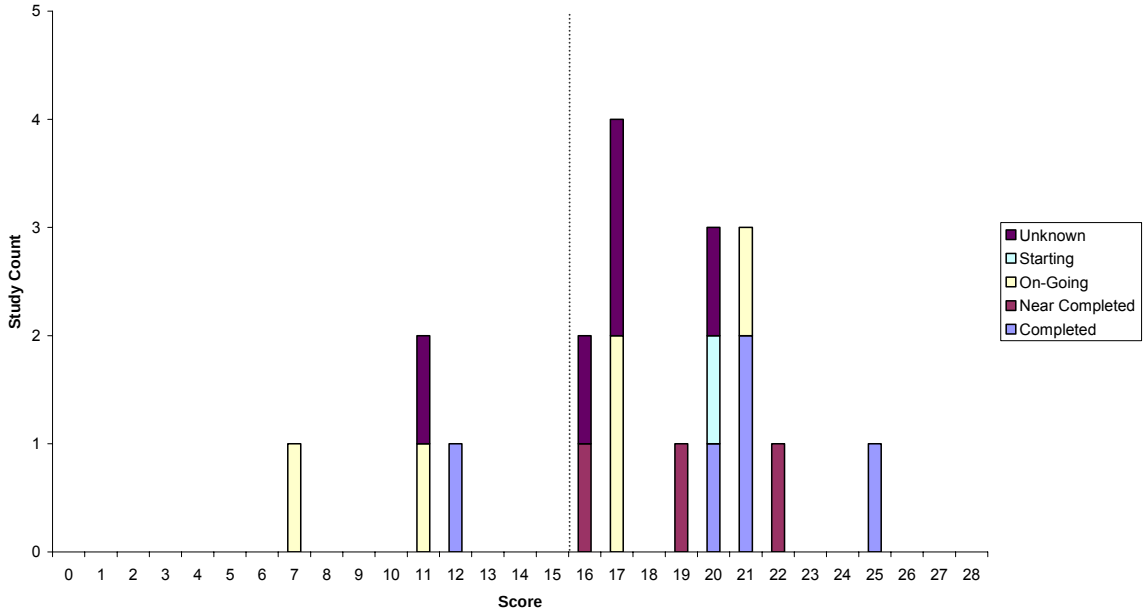

Figure A3.5: Research Objective 6

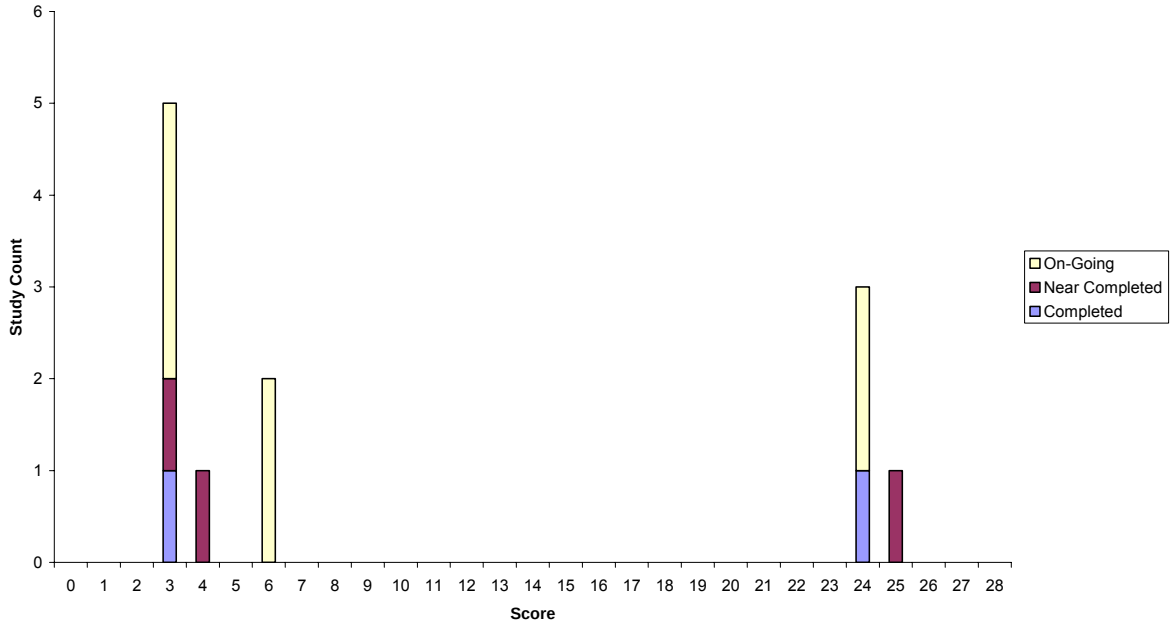

Figure A3.6: Research Objective 7

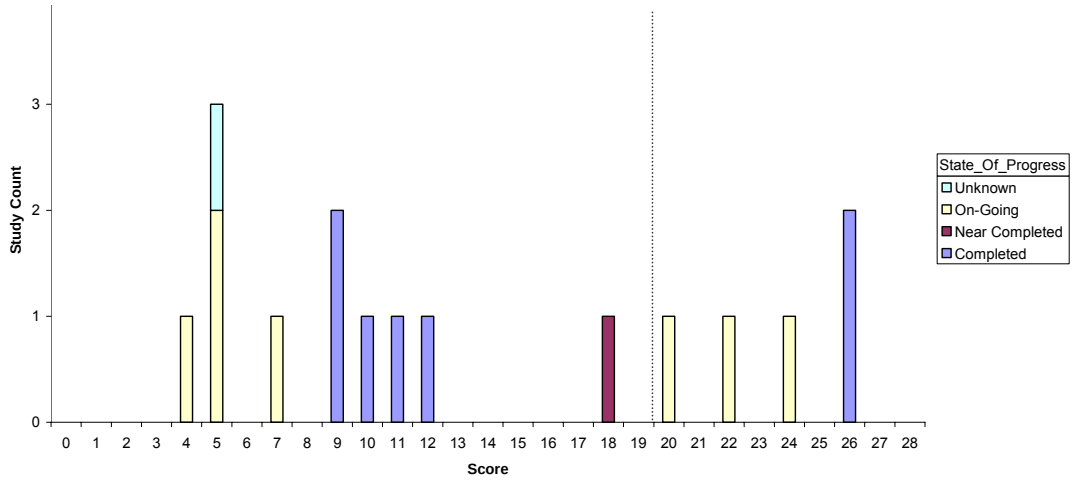

Figure A3.7: Research Objective 8

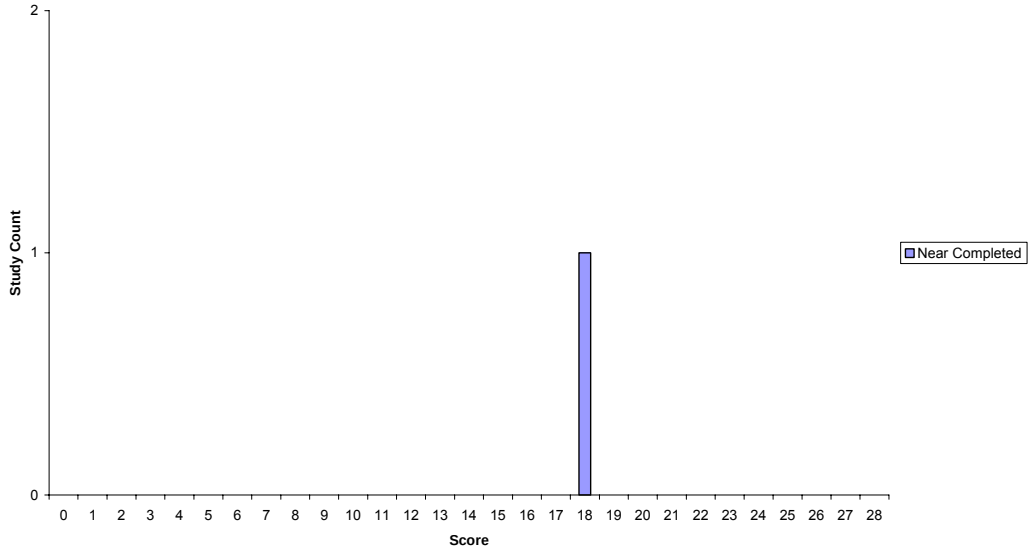

Figure A3.8: Research Objective 9

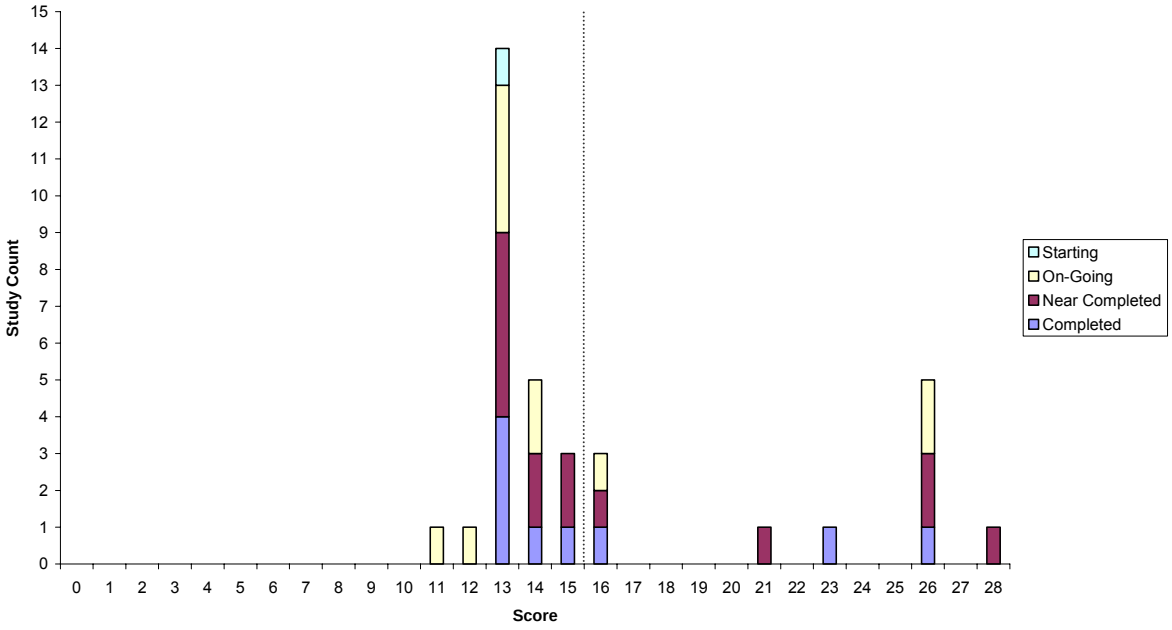

Figure A3.9: Research Objective 10

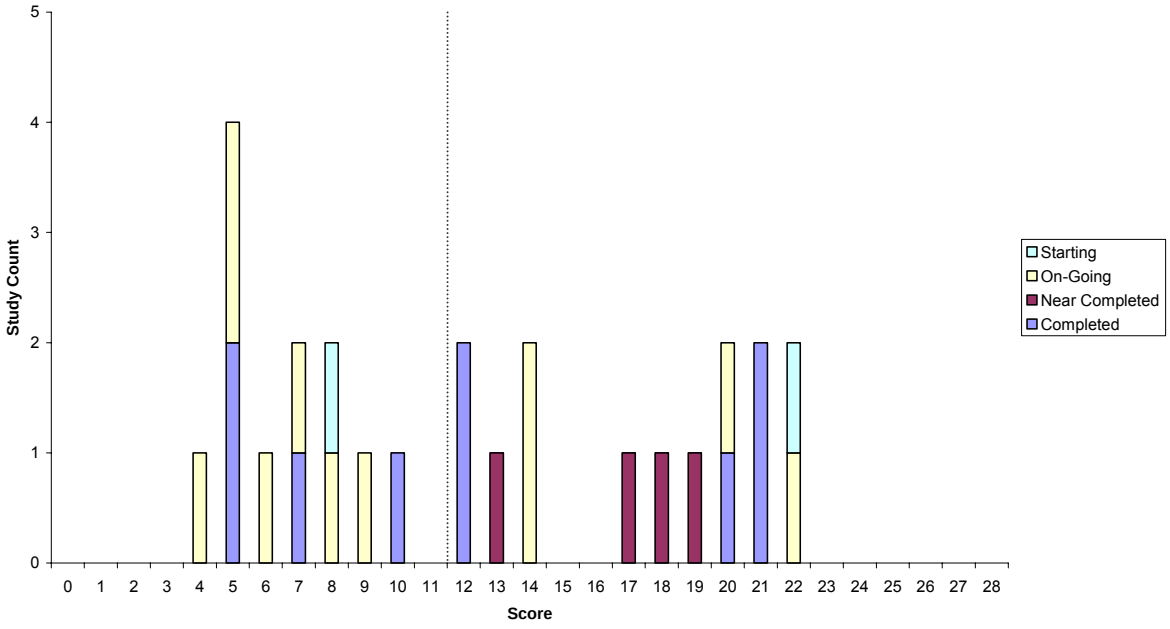

Figure A3.10: Research Objective 11

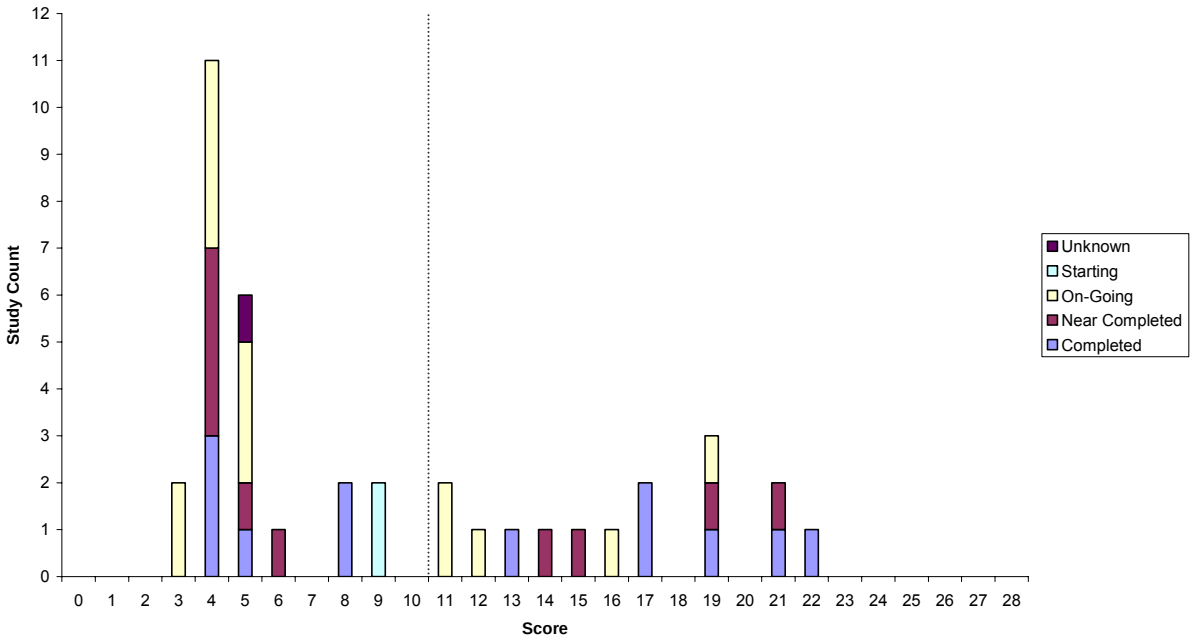

Figure A3.11: Research Objective 12

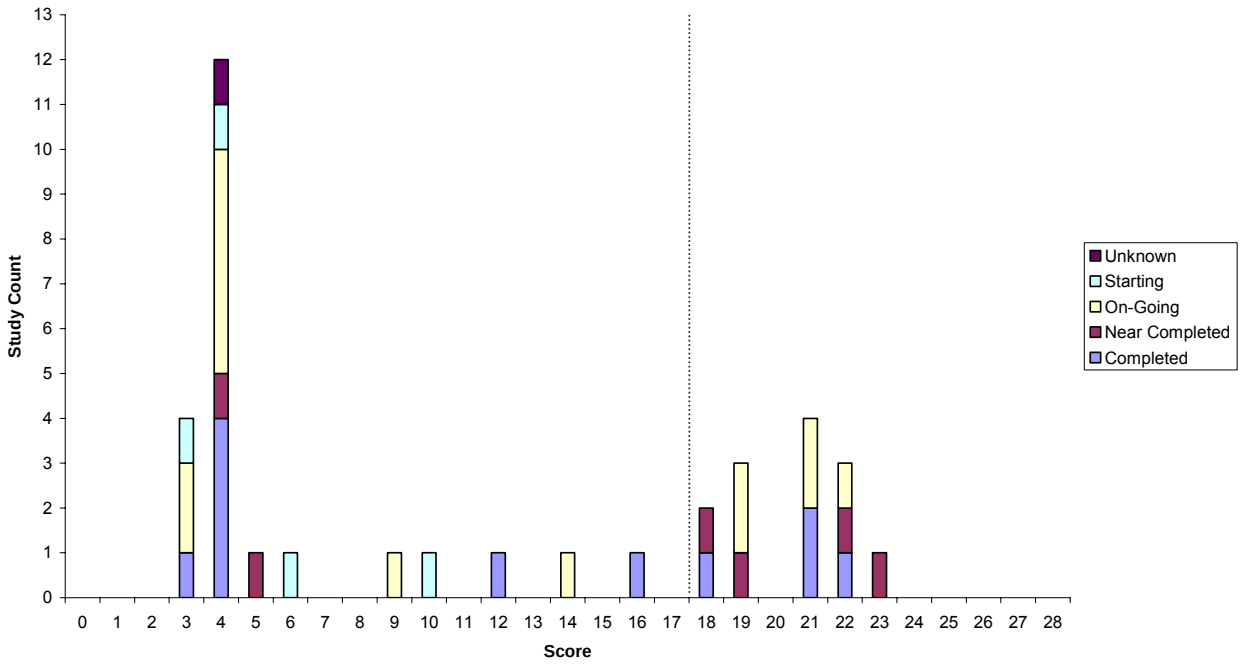

Figure A3.12: Research Objective 13

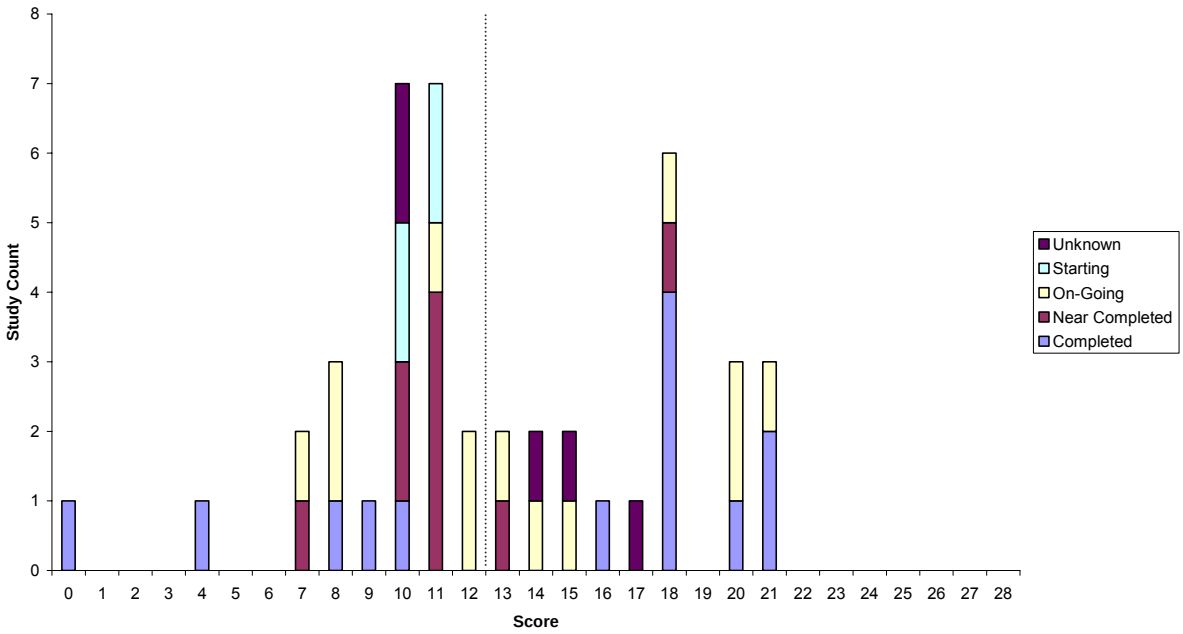

Figure A3.13: Research Objective 14

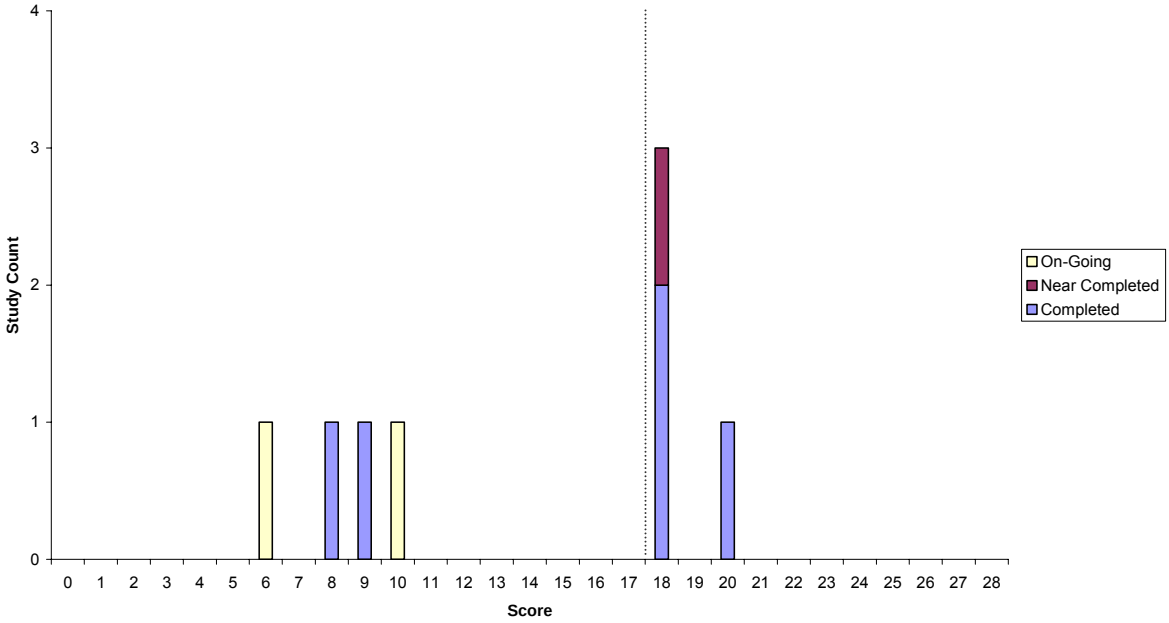

Figure A3.14: Research Objective 15

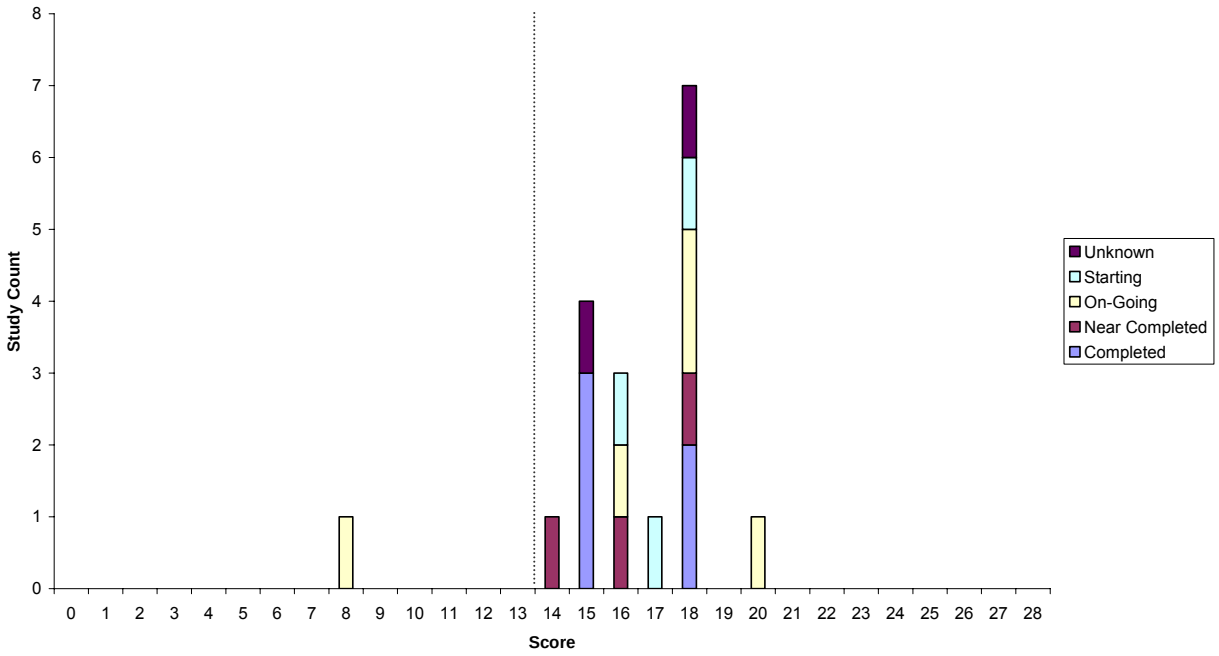

Figure A3.15: Research Objective 16

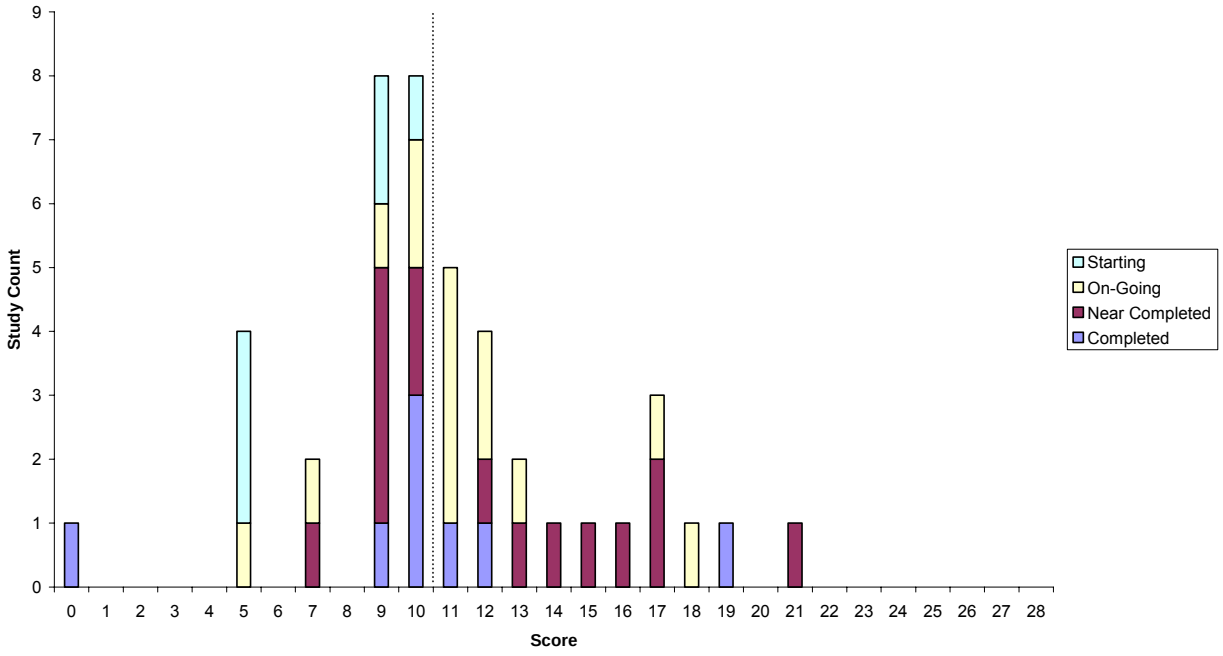

Figure A3.16: Research Objective 17

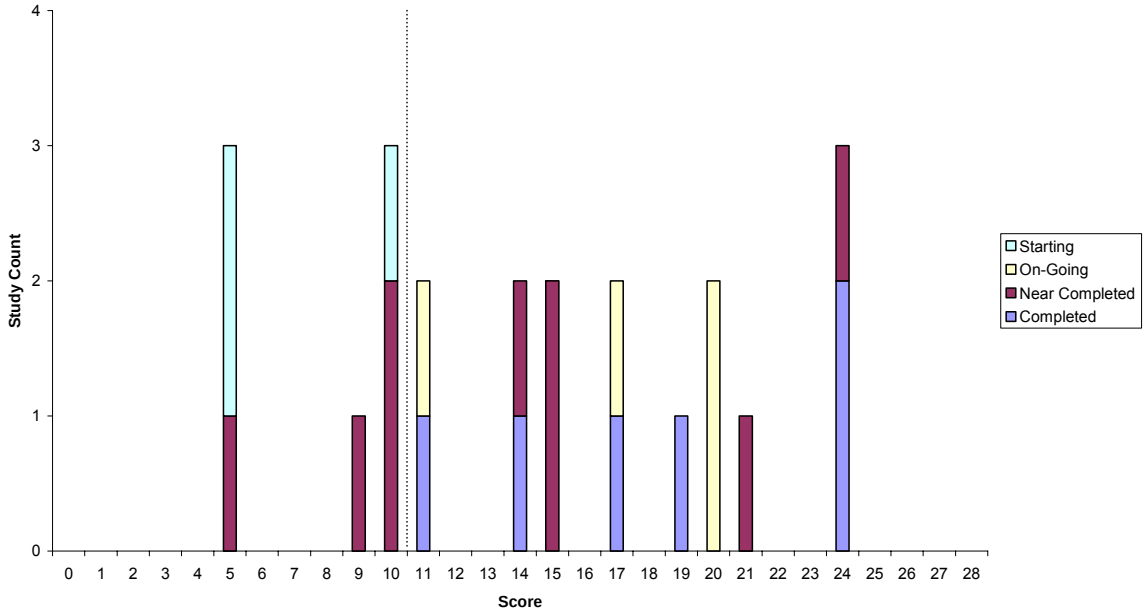

Figure A3.17: Research Objective 18

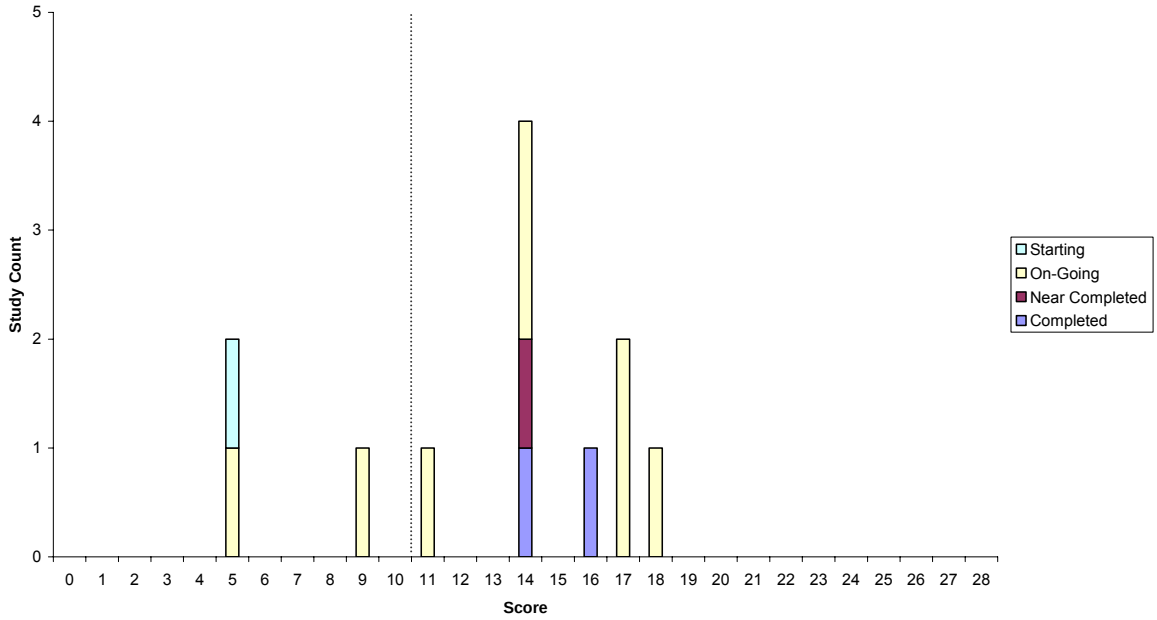

Figure A3.18: Research Objective 19

APPENDIX 4 - FINANCIAL INFORMATION FOR STUDIES

Tables A4.1 – A4.18 provide a tabular representation of study funding value over the project lifetime, according to both the state of progress and whether a study fell above or below the threshold set by the RO's assessor. Study value was available for many, but not all, of the studies. Therefore, the number of studies which were summed to provide each indication of grouped study value has been provided in brackets underneath the value listed.

Table A4.1: Research Objective 2

	State of progress					
	Unknown	**Starting (+/- 3 months)**	**On-Going**	**Near Completed (expected 2008)**	**Completed**	**Total**
No. studies > threshold score	3	2	4	2	7	18
Value / £	none listed	none listed	7,697,661 (n=3)	822,475 (n=2)	1,016,594 (n=4)	9,536,731 (n=9)
No. studies < threshold score	1	0	6	3	0	10
Value / £	none listed	-	6,363,303 (n=3)	4,043,604 (n=3)	-	10,406,907 (n=6)
TOTAL No. studies	4	2	10	5	7	**28**
Total Value / £	-	-	14,060,964 (n=6)	4,866,079 (n=5)	1,016,594 (n=4)	**19,943,638 (n=15)**

Table A4.2: Research Objective 3

	State of progress					
	Unknown	**Starting (+/- 3 months)**	**On-Going**	**Near Completed (expected 2008)**	**Completed**	**Total**
No. studies > threshold score	1	0	1	4	2	8
Value / £	none listed	-	none listed	243,351 (n=3)	167,999 (n=1)	411,350 (n=4)
No. studies < threshold score	0	0	0	0	0	0
Value / £	-	-	-	-	-	-
TOTAL No. studies	1	0	1	4	2	**8**
Total Value / £	-	-	-	243,351 (n=3)	167,999 (n=1)	**411,350 (n=4)**

Table A4.3: Research Objective 4

	State of progress					
	Unknown	**Starting (+/- 3 months)**	**On-Going**	**Near Completed (expected 2008)**	**Completed**	**Total**
No. studies > threshold score	0	0	1	1	1	3
Value / £	-	-	5,567,543 (n=1)	305,621 (n=1)	10,000. (n=1)	5,883,164 (n=3)
No. studies < threshold score	0	0	0	0	0	0
Value / £	-	-	-	-	-	-
Total No. studies	0	0	1	1	1	**3**
Total Value / £	-	-	5,567,543 (n=1)	305,621 (n=1)	10,000 (n=1)	**5,883,164 (n=3)**

Table A4.4: Research Objective 5

	State of progress					
	Unknown	**Starting (+/- 3 months)**	**On-Going**	**Near Completed (expected 2008)**	**Completed**	**Total**
No. studies > threshold score	1	0	6	2	7	16
Value / £	none listed	0	7,766,637 (n=4)	212,723 (n=2)	487,919 (n=6)	8,467,279 (n=12)
No. studies < threshold score	0	0	5	2	2	9
Value / £	0	0	3,807,290 (n=4)	1,103,683 (n=2)	248,547 (n=2)	5,159,520 (n=8)
TOTAL No. studies	1	0	11	4	9	**25**
Total Value / £	-	-	11,573,927 (n=8)	1,316,405 (n=4)	736,466 (n=8)	**13,626,799**

Table A4.5: Research Objective 6

	State of progress					
	Unknown	**Starting (+/- 3 months)**	**On-Going**	**Near Completed (expected 2008)**	**Completed**	**Total**
No. studies > threshold score	5	1	3	3	4	16
Value / £	none listed	none listed	5,967,543 (n=2)	522,731 (n=3)	429,215 (n=3)	6,919,489 (n=8)
No. studies < threshold score	0	0	2	0	1	3
Value / £	0	0	none listed	0	52,719 (n=1)	52,719 (n=1)
TOTAL No. studies	5	1	5	3	5	**19**
Total Value / £	-	-	5,967,543 (n=2)	522,731 (n=3)	481,934 (n=4)	**6,972,208 (n=9)**

Table A4.6: Research Objective 7

	State of progress					
	Unknown	**Starting (+/- 3 months)**	**On-Going**	**Near Completed (expected 2008)**	**Completed**	**Total**
No. studies > threshold score	0	0	7	3	2	12
Value / £	-	-	1,234,159 (n=5)	1,103,764 (n=3)	809,942 (n=2)	3,147,865 (n=10)
No. studies < threshold score	0	0	0	0	0	0
Value / £	-	-	-	-	-	-
TOTAL No. studies	0	0	7	3	2	**12**
Total Value / £			1,234,159 (n=5)	1,103,764 (n=3)	809,942 (n=2)	**3,147,865 (n=10)**

Table A4.7: Research Objective 8

	State of progress					
	Unknown	**Starting (+/- 3 months)**	**On-Going**	**Near Completed (expected 2008)**	**Completed**	**Total**
No. studies > threshold score	0	0	3	0	2	5
Value / £	-	-	5,567,543 (n=1)	-	248,676 (n=1)	5,816,219 (n=2)
No. studies < threshold score	1	0	4	1	5	11
Value / £	none listed	-	none listed	198,941 (n=1)	910,027 (n=5)	1,108,968 (n=6)
TOTAL No. studies	1	0	7	1	9	**16**
Total Value / £	-	-	5,567,543 (n=1)	198,941 (n=1)	1,158,703 (n=6)	**6,925,187 (n=8)**

Table A4.8: Research Objective 9

	State of progress					
	Unknown	**Starting (+/- 3 months)**	**On-Going**	**Near Completed (expected 2008)**	**Completed**	**Total**
No. studies > threshold score	0	0	0	1	0	1
Value / £	-	-	-	56,564 (n=1)	-	56,564 N=(1)
No. studies < threshold score	0	0	0	0	0	0
Value / £	-	-	-	-	-	-
TOTAL No. studies	0	0	0	1	0	**1**
Total Value / £	-	-	-	56,564 (n=1)	-	**56,564 (n=1)**

Table A4.9: Research Objective 10

	State of progress					
	Unknown	**Starting (+/- 3 months)**	**On-Going**	**Near Completed (expected 2008)**	**Completed**	**Total**
No. studies > threshold score	0	0	3	5	3	11
Value / £	-	-	496,804 (n=3)	536,668 (n=5)	561,975 (n=3)	1,595,447 (n=11)
No. studies < threshold score	0	1	8	9	6	24
Value / £	-	none listed	1,013,499 N=(6)	2,415,399 (n=9)	896,215 (n=6)	4,325.113 (n=21)
TOTAL No. studies	0	1	12	14	9	**35**
Total Value / £	-	-	1,510,303 (n=9)	2,952,067 (n=14)	1,458,190 (n=9)	**5,920,560 (n=32)**

Table A4.10: Research Objective 11

	State of progress					
	Unknown	**Starting (+/- 3 months)**	**On-Going**	**Near Completed (expected 2008)**	**Completed**	**Total**
No. studies > threshold score	0	1	4	4	5	14
Value / £	-	596,823 (n=1)	459,307 (n=2)	869,584 (n=3)	797,936 (n=4)	2,723,650 (n=10)
No. studies < threshold score	1	0	7	0	4	12
Value / £	none listed	-	1,447,073 (n=7)	-	1,662,033 (n=3)	3,109,106 (n=10)
TOTAL No. studies	1	1	11	4	9	**26**
Total Value / £	-	596,823 (n=1)	1,906,380 (n=9)	869,584 (n=3)	2,459,969 (n=7)	**5,832,756 (n=20)**

Table A4.11: Research Objective 12

	State of progress					
	Unknown	**Starting (+/- 3 months)**	**On-Going**	**Near Completed (expected 2008)**	**Completed**	**Total**
No. studies > threshold score	0	0	5	4	6	15
Value / £	-	-	7,562,304 (n=5)	930,439 (n=4)	415,070 (n=6)	8,907,813 (n=15)
No. studies < threshold score	1	2	9	6	5	19
Value / £	none listed	none listed	8,414,850 (n=8)	1,430,175 (n=6)	658,304 (n=6)	10,503,329 (n=20)
TOTAL No. studies	1	2	14	6	11	**34**
Total Value / £	-	-	15,977,154 (n=13)	2,360,614 (n=10)	1,073,374 (n=12)	19,411,142 **(n=25)**

Table A4.12: Research Objective 13

	State of progress					
	Unknown	**Starting (+/- 3 months)**	**On-Going**	**Near Completed (expected 2008)**	**Completed**	**Total**
No. studies > threshold score	0	0	5	4	4	13
Value / £	-	-	219,524 (n=4)	312,461 (n=4)	615,024 (n=4)	1,147,009 (n=12)
No. studies < threshold score	1	4	9	2	7	23
Value / £	none listed	none listed	9,908,528 (n=9)	3,485,521 (n=2)	765,563 (n=6)	14,159,612 (n=17)
TOTAL No studies	1	4	14	6	11	**36**
Total Value / £	-	-	10,128,052 (n=13)	3,797,982 (n=6)	1,380,587 (n=10)	15,306,621 **(n=29)**

Table A4.13: Research Objective 14

	State of progress					
	Unknown	**Starting (+/- 3 months)**	**On-Going**	**Near Completed (expected 2008)**	**Completed**	**Total**
No. studies > threshold score	3	0	7	2	8	20
Value / £	none listed	-	494,081 (n=5)	635,968 (n=1)	2,295,278 (n=7)	3,425,327 (n=13)
No. studies < threshold score	2	4	6	7	5	24
Value / £	none listed	596,823 (n=1)	15,897,018 (n=6)	962,148 (n=7)	697,246 (n=5)	18,153,235 (n=19)
TOTAL No studies	5	4	13	9	13	**44**
Total Value / £	-	596,823 (n=1)	16,391,099 (n=11)	1,598,116 (n=8)	2,992,524 (n=12)	**21,578,562 (n=32)**

Table A4.14: Research Objective 15

	State of progress					
	Unknown	**Starting (+/- 3 months)**	**On-Going**	**Near Completed (expected 2008)**	**Completed**	**Total**
No. studies > threshold score	0	0	0	1	3	4
Value / £	-	-	-	68,500 (n=1)	1,023,523 (n=3)	1,092,023 (n=4)
No. studies < threshold score	0	0	2	0	2	4
Value / £	-	-	8,148,590 (n=2)	-	873,324 (n=1)	9,021,914 (n=3)
TOTAL No studies	0	0	2	1	5	**8**
Total Value / £	-	-	8,148,590 (n=2)	68,500 (n=1)	1,896,847 (n=4)	**10,113,937 (n=7)**

Table A4.15: Research Objective 16

	State of progress					
	Unknown	**Starting (+/- 3 months)**	**On-Going**	**Near Completed (expected 2008)**	**Completed**	**Total**
No. studies > threshold score	2	3	4	3	5	17
Value / £	none listed	2,381,678 (n=1)	3,942,894 (n=3)	878,625 (n=3)	695,814 (n=5)	7,899,012 (n=12)
No. studies < threshold score	0	0	1	0	0	1
Value / £	-	-	none listed	-	-	-
TOTAL No studies	2	3	5	3	5	**18**
Total Value / £	-	2,381,678 (n=1)	3,942,895 (n=3)	878,625 (n=3)	695,814 (n=5)	**7,899,012 (n=12)**

Table A4.16: Research Objective 17

	State of progress					
	Unknown	**Starting (+/- 3 months)**	**On-Going**	**Near Completed (expected 2008)**	**Completed**	**Total**
No. studies > threshold score	0	0	9	8	3	20
Value / £	-	-	899,330 (n=6)	1,221,774 (n=8)	346,704 (n=3)	2,467,808 (n=17)
No. studies < threshold score	0	6	5	7	5	23
Value / £	-	240,034 (n=5)	993,267 (n=5)	771,207 (n=7)	1,131,293 (n=5)	3,135,801 (n=22)
TOTAL No studies	0	6	14	15	8	**43**
Total Value / £	-	240,034 (n=5)	1,892,597 (n=11)	1,992,981 (n=15)	1,477,997 (n=8)	**5,603,609 (n=39)**

Table A4.17: Research Objective 18

	State of progress					
	Unknown	**Starting (+/- 3 months)**	**On-Going**	**Near Completed (expected 2008)**	**Completed**	**Total**
No. studies > threshold score	0	0	4	5	6	15
Value / £	-	-	698,316 (n=4)	205,496 (n=5)	914,741 (n=5)	1,818,553 (n=14)
No. studies < threshold score	0	3	0	4	0	7
Value / £	-	133,554 (n=2)	-	489,142 (n=4)	-	622,696 (n=6)
TOTAL No studies	0	3	4	9	6	**22**
Total Value / £	-	133,554 (n=2)	698,316 (n=4)	694,638 (n=9)	914,741 (n=5)	**2,441,249 (n=20)**

Table A4.18: Research Objective 19

	State of progress					
	Unknown	**Starting (+/- 3 months)**	**On-Going**	**Near Completed (expected 2008)**	**Completed**	**Total**
No. studies > threshold score	0	0	6	1	2	9
Value / £	-	-	3,007,699 (n=5)	180,877 (n=1)	506,912 (n=2)	3,695,488 (n=8)
No. studies < threshold score	0	1	2	0	0	3
Value / £	-	None Listed	1,059,878 (n=2)	-	-	1,059,878 (n=2)
TOTAL No studies	0	1	8	1	2	**12**
Total Value / £	-	-	4,067,577 (n=7)	180,877 (n=1)	506,912 (n=2)	**4,755,366 (n=10)**

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