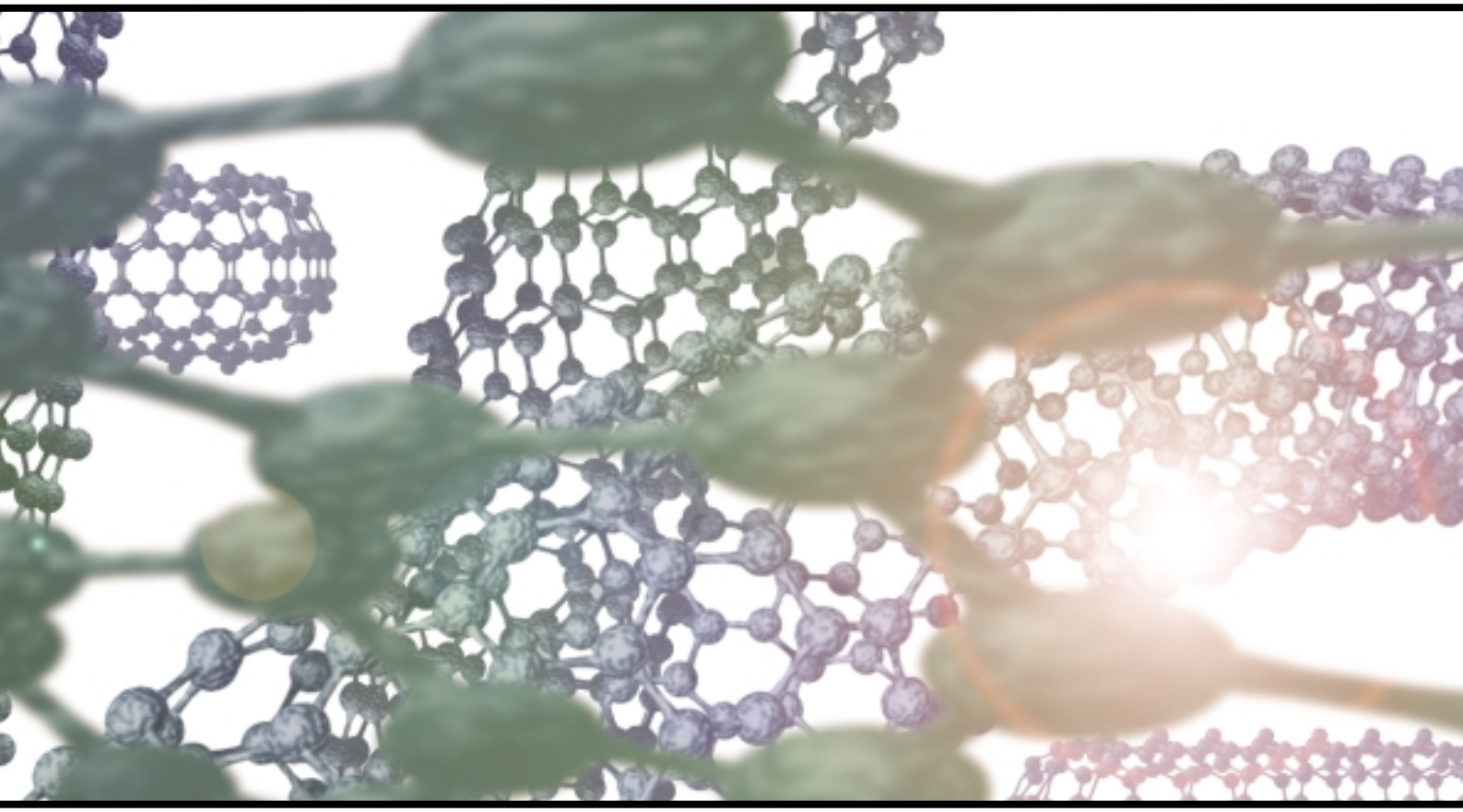
Nanomaterials

a risk to health at work?



First International Symposium on Occupational Health Implications of Nanomaterials

12-14 October 2004
Palace Hotel, Buxton, Derbyshire,UK

Report of Presentations at Plenary and Workshop Sessions and Summary of Conclusions

David Mark
Chair of Symposium
Health and Safety Laboratory,
Buxton, SK17 9 JN

HEALTH & SAFETY LABORATORY

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www.hsl.gov.uk

FOREWORD

The British Health and Safety Executive (HSE) and the US National Institute for Occupational Safety and Health (NIOSH) are pleased jointly to publish these proceedings from the First International Symposium on Nanotechnology and Occupational Health, which was organised by the British Health and Safety Laboratory (HSL) and held under NIOSH co-sponsorship on 12-14 October 2004, in Buxton, England. The symposium represented a historic gathering of scientists and engineers from many disciplines and many countries. These proceedings, resulting from the intensive three-day meeting, offer a significant addition to the growing body of literature on this emerging field of occupational health study. This report was compiled by HSL from abstracts authored by the invited speakers and contributions made by participants at the symposium. The views and recommendations expressed in these proceedings are solely those of the authors and workshop participants in the First International Symposium on Nanotechnology and Occupational Health. Inclusion in this document does not constitute endorsement by NIOSH or HSE.

Nanotechnologies are poised to revolutionise medicine, manufacturing, energy production, and other fundamental features of everyday life in the 21st Century. But they also pose important questions that stem from the unique nature of materials and processes at the nanometer scale. In the realm of occupational health, much is unknown about the ways in which people may be exposed to nanomaterials through their manufacture and use in the workplace, and the potential health implications of such exposure. We are pleased to be able to work together, and with many other partners, to address these questions as an integral part of efforts internationally to help nanotechnologies realise their full potential. The report of proceedings will be used to inform the development of associated research programmes.

Finally, we invite you to take note of the Second International Symposium on Nanotechnology and Occupational Health, which NIOSH and HSE, amongst others, will co-sponsor on 23-26 October 2005, in Minneapolis, USA, where we and our colleagues will assess progress that has been made since the First International Symposium.

Paul Davies
Chief Scientist,
HSE,
UK.

John Howard
Director,
NIOSH,
USA

CONTENTS

Disclaimer

The views expressed in the extended abstracts and the poster abstracts are those of the authors only. The summaries from the workshop sessions represent the views of those delegates who attended the workshops and do not necessarily reflect HSE or NIOSH opinion or official policy. HSE and NIOSH did not seek the collective or consensus advice, opinions, or recommendations of the participants.

1. INTRODUCTION

1.1 Nanotechnologies and nanomaterials

In the recently published Royal Society / Royal Academy of Engineering report 'Nanoscience and nanotechnologies: opportunities and uncertainties' (http://www.nanotec.org.uk/finalReport.htm) a definition of the nanotechnologies was proposed:

'***Nanotechnologies*** *are the design, characterisation, production and application of structures, devices and systems by controlling shape and size at nanometre scale.'*

The plural was used because there are many areas and scientific disciplines in which current research activity is at the nanometre scale. They include: nanomaterials, metrology, electronics, optoelectronics, information and communication technology and bionanotechnology and nanomedicine.

Interest in the unique properties associated with materials having structures on a nanometer scale has been increasing at an exponential rate. By restricting ordered atomic arrangements to increasingly small volumes, materials begin to be dominated by the atoms and molecules at the surfaces, often leading to properties that are strikingly different from the bulk material. For instance, a relatively inert metal or metal oxide may become a highly effective catalyst when manufactured as nanoparticles; opaque particles may become transparent when composed of nanoparticles, or vice versa; conductors may become insulators, and insulators conductors; nanophase materials may have many times the strength of the bulk material. Nanoparticles can comprise a range of different morphologies including nanotubes, nanowires, nanofibres, nanodots and a range of spherical or aggregated dendritic forms. These materials have seen application in a wide range of industries including electronics, pharmaceuticals, chemical-mechanical polishing, catalysis, and it is likely that the next few years will see a dramatic increase in the industrial generation and use of nanoparticles. When this occurs, the workforces in these industries may be exposed to unknown levels of airborne nanoparticles (these are also known as ultrafine particles with aerodynamic diameters < 100 nm), and unique health outcomes associated with these particles may result, if work is not carried out at an early stage to develop a better understanding of the risks, and to develop guidance on how to measure and control worker exposure to these particles.

Nanoparticles are encountered in ambient air as well as in the workplace, and in terms of particle number and surface, they totally dominate the ambient particle levels. Epidemiological studies have shown an association between increased particulate air pollution and adverse health in susceptible members of the population, in particular the elderly with respiratory and cardiovascular diseases. This association has been found to be particularly relevant for the finer fractions of the airborne particles (PM2.5, and PM1).

Estimation of the potential health risks associated with these new materials requires understanding of the mechanisms of ill health, the identification of some property or metric of the material which relates exposure to the material to health risk and some method for measuring exposure in relation to that metric. Once these are in place, it is potentially possible to define safe levels of exposure to these materials and if needed to design control methodologies to enable exposures to be maintained at or below these safe levels. For nanoparticles, there is currently poor understanding of all of these issues.

The aim of the symposium discussed in this report was to provide a forum in which the latest understanding of the potential health implications of the production and handling of nanomaterials in the form of nanoparticles was presented and discussed. It was particularly timely coming just 2 months after the publication of the Royal Society / Royal Academy of Engineering report in the UK and was part of the UK Health and Safety Executive's activities in addressing the occupational health implications of nanotechnology especially the production and use of new nanomaterials. The summaries from the workshop sessions represent the views of those delegates who attended the workshops and do not necessarily reflect HSE or NIOSH opinion or official policy. HSE and NIOSH did not seek the collective or consensus advice, opinions, or recommendations of the participants.

1.2 Organising Committee

The main organizing committee for the symposium was comprised the following members:

David Mark	HSL/HSE (UK)	(Chair)
Andrew Maynard	NIOSH (USA)	
Christine Northage	HSE (UK)	
Carsten Möhlmann	BIA (Germany)	
Olivier Witshger	INRS (France)	

The symposium coordinating team comprised the following members from HSL/HSE:

Karen Wilkinson	(Chair)
Ursula Haslam	
Liz Woods	
Linda Heritage	

Sponsorship for the symposium was afforded by HSE and NIOSH.

2 PROGRAMME

The two and a half day symposium comprised a mixture of three sessions of presentations by invited experts, a poster session of latest research and two workshop sessions. The final programme is given below:

Day 1: Tuesday 12 October 2004

SESSION 1: Introduction to Nanotechnology

13.30	Welcome and Introduction	Norman West (HSL, UK)
13.45	Nanotechnology - The Challenge to Occupational Health and Hygiene	John Ewins (Head of the Science Strategy and Statistics Division, HSE presenting on behalf of Paul Davies Chief Scientist (HSE, UK)
14.30	Nanotechnology in US: Research & Education & Risk Governance	Mike Roco (National Science Foundation, USA)
15.15 - 15.45	TEA	
15.45	Nanotechnology – EU Perspective including Research Programmes	Angela Hullmann (European Commission, DG Research) - presenting on behalf of Renzo Tomellini
16.30	The Cutting Edge of Materials	Markus Pridöhl (Degussa Advanced Nanomaterials, Germany)
17.00	CLOSE	
18.00	Leave the Hotel for Tour and Dinner at Chatsworth House	

Day 2: Wednesday 13 October 2004

SESSION 2: Toxicity and Health Effects

08.30	Chairperson's Introduction	Eileen D Kuempel (NIOSH, USA)
08.35	Toxicity of Nanoparticles	Ken Donaldson (Univ. of Edinburgh, UK)
09.20	Inhaled Nano-sized Particles: Potential Effects & Mechanisms	Günter Oberdörster (Univ. of Rochester, USA)

10.05 – 10.35	COFFEE	
10.35	Skin Exposure to Nanoparticles: Is It a Problem?	Sally Tinkle (NIH, USA)
11.10	Epidemiology of Nanoparticles	Irene Brüske-Hohlfield (GSF, Germany)
11.45	Impact of Single Wall Carbon Nanotubes on Respiratory Health Effects	David B Warheit (Dupont, USA)
12.20 – 13.40	LUNCH	

SESSION 3: Exposure and Control Issues

13.40	Chairperson's Introduction	Rob Aitken (IOM, UK)
13.45	Hazards, Exposures and Risks of Nanoparticles: A Look Forward	Paul Borm (CEL, Netherlands)
14.15	Monitoring Nanoparticles in the Workplace	Andrew Maynard (NIOSH, USA)
14.45 – 15.15	TEA	
15.15	Control of Exposure to Nanoparticles	Dave Mark (HSL, UK)
15.45	Industry Perspectives: Risk Evaluation and Control – Current Perspectives and Approaches	Gordon Peters (Proctor and Gamble, USA)
16.15	Problems and Solutions of Current Manufacture of Nanoparticles	Alan Shakesheff (QinetiQ Nanomaterials, UK)
16.45 – 17.45	Poster Session	
19.30	Dinner at Palace Hotel	

Day 3: Thursday 14 October 2004

WORKSHOPS

08.30	Chairperson's Introduction	Brian Fullam (HSE, UK)
08.45 – 10.00	Current Occupational Health-Related Research Programmes. Five bodies give overview of their programmes: HSE, NIOSH, INRS, BIA, TNO	
10.00 – 10.30	COFFEE	
10.30	Workshop Sessions – Research Gaps	
12.00	Report Back	
12.30 – 13.30	LUNCH	
13.30	Workshop Sessions – Regulatory Implications	
15.00 – 15.15	TEA (taken in Peak Ballroom)	
15.15	Report Back Discussion on Way Forward	
16.20	Closing Remarks	Brian Fullam (HSE, UK)
16.30	END	

3. EXTENDED ABSTRACTS FROM PLENARY SESSIONS

3.1 SESSION 1: INTRODUCTION TO NANOTECHNOLOGY

3.1.1 Nanotechnology – the challenge to occupational health and hygiene

John Ewins,
Head of the Science Strategy and Statistics Division, Health and Safety Executive, UK

The technologies falling under the umbrella term "Nanotechnology" have the capacity to transform many aspects of our lives. At present theoretical modelling and research and development are dominating much of the work on nanotechnologies, but commercial exploitation of these innovations is growing rapidly. Since its creation in 1974 the HSE has seen many new technologies develop, some of which have created new hazards and risks requiring novel controls. Close working between the regulators, industry, workers and the public to solve these challenges has resulted in safe and useable devices and materials that we now take for granted e.g. new medicines, plastics, computers and mobile phones.

There are many arguments as to exactly what and when nanotechnologies will deliver new products and manufacturing techniques. Some products, self-cleaning glass and sun protection creams are already here, others such as higher capacity hard disks, enhanced solar panels or fuel cells are near and the really exciting super strong but light materials or 'point of care' medical intervention are somewhat further into the future. Nanotechnologies promise to be the next industrial revolution and possibly could change the workplace and society as much as the last one.

In July 2004 the Royal Society / Royal Academy of Engineering report 'Nanoscience and nanotechnologies: opportunities and uncertainties' was published (http://www.nanotec.org.uk/finalReport.htm). This report covered the technical, ethical, health, environmental and regulatory issues raised by the potential of nanotechnology. The report starts with their definition of the nanotechnologies

'***Nanotechnologies*** *are the design, characterisation, production and application of structures, devices and systems by controlling shape and size at nanometre scale.'*

and ends with 21 recommendations. These include some specific to issues that impact upon occupational health. Anyone with an interest in nanotechnology should read this report and consider the implications of the recommendations.

The Health and Safety Executive (HSE) is the primary regulator of health and safety in British industry. HSE recognises that changes in industry can create new hazards and risks. HSE's mission is to make sure that even in today's changing workplace people's

health and safety are protected. In fact HSE's vision is for sensible health and safety to be recognised as one of the cornerstones of a civilised society. HSE, acting as a responsible regulator, aims to work with those in control of workplaces to get it right from the start. Whilst HSE will always take the appropriate action against those that disregard their duties, this is can be too late to help those harmed. That is why HSE has a long history of giving advice, publishing advice and helping industry to find and share its own solutions. Whatever the industry, getting health and safety right from the start is cheap in terms of human harm and the money spent to achieve it. To do this industry, researchers and regulators must work together, through a process of international collaboration, to develop the technical, scientific and regulatory framework that will ensure the risks can and will be controlled. This is the first international event devoted to looking at the occupational health implications posed by the nanotechnologies and is a step in developing that collaboration. HSE/HSL and NIOSH have collaborated to host this event as part of the process of gaining a shared understanding of the hazards and risks of nanotechnologies and of the key gaps in our knowledge.

Three preliminary studies have been funded by HSE as part of its horizon scanning activities to look at the potential hazards and risk of nanotechnology. These 'snapshot' reviews considered:

- Fire and explosion – undertaken by the Health and Safety Laboratories, Buxton.
- Occupational hygiene – undertaken by the Institute of Occupational Medicine, Edinburgh.
- Toxicological hazard - carried out internally by HSE's own toxicologists

These all reported how limited the data available was and the difficulty of reading across from existing data and that the hazards from nanoparticles and fibres are sufficiently different from bulk materials to require further careful study. They also indicated that the areas of initial occupational health concern should be:

- potential for enhanced toxicity
- potential to cross the skin barrier
- existing control measures are unproven
- possible persistence in the workplace

These reports are available from the HSE website (www.hse.gov.uk).

In the main, regulation of health and safety in the UK is fundamentally a risk based system from the bed rock of the Health and Safety at Work Act etc. 1974 to the regulations that clarify and qualify the duties of employers and those in positions of control. The Control of Substances Hazardous to Health Regulations 2002, requires assessment of risk and suitable controls to be put in place of any substance, including biological agents that are hazardous to health. This covers the nanotechnologies just as much as any other chemical. However such risk assessments require a body of information to define the hazard, determine the level of risk and identify suitable means of control. Currently much of the original core data regarding a new chemical substance

placed on the market is presented to meet the requirements of the Notification of New Substances Regulations (UK's implementation of the European Dangerous Substances Directive). Currently many new nanomaterials do not qualify as new materials so this data will not be generated in the normal manner. This means there could be ever increasing gaps in our knowledge.

Whenever a new technology arises there are gaps in the knowledge base relating to its potential health and safety hazards and risks. In some cases extrapolating from what is already known can easily fill these, but in others such as nanotechnology new techniques and or data must be generated to fill those gaps. Some of the more significant gaps in the 'nano knowledge base' are:

- Nomenclature – the current chemically based naming systems are not adequate to describe the new nanoproducts. A new agreed nomenclature is needed so materials can be accurately described and specified.
- Measurement – Metrology is a key issue for researchers and manufacturers but is also important for regulators – if a material cannot be reliably measured, workplace monitoring and exposure standards are unachievable.
- When is a nanomaterial new? – It has been suggested by a number of sources that nanomaterials should be considered to be "new chemicals" for the purposes of existing and future chemical regulatory systems. Also if nanotechnology starts at 100nm is a 101nm product not nanotechnology? Or if a product is 100's of microns across but has 20nm features that are important to its function is that a new 'nano' chemical or a device?
- How different is different – e.g. If data etc exists for a 50nm version of a chemical should a 60nm version require a complete new set of safety data?
- What test data is needed – Are new tests required for nanomaterials? Different animals tests? New RPE/PPE standards? New engineering control standards?
- Are new toxicological models needed?
- This leads to the ultimate question - What exposure will cause harm?

With the risk based regulatory structure that the UK has for health and safety any technology with gaps it its knowledge base is a challenge to undertaking systematic risk assessments. In these cases the concept of the precautionary principle is applicable. There is some confusion as to what the precautionary principle means HSE has some guidance on what we mean by this term and when exactly it should be applied (http://www.hse.gov.uk/aboutus/meetings/ilgra/pppa.htm). In very crude terms it means not 'do nothing until you know everything' but to act in a cautious manner, assume the worst and take steps such that the potential for harm is drastically reduced. However, even adopting this approach it must be recognised that you are still left to deal with a degree of uncertainty.

As mentioned above uncertainty is a challenge to a risk based regulatory system, without a detailed risk assessment how can a duty holder or a regulator be sure that controls are adequate to the risk. HSE's view is that in the short term one must adopt a precautionary approach, this precaution is not to not do it but to use your best judgement based on what

you already know and put controls in place such that you believe the risk will be suitably controlled even if the hazard is ultimately shown to very nasty indeed. However, judgement is not perfect and there is the risk of either being over cautious and ultimately losing the benefits of the new technologies or having not done enough and people suffering significant health effects. The nanotechnologies are in this way no different from any other new technology that has been introduced in the past. The challenges for a responsible regulator is helping to ensure those in control of nanotechnologies are suitably precautionary without being unacceptably risk averse.

The other parts of UK Government have published some guidance on dealing with scientific uncertainty (e.g.www.parliament.uk/documents/upload/POSTpn220.pdf). In general terms when you have more gaps in your knowledge base you must increasingly rely on expert judgement and use generic controls. What you must also do is also seek to reduce those gaps in your knowledge until they no longer have a significant bearing upon the risks remaining. Whilst there is much speculation and promotion surrounding the potential benefits and hazards of nanotechnology we must consider what this means in terms of potential harm to those in the workplace. The potential risk and hazards are not fundamentally new there are already some very toxic chemicals in use every day by industry and yet ill effects due to chemicals are relatively rare. The pharmaceutical industry deals with materials of small often sub-micron particle size that are extremely biologically active and yet they have one of the best safety records in manufacturing industry. This is not by accident but due to suitable controls being put in place based upon detailed risk assessment when the data is available or by using a precautionary level of control where there are data gaps.
HSE has identified the following initial priorities in its response to the nanotechnologies:

- Engage with researchers and workers in the field– this symposium is part of that process.
- Promote a balanced cautious approach – currently there is an information note available for download (www.hse.gov.uk/horizons/nanotech/index.htm).
- Reduce uncertainty - Controls based on judgement are never going to be as satisfactory as those based on a risk assessment with a foundation in fact. There must be cooperation between researchers, industry and regulators to reduce the uncertainty.
- Fill the knowledge gaps - New data must be generated and models validated to fill those knowledge gaps. And as the pool of knowledge grows the regulatory framework must be kept under constant review to ensure that the nanotechnologies are suitably regulated.
- Review the regulatory framework

Ultimately nanotechnologies will just be another part of the manufacturing industries we know today. Industry will have a knowledge base to draw upon and industry guidance and international standards to guide them.
What must always be borne in mind is that if the public lose confidence in the safety of a new or particular technology their pressure will ultimately result in loss of its ‘licence to operate’. GMO’s for food production are currently not a commercially viable option in Europe and industry have closed their production in the light of massive public concern.

Public trust can only be earned by engagement, the deficit model of 'if I teach them they will think like me' has been shown to not work. We must all remember that ultimately the public decide via consumer power and the political process if 'safe' is 'safe enough' and if they do not see the benefits of a technology then their willingness to accept it much less. HSE want the social and ethical debate to occur because at the end of the day we deliver the level of safety that the public want – if we don't then we lose our licence to operate.

To get real public debate and engagement, regulators (we in HSE) must be honest and clear about what we know and what we don't and industry must be open and trust the public with a real dialogue. We must all remember that the public may wish to talk about different things and have their own agendas, these must be addressed. If you do not engage on these terms they will walk away and form their opinions in isolation.

So for all new technologies not just the nanotechnologies government and regulators must now hope to 'get in' early to catalyse the activity to fill the gaps in knowledge.

The public must always be engaged and their views identified. Industry should always consider if they are just selling their new technology or if they have identified the needs of the public and are fulfilling them. Industry and the public must both be fully involved. If communication breaks down and positions become polarised, no one wins and we ,at HSE as a regulator, find operating in such an environment much more difficult. Ultimately those creating and controlling any new technologies must always remember they have the responsibility to identify and control the risks.

If regulators, industry and the public get it wrong we could see:

- controls that are too lax, significant health effects harming many people, the history of asbestos should warn all of society of the human and financial costs of this possibility.
- controls that are too stringent and the nanotechnologies never be develop and society does not gain the potential benefits.
- duplication of effort diverting research resources from where they are needed; such wasted resources will slow development of controls and benefits. a polarised debate leading to a lack to effective communication and loss of public trust.

If regulators, industry and the public get it right we could see a bright future with:

- new benefits to society from new medicines, better safety devices etc.
- joined up research programmes leading to limited research monies being used as efficiently as possible.
- a growth in public confidence in science and industry.
- proactive control preventing unnecessary human suffering.
- controls at the design stage, leading to intrinsically safer designs and also saving industry money.

In conclusion, HSE is committed to playing its part of responsible regulator. The discussions at this symposium will help identify the gaps in everyone's knowledge and start to address how they should be filled. It is hoped that the presentations and the workshop sessions will further the debate, build cooperation, frame the scientific questions and suggest routes to the solutions. The results will be made freely available to help inform the future direction for research, policy development and the start of the dialogue with the public.

3.1.2 Nanotechnology in the United States –Research and Development and Risk Governance.

Dr. M.C. Roco,
Chair, U.S. National Science and Technology Council (NSTC)'s Subcommittee on Nanoscale Science, Engineering and Technology (NSET); Senior Advisor for Nanotechnology, National Science Foundation (NSF), 4201 Wilson Blvd., Arlington, VA 22230, mroco@nsf.gov; www.nano.gov and www.nsf.gov/nano

ABSTRACT

Nanotechnology research and development (R&D) is expanding rapidly—which makes it all the more critical that we strike a proper balance between the promised benefits and the necessary measures to mitigate and prepare for possible undesirable secondary effects. Industry, specialized government agencies, civic organizations and the public already have recognized the need to address the immediate issues related to environmental, health and safety (EHS) implications. The longer term issues of responsible development of nanotechnology are at least as important for their social and economic outcomes, and are expected to affect essential aspects of human activity. Nanotechnology implications on human welfare, cognition, and sustainable growth require a longer time to be recognized, and need to be understood and addressed by governments and civic organizations. Federal government investments and administrative measures in the area of EHS in the United States are outlined for illustration, including aspects of risk governance. Since collaboration with industry is an indispensable component of the National Nanotechnology Initiative (NNI), this paper also outlines the current interaction in the areas of EHS with various industry sectors, including the electronic, chemical, and organization and business sectors.

Keywords: nanotechnology, societal implications, environment, health, human development, international

INTRODUCTION

Human potential and technological development are coevolving, and quality of life has increased tremendously because of technological advancements. However, technological progress raises societal concerns. Such concerns seem to have particular resonance when it comes to nanotechnology—not the least because nanotechnology allows us to work at the very foundation of matter, the first level of organization for both living and manmade systems. The potential benefits are large—and so are the potential transforming tools and risks that need to be addressed. For this reason, societal aspects need to be fully considered from the beginning by humankind as a whole. In the United States we have taken a proactive approach to this challenge. The National Science Foundation (NSF) has prepared a report on societal implications in 2000 (1, 2), and called for research and education proposals in the same year (the first program solicitation NSF 01-157 was published in August 2000). More recent National Nanotechnology Initiative (NNI) reports on environment (3), nanobiotechnology (4) and societal implications (5) have

been issued in 2004. In several previous papers (6-8) unexpected consequences of nanotechnology and public attitudes were outlined. In 2003 and 2004, relevant reports were published by VDI in Germany (9), EC (2004) (10), Royal Society in UK (2004) (11), Green Peace (12), ETC Group (13), the insurance industry (Swiss RE) (14), and Meridian Institute in conjunction with NSF (15).

IMMEDIATE AND CONTINUING ISSUES

The immediate and continuing issues need to be addressed concurrently with the development of nanoscale R&D projects and the creation of nanotechnology products. They may be separated into three groups:

a). Environmental, Health and Safety (EHS) knowledge and measures specific to nanotechnology in both research and industrial units.

b). cross-sectors and internationally accepted nomenclatures, norms, standards and regulations for the development of science, engineering, technology and new markets.

c). management of risk analysis for the private sector and government.

KEY ISSUES IN THE LONG TERM

Long-term issues for responsible development of nanotechnology are related to its broader social and economic outcomes, require longer time intervals to be recognized and changed, and must be on the radar of the governments and civic organizations that work to ensure an equitable and responsible growth. Those issues include:

A). Respect of human nature, dignity and physical integrity. The harmonious co-evolution of human potential and new technology based on nanoscale control is a main goal (16, 17). Human right to welfare (quality of life, long term health and safety issues) and access to knowledge must be respected. Several groups call for cultural changes and a “code of conduct”.

B). Balanced and equitable R&D nanotechnology investment in society. The investments must be done in such a way that the benefits and secondary consequences are properly distributed in society, including for opportunities for education and training and development of knowledge needed to address EHS.

C). Human health and environment protection and improvement. This includes:
- Approaches and criteria for sustainable development of technology, energy supply and transportation, including life-cycle analysis of products, materials flow analysis, clean-up techniques on new principles, weather implications, and other global effects. Examples are environmental benign manufacturing methods.
- Control and mitigation of industrial by-products and natural nanomaterials. Examples of such nanomaterials include by-products from combustion engines, furnaces and welding, as well as natural nanoparticles in sand storms and forest

fires.
- Providing clean water, air and food, including R&D and production facilities. Examples include nanoparticle monitoring and filtration in drinking water, air, and food (3)
- Development of a system to monitor and label the nanostructures that may move from benign to toxic because of nanostructuring (surfaces, materials, nanoparticles, etc.);

D). Economic, legal, ethical, moral, and other social aspects to adjust - and when possible - anticipate socio-economic changes caused by nanotechnology. The necessary knowledge should be developed through research, creation of databases, and dissemination, including two-way interaction with the public and various interested organizations. Adaptive/corrective approaches in government organizations for long-term risk governance need to be developed in complex societal systems.

NANOTECHNOLOGY R&D PROGRAMS IN UNITED STATES

The National Nanotechnology Initiative (NNI) is a long-term research and development program that currently coordinates 21 federal departments and agencies. The investment is about $960 million in the fiscal year 2004 (ending on September 30, 2004). The NNI was established in October 2000, and has been coordinated by the Nanoscale Science, Engineering and Technology (NSET) Subcommittee of the National Science and Technology Council (NSTC). The main goals of NNI are to extend the frontiers of nanoscale science and engineering through R&D support; facilitate the development of beneficial applications of nanotechnology; to establish a balanced and flexible infrastructure, including a skilled workforce; and to address the societal implications of nanotechnology. The annual implementation plan for fiscal year (FY) 2004 is balanced between fundamental research, nine Grand Challenges, centers of excellence and networks, infrastructure, and societal and educational implications of nanotechnology. About 65 % of NNI funds are invested to academic research, 25 % to research laboratories, and 10 % to industry (about 7 % to small businesses and 3 % to other investments). Industry investment in long-term R&D is estimated to be equal to the Federal government funding in FY 2004. In addition, state, local, and private organizations have regional nanotechnology investments in infrastructure and education, as well as support for business. Their contribution is estimated in fiscal year 2004 to be about half of the federal investment in NNI.

The NNI definition of nanotechnology includes - besides the size range between about 1 and 100 nm - three other conditions: exploiting specific phenomena and functions that do not extrapolate outside of the nanoscale domain; ability to measure, control and change the structure at the nanoscale for a given purpose; and ability to integrate the nanostructures with larger structures while maintaining the properties at the nanoscale. This definition encourages new developments in the field that would have not been possible without new tools and understanding. The interagency nanotechnology group established in November 1996 organized an international benchmarking in 1997-1999. The result of that activity was the estimation that the worldwide marked for products with

nanotechnology components will reach $1 trillion by 2015. I had the opportunity to formally propose the NNI at the White House's Office of Science and Technology Policy on behalf of the interagency group in March 1999. NNI was funded by Congress beginning with fiscal year 2001. The "21st Century Nanotechnology R&D Act" for (FY) 2005-2008 passed Congress in December 2003. The worldwide nanotechnology R&D investment made by government organizations has increased approximately eight times in the last seven years between 1997 and 2004, exceeding $3.6 billion. About 40 countries have initiated national activities in this field, partially inspired and stimulated by the National Nanotechnology Initiative.

The key NNI activities for environmental, health and other societal implications may be separated into three groups.

a). Balanced R&D Investment aligned with societal implications

About 10 percent of the NNI budget in FY 2003 and FY 2004 supports interdisciplinary projects that relate to environment (in various areas of basic research, implications and applications), health (basic research and implications). The R&D efforts are funded by several agencies, including NSF (about $41 million in FY 2004), NIH (about $33 million), EPA (about 5 million), DOE, NIOSH (National Institute for Occupational Safety and Health), USDA, and DOD. NSF has a focus on nanoscale processes in the environment and on societal implications in its programs since August 2000. NSF has awarded about $16 million in 2004 for grants with primary focus on the environment and nanotechnology, and additionally about $20 million for multidisciplinary projects including related environmental issues, and about $4 million for fundamental biomedical aspects. A list of 100 environment-related grants made in 2001-2003 is available on www.nsf.gov/home/crssprgm/nano/nni01_03_env.htm .

NSF has identified "Nanoscale Processes in the Environment" and "Societal and Educational Implications of Nanotechnology" as two of the main research and education themes of its program since July 2000 (annual program solicitations NSF 00-119, 01-157, 02-148, 03-043, 04-043). Table 1 shows examples of interdisciplinary group awards made by NSF with a focus on basic aspects such as transport of nanoparticles in soil, water and air. EPA has annual program announcements with a focus on nanotechnology and the environment since 2002, from where 22 awards (with a focus on applications) were made in fiscal year 2003 and about 12 (with a focus on nanoparticle implications on health) in 2004. A joint EPA-NSF-NIOSH program announcement on health implications of nanomaterials is under competition in fiscal year 2005. DOE has included nanoscience in the environmental research performed at several National Laboratories such as Oak Ridge in Tennessee and Environmental Molecular Laboratory in Washington State. SBIR/STTR awards related to nanoscale processes in the environment have made by NSF and DOD since 1999 when nanotechnology was specifically targeted in the annual program announcements. EPA supported an SBIR solicitation on "Nanomaterials and Clean Technology" in 2004. FDA, EPA and other regulatory agencies are following very closely the research results.

The support for social, ethical, and economic implications is an area of growing interest. NSF supported projects of about $4 million in fiscal year 2004. Information on two grants of over $1 million each with a focus on the interaction with the public and the creation of databases is available on http://www.nsf.gov/od/lpa/news/03/pr0389.htm. All 14 of NSF's Nanoscale Science and Engineering Centers (NSEC) and the National Nanotechnology Infrastructure Network (NNIN) are required to have research and education components addressing the environmental and societal implications. In August 2004, NSF announced competition for a $2.6 million/year NSEC "Center for Nanotechnology in Society" (program announcement NSF 04-043). The total NSF investment for educational implications including contributions for student assistantships is about $37 million.

Table 1. NSF environmental interdisciplinary groups with research and education at the nanoscale

Centers and interdisciplinary groups	**Institution**
Fundamental Studies of Nanoparticles Formation in Air Pollution	*Worcester Polytechnic Institute*
Center for Advanced Materials for Water Purification	*University of Illinois at Urbana*
Center for Environmentally Responsible Solvents and Processes	*University of North Carolina at Chapel Hill*
Nanoscience in Biological and Environmental Engineering (NSF's Nanoscale Science and Engineering Center, NSEC) (estimated 50% in environment)	*Rice University*
Nano-bio with health implications	*University of Pennsylvania*
Environmental Molecular Science Institute	*University of Notre Dame*
Institute of Molecular	*University of Washington*
NIRT (Nanoscale Interdisciplinary Research Team): Investigating Nano-carbon Particles in the Atmosphere: Formation and Transformation	*University of Utah*
NIRT: Nanoscale Processes in the Environment – Atmospheric Nanoparticles	*Harvard University*
NIRT: Nanoscale Sensing Device for Measuring the Supply of Iron to Phytoplankton in Marine Systems	*University of Maine*
NIRT: Combustion-generated Nanoparticles: The role of Transition Metals in Nanoparticles and Pollutant Formation	*Louisiana St. University*
NIRT: Nanoparticle-environment Interfaces: Interactions in Natural Systems	*University of Michigan*
NIRT: The Role of Nanoscale and Molecular Structures in Dictating Environmental Reactivity	*University of Alaska, Fairbanks*
NIRT: Response of Aquatic and Terrestrial Microorganisms to Carbon-based Manufactured Nanoparticles	*Purdue University*
NIRT: Social and Ethical research and Education in Agrifood Nanotechnology	*Michigan State University*

In FY 2004, the NNI annual investment in multidisciplinary research with educational and societal contributions is estimated at about $45 million (of which NSF awards about $35 million including contributions to student fellowships), and in nanoscale research with relevance to environment and health (EHS, without research dedicated to health applications) at about $95 million.

b). Evaluate and implement regulatory standards

Several federal agencies have focused efforts to study the potential risks of exposure to nanomaterials, including the National Toxicology Program (NTP) under the lead of NIH, the National Institute for Occupational Safety and Health (NIOSH), the Environmental Protection Agency (EPA), and Department of Defense (DOD). The NTP, a multiagency effort established in the Department of Health and Human Services, will focus its studies on the potential toxicity of nanomaterials, beginning with titanium dioxide, several types of quantum dots, and fullerenes. The first studies will be of the distribution and uptake by the skin of titanium dioxide, fullerenes and quantum dots. The NTP is also considering conducting inhalation studies of fullerenes, and is exploring ways to assist NIOSH in the development of inhalation exposure capability for carbon nanotubes. The NTP has about $0.5 million in FY 2004 and plans to ramp up to $5 million in FY 2008.

The NIOSH provides research, information, education and training in the field of occupational safety and health. In 2004, NIOSH initiated several research projects focusing on nanotechnology, including a five-year program to assess the toxicity of ultrafine and nanoparticles. NIOSH has an investment of $1.7 million in FY 2004 and the plans are for $2.3 million in FY 2005.

The EPA is funding research at universities to examine the toxicity of manufactured nanomaterials such as quantum dots, carbon nanotubes, and titanium dioxide. The agency is also providing information on the effects of nanoparticles on human health through its current and past work in ultrafine particulates, which has been carried out at the EPA's own labs and funded through its extramural program. DOD has announced a $1 million per year center for five years on toxicological studies at the University of Rochester starting this year (2004-).

NSF supports three Nanoscale Science and Engineering Centers that support toxicology studies from various perspectives: the center at Rice University (2001-) is focused on dry-wet interface and biological impact of nanostructures released in the environment, the center at the University of Pennsylvania (2004-) is focused on the individual cell response to nanostructures, and the center at Northeastern University (2004-)is focused on safety during nanomanufacturing. Exploratory research on size dependent neural translocation of nanoparticles towards the brain is supported at the University of Rochester, and on reverse engineering cellular pathways from human cells exposed to nanomaterials at the University of Houston.

In addition, scientists funded by the NIH and NSF are studying the chemistry, biology, and physics of nanoscale material interactions within the environment at the molecular

and cellular level, using both in vitro experiments and models. This research is creating a significant body of knowledge of nanoscale materials reactions with biological materials.

c) Coordinated measures for EHS

The NSF's report on "Societal Implications of Nanoscience and Nanotechnology" based on the workshop held in September 2000 (1) has identified that environmental and health issues are important for the development of the field. The report has been revisited in 2004 (5) with a reinforced message about EHS.

NSET/NSTC has established the National Nanotechnology Coordinating Office (NNCO) in January 2001 as its secretariat, with one of its roles to monitor potential unexpected consequences of nanotechnology. The NSTC, through NSET and NNCO, has certain responsibilities pursuant to Public Law 108-153, including reporting to the executive and legislative branch, as well as to the public, on this topic. In this context, the NSET has establish the Nanomaterials Environmental and Health Implications (NEHI) working group in August 2003 to address environment, health and safety (EHS) issues, including risk assessment, identification and prioritization of EHS research needs, and communication of information pertaining the EHS of nanomaterials to researchers and others who handle and use nanomaterials. A "best practices" document will be prepared in 2004 by NIOSH and OSHA for handling and use of nanomaterials by researchers and workers. A list of agencies with regulatory jurisdictions and will be established and made available to the public. The needs for specific tools and methods will be communicated to appropriate agency or agencies to enable risk analysis and regulatory decision-making for nanomaterials. NEHI will support nomenclature activities within the NSET Subcommittee and American Nomenclature and Standards Institute (ANSI).

CONCLUDING REMARKS

The multifaceted co-evolution of technology and society require an increased level of planning and measures as technology is more transforming and changes accelerate. Because nanotechnology may affect the foundation of all manmade things and living systems, special attention is needed for the long-term aspect of human development, an equitable distribution of benefits, and addressing unintended consequences.

The long-term issues related to the human condition and sustainable development are at least as important as those needing immediate and continuing attention, such as toxicity, and both should be addressed from the beginning of nanotechnology R&D programs.

The U.S. NNI has devoted about 10% of its funds for projects with relevance to environment and health basic understanding and implications. Better knowledge about the health effects and transport phenomena of nanostructures in the environment, as well as risk management, need to be developed. Beneficial and potential, real and perceived risks need to be presented in balance to the public. Since nanoscience knowledge and nanotechnology products do not have borders, an international dialogue, exchange of information and eventually coordinated activities are necessary. Because we are entering

a new phase of development of nanotechnology R&D from single new phenomena and nanocomponents to creation of large nanosystems, one may expect that new issues will be raised in the next few years.

ACKNOWLEDGEMENTS

Opinions expressed here are those of the author and do not necessarily reflect the position of U.S. National Science and Technology Council or National Science Foundation.

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3.1.3 Nanotechnology: EU perspective, including research programmes

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WHY IS NANOTECHNOLOGY IMPORTANT?

Nanotechnology is supposed to become the key technology of the 21st century. New products and processes in potentially all sectors make this emerging technology economically and socially promising and disruptive at the same time. Estimates of the market potential go up to optimistic scenarios of one trillion Euro by 2015 (Fig. 1). At the same time, public funding of nanotechnological research is increasing significantly in all world regions (data for 2003: Fig. 2).

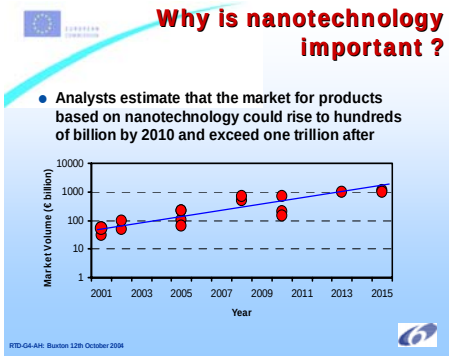

Figure 1: Estimates of market volumes

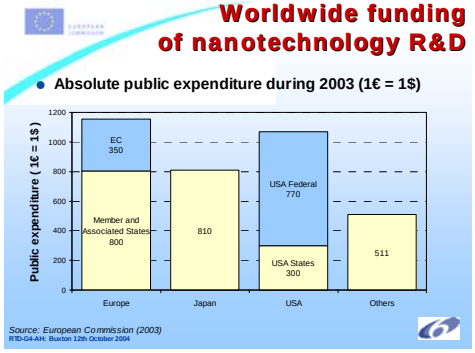

Figure 2: Public funding: world regions 2003

Where do we stand in Europe?

Europe enjoys a strong position in terms of producing knowledge in nanotechnology (e.g. publications). However, Europe is weaker in transforming this knowledge into products and services (e.g. patents, start-ups). Europe already has a commercial deficit for high-tech products of around 23 billion Euro per year and has only few “centres of excellence” on the scale of those being developed in other regions e.g. the USA. The level of private R&D funding from industry is lower in comparison to e.g. the USA and Japan.

EUROPEAN RESEARCH POLICY AND NANOTECHNOLOGY

European Research policy is responding to following objectives of the European Union:

- **Objective "Lisbon":** to become the most dynamic and most competitive knowledge-based economy of the world whereas research is one of the core elements of this strategy
- **Objective "Göteborg":** sustainable development (environment, economy, employment)
- **Objective "Barcelona":** education and training, innovation, "3%" (3% of GDP to be spent for R&D, whereas two third from industry)
- **ERA:** Integrating, reinforcing, structuring an "internal market" for research (Fig. 3).

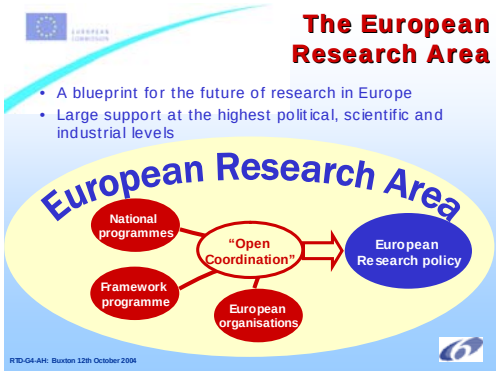

Figure 3: the European Research Area

The European Commission supports a strong portfolio of activities in nanotechnology since 1994, with significant increases in the current Framework Programme (FP6). The funding of projects related to nanotechnology in the **4th Framework programme (1994 - 1998)** amounted to annually 30 million Euro, spent in the Brite-Euram, Esprit, SMT, BioMed and Biotechnology programmes. In the **5th Framework programme (1998 - 2002)** the funding increased to approximately 45 million Euro per year, especially within the Quality of life, Information Society Technologies (IST), Growth, EESD and Improving the human potential (education and training) priorities. Only in the **6th Framework programme (2002 - 2006),** a thematic priority was dedicated to nanotechnology, together with materials and production processes (NMP) and raised to approximately 350 million Euro annually. Besides NMP, the IST, Infrastructures, Marie Curie fellowships as well as the ERA NET activities do finance nanotechnology projects and contribute to this great amount with around 100 million Euro. Furthermore, more than **30 COST Actions** to co-ordinate National Research Activities were financed that are related to nanotechnology (see http://cost.cordis.lu)

FP6: A wide range of differentiated instruments

The 6th Framework Programme (fig. 4) introduced two **New Instruments**, in order to support the integration of European research and to enable industrial participation in promising but risky research projects from the beginning: Networks of excellence (NoE) and Integrated projects (IP). As a "Stairway to excellence".

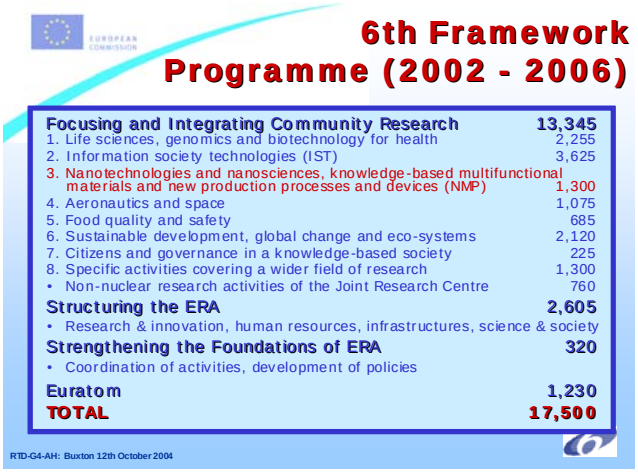

Figure 4: The 6th Framework Programme

Traditional Instruments are the 'classical research projects in form of Specific Targeted Research Projects (STREP), Co-ordination Actions (CA) for a better co-ordination of ongoing research activities as well as Specific Support Actions (SSA) that are supposed to support the Commission's policy with foresight and roadmap projects.

Current open calls are the joint calls with IST and the co-ordinated call with NSF. The IST-NMP-2 calls are focused on Nano-Photonic and Nano-Electronic Devices, Bio-sensors for Diagnosis and Healthcare, and Fast and flexible manufacturing. The NMP-NSF-1 call focuses on Computational materials research.

The Commission will decide be end of October 2004 about the NMP priority **Work programme 2005** (fig. 5), that may devote 370 million Euros to research projects. Possible headlines for nanotechnology research projects are:

Figure 5: The European strategy for nanotechnology

Area 1 (Nano):

- 3.4.1.1-1 Towards "converging" technologies – STREP
- 3.4.1.1-2 Standardisation for nanotechnology – SSA
- 3.4.1.2 Using nature as model for new nanotechnology-based processes – STREP
- 3.4.1.3 Three dimensional nano-structures based on elements other than carbon - STREP
- 3.4.1.5-1 Nanotechnology-based targeted drug delivery – IP
- 3.4.1.5-2 Interaction of engineered nanoparticles with the environment and the living world – STREP
- Area 2 (materials): 1 IP, 5 STREPs, 2 CAs
- Area 3 (production processes): 2 IPs, 1 STREP, 1 CA, 1 SSA
- Area 4 (sectoral applications, SMEs): 3 IPs, 4 IPs for SMEs
- 3.4.4.7 Nanotechnological approaches for improved security systems – IP dedicated to SMEs
- Area 5 (International Cooperation): 2 STREPS, 1 SSA
- 3.4.5.3 Cooperation with Third Countries in the field of nanotechnology, advanced multi-functional materials and new ways of production research – SSA

The indicative timetable of the next (and presumably last) call is as follows:

August 2004	Drafting of work programme
September	Opinion of Programme Committee
November	Adoption by the Commission
December	Launch of 3rd Call
March 2005	First deadlines for Integrated Projects (IPs)
September	deadlines STREPs, CAs, SSAs and IPs' 2nd stages

TOWARDS A EUROPEAN STRATEGY FOR NANOTECHNOLOGY

On 12th May 204, the Commission adopted the European strategy for nanotechnology (COM(204)338). The European strategy follows an integrated and responsible approach by embedding nanotechnological R&D in a wider context (infrastructures, innovation, human resources, society) and by putting an emphasis on the importance of international co-operation and risk assessment (Fig. 5). In the following sections, the three elements Societal issues, Health, safety, environmental and consumer protection as well as International co-operation are mentioned.

Integrating the societal dimension

Due attention should be paid to the integrating societal aspects and Europe should pursue an open and proactive approach to governance in nanotechnology R&D. A dialogue with EU citizens and consumers should be encouraged to promote informed judgement. In addition, the Commission reaffirms its commitment to ethical principles. The responsible and transparent development of nanotechnology is essential for public confidence. In particular it is important is to avoid public opinion making just by science fiction-like but unrealistic visions and fears such as the novels showed in the pictures (Fig 6).

Figure 6: Nanotechnology in Science Fiction novels

Public health, safety, environmental and consumer protection

A high level of public health, safety, environmental and consumer protection requires identification of safety concerns (both real and perceived) and action at the earliest stage, toxicological and ecotoxicological data and evaluation of human/environmental exposure, adjustment, if necessary, of risk assessment procedures for issues of nanotechnology and an integration of risk assessment at all stages of the life cycle of the technology.

Dedicated projects on risk assessment for health and environment funded in FP5 are:

- Nanosafe: Risk assessment in production and use of nanoparticles with development of preventive measures and practice codes
- Nanopathologies: nano-particles in biomaterial-induced pathologies
- Nanoderm: Skin as a barrier to ultra-fine particles

In FP6, other projects are currently in negotiation, dealing with nanosafety and toxicological impact of nanoparticles on human health and the environment.

A Further Step: International Cooperation

An international debate on issues such as public health, safety, environment, consumer protection, risk assessment, metrology, norms will be encouraged. It should promote the monitoring and sharing of information related to the scientific, technological, economical and social development of nanotechnology. The goal is to define an international “code of good conduct” for the responsible development of nanotechnology and to prepare for a possible international agreement on this issue.

Response to the proposed strategy

Estimate that around 10,000 copies of the Commission Communication have been accessed from the Commission's websites. More than 800 persons and organisations have replied to the open consultation on the Communication. It was discussed in the European Council and the proposed integrated and responsible strategy has been welcomed in the Council conclusions adopted on 24 September. Further actions to be taken are to analyse and publish the results from the open consultation (November 2004) and an Action Plan has to be developed by Spring 2005. It is very likely that nanotechnology will also play an important role in the upcoming 7th Framework Programme and is considered by the Commissioner designate for Science and Research, Janez Potocnik as one of the most important activities in research (Fig. 7).

THE NEXT, 7TH FRAMEWORK PROGRAMME (2006-2010)

Figure 7: The Commissioner Designate for science and research supports nanotechnology

The Commission on the future of research (COM(2004) launches the proposal of having six key axes Collaborative research, Human resources, Research infrastructures, Co-ordination of national programmes and newly introduced Technology initiatives as well as Basic research. The expected timetable of FP7 is as follows:

June 2004	Communication on the future of EU research policy
- Oct 2004	Open consultation on the Communication
	http://europa.eu.int/comm/research/future/index_en.html
2004 - onwards	Development work in consultation with stakeholders
	(e.g. research topics, instruments, procedures, management)
	National (and regional) debates on FP7
2005	Decision on Financial Perspectives of the European Union
2005	FP7 proposal, extended Impact Assessment
2006	Decision on FP7

For further information on nanotechnology and the European Research Policy, please check following websites:

- General information on nanotechnology in EC programmes: www.cordis.lu/nanotechnology
- The Commission Communication "Towards a European Strategy for nanotechnology": www.cordis.lu/nanotechnology/src/communication/htm

The views expressed are purely those of the writer and may not in any circumstances be regarded as stating an official position of the European Commission.

4.3.4 The cutting edge of materials

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Abstract not received

3.2 SESSION 2: TOXICITY AND HEALTH EFFECTS

3.2.1 The toxicology of airborne nanoparticles

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AIRBORNE NANOPARTICLES

There is a long history of lung disease epidemics arising from exposure to dust particles in the workplace. Most famous amongst these are silica (Quartz) and silicosis and asbestos and asbestosis/cancer. Nanoparticles and nanotubes represent a new class of materials that may become airborne as particles and so require adequate study in order to assess the risk that they pose. Nanotubes are not discussed in this paper. In fact humans have been exposed to NP for a considerable time since they are generated by combustion processes. However their impact on health has not been addressed until recently in response to the problem of air pollution particles and health. This research has focused attention on the combustion-derived NP produced from fossil fuel, especially from vehicles. The research emanating from this problem has generated considerable information regarding the effects of selected NP (e.g. diesel soot, NP carbon black) on the lungs. The main question is whether the paradigm for the toxicity of 'engineered NP' will differ greatly from the 'combustion-derived NP ' paradigm.

WHAT WE KNOW OF THE TOXICOLOGY OF COMBUSTION-DERIVED NP

Table 1 shows 4 types of combustion-derived NP that cause a range of pathologies in humans or animals.

Nanoparticle type	Origin	Adverse health effects	
		animals	humans
Diesel exhaust particles	Combustion of diesel oil	Inflammation , fibrosis, cancer, adjuvant effects	Inflammation, cancer?
Welding fume	High temperature welding	Inflammation	Metal fume fever, fibrosis, cancer?, bronchitis
Fly-ash	Combustion of coal	Inflammation	?
NP Carbon black	Combustion of heavy fuel oil	Inflammation, lung cancer	?

.

As can be seen these have been studied sufficiently to identify their pathological effects in animals at least and some have effects in humans that can be understood in terms of these experimental effects. Particle toxicologists have studied the mechanisms of these effects and these are described in Figure 1 and some generalities can be drawn as to the mechanism of their effects. Central to their effects if the process of inflammation and oxidative stress can be identified as a dominant mechanism in the production of the inflammation . The pathway to the oxidative stress may differ between particles e.g.

transition metals may dominate (welding fume) or organics (diesel NP) or surface (NP carbon black). The end result however in inflammation and this can be considered to drive pathological effects.

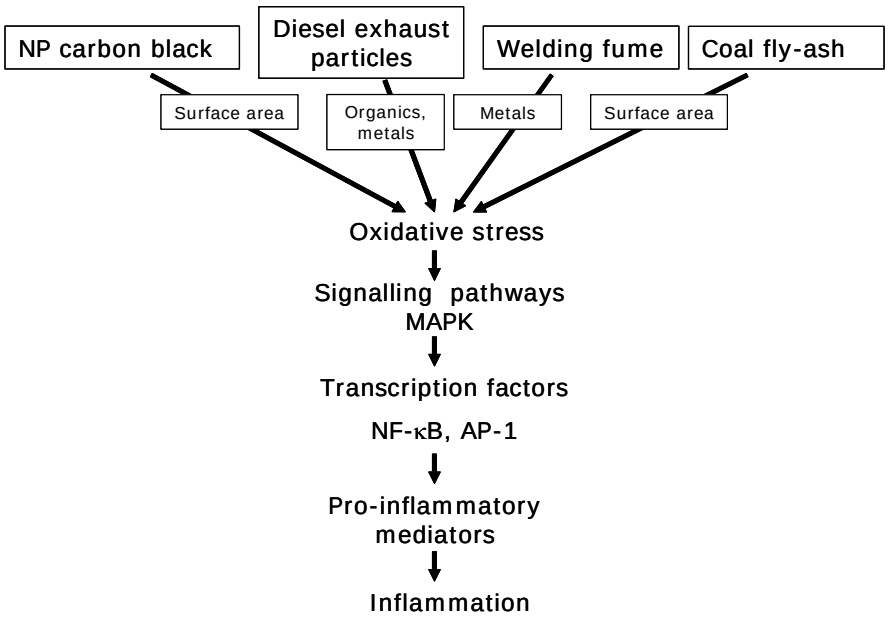

Figure 1 General hypothesis for pro-inflammatory gene expression caused by PCDNP

MECHANISMS OF INFLAMMATION CAUSED BY COMBUSTION-DERIVED NP

Diesel

There is considerable evidence that the 4 NP types shown in Table 1 act in this manner. DEP causes inflammation in rat lungs and in human lungs following short-term high level exposure and DEP display oxidative properties of *in vivo*. DEP causes oxidation of LDL and oxidative stress in exposed epithelial cells. The component responsible for the oxidative stress is mainly the organic fraction, and transition metals; high surface area may also be involved. Activation of signalling pathways have been documented including MAPK and NF-kB activation .

NP carbon black

At low lung burden NPCB showed evidence of mild pro-inflammatory effects whilst larger respirable CB did not . Oxidative stress effects have been measured with NPCB in vitro in cell-free systems and in exposed cells. NPCB oxidative stress may be related to increased influx of extracellular Ca^{++} ions seen with NPCB. Oxidative stress caused by NPCB is translated into activation of NF-κB and IL-8 gene expression in epithelial cells *in vitro* and with activation of AP-1 and TNFα production in macrophages. A recent

study reports that NPCB causes oxidative stress-mediated proliferation of airway epithelium, involving the Epidermal Growth Factor Receptor and the ERK cascade .

Welding fume

Welding fume exposure in humans is associated with inflammatory cytokine increases in the BAL and systemic oxidative stress whilst rats exposed to welding fume show marked pulmonary inflammatory responses. In a comprehensive study of the molecular signalling pathways leading to inflammation with welding fume our laboratory demonstrated that the pro-inflammatory effects of welding fume *in vitro* and *in vivo* were entirely driven by the soluble transition metal component. Epithelial cells treated with welding fume or the soluble transition metals from them showed oxidative stress leading to MAPK dependent (manuscript in preparation) NF-κB and AP-1 activation leading to IL-8 gene transcription

Coal fly-ash

Gilmour et al recently demonstrated that the nanoparticulate fraction of sub-bituminous coal fly-ash was much more potent than any other fraction in causing lung inflammation and cytotoxicity *in vitro*, when compared on a mass basis. This was not obviously linked to enrichment of Fe or any other toxic metals in the ultrafine fraction. It is possible that this effect is driven by a high surface area, as per NP CB. There are no further studies on the ability of this NP fraction of CFA to cause oxidative stress or signal for inflammatory gene expression but such studies are warranted and we would predict that, along with the other PCDNP discussed here, the pathway shown in Figure 2 would be activated, leading to inflammation.

MECHANISM OF TOXICITY OF ENGINEERED NP

Although there are few studies on the new engineered NP, C60 fullerene has recently been shown to cause toxic effects to cells in culture through an oxidative stress mechanism. In our own studies (unpublished) a range of NP produced different levels of oxidative stress in an acellular model of plasmid DNA scission.

NP AND THE CARDIOVASCULAR SYSTEM

Epidemiological studies suggest that inhaled particles (PM_{10}) increases are associated with cardiovascular deaths and hospital admissions in time-series and population studies. Cohort studies have documented an association between elevated particulate and the onset of acute myocardial infarction, increased heart rate and decreased heart rate variability. Human chamber studies delivering concentrated ambient particles (CAPs) have confirmed that particulate can have direct effects on cardiovascular physiology with alterations in heart rate variability and brachial artery diameter.

It is important to note that here is little evidence to support the contention that it is necessarily the (combustion-derived) NP component that drives these effects. However there may be some clues in the fact that the Copenhagen Male Study the influence of

occupational exposure on cardiovascular risk was assessed. In these men, 5 years or more of occupational exposure to welding fumes doubled the risk of myocardial infarction (OR 2.1, 1.05-4.2, P = 0.002), with exposure to solder and plastic fumes conferring similar increases in risk. The ability of NP to gain access to the bloodstream has been demonstrated in animal studies for a range of nanoparticles delivered by inhalation and instillation. Once circulating, particles could interact with the vascular endothelium, or have direct effects on atherosclerotic plaque. Local inflammation could destabilise a coronary plaque, resulting in rupture, thrombosis, and acute coronary syndrome. Furthermore, particles may interact with circulating coagulation factors to promote thrombogenesis. There is, as yet no published data demonstrating that the NP described here gain access to the blood in humans, but the animal studies suggest that this is a plausible hypothesis.

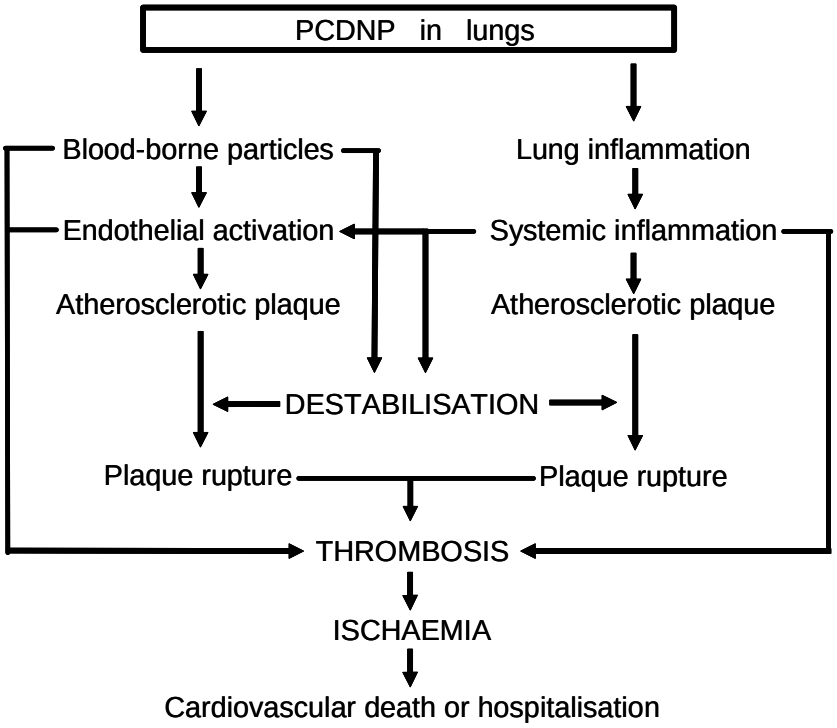

Figure 3. Some of the potential pathways for the action of NP on the cardiovascular system with special emphasis on coronary artery disease.

NANOPARTICLES AND THE BRAIN

Recent work by Oberdorster and colleagues has demonstrated the transfer of radio-labelled nanoparticulate carbon from the nose of rats directly into the olfactory lobes of the brain via the olfactory nerves. If size is the factor that drives these effects then there is some concern that new, engineered NP may have the general property of tropism to the brain. Rats exposed to stainless steel welding fume showed accumulation of manganese in the blood and liver but most importantly in various areas of the brain over 60 days of exposure. Studies of workers exposed to welding fume, however, show clear evidence of

neurological disease and Mn is implicated in these effects. It is not known whether the welding fume particles themselves are transferred to the brain or only the soluble Mn and other metals. However, soluble metals are very rapidly lost from welding fume particles and a soluble salt of Mn was more efficient than an insoluble Mn salt in gaining access to the brain following inhalation exposure in rats. Further work is required to improve our understanding of the factors dictating the transfer of PCDNP and their associated soluble contaminants to the brain.

NANOPARTICLES AND THE LIVER AND SPLEEN

As discussed above, NP of various types are reported to gain access to the blood. In coalworkers, who receive considerable exposure to particles, there are more particles in the spleen and liver than in non-coalworkers. The amount of particulate in the spleen and liver was greater in coalworkers with more severe lung disease, suggesting that inflamed/damaged lungs may be more susceptible to egress of particles into the blood than normal lungs. The normal function of the littoral macrophages of the spleen and liver includes quick removal of any bacteria that gain access to the blood. We may therefore anticipate that NP that gain access to the blood will be taken up by these littoral macrophages in the spleen and the liver. Particles may also reach hepatocytes and other spleen cells with consequences that are presently unknown. In the single study that has so far been published concerning the effects of bloodborne NP on liver function of healthy mice, UFPs induce platelet accumulation in the hepatic microvasculature that is associated with pro-thrombotic changes on the endothelial surface of hepatic microvessels. The accumulation of particles in the liver exerted a strong pro-coagulatory effect but did not trigger an inflammatory reaction. The effects of a particle burden on the spleen are unknown but could include adjuvant effects.

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3.2.2 Inhaled Nano-sized Particles: Potential Effects and Mechanisms

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The ubiquitous occurrence of airborne ultra fine particles results in significant human exposures under environmental and certain occupational conditions. Once deposited, the disposition of these particles appears to be unique. In addition to the classical clearance processes known to exist for fine and coarse particles, experimental animal models suggest that solid ultra fine particles can translocate to extrapulmonary organs. This involves movement across epithelial layers, or *via* sensory nerve endings along neuronal axons to the central nervous system circumventing the tight blood-brain barrier. The likely adverse effects of ultra fine particles will depend on their chemical composition, their bioavailability, and their toxic effects on mucosal and neuronal cells as well as other tissue sites they enter from the general circulation. The likely health impact of ultra fine particles include alveolar inflammation, the blood coagulation pathway, and cardiovascular function. Modifying factors for these effects may include age, preexisting disease susceptibility and other co-pollutants.

Several epidemiological studies have found associations between exposure to ambient ultra fine particles (ultra fine particles) and adverse respiratory and cardiovascular effects. This results in morbidity and mortality amongst susceptible parts of the population (Wichmann *et al*., 2000; Peters *et al*., 1997a, b; Penttinen *et al*., 2001; Klot *et al*., 2002; Pekkanen *et al*., 2002) whereas other epidemiological studies have not seen such *associations* (Pekkanen *et al*., 1997; Tiittanen *et al*., 1999). The effect of laboratory generated ultra fine particles has been studied in controlled clinical studies and has shown high deposition efficiencies in the whole respiratory tract accompanied by cardiovascular defects (Anderson *et al*., 1990; Daigle *et al*., 2003; Brown, *et al*., 2002; Kim and Jaques, 2000; Pietropaoli *et al*., 2004). Studies in animals using laboratory generated surrogate ultra fine particles (or ambient ultra fine particles) showed consistent mild but significant pulmonary inflammation and effects in the extra pulmonary organs. These animal studies reported localised lung inflammation and pathological changes, increased blood coagulation, and translocation of the particles to extra pulmonary tissues (Nemmar *et al*., 2002a, b; Elder *et al*., 2000, 2002, 2004; Zhou *et al*., 2003; Oberdörster *et al*., 2000; Kreyling *et al*., 2002). Tissue culture studies have shown to varying degrees oxidative stress as the major cellular response to laboratory or ambient ultra fine particles (Li *et al*., 2003; Brown *et al*., 2001; Donaldson *et al*., 2002; Finkelstein *et al*., 2003).

This review will summarise evidence regarding the deposition, clearance, and translocation mechanisms of ultra fine particles across mucosal and neuronal tissues. The weight of experimental data is currently based upon animal and tissue culture models but the potential implications for human toxicology will be discussed.

The Concentration Of Inhaled Particulate Matter (PM)
This can vary by orders of magnitude (from ng/m^3 to mg/m^3) depending on the source material, particle size, and proximity to the source. Particle number concentrations (expressed as $particles/cm^3$) can also span orders of magnitude with ultra fine particles (<100 nm) accounting for the highest number yet lowest mass concentration. The nose is a very efficient filter for large >2µm particles (that impact) and smaller ultra fine <5nm particles (that diffuse). In the respiratory tract overall there is minimal deposition at 0.2–0.5µm where settling and displacement by diffusion is minimal. Whilst the chemical composition does not influence deposition (assuming no hygroscopicity), the disposition of deposited particles is highly dependent on their chemical characteristics. Particulate matter is a chemically complex mixture and different

mechanisms of dissolution, leaching, chemical binding and mechanical transport are involved in particle clearance. The classical pathways for clearance of poorly soluble solid particles include the mucociliary escalator (in the nasal and tracheobronchial airway), alveolar macrophages, and the interstitial lymphatic transport to regional lymph nodes. For poorly soluble ultra fine particles two additional pathways have recently been recognized, transit to the extrapulmonary organs via blood circulation and transport along axons of sensory nerves to the central nervous system. These additional pathways provide a plausible mechanism for adverse cardiovascular and central nervous system health effects of ultra fine particles.

Deposition in the Respiratory Tract

The main mechanism of deposition of inhaled ultra fine particles in the respiratory tract is by diffusion due to displacement when colliding with air molecules. The predictive ICRP model (1994) gives the fractional deposition of inhaled particles in the nasopharyngeal, tracheobronchial and alveolar region of the human respiratory tract under conditions of resting nasal breathing. For ultra fine particles (1-100 nm) different amounts of a given size of these particles are deposited along the respiratory tract. For example, 90 % of inhaled 1nm particles are deposited in the nasopharyngeal compartment, ~10 % in the tracheobronchial region, and essentially none in the alveolar region. 5nm particles show about equal deposition with ~30 % in all three regions. In contrast, 20nm particles deposit most efficiently in the alveolar region (~50 %) but with an ~15 % efficiency in the tracheobronchial and nasopharyngeal regions. These different deposition efficiencies should have consequences for the health effects of inhaled ultra fine particles of different sizes and for their disposition to extrapulmonary organs. The ICRP model predictions are based on experimental evidence of well-conducted human studies for particles >0.1 µm for the three regions of the respiratory tract (for review see EPA, 1996). Experimental data for nasal deposition of ultra fine particles in humans have been published (Swift *et al*., 1992; Cheng *et al*., 1996) and served as a basis for the ICRP model. Data on deposition of ultra fine particles in the human tracheobronchial and alveolar region of the respiratory tract (Kim and Jaques, 2000) are available as well as deposition data for the total respiratory tract (Schiller *et al*., 1988; Jaques and Kim, 2000; Daigle *et al*., 2003).

A high deposition fraction in one region of the respiratory tract does not necessarily equate to a high dose delivered to individual cells lining that region of the tract since there are large differences in the epithelial surface area between these regions. Inhaled 20nm particles have their highest deposition efficiency in the alveolar region and modeling predicts the greatest mass of particles deposited beyond generation 16 of the tracheobronchial region (Fig. 1a). However, the epithelial surface areas in tracheobronchial and alveolar regions differ greatly. Expressing the deposition data normalized per unit surface area (Figure 1b) shows that the upper generations of the tracheobronchial region receive the highest doses per unit surface area. During inhalation hot spots of deposition can also occur at bifurcations in the airway tracts (Balaschazy *et al*., 1999, 2003) where groups of ~100 cells ($10mm^2$) on the Carinal ridges increase the focal concentration of deposited particles by ~1-2 orders of magnitude.

The MPPD model (CIIT/RIVM, 2002) has been used to study the deposition of inhaled poly-dispersed ultra fine (20nm) and fine (250nm) particles in the human respiratory tract (Figure 2). In this study, an inhaled concentration of 100 $\mu g/m^3$ over a 6-hr exposure period was assumed (with a geometric standard deviation of particle size distribution of 1.7) and the deposited amount (or dose) per unit surface area for all three regions of the respiratory tract was predicted. On a mass basis the ultra fine particle deposition is more than twice in each region of the respiratory tract compared to deposition of 250nm particles. The dose deposited increases from the nasal to the alveolar region for both particle types but in terms of the deposited dose per unit surface area, the picture is reversed. For the ultra fine particles the highest surface area dose is received by the

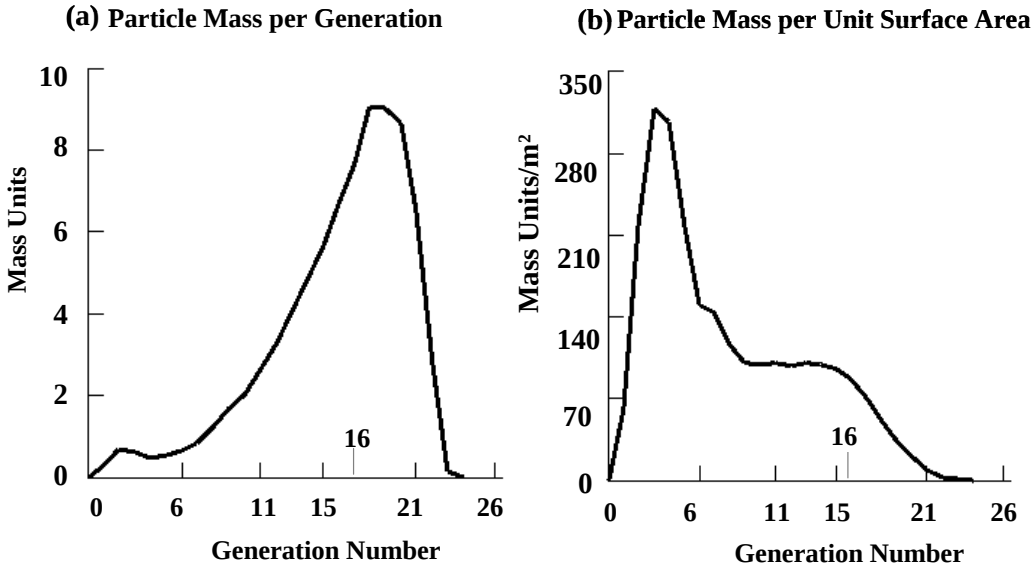

Figure 1: Deposition of inhaled 20 nm particles in human tracheobronchial and alveolar region showing deposited mass per airway generation and per unit surface area of each generation. Generation 1 = trachea; Generation 16 = terminal bronchioles *(predicted by MPPD model, CIIT/RIVM, 2002).*

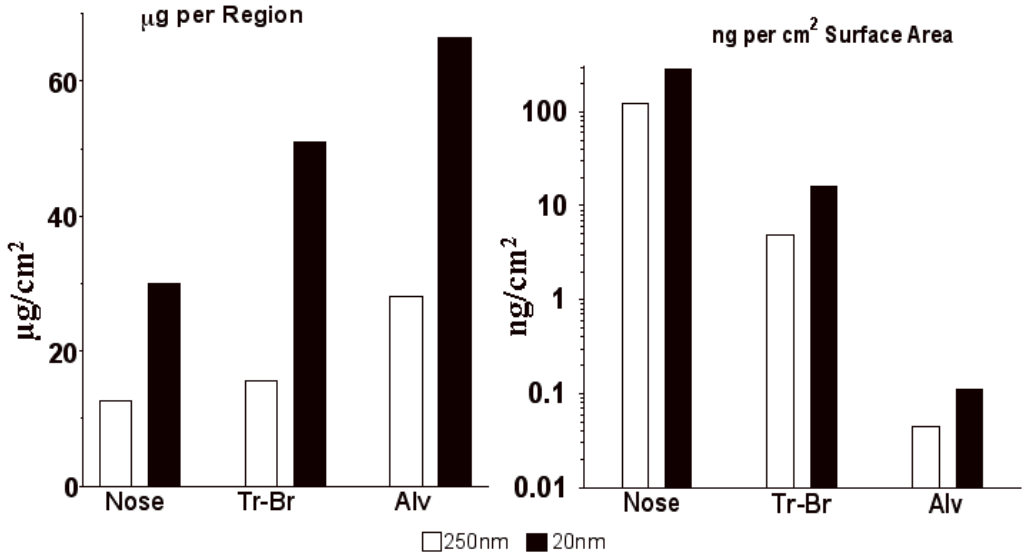

Figure 2: Deposited doses in human naso-pharyngeal, tracheobronchial and alveolar region of aerosol with 20 and 250 nm (CMD) particles (GSD = 1.7) inhaled over 6 hrs at 100 μg/m^3 *(predicted by MPPD model).*

nasal passage and the least by the alveolar region (Figure 2) with 5,000 times higher 20nm particles deposited per unit surface area compared to 250nm particles. This may have significant implications for the translocation of ultra fine particles to extrapulmonary sites.

Respiratory Tract Clearance

Several reviews describe the classic clearance mechanisms for removal of deposited particles (e.g. EPA, 1996; Kreyling and Scheuch, 2000) in the airways but this article will highlight the not so generally well understood mechanisms that appear to be specific for translocation of ultra fine particles across the respiratory tract and into the central nervous system.

The clearance of deposited particles in the respiratory tract basically involves two processes, physical translocation and chemical dissolution (or leaching). The latter mechanism is directed at biosoluble particles, or components of particles that are either lipid soluble or soluble in intracellular and extra cellular fluids. These eventually may be adsorbed and diffuse into cellular spaces or into the blood and lymphatic circulation. The clearance of biosoluble materials can happen at any location within the respiratory tract. In contrast, a number of diverse physical processes for translocation of inhaled particles exist which differ in the three regions of the respiratory tract (see Figure 3) and these show significant particle size-dependent differences, making them either effective, or ineffective, for particles of a given size.

The nasal and tracheobronchial mucosa is supplied with ciliated cells that form an escalator moving mucus towards the oropharynx, resulting in a fast clearance for solid particles within ~24 hours. It operates most likely also for ultra fine particles (Kreyling *et al*., 2002). From the oropharynx, particles are then swallowed into the gastrointestinal tract thus being eliminated from the respiratory tract. A more detailed review of these nasal mechanisms is provided by Kreyling and Scheuch (2000).

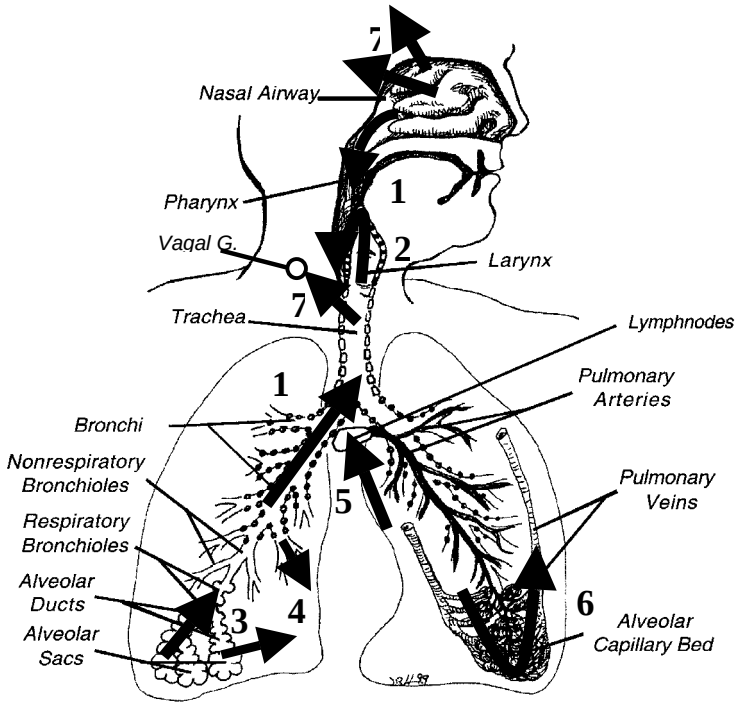

Figure 3: Respiratory tract particle clearance pathways. (1) Mucociliary escalator; (2) GI tract; (3) AM-mediated clearance; (4) Interstitium (*via* epithelium); (5) Lymphat. circulation; (6) Blood circulation; (7) Sensory neurons (olfactory, trigeminus, t-bronchial).

The most common mechanism for clearance of solid particles is via phagocytosis by alveolar macrophages and this process appears to be facilitated by chemotactic attraction (Warheit *et al*.,

1988) . For example activation of complement C5a in serum proteins present in the alveolar surface (Warheit *et al*., 1986; Warheit and Hartsky, 1993) results in chemotactic attraction. Once the particles are internalized by the macrophages, the laden cells move towards the mucociliary escalator. These solid particles have a retention halftime in the alveolar region of ~70 days in rodents and ~700 days in humans. Within 6-12 hours after deposition in the alveoli, essentially all of the particles are phagocytosed but it appears that there are significant particle size-dependent effects on the effectiveness of this process.

Studies in rats exposed to polystyrene beads of different size (with intra tracheal instillation of 40µg or 10µg) showed that twenty-four hours later ~80 % of 0.5, 3 and 10 µm particles could be retrieved within the macrophages by brief lavage of the lung but only ~20 % of ultra fine (15-20nm and 80 nm) particles. After exhaustive lavage, ~80 % of the ultra fine particles were recovered but ~20 % of particles >0.5µm (see Figure 4). This indicated that single ultra fine particles deposited in the alveoli were not efficiently phagocytosed by the macrophages. This was either due to their inability to phagocytose small particles, or the lack of an effective chemotactic gradient at the site of their deposition. The optimal particle size for phagocytosis by alveolar macrophages has been estimated at 1-3µm and particles smaller than this result in a rate of phagocytosis that is progressively slower (Hahn *et al*., 1977; Tabata and Ikada, 1988; Green *et al*., 1998). In contrast, in cell culture alveolar macrophages are able to phagocytose ultra fine particles and consequently are activated (Brown *et al*., 2001; Stone *et al*., 1998; Donaldson *et al*., 2002; Li *et al*., 2003). This suggests that the absence of chemotactic gradients may also play a part in the poor clearance of ultra fine particles by macrophages. Experimental proof to explain the results of the inefficient clearance of ultra fines by alveolar macrophages is still required.

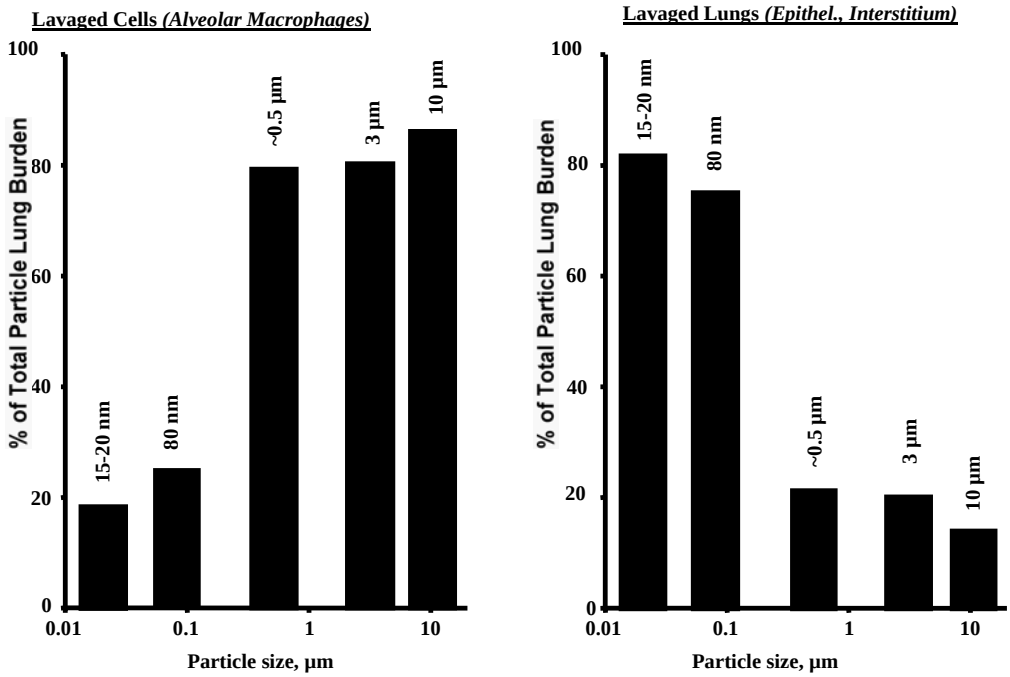

Figure 4: Retention of ultra fine, fine and coarse particles in alveolar macrophages of rats determined 24 hrs post-exposure by exhaustive lung lavage.

Translocation of Ultrafine Particles Across the Respiratory Tract Epithelium.

The inefficient uptake of ultra fine particles could lead to greater interaction of these particles with the epithelial lining of the respiratory tract. Several studies have shown that ultra fine

particles can readily gain cross the epithelium to the interstitial tissue (Kreyling and Scheuch, 2000). For PTFE fumes after a 15-min exposure the fluorine containing particles were found in the interstitial and submucosal tissue of the conducting airways and interstitial tissue close to the pleura (Oberdörster *et al*., 2000). Ultra fine and fine TiO_2 particles (ranging in size from 12 nm to 250 nm) show a strongly particle size-dependent translocation across the epithelium to the interstitial tissue (Oberdörster *et al*., 1992). The administered particle number is three orders of magnitude higher for ultra fines (12 and 20 nm) compared to larger particles (220 and 250nm). This is an important determinant for particle translocation across the alveolar epithelium and for the total dose and rate of delivery (Ferin *et al*., 1992). This translocation of fine particles across the alveolar epithelium is more prominent in large species (dogs, non-human primates) than rodents (Nikula *et al*., 1997; Kreyling and Scheuch, 2000), suggesting that a high rate of translocation is likely to occur in humans.

Once in the interstitium, translocation to regional lymph nodes either as free particles or within macrophages can occur and particles may proceed further into post-nodal lymph and the general circulation. For example, amosite fibers instilled in the right thoracic duct found to enter the post-nodal lymph sites of the neck area and the venous circulation (Oberdörster *et al*., 1988). However, only the short thinner fibers (<500nm diameter) appeared in the post-nodal lymph sites. In non-human primates fine crystalline silica particles were reported to translocate to the liver of exposed monkeys following chronic exposure to high concentrations (Rosenbruch, 1990; Rosenbruch and Krombach, 1992). This suggests that clearance from lymph nodes to the circulation is not restricted to ultra fine particles but the rate of their movement may be dependent upon their. Transcytosis of large particles across the alveoli into the pulmonary interstitium seems to occur only in situations of high load when the capacity of alveolar macrophages to phagocytose these particles is overwhelmed.

Recent studies have shown that rapid translocation of inhaled ultra fine particles into the blood circulation can occur. In humans, inhalation of ^{99m}Tc-labeled ultra fine carbon particles (Technegas®) resulted in the label rapidly appearing in the blood circulation and liver (Nemmar *et al*. (2002a). In contrast, studies in humans with ^{99m}Tc-labeled carbon particles (33 nm) by Brown *et al*. (2002) did not confirm such an uptake and these authors suggested that the findings by Nemmar *et al*. (2002a) were probably representative of soluble pertechnetate rather than labeled ultra fine particles. In rats, ultra fine elemental ^{13}C particles (CMD ~30 nm) accumulated in the liver 24 hours after exposure indicating efficient translocation into the circulation (Oberdörster *et al*., 2002). In contrast, intra tracheal inhalation of ultra fine ^{192}Ir particles resulted in minimal translocation (<1 %) from the lung to extra pulmonary organs although there was a 10 fold greater translocation of small (15 nm) particles versus large (80 nm) particles (Kreyling *et al*, 2002)

Additional studies are needed to determine how particle size and surface chemistry affects this translocation across the alveolar epithelial and endothelial barriers. It has been suggested that Caveolae in alveolar epithelial and endothelial cell membranes support this transcytosis (Oberdörster and Utell, 2002). During the inspiratory and expiratory cycle of the alveolar walls, Caveolae (~40 nm) disappear and reappear and forming vesicular transport pathways for macromolecules across the cells (Patton, 1996).

Translocation of Ultrafine Particles Along Respiratory Neurosensory Cells

Translocation along respiratory neuronal axons may be specific to ultra fine particles (see Figure 3) and could involve nasal and tracheobronchial sensory nerve endings as well as the olfactory and trigeminus nerves. Studies in the early 1940's (Bodian and Howe, 1941; Howe and Bodian, 1941) showed that 30 nm poliovirus particles instilled intranasally in chimpanzees translocated

along olfactory nerve axons into the olfactory bulb. DeLorenzo (1970) provided excellent electron microscopy photographs of colloidal 50nm gold particles translocating along these axons into the olfactory bulb of squirrel monkeys following intranasal instillation. This process appeared to involve retrograde movement along the neural dendrites, and anterograde movement along the olfactory nerve axons to the olfactory bulb. Transport velocities of 30nm for viral particles and 50nm for gold particles were measured at 2.4 and 2.5 mm/hour respectively, with particles appearing in the olfactory bulb within 30-60 mins after nasal inoculation.

Intranasal and intratracheal instillation of rhodamine-labeled microspheres has been used to demonstrate translocation *via* trigeminus nerves in the nasal region (Hunter and Dey, 1999; Hunter *et al*, 1998) and to the trigeminal ganglion at the internal base of the skull and ganglion nodosum of the neck. The use of 20-200nm microspheres to visualize neuronal transport is well documented and was first described by Katz *et al*. (1984). The size and surface properties of these microspheres influences their translocation along axons, with an ~30 nm particle size being optimal (*Katz, personal communication*).

Inhalation of ultra fine elemental ^{13}C particles (35nm) results in significant accumulation in the olfactory bulb of rats (Fig. 5) 7 days post-exposure. A significant increase in ^{13}C accumulation also occurs in the cerebrum and cerebellum but within 2 days post exposure (Oberdörster *et al*., 2004). Preliminary results of another inhalation study using ultra fine (30nm) manganese oxide particles in rats again showed significant increases of Mn in the olfactory bulb and striatum and frontal cortex. These results are consistent with a translocation route operating for inhaled ultra fine particles deposited in the nasal region of rats.

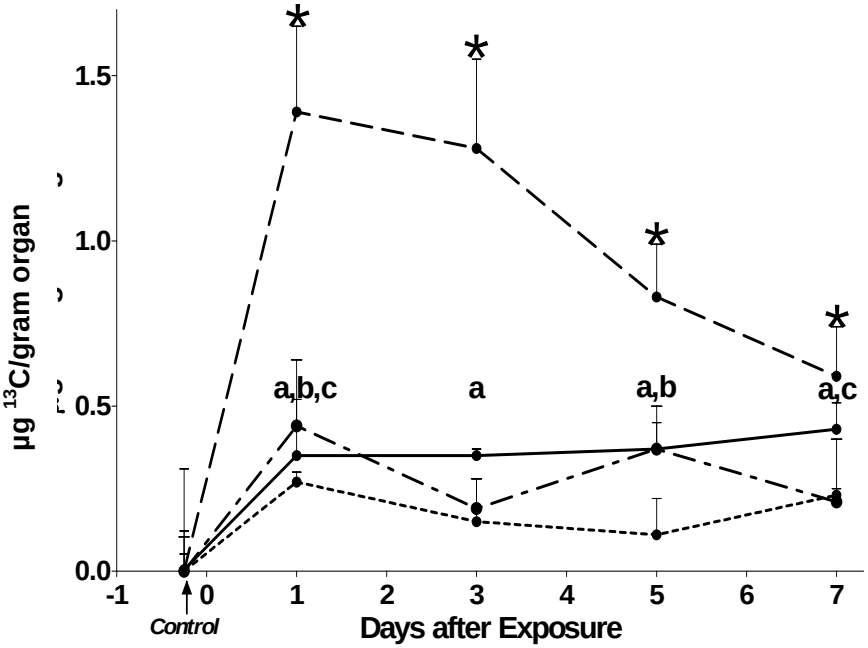

Figure 5: Time course of ^{13}C tissue concentrations in lung, olfactory bulb, cerebrum and cerebellum of rats following a 6-hr inhalation exposure to ultra fine (36 nm CMD) elemental ^{13}C particles (n = 3 rats per timepoint). *- - - = lung; ── = olfactory; - ·- = cerebrum; ···· = cerebellum *and a, b, c indicate values significantly greater than controls, p<0.05 (ANOVA) (*= for lung; a = for olfactory bulb; b =for cerebellum; c = for cerebrum).*

The relevance of these observation and whether these mechanisms of neuronal uptake and transport occur in human need to be addressed. In rodents, numerous studies have demonstrated that inhaled (or intranasally instilled) soluble metal compounds translocate *via* olfactory neurons to the olfactory bulb (Tjälve and Henriksson, 1999; Gianutsos *et al*., 1997; Dorman *et al*., 2002; Brenneman *et al*., 2000). The olfactory mucosa in the human nose comprises only 5 % of the surface compared to 50 % in the rat nose (rats are obligatory nasal breathers). 10 % of the total nasal airflow is directed to the olfactory mucosa in humans (Keyhani *et al*., 1997) compared to 15 % in rats (Kimbell *et al*., 1997). Given the larger surface area of the olfactory tissue in the rat nose, the relative amount deposited per unit surface area is probably lower in rats than humans. The Multiple Path Particle Deposition (MPPD; CIIT/RIVM, 2002) model predicted the deposition of inhaled 20nm particles as 5 times greater per unit nasal surface area in humans compared to rats (i.e. ~60ng/cm^2 for rats vs ~300ng/cm^2 in humans). This was based upon an assumption of a 6-hr exposure at 100 µg/m^3 with normal resting breathing conditions in both species. Assuming an even distribution of particles across the nasal mucosa (which may not be correct) ~480ng would deposit on the rat olfactory mucosa compared to ~1575ng on the human mucosa. This theoretical assessment has been supported by an experimental study in rats [Turetsky *et al*., 2003]). If instead of assuming an even distribution of deposited ultra fine particles one assumes that inhaled 20nm particles deposit on the olfactory mucosa proportionate to the airflow directed to that region, the result changes towards a higher surface area dose in the human olfactory mucosa and a lower one in rats (20ng/cm^2 in rats and 600 ng/cm^2 in humans), a 30-fold difference.

These modeling exercises do not prove that an efficient olfactory translocation of inhaled solid ultra fine particles occurs in humans, but primates studies together with these dosimetric arguments strongly support the existence of this mechanism. Even if the surface loading of the human olfactory mucosa is much less than predicted an exposure over many years (or decades) conceivably could result in significant accumulation in the olfactory bulb. Translocation into deeper brain structures may also occur as has been reported for soluble manganese (Gianutsos *et al*., 1997), but this requires further confirmatory studies.

Other neuronal translocation pathways in the respiratory tract need to be considered and it is as the nasal and oro-pharyngeal mucosa have sensory nerves derived from the maxillary and ophthalmic branches of the trigeminus nerve. These come together at the trigeminal ganglion and continue to the base of the brain. There is also a dense network of sensory nerves in the mucosa of the tracheobronchial region. These may represent alternative translocation pathways for solid ultra fine (and perhaps fine) particles as Hunter and Dey (1998) and Hunter and Undem (1999) have demonstrated in rodents. No evidence is available that these two additional particle translocation routes operate in humans (or non-human primates), and this is an open field for future research. It may be that the reported cardiovascular effects attributed to ambient pollutant particles (Pope, 2000) are in part due to their direct effects upon the autonomic nervous system.

The chemistry of ambient ultra fine particles changes depends on particle size and their source of origin. The smaller ambient ultra fine particles approaching the nanoparticle range consist increasingly of organic compounds (Kittelson, 1998) that are lipid or water soluble. It is conceivable that these, too, could be translocated *via* neurons since neuronal transport of proteins, lipids, and cellular organelles is a well known phenomenon (Grafstein and Forman, 1980). A likely mechanism for neuronal transport of solid ultra fine particles is the axonal and dendritic microtubules (Hirokawa, 1998) whose movement involves the binding proteins of the kinesin superfamily. In the context of potential central nervous system effects of air pollution, including ambient ultra fine particulates a study by Calderon *et al*. (2002) may point to an interesting link: These authors describe significant inflammation of the olfactory mucosa, olfactory bulb, cortical

and subcortical brain structures in dogs in heavily polluted areas of Mexico City. These changes were not seen in dogs from a less polluted control city. Whether direct effects of airborne ultra fine particles are the cause of these effects remains to be determined.

The Significance of Systemic Circulation of Ultrafine Particles from the Respiratory Tract

The translocation of inhaled ultra fine particles into the blood circulation may affect endothelial function by promoting thrombosis. This has been demonstrated in a hamster model where 60nm ultra fine polystyrene particles were injected intravenously or instilled intratracheally (Nemmar et al; 2002b; 2003) Only positive, but not negatively, charged particles increased thrombus formation in a peripheral vein and large (400nm) positively charged particles instilled intratracheally at a dose which induced pulmonary inflammation did not influence thrombus formation. Whilst high doses were used in this study, the results are consistent with ultra fine (but not fine particles) translocating across the lung surface into the circulation and subsequently affecting endothelial cell surface functions (Kato et al., 2003; Oberdörster, 2000)

Translocation into the general blood circulation could help to explain the epidemiological findings of cardiovascular defects associated with exposure to ambient ultra fine particulates as well as results of clinical studies that show vascular responses to inhaled ultra fine elemental carbon particles (Wichmann *et al*., 2000; Pekkanen *et al*., 2002; Pietropaoli *et al*., in press). The evidence in humans for the translocation of inhaled ultra fine particles is ambiguous (see Nemmar *et al*., 2002a and Brown *et al*., 2002). The cardiovascular effects may be caused by a sequence of events starting with particle induced alveolar inflammation that results in acute changes in the coagulation properties of bloody, subsequently leading to cardiovascular effects (Seaton *et al*., 1995).

Conclusions

The ubiquitous occurrence of airborne ultra fine particles results in significant human exposures under environmental and certain occupational conditions. Once deposited, the dispersion of these particles appears to be unique. In addition to the classical clearance processes that remove fine and coarse particles, in animal models solid ultra fine particles can also translocate to extra pulmonary organs across the epithelium and via sensory nerve endings of the upper and lower airways. Translocation of nasally deposited ultra fine particles along nerve axons into the olfactory brain has been well demonstrated in primates and rodents, thereby circumventing the tight blood-brain barrier around central nervous structures. It is very likely that ultra fine particles reach the central nervous system via this neuronal pathway in humans and thus may cause adverse effects depending on their chemical composition and bio availability. However, conclusive proof that this pathway exists in humans is still lacking and there is a need for more research into the potential for airborne ultra fine particles to affect human health. Figure 6 summarizes our working hypothesis of ultra fine particles and their effects upon the respiratory mucosa, the cardio-vascular system and the peripheral and central nervous system. Modifying factors for these events most likely include age, underlying disease and other co-pollutants. Future research addressing these hypotheses requires close collaborations between toxicologists (animal, cellular, molecular), epidemiologists, clinicians (pulmonary, cardiovascular, neurological), and atmospheric scientists**.**

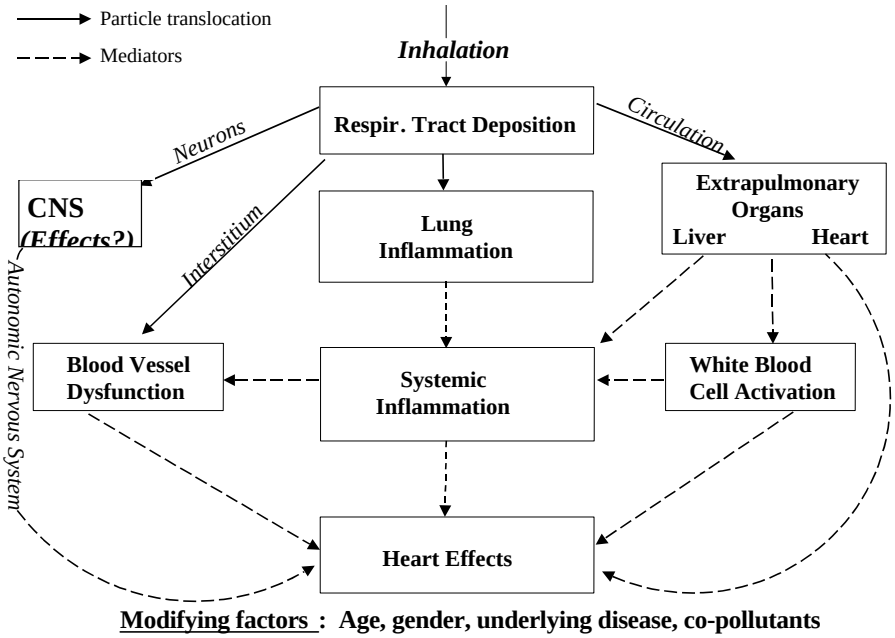

Figure 6: Potential mechanisms of effects of inhaled ultra fine particles.

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3.2.3 Dermal penetration of nanoparticles

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Responsible development of nanotechnology requires evaluation of human and environmental exposure. While significant fine and ultrafine particle literature addresses inhalation exposures, less is known about skin exposure and its biological consequence.

The external layer of the skin, the stratum corneum, is considered a mechanically strong and resilient structure that can withstand physical strain and stress. It is considered an effective barrier essential to the protection of the internal milieu from the external environment. The stratum corneum is composed of several layers of corneocytes with an interdigitating lipid structure that Schatlein and Cevc have described as an intercorneocyte lipid network (Schatzlein and Cevc, 1998). This network extends from the skin surface through the stratum corneum. The epidermis lies under the stratum corneum and contains the keratinocytes and the antigen presenting cells, Langerhans cells. Langerhans cells initiate the cutaneous antigen-specific immune response, and *in vitro* studies have demonstrated their particle phagocytic capacity (Reis y Sousa, 1993). The dermis lies under the epidermis and contains cells that support the inflammatory response. Several reports provide evidence for fine and ultrafine particle translocation through the stratum corneum or into hair follicles. Titanium has been reported in the epidermis following topical application of titanium dioxide-containing sunscreens (Lademann et al., 1999; Tan et al., 1996), although this remains controversial. Additionally, soil microparticles have been identified in the lymph nodes and dermis of individuals who walk barefoot in the African rift valleys and have elephantiasis. In combination, these studies suggest that particles accompanied by an external pressure, such as rubbing or walking, may penetrate the stratum corneum, perhaps through the intercorneocyte lipid network.

To test this possibility, we developed a proof of concept paradigm in which we tested flexing motion, as at the wrist, and translocation of size selected, fluorescent dextran spheres (fluorospheres) through intact stratum corneum. Recovered human skin was applied to a flexing apparatus using a 45 degree flex and 20 flexes per minute. One hundred microliters of a 25% solid solution was applied to the skin over the flexing area for 15, 30 or 60 minutes. Control tissues taken from the same skin sample were handled identically but left flat. Twenty micron thick samples were evaluated by laser fluorescent confocal microscopy with optical images obtained at one micron intervals. To avoid edge effects, only the 5^{th} – 15^{th} optical images taken at the center of the tissue were evaluated.

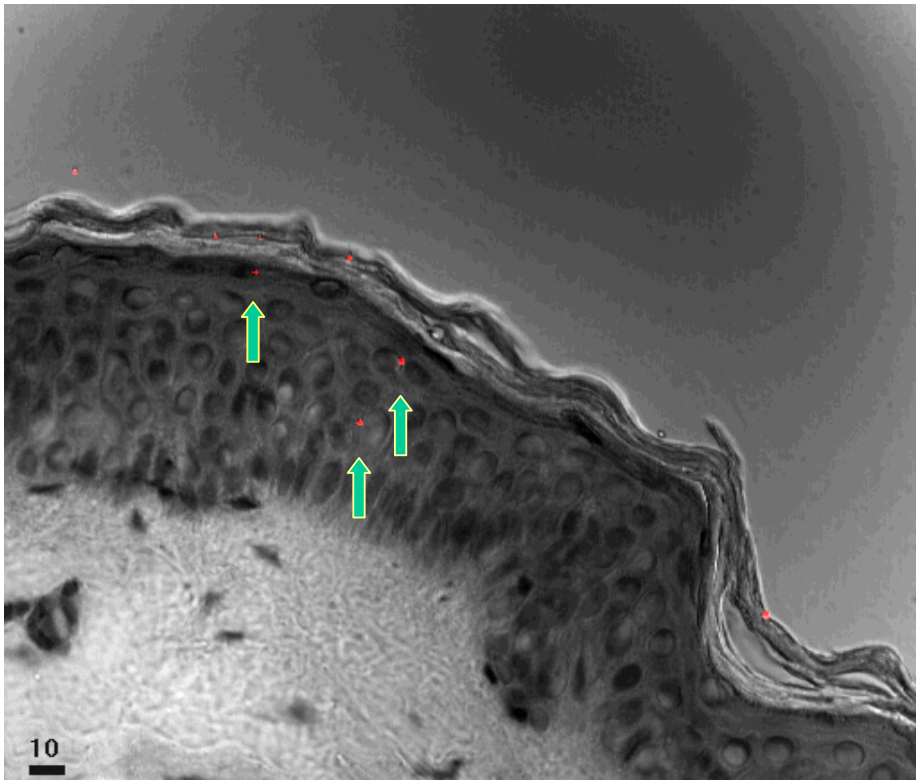

Figure 1. One micron fluorospheres identified in the epidermis at a ten micron depth; tissue flexed for 30 minutes.

One-micrometer red fluorospheres are clearly visible in the stratum corneum and epidermis after the 30-min application of beads and flexing motion (blue arrows, Figure 1), and in the stratum corneum, epidermis, and dermis at 60 min (all arrows, Figure 2). Occasional clusters of fluorospheres were observed in the epidermis and the dermis (purple arrows, Figure 2). Clustering may represent accumulation of fluorospheres at bifurcations in the intercorneocyte network. To test the relationship of particle size to penetration, we also evaluated 0.5-, 2-, and 4-µm spheres. We observed penetration of 0.5-µm beads in addition to the 1-µm beads, and exclusion of 2- and 4-µm beads (Figure 3). A summary of all data in this study revealed a time-dependent increase in particle translocation: 0.5- and 1-µm beads penetrated into the epidermis in 2 of 11 skin samples (18%) flexed for 15 min, in 5 of 12 samples (41%) flexed for 30 min, and in 9 of 16 samples (56%) flexed for 60 min. Penetration into the dermis occurred in two samples after flexing 60 min. The fluorospheres that penetrated through the stratum corneum represent only a small percentage of the applied beads, and the pattern of penetration was random. In contrast to skin samples with intact stratum corneum, discontinuous stratum corneum permitted entry of a bolus of beads directly under the tear. No particle penetration was observed in unflexed tissues at any time point.

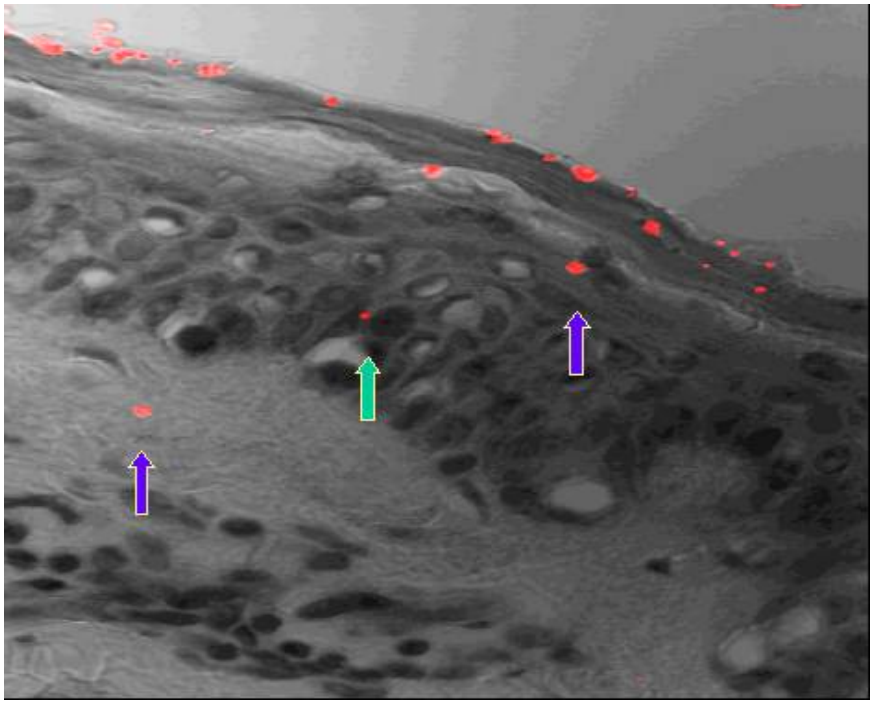

Figure 2. One micron fluorospheres identified in the epidermis and dermis at a ten micron depth; tissue flexed for 60 minutes.

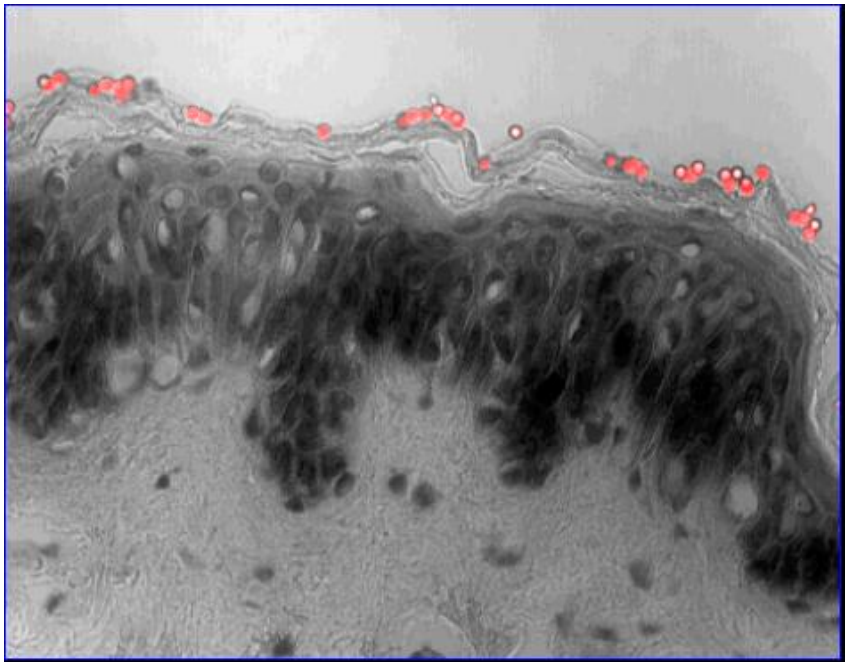

Figure 3. Two and four micron fluorospheres were excluded from entry into the stratum corneum, even with 60 minutes of flexing.

We repeated this study by combining tape stripping of bead-exposed, flexed skin with scanning electron microscopy. Although the technique does not permit the more precise tissue localization possible with the laser scanning confocal procedure, we observed beads in the skin after no tape stripping and tape stripping 10 times and 20 times to an approximate depth of 6–7 μm into the stratum corneum.

We next asked if cutaneous application of a particulate material could initiate a specific immune response in C3H/OuJ mice. For these experiments we used beryllium compounds that cause an immune hypersensitivity response in a subset of workers in the beryllium industry. Beryllium sulphate (BeSO4) solution was used as a positive control and beryllium oxide (BeO), a material with a dissolution half-life of several hundreds of days, as the experimental particulate.

We tested the skin antigen recall response which measures cutaneous antigen-specific immunity as increased ear thickness. We tested the irritant/inflammatory properties of beryllium salts with a single application of this solution on the ear (dibutylphthalate (DBPT)/BeSO4-treated mice) and, compared to mice receiving vehicle on the ear (BeSO4/DBPT-treated mice), we measured no significant increase in ear thickness at any time point (Figure 4). In contrast, sensitization and challenge with beryllium salts (BeSO4/BeSO4-treated mice) increased ear thickness 25–30% at 24 and 48 hr ($p < 0.04$). Mice receiving epicutaneous BeO and challenged once on the ear with the salts (BeO/BeSO4-treated mice) displayed a 30% increase at 24 hr that remained elevated significantly at 48 hr ($p < 0.04$). Because a single application of beryllium to the ear does not increase ear thickness unless preceded by beryllium sensitization on the back, these data illustrate that BeO particles induced sensitization in the BeO/BeSO4-treated mice, analogous to the increase measured for BeSO4/BeSO4-treated mice.

To further confirm a beryllium specific cell-mediated immune response, we tested antigen-specific changes in T cell activation markers. CD44 is a membrane glycoprotein involved in cell adhesion and extravasation and is up-regulated on activated and memory T cells. CD62L, or L-selectin, is expressed on the majority of B and T lymphocytes, and its expression is rapidly lost upon T-cell activation. We determined by flow cytometry that a single application of BeSO4 to the mouse ear did not change the percentage of LN cells expressing CD44/CD62L, however mice treated with BeO on the back before application of BeSO4 on the ear displayed a 10% increase in CD44+ LN cells ($p < 0.006$) and a 20% decrease in CD62L+/CD3+ cells ($p < 0.001$; data not shown).

The data presented in this study demonstrate that, in conjunction with motion, fine particles less than or equal to one micron, are able to penetrate the stratum corneum and lodge in the epidermis, the anatomical location of the cutaneous antigen-presenting cell, the Langerhans cell. Further, we provided evidence for a murine beryllium-specific, cell-mediated immune response following cutaneous application of BeO and BeSO4. These results, coupled with analysis of the surface features of the skin (Figure 5) suggest that skin exposure to nanoparticles, which are at least ten fold smaller in diameter than the particles used in this study, merit further study.

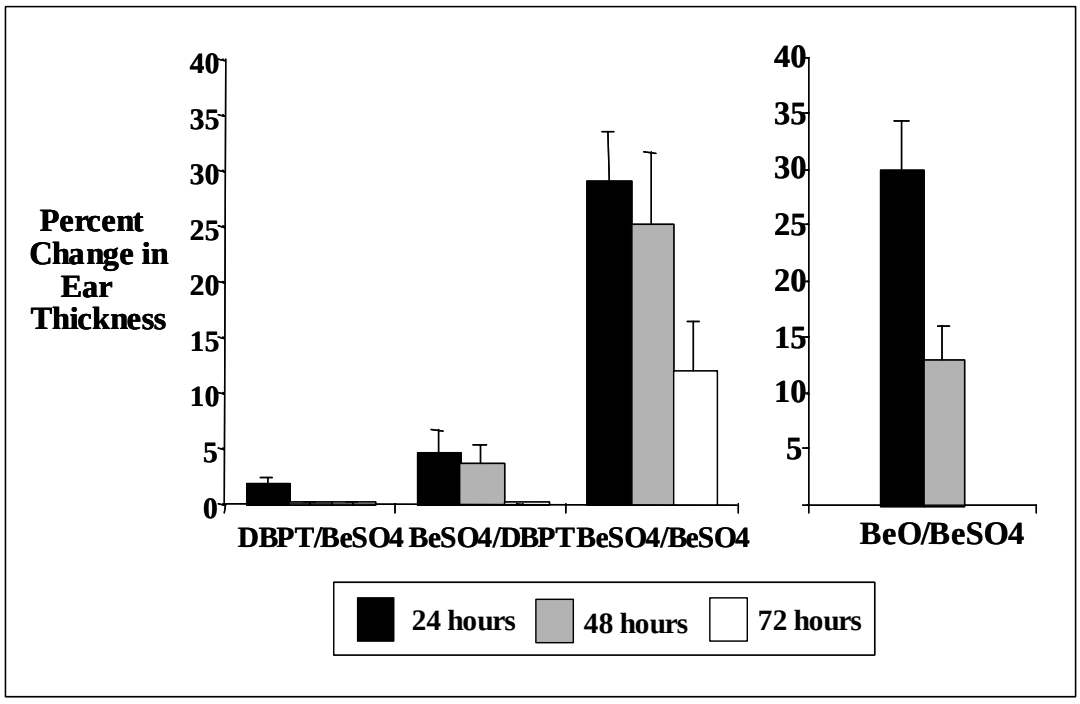

Figure 4. Topical application of beryllium oxide particulate initiated a specific immune response (left panel) that is documented by antigenic recall with beryllium sulphate in C3H/OuJ mice. Beryllium sulphate was used as the positive control (right panel).

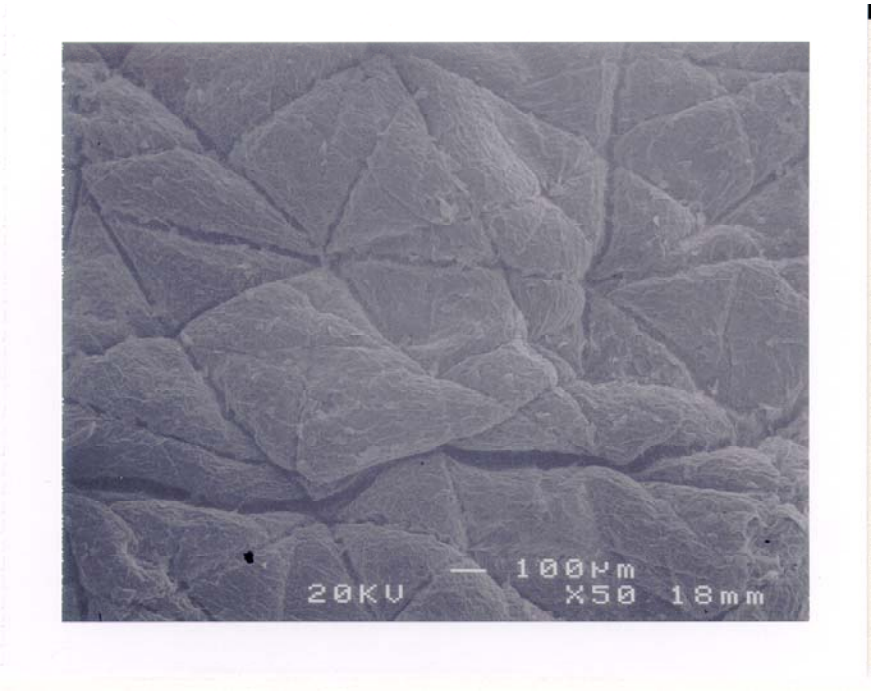

Figure 5. Scanning electron micrograph of the surface of the skin at 50X magnification, demonstrates the surface irregularities that may contribute to particle sequestration.

Experimental details for this study are located at Tinkle et al., (2003) Skin as a Route of Exposure and Sensitization in Chronic Beryllium Disease. Environ Health Perspect 111:1202–1208.

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3.2.4 Epidemiology of Nanoparticles

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Vicky Colvin from the Rice University (Center for Nanoscale Science and Technology Houston, TX, USA) was very surprised to find out that in the field of fast expanding "nanotechnology", there was no prior research regarding the risk to health. Are we really diving into the unknown? It turns out that we already have a fair amount of data on the dangers of airborne particles. First data stem from smog episodes. They were later refined in epidemiological studies that have provided a strong body of evidence that increased cardiovascular and respiratory morbidity and mortality are associated with unexpectedly low levels of ambient particulate air pollution. Also, we know a bit about dangerous dusts at the work place. Dust from mechanical processes usually generates particle sizes of 1 µm or greater. This is the area of classical occupational hygiene. We have plenty of knowledge accumulated about particles that cause diseases like silicosis or asbestosis, but usually, they are a magnitude larger than 'nano'. Hot processes, involving the vaporisation of material and inevitable subsequent cooling like welding, have the potential to generate significant number concentrations of nanoparticles. Epidemiologists use a different terminology: they call particles with diameters less than 100 nm as ultrafine, but it is essentially the same as nanoparticles. Particles we meet in the environment are mostly generated from combustion processes (traffic, heating, industry). The first part of the presentation will focus on the present knowledge that predominantly comes from epidemiological studies that have been conducted in the environmental context. The second part will give a short and by no means comprehensive overview about potential occupational work place hazards.

Smog episodes may not be common any more in Europe or the US, but they are still an enormous problem in other parts of the world. This is the view from the Beijing TV Tower about 350 metres above the ground on a typical sunny day in winter.

It was not a smog day, rather it was considered to be a clear day with a good view to see the mountains. Fine and ultrafine particles in the air create the haze. Formerly it used to be mainly smoke from coal burning, but nowadays it mainly stems from traffic emissions. Currently, Beijing has more than 2 million automobiles. The number of cars has increased at an annual average of 15 per cent in recent years. The average accumulates 47,400 kilometres per year - almost three times the figure for drivers in the United States.

Ultrafine particles are ubiquitous in Europe, even in regions with comparatively low air pollution measured as PM 2.5. Although the net amount of urban particulate air pollution, as expressed in terms of airborne mass concentration PM 2.5, has decreased with reductions in particulate emissions from industry and power stations, the number concentrations of very small particles has increased mainly from traffic emissions. We have been measuring particles in Erfurt for our epidemiological studies since the German unification. The black curve shows the distribution of ultrafine particles in the winter 1991/92. You see an increase of ultrafine particles for the later time periods, whereas larger particles decreased. These changes mainly reflect a decrease of coal burning for heating in winter and an increase of emissions from cars, as you can tell from the different concentrations of ultrafine particles for workdays and weekend.

Over more than a decade, time-series epidemiological studies conducted in many cities from the US and Europe show fairly consistently an increase in mortality of about 1 % per 10 µg increase of PM 10. The bars represent the 95% confidence interval. For PM 2.5 we only have studies from the US, and you see that the increase in mortality is even more pronounced. Cardiovascular disease is responsible for almost 30% of all deaths (men 27%, women 32%) and is the leading cause of death worldwide. Pulmonary diseases contribute about 6% to all deaths in Germany, but probably also in other European countries. This is the reason, why the impact of particulate air pollution is higher on cardiovascular deaths than on respiratory deaths. Let us assume that out of 100 people 6 are dying of respiratory diseases. If their relative risk increases by about 25%, that would be 1.5 more deaths per 100 deaths. The relative risk of dying from cardiovascular disease is increased by about 10 %, this means about three additional deaths, attributable to particulate air pollution.

Epidemiological studies have provided valuable information on the adverse health effects of particulate air pollution in the community, indicating that nanoparticles act as an important environmental risk factor for cardiopulmonary mortality. Particle-induced pulmonary and systemic inflammation, accelerated atherosclerosis, and altered cardiac autonomic function may be part of the patho-physiological pathways, linking particulate air pollution with cardiovascular mortality. Also, it has been shown that particles deposited in the alveoli lead to activation of cytokine production by alveolar macrophages and epithelial cells and to recruitment of inflammatory cells. An increase in plasma viscosity, fibrinogen and C-reactive protein has been observed in samples of randomly selected healthy adults in association with particulate air pollution. The potential mechanism leading to cardiovascular effects is:

- Increased sympathetic activation and /or withdrawal of parasympathetic tone
- Imbalance of sympathetic and parasympathetic control
- Decreased heart rate variability
- Increased risk for cardiac events (alteration of myocardial substrate, increased myocardial vulnerability)

We conducted a case–crossover study in which cases of myocardial infarction were identified with the use of data from the Cooperative Health Research in the Region of Augsburg Myocardial Infarction Registry in Augsburg, in southern Germany, for the period from February 1999 to July 2001. There were 691 subjects for whom the date and time of the myocardial infarction were known who had survived for at least 24 hours after the event, completed the registry's standardized interview, and provided information on factors that may have triggered the myocardial infarction. Data on subjects' activities during the four days preceding the onset of symptoms were collected with the use of patient diaries. An association was found between exposure to traffic and the onset of a myocardial infarction within one hour afterward (odds ratio, 2.92; 95 percent confidence interval, 2.22 to 3.83; $P<0.001$). The time the subjects spent in cars, on public transportation, or on motorcycles or bicycles was consistently linked with an increase in the risk of myocardial infarction. Adjusting for the level of exercise on a bicycle or for getting up in the morning changed the estimated effect of exposure to traffic only slightly (odds ratio for myocardial infarction, 2.73; 95 percent confidence interval, 2.06 to 3.61; $P<0.001$). The subject's use of a car was the most common source of exposure to traffic; nevertheless, there was also an association between time spent on public transportation and the onset of a myocardial infarction one hour later. Transient exposure to traffic may increase the risk of myocardial infarction in susceptible persons. (Peters A. et al., 2004).

The association between fine and ultrafine particles and respiratory health was studied in adults with a history of asthma in Erfurt, Eastern Germany. Twenty-seven non-smoking asthmatics recorded their peak expiratory flow (PEF) and respiratory symptoms daily. The size distribution of ambient particles in the range of 0.01 to 2.5 µm was determined with an aerosol spectrometer during the winter season 1991-1992. Most of the particles (73%) were in the ultrafine fraction (smaller than 0.1 µm in diameter), whereas most of the mass (82%) was attributable to particles in the size range of 0.1 to 0.5 µm. Because these two fractions did not have similar time courses (correlation coefficient $r = 0.51$), a comparison of their health effects was possible. Both fractions were associated with a decrease of PEF and an increase in cough and feeling ill during the day. Health effects of the 5-d mean of the number of ultrafine particles were larger than those of the mass of the fine particles. In addition, the effects of the number of the ultrafine particles on PEF were stronger than those of particulate matter smaller than 10 µm (PM10). Therefore, the present study suggests that the size distribution of ambient particles helps to elucidate the properties of ambient aerosols responsible for health effects. (Peters A. et al., 1997).

The association between particulate air pollution and asthma medication use and symptoms was assessed in a panel study of 53 adult asthmatics in Erfurt, Germany in winter 1996/1997. Number concentrations of ultrafine particles in the size range 0.01-0.1

µm with mean values of 17,300 per cm^{-3}, and mass concentrations of fine particles in the size range 0.01-2.5 µm with mean values of 30.3 µg m^{-3}, were measured concurrently. They were not highly correlated (r=0.45). The associations between ambient particle concentrations and the prevalence of inhaled beta2-agonist, corticosteroid use and asthma symptoms, were analysed separately with logistic regression models, adjusting for trend, temperature, weekend, holidays, and first order autocorrelation of the error. Cumulative exposures over 14 days of ultrafine and fine particles were associated with corticosteroid use. Beta2-agonist use was associated with 5-day mean number concentration (0.01-0.1) and mass concentration (0.01-2.5). The prevalence of asthma symptoms was associated with ambient particle concentrations. The results suggest that reported asthma medication use and symptoms increase in association with particulate air pollution and gaseous pollutants such as nitrogen dioxide (S. von Klot et al., 2002)

We hypothesize that the effect for ultrafine particles will be even stronger than that of PM 2.5 for the following reasons:

- Ultrafine particles are deposited in the alveolar region with high efficiency.
- The large surface of ultrafine particles can increase toxicity.
- Decreased phagocytosis allows enhanced interaction between ultrafine particles and the epithelium
- Ultrafine particles are dislocated from the alveolar space and might therefore elicit systemic effects.

The lungs may be both a target organ and a route of exposure for systemic toxicity. For particulate respiratory hazards, size is a major determinant of penetration into the respiratory tract and deposition in different areas of the system. For practical purposes the respiratory tract is divided into three anatomic and functional regions: (1) the upper airways, including the nasal passages, mouth and oropharynx, (2) the tracheobronchial region including the larynx, main and segmental bronchi, and terminal bronchioles, and (3) the gas-exchange region consisting of the respiratory bronchioles and alveoli. The particle size is a major determinant of its ability to enter the lung and to predict where it will be deposited in specific functional regions of the respiratory tract. Particles with an aerodynamic diameter >10 µm are deposited in the nasal passages, mouth and oropharynx and are rapidly cleared. However, smaller particles - referred to as respirable aerosol – may be deposited in the gas-exchange region, where the clearance via phagocytosis and the lymphatic system is not quite as effective. Deposition in the lung parenchyma may eventually lead to the development of a chronic diffuse interstitial fibro-nodular lung disease, like silicosis and asbestosis, or to lung cancer.

What do we know about adverse health effects of particle exposure at the work place?

Silicosis is one of the commonest occupational lung diseases. It develops over time when dust-containing silica is inhaled into the lungs. Silica in crystalline form is toxic to the lining of the lungs. When they come into contact a strong inflammatory reaction occurs. Over time this inflammation causes the lung tissue to become irreversibly thickened and

scarred - called fibrosis. The crystalline silica is commonly found in sandstone, granite, slate, coal, and pure silica sand so people who work with these materials, as well as foundry workers, potter's, and sandblasters are at risk. Other forms of silica, such as glass, are less of a health risk as they are not as toxic to the lungs. Crystalline silica is considered as carcinogenic to humans (Group 1, IARC monographs Vol. 68; 1997)

Asbestos is a naturally occurring mineral fibre that has been used in more than 3,000 different construction materials and manufactured products. It is commonly found in heating system insulation, decorative spray-on ceiling treatments, vinyl flooring, cement shake siding and a variety of additional materials. Some asbestos-containing materials were still being installed into the late 1980s. All types of asbestos tend to break into very tiny fibres. Because asbestos fibres are so small, once released into the air, they may stay suspended there for hours or even days. Asbestos fibres are also virtually indestructible. They are resistant to chemicals and heat, and they are very stable in the environment. They do not evaporate into air or dissolve in water, and they are not broken down over time. Asbestos belongs to the group 1 carcinogens (IARC monographs Vol. 14, Suppl. 7; 1987). Projections for 1995-2029 suggest that the number of men dying from mesothelioma caused by asbestos in Western Europe will almost double from 5000 in 1998 to about 9000 around 2018, and then decline, with a total of about a quarter of a million deaths over the next 35 years. The highest risk will be suffered by men born around 1945-50, of whom about 1 in 150 will die of mesothelioma.

Carbon black is a powdered form of elemental carbon manufactured by the vapour-phase pyrolysis of hydrocarbon mixtures, such as heavy petroleum distillates and residual oils, coal-tar products, natural gas and acetylene. Worldwide production of carbon black in 1993 was approximately 6 million tonnes. The primary use of carbon black is in rubber products, mainly tyres and other automotive products, but also in many other rubber products such as hoses, gaskets and coated fabrics. Much smaller amounts of carbon black are used in inks and paints, in plastics and in the manufacture of dry-cell batteries. Studies on the pulmonary retention of inhaled carbon blacks in rats and mice have shown that these particles behave very similarly to other low-solubility, low-toxicity particles. Impaired particle clearance due to high loading of carbon black in experiments with rats results in increased accumulation of particles. Subsequent inflammatory responses occur which develop into chronic active inflammation. Increased collagen deposition from proliferating fibroblasts, increased epithelial cell proliferation and metaplasia have been found at high lung burdens of carbon black. It appears that the high specific surface area of most carbon blacks may be an important parameter in the induction of inflammatory and subsequent other responses in the lung. Carbon black is possibly carcinogenic to humans (Group 2B, IARC monographs Vol. 65; 1996).

Depending on their particle size, inhaled ultrafine particles (UFP) are efficiently deposited in nasal, tracheobronchial, and alveolar regions due to diffusion. Oberdörster's previous rat studies have shown that UFP can translocate to interstitial sites in the respiratory tract as well as to extrapulmonary organs such as liver within 4 to 24 h post exposure. There was also a significant and persistent increase in added 13C in the olfactory bulb of 0.35 µg/g on day 1, which increased to 0.43 µg/g by day 7. The increases in olfactory bulbs are consistent with earlier studies in nonhuman primates and rodents that demonstrated that intranasally instilled solid UFP translocate along axons of

the olfactory nerve into the central nervous system. If this route of internal exposure proves to be functional, it may explain manganism and Parkinsons disease with relatively low levels of manganese in the blood of exposed welders. Until the last decade, little attention has been given to the neurometabolism of metals, however, the neurobiology of heavy metals is now of growing interest, since they have been linked to major neurodegenerative diseases. Occupational and environmental exposure to these metals has been suggested as a possible cause of neurodegenerative disorders. Manganese exposure can probably play a role in the determination of Parkinsonian disturbances. There are several differences between manganism and Parkinson's disease, but it is important to consider that manganism is the result of exposure to very high doses of Mn. Prolonged exposure to lower exposure levels of Mn may act differently, and enhance the onset of Parkinsonian disturbances.

Overall, merging the present knowledge gathered from environmental epidemiological studies on the adverse health effects of ultrafine particles and the long standing experience with occupational diseases associated with exposure to respirable dust, it seems fair to warn against a naïve attitude towards nanotechnology. Preventive measures to avoid exposure in the production and handling of nanomaterial are certainly warrantable.

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3.2.5 Pulmonary Bioassay Toxicity Study in Rats with Single Wall Carbon Nanotubes

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The aim of this study was to assess the acute pulmonary toxicity of intratracheally instilled single wall carbon nanotubes (SWCNT) in rats. The lungs of rats were instilled either with 1 or 5 mg/kg of the following control or particle-types: 1) SWCNT; 2) quartz-crystalline silica particles (positive control), 3) carbonyl iron particles (negative control); 4) Phosphate-buffered saline + 1% Tween 80; or 5) graphite particles (lung tissue studies only). Following exposures, the lungs of PBS and particle-exposed rats were assessed using a variety of methods at 24 hrs, 1 week, 1 month and 3 months post-instillation exposure including bronchoalveolar lavage (BAL) fluid biomarkers, cell proliferation methods, and histopathological evaluation of lung tissue.

High dose exposures (5 mg/kg) to SWCNT produced mortality in ~15% of instilled rats within 24 hrs post-instillation exposure. This lethal effect was due to mechanical blockage of the large airways by the instillate, and did not result from inherent lung toxicity per se of the instilled SWCNT particulate.

Results from the bronchoalveolar lavage and cell proliferation studies indicated that pulmonary exposures to quartz particles resulted in the persistent enhancement of pulmonary inflammation, cytotoxicity, and lung cell parenchymal cell proliferation indices. In contrast, exposures to single wall carbon nanotubes produced transient inflammatory and cytotoxic effects at 1 day post exposure, due primarily to the blockage of airways and resulting injury by the instillate.

Histopathological evaluations revealed that exposures to quartz particles (5 mg/kg) resulted in dose-dependent lung inflammatory responses, concomitant with accumulation of foamy alveolar macrophages and early development of lung fibrosis at the sites of normal particle deposition. Lung exposures to carbonyl iron particles produced no significant adverse effects. Pulmonary exposures to SWCNT in rats produced lesions characterized by multifocal mononuclear cell granulomas. The granulomas contained black SWCNT agglomerated nanotubes (i.e., nanoropes or nanomats) in the center of the lesion, and were surrounded by macrophage-like giant cells. The lesions did not appear to progress beyond 1 month post exposure. Surprisingly, the bronchoalveolar lavage and cell proliferation results were not predictive biomarkers of the SWCNT-induced granulomatous lesions, unlike pulmonary responses to quartz particles.

The observation of SWCNT particulate-related granulomas as a result of pulmonary inflammatory effects are not consistent with the following: 1) lack of lung toxicity by assessing lavage parameters; 2) lack of lung toxicity by measuring cell proliferation parameters; 3) an apparent lack of a dose response relationship; 4) non-uniform distribution of lesions; 5) the paradigm of dust-related lung toxicity effects; 6) possible regression of effects over time. Moreover, recently reported data from two exposure

assessment studies at the workplace have indicated very low respirable aerosol SWCNT exposure levels. As a consequence, the physiological relevance of these findings remains to be determined. Accordingly, to reconcile the apparent discrepancies in this lung bioassay study, it is critical that the pulmonary effects of SWCNT soot in rats be assessed by generating SWCNT aerosols in an inhalation toxicity study**.**

INTRODUCTION

Carbon nanotubes are known to have superior mechanical, electrical and magnetic properties. Single-wall nanotubes (SWCNT), due to electrostatic properties and strong chemical van der Waals forces, self-organize into rope-like structures which can range in lengths up to several microns. The potential health risks and corresponding hazards of inhalation exposure to carbon nanotubes have not been elucidated. Recent experimental studies in rats indicate that inhaled, ultrafine-sized carbon black particles may produce significant lung toxicity in rats at high aerosol concentrations and the toxicity potential is inversely related to particle size. Thus, smaller ultrafine or nano-sized carbon black particles [furnace black particles - mean diameters = 14 nm (surface area = 270 m^2/g)] produced enhanced lung toxicity in rats when compared to larger nano-sized carbon black particles [(lamp black particles - mean diameters = 95 nm (surface area = 22 m^2/g)]. In one study, intratracheal instillation of 15 mg of 14 nm-sized carbon black particles produced 21% lung tumors in exposed female rats while 15 mg of instilled lamp black particles (95 nm) produced only 8% lung tumors.

This study was designed as a hazard screen to evaluate whether SWCNT particulate exposures produce significant toxicity in the lungs of rats by comparing the activity of the carbon-derived particulates with other reference particulate materials. Thus, the aim was to evaluate in rats, using a well-developed, short-term lung bioassay, the pulmonary toxicity impact of intratracheally instilled SWCNT samples and to compare the pulmonary effects with low and high toxicity particulate samples.

Bridging studies (i.e., bridging instillation studies with inhalation studies) can be useful in generating an inexpensive preliminary safety screen when evaluating the hazards of a variety of new developmental compounds or when making small modifications to an existing chemical product. The strength of the bridging strategy is dependent upon having good inhalation toxicity data on one of the compounds utilized as a reference material. The material for which there is inhalation data can then be used as a control material for an intratracheal instillation bridging study (see Figure 1). The basic idea for the bridging concept is that the effects of the instilled material serve as a control (known) material and then are “bridged” on the one hand to the inhalation toxicity data for that material, as well as to the new materials being tested. The results of bridging studies in rats are then useful as preliminary pulmonary toxicity screening (i.e. hazard) data, because consistency in the response of the inhaled and instilled control material serves to validate the responses with the newly tested dust.

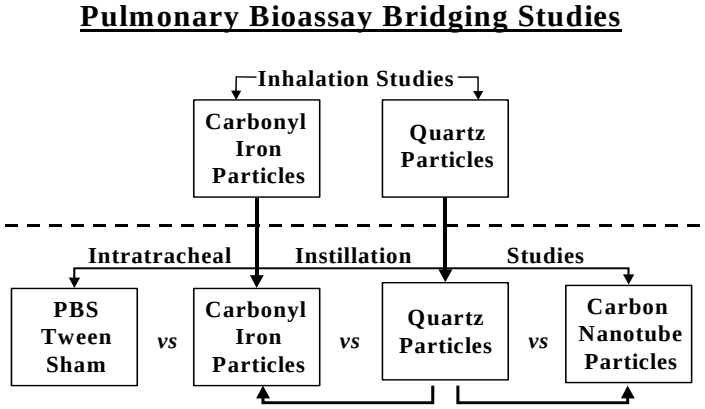


Figure 1. Schematic demonstrating the strategy for conducting pulmonary bioassay bridging studies. Bridging studies can have utility in providing an inexpensive preliminary safety screen when evaluating the hazards of new developmental compounds. The basic idea for the bridging concept is that the effects of the instilled material serve as a control (known) material and then are "bridged" on the one hand to the inhalation toxicity data for that material, and on the other hand to the new materials being tested.

METHODS

General Experimental Design

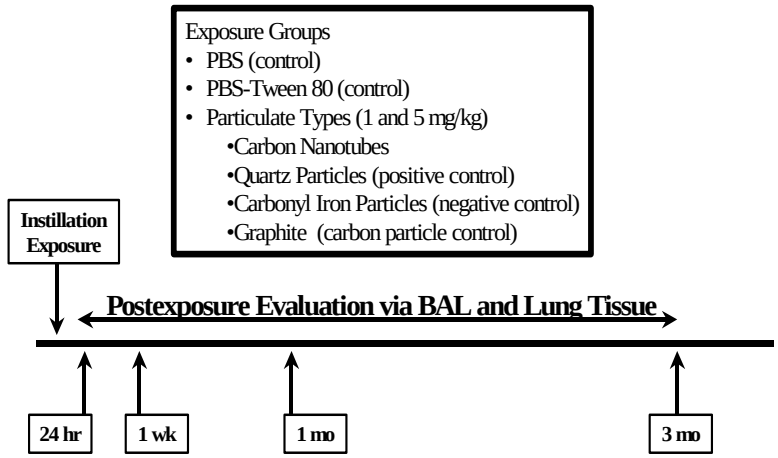
The major features of the pulmonary bioassay bridging study are 1) dose response evaluation, and 2) time course assessments to determine the persistence of any observed effect. Thus, the major endpoints of this study were the following: 1) time course and dose/response intensity of lung inflammation and cytotoxicity; 2) airway and lung parenchymal cell proliferation indices; and 3) histopathological airway and lung parenchymal cell proliferation indices; and 4) histopathological evaluation of lung tissue.

Table I. Experimental Groups for SWCNT Pulmonary Bioassay Study

Group	Description	Purpose	Dose (mg/kg)
1	Phosphate Buffered Saline (PBS)	Negative Control	
2	PBS + 1% Tween-80	Surfactant Control	
3	Carbon nanotubes (SWNT) + 1% Tween-80	Test Compound	1 and 5
4	Crystalline silica particles (Quartz) + 1% Tween-80	Positive Particle Control	1 and 5
5	Carbonyl iron particles + 1% Tween-80	Negative Particle Control	1 and 5

Groups of rats were intratracheally instilled with 1 or 5 mg/kg of carbon nanotubes (CNT), quartz-crystalline silica particles (Q), or carbonyl iron (CI) particles. All particles were prepared in a volume of 1.0 % Tween 80 and phosphate-buffered saline (PBS) and subjected to polytron dispersement. Groups of PBS and PBS-Tween instilled rats served as controls. The lungs of PBS, PBS- Tween and particle-exposed rats were evaluated by bronchoalveolar lavage, and lung tissue evaluations such as cell proliferation studies, and histopathology.

Table II. Protocol for Carbon Nanotube Bioassay Study



Single Wall Carbon Nanotube Material

Single wall carbon nanotube soot generated via a laser ablation process was obtained from DuPont Central Research. The nominal size of single wall nanotubes are 1.4 nm diameter x >1 um length. However, the nanotubes rarely exist as individual units and exist primarily as agglomerated "ropes" of nanotubes of ~30 nm in diameter. The soot is comprised of about 30-40 weight % amorphous carbon and 5 weight % each of nickel and cobalt with the balance being the carbon nanotube agglomerates.

Histopathological Evaluation

Histopathological assessment of lung tissues from exposed animals revealed that exposures to carbonyl iron particles in rats produced no significant adverse effects when compared to PBS-Tween exposed controls. In contrast, pulmonary exposures to quartz particles in rats produced a dose-dependent lung inflammatory response, foamy (lipid-containing) alveolar macrophage accumulation, and the early development of lung fibrosis. Exposures to SWCNT produced a non dose-dependent foreign tissue body reaction in the form of multifocal mononuclear cell granulomas. The lesions were initially observed at 1 week post exposure. There seemed to be little progression of the lesion after the 1 month post exposure time period.

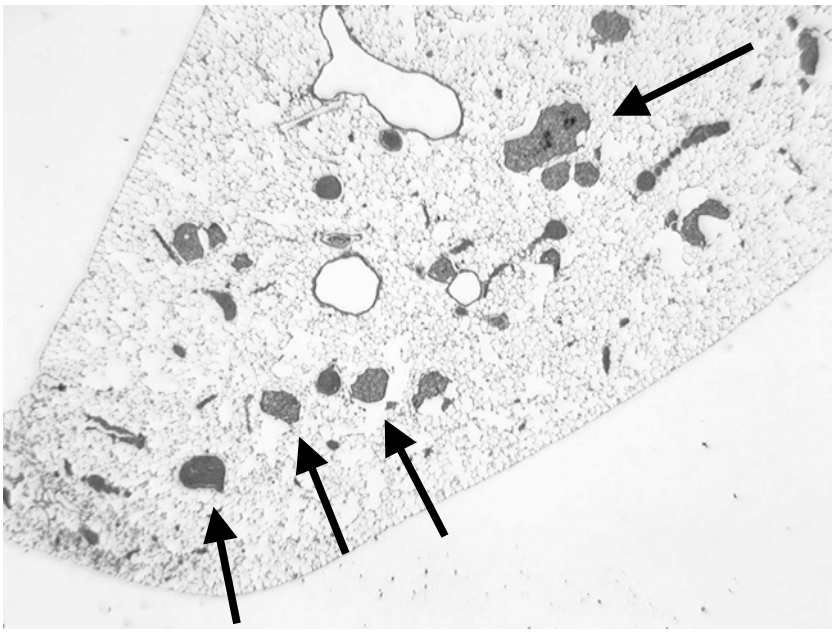


Figure 2. Low magnification micrograph of lung tissue from a rat exposed to single wall carbon nanotubes (1 mg/kg) at 1 month post-instillation. *Note the diffuse pattern of granulomatous lesions (arrows). It was interesting to note that few, if any, lesions existed in some lobes while other lobes contained several granulomatous lesions – and this was likely due to the nonuniform deposition pattern following carbon nanotube instillation. Magnification = x20.(reprinted from DBW ACS manuscript).*

To summarize these findings, pulmonary instillation of SWCNT in rats produced a non dose-dependent foreign tissue body reaction. The distribution of the multifocal granuloma lesions was nonuniform. There appeared to be no dose response relationship, and a possible regression of lesions occurring from the 1-month to 3-month post exposure periods was noted. The development of these lesions is not consistent with lung effects observed with fibrogenic dusts, which generally develop anatomically at bronchoalveolar junctions. Moreover, it was interesting to note that the pulmonary biomarkers measured in this study were not predictive of this granulomatous lesion. In addition, two occupational exposure assessment studies of carbon nanotube operations have recently been conducted which report very low aerosol exposure levels of respirable-sized carbon nanotubes, ranging from not detectable to < 0.1 mg/m^3. These findings give support to the postulation that, due to their electrostatic attraction and potential to agglomerate into nanorope structures, aerosol exposures at the workplace to respirable-sized carbon nanotubes are extremely low. Therefore, the study findings of multifocal granulomas reported herein may have little physiological relevance. As a consequence, we have commenced an aerosol exposure study to assess the exposure potential and corresponding pulmonary toxicity of aerosolized SWCNT in rats.

(Figures and Tables reprinted from a DBW manuscript entitled "**Lung Toxicity Bioassay Study in Rats with Single Wall Carbon Nanotubes",** American Chemical Society Journal, 2004.

3.3 SESSION 3: EXPOSURE AND CONTROL ISSUES

3.3.1 Hazards and risks of nanomaterials: a look forward.

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INTRODUCTION

Nanoscience and its emerging technology is expected to bring a fundamental change in manufacturing in the next few years and will have an enormous impact on Life Sciences, including drug delivery, diagnostics, nutraceuticals and production of biomaterials. Engineered nanoparticles (< 100 nm) are an important tool to realize a number of these applications. The reason why these nanoparticles (NP) are attractive for such purposes is based on their important and unique features, such as their surface to mass ratio which is much larger than that of other particles, their quantum properties and their ability to adsorb and carry other compounds. NP on one hand have a large (functional) surface which is able to bind, adsorb and carry other compounds such as drugs, probes and proteins. On the other hand, NP have a surface that might be chemically more reactive as compared to their fine (> 100 nm) analogues. Many of these special purpose engineered NP are produced in small quantities. In 2003, Single-Walled and Multi-Walled Nanotubes had a world-wide production of 2954 kg. However, the Carbon Nanotechnology Research Institute (Japan) plans on expanding their production from ~1000 kg in 2003 to 120,000 kg per year within the next five years. Although current production of engineered nanomaterials is small, it is evident that production rates will accelerate exponentially in the next few years.

In addition to these specifically engineered nanomaterials, nano-sized particles are also being produced non-intentionally in diesel exhaust and other combustion processes. It is estimated that 50,000 kg/year of nano-sized materials are being produced through these un-intended anthropogenic sources. These combustion NP are included in particulate matter (PM) which is measured by mass and related to adverse effects in patients with lung and cardiovascular disease. Combustion NP have also been denominated as ultrafine particles, and are primary particles or agglomerates with a diameter smaller then 100 nm. These ultrafine particles are a small mass fraction of total anthropogenic particulate emissions, described with total suspended particles (TSP), particulate matter (PM) or PM beyond a specific size in micrometers (PM10, PM2.5, PM1). It is estimated that 50,000 kg/year of nano-sized materials are being produced through these un-intended anthropogenic sources. The first publication on this topic was the so-called Six Cities study that described an association between mortality in six US cities and the annual mean of particulate mass sampled by convention with a 50 % cut-off at 2.5 µm (PM2.5). From this and later studies it is estimated that per 10 µg m^{-3} increase in the concentration of PM2.5, overall mortality increases by 0.9 %, while deaths from specific respiratory diseases can increase by as much as 2.7 %. There is ample evidence that a small proportion of the mass but a large proportion of the number of the particles in ambient air are ultrafine in size. Numerous toxicological studies have now forwarded these ultrafine

particles to be responsible for adverse effects, but so far few human studies have been able to investigate this.

Interestingly most of the toxicological work on NP has been generated with a small set of bulk nanoparticles, that have been around in industry for some decades and are produced in quantities that currently exceed many tonnes per year. According to the National Nanotechnology Initiative (USA), the largest production volume in 2004 was for colloidal silica, titanium dioxide, and various iron-oxides. All these bulk NP were considered to be so-called nuisance dusts until it was observed that upon prolonged exposure in rats inflammation and lung tumors can occur [reviews: Donaldson et al, 2002; Oberdorster, 2001; Borm et al, 2004; Borm & Kreyling, 2004). The question now is whether in this triangle of different applications and sources of NP (Figure 1) the different pieces of toxicological and epidemiological evidence on different NP can be mutually used or whether a more sophisticated approach is necessary.

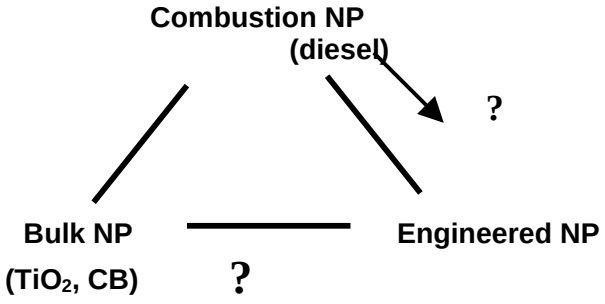

Fig 1. Illustration of the different sources and applications of Nanoparticles (NP) and the evidence for their relation with adverse effects in humans or animals.

Epidemiology and toxicology have demonstrated acute effects of combustion NP in humans, as well as chronic effects of NP in animals. It remains an open issue whether the hazards and risks found with those types of NP can be extrapolated to engineered NP, which is illustrated by the question marks.

HAZARDS OF INHALED NANOPARTICLES

Pulmonary deposition and translocation of nanoparticles

Although the deposition of inhaled NP in the respiratory tract follows largely the same distribution as fine particles, the underlying mechanisms are different. Nanoparticles (< 100 nm) have a size dimension that makes them less subject to gravity and turbidometric forces and therefore their deposition occurs mostly by diffusion. In addition their size makes them more likely to interact with other potential targets than conventional fine particles. As a result of their small size, defence is less efficient since recognition by macrophages is suggested to be impaired or less effective. In addition for drug delivery, particle surfaces have been treated to behave as "stealth" particles and remain

unrecognised by phagocytosing cells. Because of their low uptake by macrophages and their diffusion behaviour, NP are suggested to be taken up by endothelial cells and they have access to cells in the epithelium, the interstitium and the vascular walls. However, It is only after increasing endothelial or epithelial permeability that particles do translocate to the blood. This maybe achieved by mediators released during an inflammatory response such as hydrogen peroxide or histamine (Nemmar et al, 2003). NP can translocate from the lung to the circulation, and exert their effects when being in the systemic circulation. However, quantitative estimates of translocation range between 50% of ^{13}C NP (26 nm size) within 24 hours in a rat model to less than 1 % using 18 nm ^{129m} Ir NP in vivo or in isolated perfused rat lungs. Apart from particle characteristics, also epithelial and endothelial permeability are considered to play a role, which was demonstrated in a study injecting (Iv) 4 nm gold NP that was found in interstitial spaces and lumen after concomitant treatment of animals with LPS.

Pulmonary inflammation and immune defence

The toxicological profile of (bulk and combustion) NP has only emerged during the past decade. An early key study demonstrated that ultrafine TiO2 (20 nm) caused more inflammation in rat lungs than exposure to the same airborne mass concentration of fine TiO2 (250 nm) (Ferin et al, 1992). Until then TiO2 had been considered a non-toxic dust and indeed had served as a inert control dust in many studies on the toxicology of particles. Therefore, this report was highly influential in highlighting that a material that was low in toxicity in the form of fine particles but could be toxic in the form of ultrafine particles. Later studies have demonstrated that the pulmonary inflammation, usually measured as the number of neutrophilic granulocytes (PMN) in bronchoalveloar lavage (BAL), is related to the instilled or inhaled surface area of particles, although at similar surface some ultrafines seem to be more inflammatory than others (Dick et al, 2003). Among mechanisms by which NP could cause an enhanced inflammatory response, direct effects have been reported on alveolar macrophages such as inward leaching of Ca^{2+}, impairment of phagocytosis and cytoskeletal changes (review: Borm & Kreyling, 2004) Epithelial and nerve cells may also contribute to airway inflammation by producing pro-inflammatory cytokines such as interleukin-8 (review: Donaldson et al, 2004) or pharmacologically active compounds such as capsacein. In this neurogenic inflammation, stimulation of sensory nerve endings releases neurotransmitters which may affect many types of white blood cells in the lung, as well as epithelial and smooth muscle cells. Another potential consequence of exposure to NP may be their effect on the capacity to defend against micro-organisms or, in contradiction, an augmentation of allergic immune response to common allergens [review: Granum & Lovik, 2002).

Pulmonary carcinogenicity

Poorly soluble particles (PSP) without specific toxicity such as carbon black and titanium dioxide (TiO_2) are known to cause fibrosis, neoplastic lesions and lung tumours in the rat [review: Borm et al, 2004). NP (TiO_2, carbon black) can induce lung tumours in rats at considerably lower gravimetric lung burdens than their larger sized analogues and actually the retained particle surface metric has been used to describe the lung tumour rate in chronic inhalation studies. It is now generally accepted that the continued

presence of high levels of particle surface leads to impairment of alveolar macrophage clearance, culminating in rapid buildup of particles, chronic inflammatory response, fibrosis and tumorigenesis, known as the so-called rat lung overload. The overall pattern is one of chronic inflammation that occurs upon saturation of lung clearance by overloading of macrophages at which point particle accumulation starts and inflammatory cell influx increases sharply. The inflammatory cell influx is held responsible for the lung tumours after chronic particle exposure to PSP due to their mutagenic activity and actions on cell proliferation. Since NP have a larger specific surface area, at similar gravimetric dose, NP cause higher tumour doses at similar mass dose. Still this surface dose concept is probably an oversimplification for several reasons:

- NP at similar surface area appear to exhibit significant differences in inflammatory activity.
- NP particles following inhalation have a different lung distribution between alveolar spaces, macrophages and interstitium.
- At high local concentrations of NP, they should be considered to penetrate target cells and enter the mitochondria and the nucleus exerting direct effects to DNA.
- The surface dose may be a derivative of other active surface properties.

SYSTEMIC EFFECTS OF NANOPARTICLES

Studies with inhaled PM have forwarded several major mechanisms by which the ultrafine component of PM may cause responses that explain the mortality in those with existing pulmonary and cardiovascular diseases (review: Pope et al, 2004). Mechanisms to explain for these effects can be discriminated into direct and indirect pathways, as effects by particles themselves or processes induced by particles (mainly in the lung). Among direct mechanisms particle translocation to the brain and other target organs have been suggested. Among indirect mechanisms the release of inflammatory mediators can trigger systemic hypercoagulability (Seaton et al, 1995) and the progression and destabilization of atheromatous plaques by inhalation of PM (Suwa et al, 2002).

Uptake routes and applications.

Apart from inhalation in occupational or environmental settings, NP can get access to the body by a number of other pathways.

Recently, carbonaceous NP and gold were shown to translocate from the nasal cavity through the olfactory epithelium (2 cm^2) along the olfactory nerves to the central nervous system (CNS), based on their presence in the olfactory bulb of rats after inhalation (Oberdörster et al, 2004). Such a mechanism was first reported for polio virus (30 nm) and colloidal gold particles (50 nm) moving into the olfactory bulb of various primates.

Uptake through the gastro-intestinal tract (40 m^2) has also been described for particles of different sizes and is actually now being employed by food industry to increase bioavailability of compounds that normally have a low bioavailability (vitamins, proteins). To do so, pure chemical substances are synthesized into nanoparticles with

crystalline structure and in this was may be taken up through the immune system in the gut. We therefore have to consider that NP that reach the gut (e.g. by mucociliary clearance of inhaled NP) can reach the circulation through a secondary pathway.

Dermal uptake (2 m^2) has been studied extensively with coated and uncoated TiO_2-NP and this material was not detected beyond the horny layer. Considering its widespread application in cosmetics and sunscreens, it is surprising that so little data is available on the uptake of NP-TiO_2 through normal skin. In addition, hardly any data is available on local effects in the skin, or uptake through skin in diseased conditions (e.g. eczema, sunburn).

A LOOK FORWARD

Toxicity testing of Nanomaterials

Although there is a considerable amount of data on the toxicity of NP, this data is mainly based on a small panel of NP (diesel, TiO_2, CB) and the assumption that a lot of effects by PM are driven by the ultrafine particles in it (Donaldson et al, 2002). Due to this background of the data and the specificity of most preparations of engineered nanoparticles, a lot of work needs to be done with regard to characterization and biological testing of engineered NP. With this regard it is recommended to perform testing driven by the anticipated application and classification by risk and not by hazard.

Whatever test will be used it needs to be realized that nanoparticles are usually surface modified to prevent aggregation. In fact, about 90 % of TiO_2 is coated by organic or mineral (SiO_2) and it needs to be considered that most suppliers apply post synthetic strategies to modify engineered and bulk NP to prevent aggregation to retain its anticipated properties. Particle coating with polyethylene glycol is a common treatment in drug delivery to prevent recognition by the reticulo-endothelial system and increase the half-life of the particle-conjugated drugs. For fullerenes such surface modifications have been shown to determine toxicological parameters (Sayes et al, 2004). Apart from modifying the surface the compounds used in post synthetic routes such as 4-dimethylaminopyridine, various thiols, fluoroalkanes, alkoxysilanes and phosphorous may be released and need to be included in testing protocols.

Exposure and distribution

The ultimate risk of NP is of course dependent on both the hazards and the risk. The risk is mainly driven by exposure to and uptake of NP at different routes of uptake. The preceding data show that different uptake routes exist and that NP can distribute from site of entry to other sites in the body. Studies with NP in drug delivery have shown that NP can accumulate in areas with increased permeability and cross barriers such as blood-brain barriers and placenta. Little is known about conditions and co-exposures that may cause increased uptake and altered distribution upon exposure to NP. In addition there is a need for simple methods to assess airborne exposure to NP, and assess contribution to the total body burden of NP.

Worker and consumer protection

Exposure to NP may occur in occupational and environmental sources as well as through (functional) food and food chains. At this moment there is little know-how as how to circumvent exposure or to protect workers to NP uptake at accidental or chronic exposure to NP. Simple techniques for online measurement of NP will help to identify industrial operations and procedures that may give rise to emissions of NP. Apart from incidental publications, the environmental distribution and effects of NP are completely missing and there is large need for studies on the life-cycle of NP especially in consumer product such a instruments, implants, coatings, and food components.

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Table 1. Different sources and applications of Nanoparticles.

Source of NP	Examples	Application/use
Combustion NP	**Diesel exhaust particles** **Fly-ashes**	**Environmental exposure**
Bulk synthetic NP	**Titanium dioxide (TiO_2)** **Carbon blacks** **Amorphous silica** **Iron oxides**	**Cosmetics** **Pigments, tires, toner** **Paints, fillers**
Engineered NP	Organic **Liposomes** **Polycyanoacrylates** **polyethene** Inorganic **Gold, dendrimers,** **Zeolites, silver**	 **Drug delivery** **Drug delivery** **Implants** **Drug delivery** **Quantum dots (imaging)**

3.3.2 Monitoring of nanoparticles in the workplace

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Introduction

Nanotechnology is a broad-based enabling technology that holds the promise of major advances in many areas. The next few years will see increasing commercialisation of products that exploit the unique properties of nanoscale materials and devices. However, these same properties present tough new challenges to understanding, predicting and managing potential adverse health effects following exposure. Among the many challenges being faced is the need to be able to monitor exposure to nanomaterials in the workplace in terms of relevant material characteristics. Historically, the mass and bulk chemical composition of materials entering the body have been used to estimate health impact. However, research over the past 15 years has indicated that the size, physical structure and surface chemistry of nanostructured materials play an important role in determining biological response (Donaldson et al. 2000; Oberdörster 2000; Tran et al. 2000; Brown et al. 2001; Oberdörster et al. 2004).

Background

Although nanomaterials may potentially enter the body via a number of routes, most toxicology and epidemiology data related to nanometer-diameter particles to date have focused on inhalation exposure. The potential exists to inhale nanostructured materials when working with suspensions or slurries of nanostructured materials (through sprays and atomisation), nanoscale powders and airborne nanoscale materials. While the composition and chemistry of insoluble inhaled nanomaterials are likely to be important factors in determining biological response, the ability to measure physical characteristics such as size, number and surface-area will be key to appropriate *in situ* exposure monitoring.

A critical step towards developing appropriate airborne exposure monitoring approaches is the definition of particle sizes of interest. Nanotechnology generally refers to the creation and use of sub-100 nm structures, and the exploitation of the unique properties associated with these structures. A wide range of nanostructured materials are formed as powders, suspensions or solutions are comprised of primary particles with diameters of less than 100 nm. Consequently, there has been a tendency to discuss airborne exposure to ‘nanoparticles’ or ‘ultrafine particles’ in terms of discrete sub-100 nm diameter airborne particles. However, the unique properties of nanostructured materials are not confined to discrete nanometer diameter particles. In the context of health risk it is important to consider whether the nanostructure of a material leads to a specific or enhanced biological response, and whether the material can interact with the body in such a way that the nanostructure is bio-available. Under these criteria, the size of discrete particles only becomes important where biological activity is associated with individual particles, and where size governs location following inhalation and subsequent translocation in the body. For instance, the increased inflammatory response to ultrafine

TiO_2 reported by Oberdörster *et al.* (Oberdörster et al. 1994) was most likely associated with nanostructured agglomerates larger than 100 nm in diameter (Maynard 2002), and other studies have demonstrated a correlation between surface-area and inflammatory response for particles significantly larger than 100 nm in diameter (Lison et al. 1997; Tran et al. 2000). These and other studies suggest that in some cases nanostructure alone (represented by surface-area) can provide an indication of biological activity. However studies indicating size-dependent particle translocation from the respiratory system to other organs (Nemmar et al. 2001; Nemmar et al. 2002; Oberdörster et al. 2004) suggest that there will be cases where discrete particle diameter strongly influences impact. If particle nanostructure rather than diameter is the primary driver behind biological response, exposure monitoring most likely needs to be carried out with respect to the impacted areas of the respiratory system, in line with the inhalable, thoracic and respirable sampling conventions. However, where discrete particle size potentially drives translocation and biological response, size-selective sampling/monitoring methods beyond these conventions are most likely required.

For an equivalent mass of material, aerosol surface-area varies inversely with particle diameter, and particle number varies inversely with the cube of particle diameter (assuming spherical particles). Thus, even at low mass concentrations, the surface-area and particle number associated with airborne nanoparticles may be substantial. Critical challenges to nanoparticle monitoring include the use of mass-based methods to reflect increasing number and surface-area with decreasing particle diameter, the use of number concentration as an exposure metric and measurement of aerosol surface-area *in situ*.

Monitoring Approaches

Mass concentration measurements offer continuity with historic and current monitoring approaches, but are by their very nature relatively insensitive to nanometer-diameter particles. In principle though, they may offer a bridge between established and new exposure monitoring approaches if high sensitivity and appropriate particle size selectivity is achievable. If particle number or surface-area is a more relevant exposure metric, it may be possible to use mass concentration as a surrogate measurement where information on particle size distribution or aerosol specific surface-area is known.

Aerosol number concentration is relatively easy to measure above 10 nm using Condensation Particle Counters (CPCs), and may be extended to particles as small as 3 nm in diameter with relative ease. Number concentration measurements are generally not size-specific though, unless made with an appropriate pre-separator for a specific particle size range. Consequently, it is difficult to distinguish between different sources of process-related aerosols, or between process and background aerosols. Kuhlbusch *et al.* found number concentration measurements in carbon black production facilities were frequently dominated by other aerosol sources, leading to difficulties in monitoring process-specific emissions using number alone (Kuhlbusch et al. 2004). Despite this drawback, the use of number concentration measurements has been proposed for crude identification of nanometer aerosol emission sources in workplaces by carrying out measurements close to potential or suspected sources (Brouwer et al. 2004).

The surface-area of insoluble nanostructured particles would appear to be an appropriate exposure metric for airborne nanostructured particles, where surface-area and activity are more important than discrete particle size. However, available methods to measure aerosol surface-area are somewhat limited. The Brunaeur, Emmett and Teller (BET) method of determining surface-area remains the standard measurement method for powders (Brunauer et al. 1938), and has been used for aerosol surface-area determination with some success (Lison et al. 1997). However, as well as being an off-line technique, there is little information on how the collection process or particle structure affect the biological applicability of measurements. A second widely used method for determining particle and aerosol surface-area is Transmission Electron Microscopy (TEM). Through the use of image processing, the projected area of sampled particles can be determined with relative ease. Once again though, this is an off-line technique, and the results are open to interpretation.

A number of on-line methods are available for estimating aerosol surface area. The most intuitively obvious perhaps is derivation of surface area from measured size distribution. The association between mobility particle diameter and surface area in the free molecular regime has been well established (Rogak et al. 1993; Ku et al. 2004), allowing size distributions measured using mobility analysis to estimate aerosol surface area reasonably well. To cover an appropriately broad particle size range however, mobility analysis needs to be coupled with techniques such as optical particle sizing or aerodynamic particle sizing. While these techniques are sensitive to particles ranging from a few tenths of a micrometer to tens of micrometers in diameter, assumptions must be made about particle shape and composition to derive surface area. Aerosol surface area estimates have been made in this way (Maynard et al. 2002), but the necessary instrumentation array and the data inversion/interpretation are not typically well suited to routine exposure monitoring.

An intriguing approach to estimating aerosol surface area on line has been proposed by Woo *et al.* (Woo et al. 2001). If an aerosol is assumed to have a unimodal lognormal distribution, the distribution, and hence an estimation of surface area, can be derived from just three independent measurements. Woo *et al.* used measurements of number concentration, mass concentration and aerosol charge to estimate surface area. Estimates made using their method correlated well with estimates derived from size distribution measurements. Recognizing that in many occupational settings aerosol number and mass concentration, but not charge, may be measured simultaneously, Maynard has estimated the anticipated errors that would arise from using just these measurements, and assuming a width for the lognormal size distribution (Maynard 2003). In the worst case, where the sampled aerosol is bimodal, it was predicted that estimates could be wrong by up to a factor of ten. However, simulations showed that in many cases, aerosol surface area estimates from number and mass concentration measurements are likely to be within a factor of four of the actual surface area. Although these errors are potentially large, they may be sufficiently small compared to the range of surface area concentrations experienced to provide semi quantitative banding of exposures.

The first instrument designed specifically to measure aerosol surface area was the epiphaniometer (Baltensperger et al. 1988). This device measures the Fuchs or active surface area of aerosols by measuring the attachment rate of radioactive ions. For particles smaller than approximately 100 nm in diameter, active surface scales as the square of particle diameter, and thus is probably a good indicator of external surface area for nanoparticles. However above approximately 1 μm it scales as particle diameter, and so the relationship with actual particle surface area is lost.(Fuchs 1964). In the transition region there is a gradual shift from active surface area varying as d^2 to d^1. Clearly, if biological impact is associated with geometric surface area, active surface area will underestimate 'biologically available' surface area. However, as the surface area of aerosols comprised predominantly of nanoparticles is dominated by particles smaller than a few hundred nanometers in diameter, it may be that active surface area provides a reasonable estimate of biologically relevant surface area in many cases.

The epiphaniometer is not well suited to widespread use in the workplace due to the inclusion of a radioactive source. However, the same principle forms the basis of diffusion charger-based aerosol monitors where the charging rate is low. Diffusion charger-based aerosol surface area monitors measure the attachment rate of positive unipolar ions to particles, and from this the aerosol active surface area is inferred (Keller et al. 2001). Following charging, usually using a corona discharge, the aerosol is collected onto a HEPA filter within a sensitive electrometer, and the aerosol charge per unit volume of air sampled measured. Evaluation of the LQ1-DC and DC-2000CE diffusion chargers (Matter Engineering, Switzerland) with spherical and fractal-like silver particles show a clear correlation with diameter squared for particles with a mobility diameter below 100 nm. In this region, agreement with TEM and mobility analysis measurement methods is good. Above 100 nm, the diffusion chargers increasingly underestimate the aerosol surface area (Ku et al. 2004).

Although instruments and methodologies for measuring aerosol exposure against a range of metrics are still at an early stage of development, it is currently possible to estimate exposures in terms of surface area and number concentration. The continued testing and development of available techniques will lead to routine exposure measurements of number and surface area that are increasingly viable. In addition, the commercialization of emerging technologies such as personal diffusion chargers, compact integrated mobility analyzers and cross-flow mobility analyzers has the potential to lead to inexpensive, real-time personal samplers over the next few years. However, as these technologies are increasingly used to monitor exposure against a range of metrics, it must be remembered that the measurements only provide an indicator of the potential biological activity of a given aerosol.

If, as is indicated, the health impact of nanostructured materials is related to a combination of physical and chemical properties, measurements need to be understood in the context of the nature of the aerosol being sampled. In the case of measurements such as aerosol surface-area, it is intuitive that these need to be related to the surface activity of a given material. Likewise, the size range of particles to which a particular measurement method is sensitive needs to be considered. As the physical characteristics

of aerosols can change through coagulation and dispersion, care needs to be taken over where measurements are made in relation to generation and inhalation locations. The surface chemistry of nanostructured particles may also be influenced by conditions during transportation, or even by the process of generation. Thus, while it is likely that metrics such as number and surface-area concentration will provide biologically relevant exposure measurements for many airborne nanostructured materials, the validity of these measurements will only be as good as our understanding of their limitations, and the underlying biological activity they represent.

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3.3.3 Control of Nanoparticles

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INTRODUCTION

The common approach to the control of chemicals and other hazardous substances is based upon assessing the risk to the health of the workforce of exposure to the substance. Once the risk has been assessed, the most appropriate control solution can be proposed and implemented. This is the basis of the Control of Substances Hazardous to Health Regulations 2002 (COSHH) regulations in the UK (HSE, 2002) and the associated COSHH Essentials risk assessment and control identification tool (www.coshh-essentials-org.uk).

Risk assessment requires information on; the toxicity, the physical and chemical properties and the hazard of the material; a description of the process and the amount of material produced or used; and the levels of exposure that are likely to arise. In the EU, there are a number of regulations in place to control the supply and transport of chemicals. The Chemicals (Hazard Information and Packaging for Supply) Regulations 2002 (HSE, 2002) controls the supply of existing chemicals requiring suppliers to identify the hazards of the chemicals they supply, give that information to their customers and package the chemicals safely. The Notification of New Substances (NONS) Regulations 1993) requires the manufacturers of new chemicals to provide information to regulators about the possible risks posed to people and the environment from the new chemical and to recommend controls to ensure its safe use.

The above regulations and associated guidance have in the main been concerned with minimising exposure to particles of size of the order of 1 μm and above. This review presents these regulations and control guidance and assesses the extent to which they are applicable to the new-engineered nanoparticles.

CURRENT GUIDANCE ON CONTROL OF HAZARDOUS SUBSTANCES

In the UK, the COSHH Regulations require employers to control exposure to hazardous substances to prevent work-related ill health in their workforce. The regulations set out a simple step-by-step approach to help assess risks; implement and control measures needed and establish good working practices. To satisfy the regulations, the employer must carry out a “COSHH assessment” of the various processes involving chemicals in his workplace. This assessment will include:

- Identification of the hazard from the chemical,
- Assessment of the risk to health from working with the chemical in the given process,
- Recommendations of measures that need to be taken to prevent and control risk
- Evaluation of the effectiveness of the control measures.

All workplaces involving the use of chemicals must have a current COSHH assessment with appropriate control measures operating effectively. Similar regulations are in force in other countries. The regulations provide a list of different options for control and place them in a broad control hierarchy. The main options are:

1) Change the process or activity so that the hazardous substance is not needed or generated
2) Replace the substance with a safer alternative
3) Use the substance in a safer form (e.g. pellets instead of powder)
4) Use appropriate work processes to minimise materials used or totally enclose the process
5) Control exposure at source using local exhaust ventilation and/or general ventilation
6) Use suitable personal protective equipment (respirators, masks, protective clothing, etc., but only as a last resort).

As a means to guide employers to the most appropriate control measure to choose for their material and process, HSE has produced COSHH Essentials. This is a web-based tool that uses information from material safety data sheets to provide control guidance (www.coshh-essentials.org.uk). The tool uses the simple risk assessment via a decision tree to arrive at four methods of control: containment, local exhaust ventilation, general ventilation and PPE.

CURRENT REGULATION ON SUPPLY OF CHEMICALS

The Chemicals (Hazard Information and Packaging for Supply) Regulations 2002 provide legal requirements for suppliers of dangerous chemicals to provide safety, health and environmental information about the chemicals that they are supplying. Included in the term ‘suppliers’ are; manufacturers, importers, distributors, wholesalers and retailers. The supplier must identify the hazard of the material and derive a label that identifies it as on the approved supply list or gives the appropriate risk phrase or symbol. The chemical must be packaged safely and a materials safety data sheet should be supplied with the package. There are some exemptions such as medicines and cosmetics that have their own specific regulations. There are no reasons to suggest any change in these regulations for nanomaterials, but as very few will appear on the approved supply list then they may come under the NONS regulations.

The Notification of New Substances (NONS) Regulations (1993) requires the manufacturers of new chemicals to provide information to regulators about the possible risks posed to people and the environment from the new chemical and to recommend controls to ensure its safe use. If the chemical is registered in the European Inventory of Existing Commercial Chemical Substances as of 1986, then it is not considered to be a new material and no action is required. The application of these regulations to nanomaterials is complicated by how we define what is a new material. For example, is an ultrafine version of TiO_2 a new material when the micrometer-sized version is not? This question and similarly for other materials will have to be considered carefully by the international regulatory bodies to ensure consistency of approach.

TYPES OF NANOPARTICLES

Nanoparticles are available with a wide range of morphologies and states of agglomeration. They include:

- nanotubes mostly of carbon fullerenes with diameters from 1 to 20 nm and lengths greater than 1mm,
- nanowires of metals, semiconductors, etc., comprising a single crystal structure with diameters of 10s of nanometres and large aspect ratio
- nanocrystals and quantum dots of semiconductors, metals and metal oxides comprising 1000 to 100,000 atoms,
- spherical and dendritic aggregated nanoparticles made from a range of materials including carbon black, fumed silica, metals, metal oxides, ceramics, semiconductors and organic materials. They can range in size from a 1-2 nm to 100s of nanometres.

Nanoparticles are very rarely found as single particles and readily form aggregates in which the particles can tightly be bound by covalent bonds. These aggregates can then clump together to form agglomerates held together by relatively weak forces including van der Waal's forces. Agglomerates can range in size from 0.5 to 100 μm .

AERODYNAMIC BEHAVIOUR OF NANOPARTICLES

The aerodynamic properties of particles is of fundamental importance to determine whether and for long they remain airborne, whether they will be inhaled and how far they will penetrate into the respiratory system, and the efficiency by which the particles are captured and filtered. Particle size is the main parameter governing the aerodynamic behaviour of airborne particles.

- For large particles (> 1 μm), inertial and gravitational forces dominate and govern the entry of particles into the mouth and where they deposit in the various regions of the respiratory system. Samplers for inhalable and respirable particles use the same forces to collect and select the particles of interest. Large particles deposit quickly and so will not spread far from the source unless there are strong wind currents.

- For particles in the nanometre size range (<100 nm), diffusion forces dominate and their behaviour is more like that of a vapour. They will enter the mouth with high efficiency and deposit in all regions of the respiratory system. Nanoparticles with high diffusion coefficients will disperse rapidly and remain airborne for long periods of time thereby exposing large numbers of workers albeit at low level.

WILL CURRENT CONTROL METHODS WORK FOR NANOPARTICLES?

Engineering Control

Containment

The main method used for the control of production of nanoparticles is containment because most production methods use high temperature. This has a beneficial effect of minimising the release of nanoparticles into the workplace air during production, but if there are any leaks in the system then nanoparticles will pass through with the efficiency of a gas and may become widely dispersed into the workplace atmosphere. By the time the particles reach the end of the process they have formed loose agglomerates that are not easily dispersed into the air. However, inhalation exposure to these agglomerates can occur in the particle recovery and bagging plants and during maintenance and cleaning processes. Materials such as carbon black, fumed silica, ultrafine TiO_2, carbon nanotubes and the dendritic metal and metal oxide nanoparticles are manufactured in total containment plants.

Local Exhaust Ventilation

LEV systems, enclosures and fume hoods are used to control emissions from materials handling processes such as bagging, mixing and weighing. For nanoparticles, the specification and quality of these systems should be similar to that used for gases. The collection efficiency is expected to be high provided that the emissions are not entrained in a high velocity jet. However for LEV systems, it is essential that the entry hood is always positioned correctly and adequate capture velocity is maintained. Again, maintenance and cleaning of the systems may pose the highest risk of exposure.

Filtration

The performance of a typical fibrous filter is shown in Figure 1

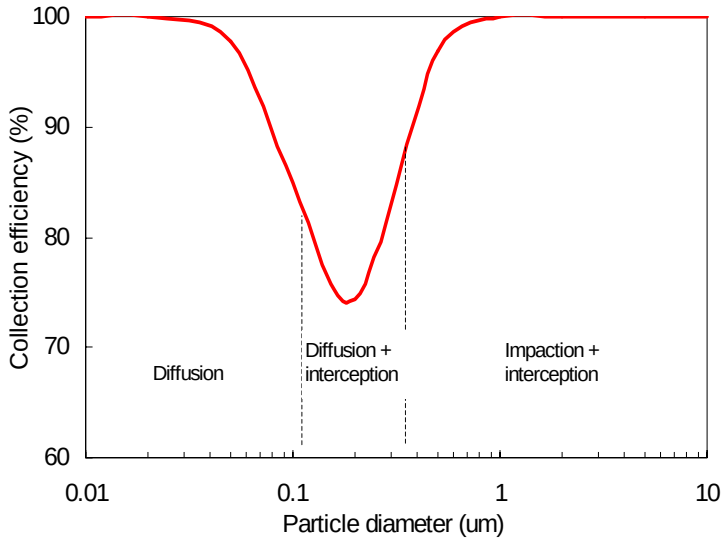

Figure 1 Filter efficiency for typical fibrous filter (from Aerosol Technology , W Hinds)

For large particles, impaction and interception forces dominate, but as particle size decreases, efficiency drops off until it increases again at about 200 nm when diffusion forces begin to become important. There is a minimum efficiency at a particle size known as the most penetrating particle size (MPPS), and most filter efficiency tests are carried out using aerosols with mass median diameters equal to the MPPS.

So, it is expected that correctly specified fibrous filters will be good collectors of nanoparticles. Problems may occur however if the filter material has pinhole leaks or if the filter housing has poor seals, because nanoparticles with behaviour close to gases will penetrate these with ease.

It has been recently suggested that filter efficiency drops again as particles become very small (< 2 nm). Work needs to be carried out to investigate this suggestion. This is particularly relevant for high efficiency vacuum cleaners used to clean production systems and materials handling areas, and filters installed into collection systems that re-circulate the air back into the workplace.

Personal protective equipment

Respiratory

Respirators for control of exposure to airborne dust use low pressure drop filters with a range of efficiencies dependent upon the particle size distribution of the airborne dust and the specific hazard that it poses. Whilst for nanoparticles it is possible to use FFP3 half-face masks with a protection factor of 20, better protection will be gained from full-face masks (PF = 40) and more comfort using powered air-fed masks. The same problems that were discussed for filters apply to respirators. The efficiency for nanoparticles will be high, apart from maybe particles less than 2 nm. For respirators, however, the problem of seal leakage is more severe around the face. It is possible that enhanced leakage will occur for nanoparticles and work should be carried out to determine the protection factors of respirators for nanoparticles.

Dermal

Evidence has been emerging that particles with diameters as large as 1000 nm can penetrate through the skin. It is reasonable to suggest therefore that nanoparticles are more likely to penetrate. The use of protective clothing such as chemical suits and gloves should be considered seriously, especially at the particle recovery and bagging stages of the process and during maintenance and cleaning. There is currently no information on the penetration of nanoparticles through glove or protective suit materials. Work should be carried to determine these parameters with particular attention being paid to the weak points such as the cuffs and the zip areas.

THE USE OF DUSTINESS AS A MEANS OF PREDICTING NANOPARTICLE RELEASE

Dustiness is the propensity of a powder to become airborne when handled. It is widely used for a range of bulk powders and there is a EU draft standard for the measurement of

the dustiness of powders (EN 15051). One of the methods recommended involves the lifting and dropping a known volume of powder in a rotating drum. Air is drawn through the drum and the airborne dust transported to a sampling section where size selective stages give dustiness values in terms of the mass of dust released in the inhalable, thoracic and respirable dust fractions. Whilst it has been used for a range of materials mostly with particles much larger than the nanometre scale, some nanopowders were tested such as fine carbon black, ultrafine TiO_2 and silica flour. Reasonable results were obtained. However, it should be remembered that if the exposure metric changes to particle number or surface area for nanoparticles, then the dustiness tester will require to be modified to receive a particle counter such as a condensation particle counter to provide maximum benefit for the information gained.

It is proposed that dustiness be quoted on the material safety datasheets and on any packaging label to aid the risk assessment process and the selection of suitable controls. In addition, it is also proposed to replace the descriptive terms in COSHH Essentials with qualitative values. However, in order to minimise particle release during the use of the nanopowders by the end users, they are increasingly being supplied as slurries in a liquid rather than as a dry powder.

FINAL THOUGHTS

- I have discussed how current regulation and guidance for the production and use of chemicals can be used to control potential exposure of producers and users of new nanomaterials to nanoparticles both airborne and deposited on surfaces.

- However, the validity of the suggestions made should be investigated in a structured research programme with actual nanoparticles and real processes.

- There should be close collaboration between those involved in toxicology, measurement and control to ensure that a consistent approach is adopted using common terminology and definitions.

- It has been implicitly assumed that once incorporated into a matrix such as a plastic or resin, then the risk of exposure to the bound nanoparticles has disappeared. However, the end use of the product should be considered, just in case the material wears by abrasion and discrete nanoparticles are released.

3.3.4 Risk Evaluation & Control: Current Perspective

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Commercial interest in nanotechnology is growing exponentially across a range of industries. Many potential new users have little or no experience of handling nano materials, which can have very different physical and chemical properties from traditional chemicals. Small and medium enterprises who wish to use nano technology may not have extensive Health Safety and Environment (HS&E) resources available in house.

Separate but related to the above there is a high level of interest in nano technology in both the popular and scientific press. The general tenor is that nano technology offers major potential benefits but there is a lot we do not yet know from a human and environmental safety standpoint.

In net, it is very important that those wishing to deploy nanotechnology develop HS&E capability before moving ahead. Not only do they need to develop capability to ensure worker and environmental safety, they also need to be able to demonstrate that they are capable to internal and external stakeholders including workers, shareholders and interest groups. This is an important aspect of Product Stewardship and miss-steps by a few companies could jeopardize the wider community's right to practice.

Workplace HS&E capability for industrial scale operations involving nano technology can be divided into five major areas:

1. Hazard identification.

2. Measurement of aerosol concentrations and particle size distribution in air and aqueous media. Thus being able to confirm extent of exposure.

3. Safe practices to control exposure to safe levels
   (The term "safe practices" is intended to include the full gamut of locating exposure sources and controlling them via the appropriate combination of engineering, administrative and personal protective equipment. Within this context we also need to be able to establish safe levels).

4. Identification, quantification and mitigation of potential Process Safety risks (over pressure, under pressure, fire and explosion).

5. Control of environmental waste streams (air, water and solid) and verification that environmental fate of wastes is not harmful to the environment.

The sum of items 1-5 can be considered an approach to Risk Assessment and Control.

By way of presenting information I will cover each area in turn using the same approach. I will start by describing current capability, the foundations we can build upon. I will then describe gaps in our knowledge or capability and finally will propose some means to close the gaps.

Of the five areas listed above human health hazards are thoroughly discussed elsewhere in these proceedings and will not be covered here. I will concentrate on areas 2-5 and will include Process Safety and Environmental hazards within the Process Safety and Environmental sections.

The discussion that follows focuses on manufacturing scale operations delivering commercially available products that contain nano technology. However it's important to point out that my company, in common with many others, are just beginning to investigate the potential value of nano technology. Our experience is with bench scale quantities. Thus the ensuing discussion is forward looking and, as we gain experience, we may modify our approach towards full- scale production versus what is stated below. However, the thing that will remain constant is our commitment to a safe and healthy workplace that protects our people, our assets, the surrounding community and the environment.

MEASUREMENT OF AEROSOL CONCENTRATIONS AND PARTICLE SIZE DISTRIBUTION IN AIR AND AQUEOUS MEDIA.

A) Current capability. The foundation we can build upon

Detecting Point sources of air emissions

In our experience the P-Trak (TSI Instruments) is an effective instrument for detecting point sources. For example it is excellent for determining if a particle source is contained within a fume hood. It is portable (hand held and weighs 3.8 lbs.), can measure total particle number concentration in the range 20-1000nm and can count up to 500,000 particles per cubic centimeter. It is also relatively low cost (circa $5,000).

As we scale up to pilot and full-scale operation we expect to continue to use the P Trak to detect point sources. Qualitative exposure assessment (based on observation and professional judgment) will identify potential sources and the P-Trak will be used to help identify the frequency and relative magnitude of release of nanomaterials. This data can help set priorities for eliminating/reducing emissions from point sources.

The P-Trak does not measure particle size distribution. However there are a number of commercially available instruments that offer this added functionality.

Instruments are available to measure size distribution and concentration

For ongoing manufacturing scale operations it will be important to understand particle size distribution as well as concentration in air. This is because health hazards are expected, at least in part, to be dictated by particle size.

There are already a number of commercially available instruments on the market. Some are based on the cascade impactor approach and others rely on light scattering. Costs range from about $5,000 up to $80,0000 or more.

B) Gaps in our knowledge or capability

Air sampling for routine manufacturing scale production

As mentioned elsewhere in these proceedings many ambient environments, inside and outside of workplaces contain quite high background levels of nano sized particulates. This is at least in part attributed to sources such as auto emissions. Within the workplace we anticipate controlling workplace emission sources from our processes. We thus face the prospect of trying to measure a small workplace contribution against a large background. In addition we may need to measure exposures at a number of sites some of which may be running 24 hours per day seven days per week.

Net, we desire low cost instrumentation that does not need high levels of expertise to operate but yet is capable of "filtering out" or compensating for ambient background.

Improved understanding of aerosol behavior

In the solid phase nanomaterials agglomerate rapidly – indeed many commercial receipts are sold in slurry form in order to stabilize the nano material against aggregation. However, what happens to solid in air aerosols? Do they agglomerate rapidly or does low concentration allow the nano material to remain nano sized for a finite length of time?

This is not an academic question. Consider that many work places currently re-circulate ambient air perhaps after passing it through an air cleaning system of some kind. If nano materials are added to the process and are present in the air will the original air cleaning system remove them? If the particles aggregate quickly it will - otherwise it may not. Removal will need to be verified and the current air cleaning may need to be upgraded or the operation may have to stop re-circulating work place ambient air. Changes in air handling can be costly.

C) Some means to close the gaps in our knowledge or capability

P&G initiated a meeting of companies interested in nanotechnology. The intent was to explore if/how we could advance general knowledge / capability with respect to solid in air aerosol behavior and measurement. DuPont indicated that they have capability to work in this area. They have put together a research proposal to deliver three things:

1. Build a test device to generate nano sized aerosols and measure aerosol behavior (solid in air) as a function of time – in particular rate of aggregation and speed of dispersion. Knowledge from this work will be critical for both consumer safety clearance and work place exposure control.

2. Develop a simple robust device to measure airborne concentration of nano materials. This is needed to ensure that work place controls are effective and emissions are below accepted limits. This work will be in collaboration with academia

3. Develop a test to measure if/how much nano materials penetrate protective clothing.

We are currently inviting interested organizations to participate. Not only does this help share costs it also makes it more likely that any methodologies coming out of the work become widely accepted and used. Referring back to the first 3 paragraphs of this article knowledge of aerosol behavior and measurement is an important element of product stewardship and many small companies lack resources – this work may help them.

SAFE PRACTICES TO CONTROL EXPOSURE IN THE WORK PLACE

This section deals with worker safety. Process and Environmental aspects are covered in following sections.

A) Current capability. The foundation we can build upon

Laboratory safety

In our experience we can effectively control exposure in laboratory work via use of laminar flow hoods or conventional fume hoods. We generally prefer laminar flow hoods because it is easier to manipulate nano materials (e.g. weigh or dispense) in the relatively low flow environment versus the higher flow rates experienced in conventional fume hoods. We confirm that containment is effective via use of a P-Trak air monitor.

In the relatively few situations where we can not work in a hood we deploy local exhaust ventilation (e.g. “elephant trunk” source capture devices) and verify effectiveness via P-Trak and sometimes also by smoke tubes.

In situations where risk assessment indicates the need for respiratory protection we generally use powered air purifying respirators equipped with HEPA filters. Based on a literature report[(1)] we believe that HEPA filters very effectively capture nanoparticles as small as 10 nm diameter. However there is a high potential for ultra fines particles to ingress via a poor face seal. This drives us towards positive pressure respirators. Obviously where the expected exposure to exceed to the protection factor of a PAPR respirator equipped with HEPA filter we would revert to a higher protecting air supplied respirator.

At the laboratory scale we capture solid and liquid wastes and dispose of them as hazardous material (incineration or chemical treatment).

Qualitative exposure assessment

As we look toward scale-up we believe that existing qualitative exposure assessment techniques will continue to be relevant and will provide a sound starting point for risk assessment of processes utilizing nanoparticles.

Many industries can use techniques such as visual inspection, back lighting, discussion with operators to assess the location and frequency of exposure sources. It is also possible to get a good feel for magnitude by observing dust build up. Whilst the approach is not quantitative it is extremely practical in that it involves employees and it provides a good understanding of how an operation is performing from a hygiene point of view. The approach helps set priorities for exposure control. If there is a tea spoon of dust below one source and cup of dust below another the cup first is fixed first.

Similarly in addition to emissions from manufacturing equipment work place exposure can also occur from work tasks. These may be off line work (e.g. weighing receipts or reclaiming scrap) or from interface with the equipment (e.g. manual dispensing). Traditionally we identify and mitigate exposure from these sources by job safety analysis. This involves breaking the task into steps. For each step a team of people identify possible risks and then identify counter measures. Behavior based operating procedures are then written based on the job safety analysis. Compliance with the Operating Procedures can be monitored by observation involving all workers.

Operational Guidelines

Some industries have experience of handling moderate to high hazard materials. In these situations the operation has to meet very high hygiene standards at all times. Similarly in say, the food or cosmetics industry, there are very high demands on the operation to ensure the integrity of the product.

In these situations “Operational Guidelines” are implemented. Sometimes this is called good manufacturing practice, GMP (e.g. in food and cosmetics industries). In other industries it is a list of specific guidance points e.g. operate to a standard of no visible dust and no recurring spills. In either case the use of this approach has a very positive effect on operational culture.

An Operational Guidelines approach has worked in a range of industries and should be a major help in sites handling nano materials.

B) Gaps in our knowledge or capability

The basis for exposure limits

Which of the following aerosol parameters correlate best with health effects? Mass, surface area or particle number concentration? This is discussed in detail elsewhere in these proceedings and a final conclusion has not yet been reached. The general consensus seems to be that surface area or number concentration are likely to be more meaningful than mass.

The key point to make here is that engineers need to know which of these units will be used to set exposure limits. This will have an important impact on design of equipment and engineering controls. As particle diameter is halved surface area doubles and particle number increase by a factor of 8. Suppose we go from micron to nano sized material – even if there is no change in toxicity, just to keep the same exposure we must:

- If based on mass - do nothing more.
- If based on surface area -reduce exposure 2X every time particle diameter is halved.
- If based on number concentration - reduce exposure 8X every time particle diameter is halved.

Also some current thinking is that nano materials behave somewhere between a gas and a solid and as such are easier captured by air handling equipment than their micron sized big brothers. Thus we will not need to invent discontinuous capability in engineering controls. This may well be true but the other thing that needs to be considered is the current process. A manufacturing process handling low hazard chemicals may be "open". i.e. present many opportunities for emissions of low hazard chemicals. If nano materials are to be added to such a process it may be necessary to change the process significantly. Said another way, addition of nano materials to existing processes may or may not require extensive process and / or engineering modification :

- If an existing process is highly enclosed and serviced with engineering controls it may be easy in practice to meet a lower exposure limit imposed by nanoparticles – irrespective of whether the limit is based on surface area or number concentration.
- If an existing process is very open it may be more difficult to meet a lower limit, and the sooner we understand the magnitude of improvement required the better. In these cases it will be important whether we base a limit on surface area or number concentration.

Fugitive releases

In all but the simplest or most enclosed processes it is not feasible to think that there will never be any release of materials into the work place air. As discussed earlier many work places re-circulate ambient air. We need to understand if/how quickly and to what extent low concentrations of nano materials aggregate. This has important implications on if/how air handling systems need to be modified to accommodate nano materials.

C) Some means to close the gaps in our knowledge or capability

The issue of fugitive releases is managed by better understanding aerosol behavior. One way to work this is via the consortium work described earlier in this document.

Deciding on the basis for exposure limits and the relevant toxicological testing to determine health hazards is a subject of very active interest. It is being worked in collaborative consortia. These efforts will need to continue in future and will be most

effective when they incorporate perspective from diverse areas e.g. academia, industry, regulatory, and NGO.

IDENTIFICATION, QUANTIFICATION AND MITIGATION OF POTENTIAL PROCESS SAFETY RISKS

As organic or metallic dusts become more finely divided they are more prone to explode. Also, when an explosion does occur it is likely to be more violent. There are well established parameters to measure likelihood of explosion and energy released e.g. KST, Minimum Ignition Energy MIE. These measurements are made experimentally in a test bomb.

Many nanoparticle receipts are available in suspension as a dilute solution to ensure the materials do not aggregate. These receipts may be a fire hazard depending on the solvent. Again there are well established parameters to assess the fire hazard e.g. flash point.

A) Current capability. The foundation we can build upon

We know how to protect process equipment against fire or explosion:

- Install explosion panels.
- Construct vessels to withstand the maximum force that can be generated from an explosion.
- Install pressure transducers that can quickly detect pressure build up and trigger release of a discharge to quench a reaction before it explodes/combusts. To prevent fire you could install temperature sensors or smoke detectors linked to a discharge system.
- Blanket the process in an inert atmosphere. In the absence of oxygen an explosion or fire will not occur. The inert atmosphere presents a different HS&E hazard (asphyxiation - but we know how to manage this risk).

The techniques are listed in order of increasing complexity to manage and cost to implement.

B) Gaps in our knowledge or capability

Let’s take the example of the explosion risk from an organic nanoparticle sold as a dilute suspension in water. In this state there is no explosion risk. The risk occurs if and only if the production process manipulates the material in a way that causes the particles to be separated from the solution and become suspended in air during the manufacturing process. For example this may occur during spray drying where you might not only evaporate the water but also produce a nano suspension at elevated temperature within the operating equipment.

In order to quantify the risk we need to be able to do two things:

1. Generate a solid in air aerosol of nano sized particles in a test bomb.

Once we have done this the second "need" is relatively easy:

2. Measure explosion parameters (e.g. KST, MIE) as a function of particle size in the nano range.

This information will allow us to calculate the likelihood of explosion and the energy released. Based on these calculations we will be able to decide the best means of protecting against explosion.

C) Some means to close the gaps in our knowledge or capability

Study of Process Safety risks is included in the Nano Safe 2 initiative being funded by the European Union. It is also a subject of interest to the UK Health & Safety Laboratory.

What about the short term if a company were considering launch of a nano project before the above studies were completed? The first question to ask is, "Is the material potentially explosive (e.g. an organic or a metal?). If the answer is "no" there is no explosion risk. If the answer is "yes" the next question is, "can a dust be generated during the production process?" This could be inside or outside the manufacturing equipment. If the answer is "no" there is no explosion risk. If the answer is "yes" then it would be necessary to find a way to generate aerosols in a test bomb to measure the explosion parameters.

There will be a fire risk associated with a raw material receipt comprising nanoparticles in an organic solvent. Measurements such as flash point should be undertaken to quantify flammability. This should be done on a case by case basis, do not rely on literature data for the solvent alone. This is because of an effect termed the "hybrid effect" whereby insoluble materials in a solvent can alter flammability versus solvent alone.

CONTROL OF ENVIRONMENTAL WASTE STREAMS (AIR, WATER AND SOLID) AND VERIFICATION THAT ENVIRONMENTAL FATE OF WASTES IS NOT HARMFUL TO THE ENVIRONMENT.

Nano materials are likely to be expensive and often will be present in products at low levels. Also, industry seeks to minimize waste by re-cycling. That said, industry can not totally eliminate waste and as nano technology becomes more widely deployed it is reasonable to expect that increasing wastes will be generated.

A) Current capability. The foundation we can build upon

<u>Air Emissions</u>

As discussed earlier, HEPA filters should be effective "end of pipe" controls provided there is a good seal between the filter and the filter housing / ducting. However there are a couple of "watch outs" to keep in mind:

1. Moisture present in some air streams can cause operational difficulties for HEPA filters (e.g. faster saturation). However this is not a nanoparticle problem, it is a problem of how to treat air in order to dry it before it reaches the filter. This is a

known problem in industry and there are various possible solutions depending on the specific process.

2. Nanoparticles are thought to behave somewhat like solids and somewhat like gases. HEPA filters do not capture gases. There is literature data [(1)] showing HEPA filters capturing 10 nm diameter sodium chloride particles, but what about smaller particles or materials other than sodium chloride?

In the case of organic air emissions, in principle Thermal Oxidizers should be effective in removing even nano materials.

The final factor we will need to ensure environmental safety with respect to air emissions is equipment and a sampling protocol to measure waste air streams.

Waste Waters

Cleaning of many industrial processes utilizes water wash downs of equipment. The waste water so generated is generally treated in some way either on site or, when it does not present a burden, in public water works.
There are well established techniques for measuring if a particular material is removed during on site or off site water treatment. These investigative approaches should still be applicable when nano materials are present in the waste water – provided we have a means to measure concentration of nanoparticles in dilute waste water receipts.

B) Gaps in our knowledge or capability

Water and Air Sampling instruments

We mentioned earlier that we need instruments to measure nano particulates in work place air to verify that exposures are at safe levels. Similarly we need instrumentation to measure nano particulates in waste water and in air emissions to verify removal from waste streams.

Better understanding of how nanoparticles behave in solid wastes

Incineration or chemical treatment of wastes containing nano materials will always be a conservative option for treating solid wastes. However nanoparticles will often be present at low levels (< e.g.1%).

The goal that produces a "win" for the environment and for the economy is to answer this question,

"What do we need to know about a specific nanoparticle in a specific solid waste so that the nanoparticle alone does not dictate the method of waste disposal?" Otherwise we let lack of knowledge about a small fraction of the waste dictate how the bulk is disposed of.

In very general terms the answer to the above is that we need knowledge in two areas:

1. The physical form of the nanomaterial in the waste - is it bound to other materials? Is it aggregated with other nano particles? If it is bound will it remain so under proposed storage conditions?

2. What is the environmental fate and environmental toxicity of the physical form/forms present in the waste?

C) Some means to close the gaps in our knowledge or capability

This is an area where instrument makers, academia and industry need to collaborate. How do nanoparticles change the game with respect to how we measure waste streams or characterize solid mixes? What new instrumentation or techniques, if any do we need?

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3.3.5 Problems and solutions of current manufacture of nanoparticles.

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INTRODUCTION

The main business of QinetiQ Nanomaterials Ltd (QNL) is to manufacture nanopowders tailored to meet the requirements of customers, combined with the ability to develop innovative solutions and targeted intellectual property around the application of nanomaterials. QNL was established in 2002 and is a wholly owned company within the QinetiQ Group Limited.

QNL is one of only a handful of companies that utilises a plasma vaporisation process to manufacture nanopowders in bulk. Currently production rates of 1-2kg/hour have been achieved. By controlling the process parameters a range of materials can be produced in a range of particle sizes. Pure metals, passivated metals, oxides, nitrides and other alloys and compounds can be produced together with more complex materials such as alloys, doped or non-stoichiometric ceramics, or layered particles can be produced. These materials have the ability to provide novel and enhanced properties across a very broad spectrum of applications ranging from biomedical and catalysts to packaging. To date examples of materials produced in the facility range from aluminium, copper and silver powders to copper oxide, zinc oxide, titania, zirconia and ceria with various different morphologies.

The production and handling of dry fine powders present a range of possible hazards from fire and explosion risks to potential long term health hazards including toxicity, respiratory and epidemiological effects. In the case of nanopowders the health risks associated with them are largely unknown.

Within the facility there are other possible hazards such as electrocution associated with the generation of a plasma via the use of high currents and asphyxiation hazards owing to possible leaks of inert protective gases during the process.

This paper will discuss the approach taken by QNL to assess and mitigate the risks identified. This includes how QNL has set up and safely operates the facility whilst meeting regulatory requirements and also the wider concerns that need to be addressed with the production, handling and use of nanopowders.

THE QNL TESIMA PROCESS

The process was originally developed under Ministry of Defence funding to produce aluminium nanopowders for energetic applications where faster burn rates were required

and subsequently expanded under EU and DTI funding. However the process offered the opportunity to enable many other nanomaterials to be produced for cross-sectoral applications. QNL set up an initial 700m^2 facility with two production units to commercially exploit the process. One of these rigs is currently dedicated to the production of aluminium powder while the second rig is used for the production of ceramic based materials such as oxides, carbides and nitrides. The latter rig can also be used to process mixed oxides or by controlling the atmosphere within the rig non-stoichiometric forms of materials can be produced. The production rates of the rigs are very dependent on the feedstock and vary between 0.5Kg/h and 5Kg/h.

The feedstock material used in the process can be in either wire or powder form which offers great flexibility and the ability to manufacture more novel materials from commodity products. The basic principle is that the feedstock material is fed into a high temperature plasma zone where it is instantaneously vaporised and then rapidly cooled by quench gases to form nanopowder which is transported to the collection system. Reactive gases can be introduced during the process to modify the surface of particles by coating or alternatively to change the nature of the particles e.g. to produce nitrides rather than oxides. Furthermore control of the atmosphere can enable non-stoichiometric materials to be produced. An example of this is reduction of white stoichiometric zinc oxide to a black conducting form that is of potential interest to the electronics industry.

The flexibility of the process in making different types of materials is illustrated in Fig 1.

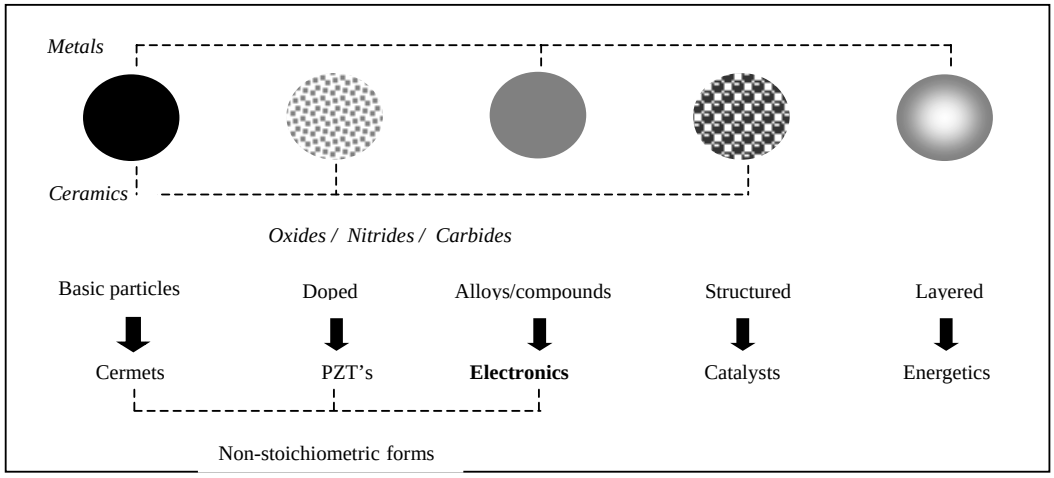

Fig 1 Schematic diagram showing range of possible types of nanopowders that can be produced.

The powders have a primary particle size in the range of 20nm to 100nm depending on the type of material and the processing parameters used. QNL uses a number of techniques to characterise the powders including scanning electron microscopy, specific surface area measurements (using BET measurements), X ray diffraction and laser particle size analysis.

Table 1 Survey of air quality conducted at QNL

Location	**No of particles cm^{-3}**
Office environment	30000-35000
Production facility	23000-52000
External to building	50000
Production rig exhaust	Up to 2000
Laminar flow booth (floor level extract)	50000-70000
Laminar flow booth (exhaust)	2000
Operators breath after smoking	Exceeded range of instrument

OUR PHILOSOPHY.

QNL aim is to manufacture powders safely without harm to our employees, neighbours and the environment. An initial survey of the air quality in the QNL facility has been conducted using a portable TSI Instruments Condensation Particle Counter, Table 1.

The survey indicated that particle concentrations within the facility tend to be lower than the outside environment or office environment and that particle emissions from rig exhaust are minimal indicating that powder collection systems are very effective. However it is currently not possible to differentiate between manufactured particles and those produced via traffic or general environmental pollution. It was quite apparent that particle concentrations associated with processing of powders are likely to be lower than those produced by cigarette smoking.

In view of the lack of hazard data for nanopowders our approach is to treat every material that is processed as a potential hazard and aim to contain powders as far as possible, use local exhaust ventilation and/or extraction facilities fitted with high efficiency filtration and ensure that our employees use suitable respiratory protection, gloves, overalls and eye protection.

All waste powder material and contaminated waste such as disposable gloves, masks and cleaning cloths are stored separately and disposed of as hazardous waste by an approved contractor. Powder is never disposed of via drainage systems.

POTENTIAL HAZARDS ASSOCIATED WITH THE PRODUCTION OF NANOPOWDERS.

The production and handling of fine powders of any description has the potential to introduce additional hazards to the workplace. These can include ingestion, inhalation, epidemiological and toxicological hazards as well as fire and explosion hazards associated with the production of fine metal powders, asphyxiation risks associated with the use of large amounts of inert gases and electrocution hazards owing to the high currents used during the QNL Tesima process. In order to safely manufacture

nanopowders QNL has had to introduce controls, systems and procedures to prevent and/or eliminate these risks.

In general a risk, COSHH and technical assessment is conducted prior to processing and the manufacturing parameters defined and controlled. A high level of cleanliness and good house keeping is an essential part of the process and a vacuum cleaning system specifically designed for collection of hazardous powders is used. To minimise uncontrolled powder release procedures have been developed to contain powders as far as is practicable and all powder handling is conducted within a dedicated area inside a laminar flow booth or alternatively within a glovebox suite.

At present the toxicological effects of nanopowders have not been determined although there are an increasing number of reports on possible adverse health effects, but without any clear conclusions being drawn. As a result this paper concentrates on the more immediate effects of fire and explosion hazards associated with the production of aluminium nanopowders.

CONTROLLING FIRE AND EXPLOSION RISKS.

Metal in finely divided form is often described in Material Safety Data Sheets as being possibly pyrophoric or explosible. In particular aluminium powder in micron sized or flake form is well known to exhibit this behaviour and experience has shown that in extreme cases complete destruction of production facilities has occurred. However despite this aluminium powders and flakes are safely used in a wide range of applications such as automotive paints, insulating building blocks and energetic applications such as rocket motors.

The relative explosibility of metal powders is illustrated in Table 2, where it can be seen that aluminium is considered to be highly explosible[1].

Table2 Relative explosibility of metal dusts[1]

Explosivity	**Metallic dust**
High	Zirconium, Magnesium, Aluminium, Lithium
Medium	Tin, Zinc, Iron,Silicon,Manganese
Low	Molybdenum, Cobalt, Lead

The explosible nature of the powders varies dependent on powder particle size and the characteristics of the production process which may result in different amounts of fine particles. Hence it is not entirely safe to solely rely on literature reported explosibility data. Typical explosibility data for aluminium powders and flake in different nominal particle sizes are shown in Table 3[2] . The data shows that in the event of an explosion the rates of pressure rise approach 1400 bar/sec for the finest powder and flake and Pmax values can reach up to 12.9 bar i.e. the maximum pressure that can be produced in standard 20 litre sphere tests.

At the time of the design of the Tesima process there was no dust explosion data available for aluminium nanopowders. Consequently the rig was designed to be operated using an inert argon atmosphere, where the oxygen content would be controlled and to contain a maximum explosion pressure of 16 bar (to take into account the highest recorded value of explosion pressure (Pmax) for conventional aluminium) and proof tested to 24 bar to meet the requirements of the Pressure Systems Directive. Furthermore water cooling of the rig would be avoided to prevent the possible formation of hydrogen and steam in the event of leakage which could lead to an explosion. It has been shown that there is a vigorous reaction between boiling water and nano-aluminium powder. The process has now been successfully and safely producing aluminium nanopowders on a regular basis.

Table 3 Influence of particle shape and size on the explosability data for aluminium powders[2]

Description of dust	MIT °C	**MEC** g/m3	**MIE** MJ	**Pmax bar**	**DP/dT bar/sec**
Flake	610	45	10	8.8	1380
6 µm	420	30	13	6.4	1331
17µm	610	40	28	7	621
29µm	610	30	ND	12.9	430
100µm	ND	ND	ND	5.4	135

A limited range of explosibility data for one batch of aluminium nanopowder produced via the Tesima process has been determined. This data is compared with data for nanopowder produced by an exploding wire process in Table 4[3,4]. These sets of data have to be treated with some caution since the age and storage conditions for the powders prior to testing are not known, and there may be differences in the particle size characterisation of the powders. Further work is underway to characterise Tesima produced powders.

Table 4 Explosibility data for aluminium nanopowder produced by two different processes[3,4]

Particle size um	**Pmax Bar**	**DP/dT Bar s^{-1}**	**Kst Bar m s^{-1}**	**St Class**	**MIE**	**MIT** °C	**LOC %**
90[3]	7.9	671	182	1	-	550	-
180[4]	9.4	1185	322		3		5

The data indicates that the rate of pressure rise is still relatively high and the St class can be classified to be either moderately explosive or severely explosive depending on the powder. The Pmax values in the range 7.9 to 9.4 bar are lower than originally anticipated but it is significant that the Minimum Ignition Energy for the 180nm powder is in the range of 1-3 MJ) which indicates that the powder is very sensitive to ignition and could be ignited from static electricity.

EQUIPMENT SELECTION CONSIDERATIONS.

The QNL facility has been set up in a large building that was previously used to house a wind tunnel and as a consequence new equipment and all new services has had to be installed to meet the new use.

In setting up the powder production facility consideration had to be given to not only producing powder in a controlled manner but also to be able to subsequently safely process and handle the powder. Initially it was considered that the use of a glove box suite would be required since this would have the advantage that the powder could be handled in an inert gas, low oxygen, low humidity environment which would be ideal for preserving the powders. However gloveboxes can be difficult to work with and can result in operator fatigue, but there were other disadvantages too as summarised in Table 5.

Table 5 Advantages and disadvantages of using glovebox facilities

- **Ensures inert low oxygen low humidity atmosphere is maintained and ideal for preserving powders in best environment but**
- **Can be difficult to work with resulting in operator fatigue**
- **End users may not have access to theses facilities and hence could be exposed to danger on opening goods.**
- **What happens inside if controlled atmosphere is lost?**
- **How do you maintain glovebox in a clean condition?**
- **What happens when unoxidised material is removed from glovebox?**
- **Inadvertent accidents could result in containers breaking inside passport system**

Consequently after considering the approaches taken by the pharmaceutical industry where laminar flow booths are often used for powder handling operations this was selected to be the best solution for QNL and a suitable system was designed and manufactured for handling and processing nanopowders. The system is designed to extract powders downwards away from the operators breathing zone and incorporates two main filters which are bag sealed on removal from the system to prevent the release of powders into the atmosphere. It also incorporates explosion venting to minimise the effect of a possible explosion, and earthing of the booth and handling table to prevent build up of static electricity which could lead to ignition of the powder.

One of the key issues in powder production facilities is to maintain good house keeping and cleanliness practices to minimise build up of powders which could at some point be ignited. Powders should be segregated to avoid the possibility of self ignition and thermite reactions. The latter reactions can be very vigorous. In general the principles outlined in the Fire/ aluminium association standard are followed[5]. Cleaning can be achieved via the use of natural bristle brushes but vacuum cleaning systems are more effective. However vacuum cleaners need to be specifically designed to handle hazardous

powders to prevent ignition of powders and possible release of particles via the filters. In the powder processing industry it is recognised that improperly designed vacuum cleaning systems can prove hazardous.
QNL was faced with the decision as to whether or not to use a vacuum system. In general vacuum systems for hazardous materials can be electrically driven or compressed air driven, but from practical experience the latter type tend to be noisier and less effective but has the advantage that there is no risk of sparks from motors. A centralised system incorporating explosion venting which could be located in a safe area seemed to be the ideal solution but this sort of system would have been prohibitively expensive for the relative size of the QNL facility.

The best solution was considered to be an electrically driven mobile system that has an induction motor to eliminate the risks of sparks, and was sufficiently powerful enough so that it could be located in a safe area external to the main facility should an uncontrolled event occur. The system was fitted with conductive hoses and suitable earthing to prevent possible ignition from static electricity and fitted with high efficiency filters. Procedures were put in place to ensure that waste material was removed from the system as soon as the cleaning operation is complete and placed in suitable storage containers to await controlled disposal. The factors influencing the selection of vacuum cleaning facility are summarised in Table 6.

Table 6. Factors influencing choice of vacuum cleaning system

- **Need to be designed to handle hazardous powders, from an ignition view point and possible release of particles.**
- **Centralised vacuum collection facility or mobile vacuum cleaner?**
- **Electrically driven or compressed air driven**
- **Induction motor to reduce /eliminate risk of sparks**
- **Conductive hoses**
- **Suitable earthing**
- **High efficiency filtration**
- **Ease of cleaning/removal of waste – as soon as cleaning operation is complete empty and store contents in suitable containers to await controlled disposal.**
- **Mobile system selected but powerful enough to be used while located in a safe area outside the facility**

GENERAL PRECAUTIONS.

Training personnel in the safe handling and understanding ignition of powders and control of powder fires is vital in the safe operation of the facility. Fire extinguishers suitable for metal powders i.e. Pyromet powder extinguishers containing Met-X agent (which consists of ~93% graphite) are provided within the facility. However experience has shown that they can prove hazardous if incorrectly applied. The extinguishing agent is very light and tends to create a dust cloud which can reduce visibility and can be

inhaled but it also settles on the ground proving very slippery and can possibly infiltrate electrical equipment causing short circuiting.

As a result additional precautions have been provided which include a steel container with a loose lid to enable smouldering/burning powder to be safely placed inside, enable oxygen to be excluded and the contents to be removed to a safe area. An inert gas supply is also readily available which can be used to exclude oxygen from the powder using a gas wand at a safe distance.

It is also important to ensure that Material Safety Data Sheets are readily available for the Emergency Services and should be located with Fire Evacuation documents.

CONCLUSIONS AND ISSUES THAT NEED TO BE ADDRESSED

1. Metal and inorganic compounds can be safely manufactured.
2. The toxicological and epidemiological hazards posed by nanopowders needs to be determined in a systematic way reflecting exposure levels likely to be encountered in industry.
3. As it is likely that the hazards testing is likely to take several years to complete a cautious approach should be taken when handling nanopowders.
4. It is unclear how exposure limits can be established for nanopowders, and how compliance can be achieved. At present it is only possible to measure numbers or concentrations of nanoparticles. Perhaps the more pragmatic approach would be to specify minimum levels of protection required for workers.
5. New standards for testing and guaranteeing suitability of filtration and respiratory protection for use in the manufacture and processing of nanopowders may be required.
6. At present nanopowders produced from existing elements and compounds are not considered to be new substances and as a result there is no requirement for registrations under the Notification of New Substances regime however the Royal Society report suggested that this may be required. The position needs to be clarified.
7. UN Dangerous Goods Regulations seem appropriate for the classification of nanopowders. The question is whether a more precise categorisation is required e.g. is it appropriate for aluminium nanopowders to be included under the under Class 4 .1 or 4.3 i.e. UN3178 or 3132 for flammable solids or UN1396 for uncoated aluminium powder or should an additional category be raised?
8. Consideration should be given as to whether additional CHIP hazard labels are required for nanopowder materials.

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5. American Fire Safety Standard, NFPA 65, Processing and finishing of aluminium.

3 POSTER ABSTRACTS

Abstracts were invited for posters on subjects relevant to the health and safety implications of the production and use of nanomaterials in the workplace. 19 abstracts were accepted and they were all presented after Session 3 on the second day.

The quality of the posters was reviewed by a small panel of judges comprising Dr Mike Roco (NSF, USA), Dr Derk Brouwer (TNO, NL) and Dr Paul Oldershaw (HSE, UK). The prize for the best poster was awarded to Julie Muller et al, Respiratory toxicity of carbon nanotubes.

The abstracts are presented in full in the following section.

A Pilot Study of Nanoparticulate Exposure in a Saturation Welding Habitat.

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University of Aberdeen Department of Environmental and Occupational Medicine and Department of Chemistry*, University of Edinburgh Centre for Inflammation Research**

There is an increased prevalence of cognitive complaint in divers for which work as a welder is an important risk factor (1). Ultra-fine particle exposure may be a causative factor. Accordingly, we carried out a pilot study to determine whether dust measurement was feasible during hyperbaric welding operations and to give a semi-quantitative estimate of exposure.

Dust monitoring was with the TSI Incorporated Dust Trak Aerosol Monitor for PM 10, PM 2.5 and PM 1. Ultrafine particles were measured as particles ml^{-1} using the TSI Incorporated P-Trak. Sampling was carried out nine times. PM1, PM2.5 and PM10 were estimated on at least one occasion and ultrafine particles were estimated on seven occasions. PM 1, 2.5 and 10 were all found to rise in the chamber during welding operations but did not exceed statutory limits. The major component of the dust was in the smaller particles. Ultrafine particle levels as high as $2x10^6$ ml^{-1} were observed with peaks of $1x10^6$ ml^{-1} being seen during four of the analysis sessions.

Samples of the dust were taken from a 30 μm filter in the chamber ventilation system. The dust was digested in nitric acid and then measured by inductively-coupled plasma mass spectrometry. Besides the major elements such as iron and manganese, each at a level of about 1%, some unexpected metals were found in high concentration such as lithium, zirconium and gallium in addition to the expected metals such as chromium, nickel and zinc.

The dust was assessed for toxicity in a human alveolar epithelial cell culture system.
The welding fume was as cytotoxic as quartz. Estimation of the inflammatory marker chemical, interleukin 8, was complicated by this. Nevertheless, Aberdeen welding fume dust was estimated to be 6-10 times more inflammatory than aged dust from Nimrod c276 rods.

High levels of ultra-fine particles were seen and the dust was unexpectedly cytotoxic. This area merits further study.

Reference

1 Macdiarmid J, Ross JAS, Taylor CL, Watt SJ, Adie W, Osman LM, Godden DG, Murray A, Crawford JR, Lawson A. Co-ordinated investigation into the possible long term health effects of diving at work. http://www.hse.gov.uk/research/rrhtm/rr230.htm HSE Research Report 230. HSE Books 2004

Integrated Research Centre on the Relative Safety of Nano-Materials

RJ Aitken[1], K Donaldson[2], J Ayres[3], V Stone[4], CL Tran[1]
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In their recently published review of the opportunities and uncertainties concerning Nanoscience and nanotechnologies, the Royal Society and Royal Academy of Engineering recommended that "Research Councils UK establish an interdisciplinary research centre to research the toxicity, epidemiology, persistence, exposure pathways and bioaccumulation of manufactured nanoparticles" and to "develop methodologies and instrumentation for monitoring them".

In response to this we have brought together a multi-disciplinary consortium of internationally recognized researchers, from the IOM in Edinburgh and from Edinburgh, Napier and Aberdeen Universities, which combines unique expertise and state-of-the art approaches to identify, characterise and estimate the relative safety of nanoparticles.

Our mission is:

- to become the UK centre for advice on the potential health, safety and environmental impacts of nanomaterials,
- to develop and maintain a national and international network of researchers and regulators,
- to maintain an accessible database of research and outcomes and to develop and implement a research program including;
- Development of suitable and practical methods for measurement of manufactured nanoparticles.
- Development of internationally agreed protocols and models for investigating the routes of exposure and toxicology to humans and non-human organisms of nanoparticles in the workplace, the indoor/outdoor human environment and the eco-system, including investigation of bioaccumulation.
- Epidemiological investigation of the inter-relations of exposure and health outcomes in those industrial and environmental processes.
- Develop an understanding of the transport and behaviour of nanoparticles and tubes in air, water and soil, including their interactions with other chemicals.
- Fundamental studies of the mechanisms of interaction of nanoparticles with cells and their components, particularly the effects on blood vessels, the skin, heart, liver and the nervous system.
- Development of protocols for in vitro and in vivo toxicological studies of any new nanoparticles and nanotubes likely to go into large-scale production and which could impact people or the natural environment.
- Investigation of the absorption through skin of different commercial nanoparticles used in dermal preparations, in particular any changes that may occur if the skin is damaged before application.
- Establishment of protocols for investigating the long-term fate of nanoparticles.
- Determination of the risk of explosion associated with bulk nanopowders.

We are currently holding dialogue with other interest groups, including industry, international governmental agencies and stakeholder organisations.

Nanoparticles: An Occupational Hygiene Review

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Nanoparticles are the end products of a wide variety of physical, chemical and biological processes some of which are novel and radically different, others of which are quite commonplace. We have reviewed processes for the deliberate development and manufacture of nanoparticle products and have considered sources and routes of exposure, levels of exposure, numbers exposed, knowledge gaps and future trends. We conclude that all of the four main groups of nanoparticle production processes may potentially result in exposure by inhalation, dermal or ingestion routes and that little is known about current levels of exposure. Control approaches are available which should be effective for exposure by inhalation but this has not been demonstrated. Control approaches for dermal and ingestion exposure may not be as effective as they are for larger particles. Surface area is probably the best exposure metric for some but not necessarily all nanoparticles but there are no effective methods by which this can be measured in the workplace. Current knowledge is insufficient for risk assessment purposes. The number of workers who may be exposed to nanoparticles in the university sector and in emerging nanoparticle companies may be as high as 2000. Around 100,000 individuals may potentially be exposed to fine powders through various powder handling processes. It is not possible to say what proportion of these may be exposed to nanoparticles although it is likely to grow. More that 1,000,000 workers in the UK may be exposed to nanoparticles via incidental production in processes such as welding and refining.

In summary, we conclude that there is little evidence to suggest that the exposure of workers arising from the production of nanoparticles has been adequately assessed.

Occurrence of Ultrafine Aerosols at Workplaces

Carsten Moehlmann
BIA, Germany

Problem

There appears to be a particular health risk from ultrafine dust particles in respiratory air. Ultrafine aerosol particles are for the most part the product of condensation in thermal and chemical reactions. Typical examples include welding fumes, metal fumes, polymer fumes, technical soot particles, amorphous silicic acids, and particulate diesel motor emissions. The primary particles that are thus created have a size of only a few nanometres (nm). They also agglomerate directly after formation and form even larger particles.

Activities

BIA (the institute for occupational safety and health of the institutions for statutory accident insurance and prevention), in conjunction with the institutions for statutory accident insurance and prevention, carried out a measurement programme at selected workplaces. The aim was to gather and catalogue technical measurement information on ultra-fine particles occurring at different work processes. The particle size distribution between approximately 10nm and 500nm and the number concentration of these particles were determined. BIA was equipped with a suitable measurement device (scanning mobility particle sizer – SMPS) for these particular measurements.

Results and Application

The poster will show some typical results from workplace measurements of ultrafine aerosols and a comparison of different industrial processes. Using the resulting data sets on ultrafine aerosols at workplaces, the institutions for statutory accident insurance and prevention hope to contribute to a helpful discussion on this topic, and improve the possible methods of prevention.

Proceedings of BIA Workshop "Ultrafine Aerosols at the Workplace", August 2002

Carsten Moehlmann
BIA, Germany

In August 2002, experts from Germany, Finland, Austria, Switzerland, and the USA presented their research results and findings on the topic of ultrafine particles at an interdisciplinary workshop held by the BG-Institute for Occupational Safety and Health (*Berufsgenossenschaftliches Institut für Arbeitsschutz*) - BIA.

The following topics were handled at the workshop:

medical aspects,
toxicology of ultrafine particles / epidemiology,
physics of ultrafine particles / measurement techniques and technology,
ultrafine aerosols at industrial workplaces, and
discussions on whether exposure limit values should be introduced.

The report relates the presentations and summary excerpts of the discussions on each topic.

The poster will give general information on the proceedings.

Effect of Cutaneous Application or Subcutaneous Administration of Zinc Oxide Particles of Different Size – A Preliminary Study

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There has been increasing concern about the possible toxicity of very small particles. Animal experiments have demonstrated that small nanoparticles (<50 nm) have greater inflammogenic potential and this is consistent with their physicochemical properties. Zinc oxide and similar particles are increasingly being used in cosmetics, however very little work has been carried out on possible cutaneous toxicity.

The preliminary experiments described below were designed to investigate whether size had an influence on penetration of ZnO into the skin or on the response to subcutaneous tissue. Zinc oxide particles of two sizes (~1000 nm and ~20 nm) were purchased from Aldritch and applied suspended in a cream or a permeablising agent (ethanol) or were injected as a saline suspension into subcutaneous tissue. Appropriate controls received the vehicles only. Each group consisted of 3 animals. Zinc levels were analysed in the skin, liver and kidneys. Skin samples were also taken for histological examination.

The study did not produce any evidence of cutaneous toxicity of either size zinc oxide particles. Zinc concentrations in the skin remained unchanged following application of zinc oxide in a cream. Administration in ethanol significantly increased zinc concentration in skin but there were no significant variations between small and large particles. Following subcutaneous administration, zinc levels were significantly increased in the body wall taken below the injection site, however again there was no difference between the large and small particulates. Finally, the granulomas induced by implantation of nano-sized powder appeared encapsulated, larger and had more extensive areas of necrosis than those conventional powders of ZnO. However the small number of animals used in these experiments, sampling problems due to the nodular nature of the lesions and the presence in both zinc oxide preparations of aggregates make interpretation difficult. Our experiments hence give no evidence that the toxicity of commercially available zinc oxide nanoparticles differs grossly from that of more conventional preparations.

Information Management for Nanotechnology Safety and Health

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Efforts to ensure the health and safety of nanotechnology workers and members of the public could be substantially enhanced by a coordinated approach to information management. Specific objectives would be to classify nanomaterials and nanoparticles with universal models, share information across industries and organizations, understand routes of exposure and the interrelationships of particle size, surface area, bioavailability, and mechanisms of action for disease; generalize control strategies across industrial processes and classes of materials; develop national and international consensus standards; and be prepared for surprises. The approach to developing an effective information management system will be to engage partners, evaluate candidate information systems, and adopt a Geographical Information Systems (GIS) approach to track multiple layers of information on a local, regional, and global basis. A GIS approach will provide compatibility with the extensive census, epidemiological, and health-based information systems that have already been developed. Candidate database elements might include material type, structural form, composition, application, industry, physicochemical characteristics, toxicology results from studies in laboratory animals, observed health effects in humans, epidemiology, opportunities for health surveillance, and applicable health risk models and exposure limits. A prototype information database has been developed to illustrate how data of interest may be organized and shared. Dialog and collaboration are being sought to refine and apply the information management system.

A Quantitative Risk Assessment in Workers Using Rodent Dose-Response Data of Fine and Ultrafine Titanium Dioxide

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As with traditional materials, compared to the general population, workers may be exposed to the highest levels of nanomaterials. Under the assumption that the rat model is a useful predictor of human risk, we used data from published inhalation studies in rats exposed to titanium dioxide (TiO_2) in an exploratory risk assessment. The available data included fine/ultrafine particle fractions, chronic/subchronic exposures, and cancer/noncancer lung responses. The dose metric selected for these analyses was particle surface area dose in the lungs (converted from particle mass lung dose) because it is a strong predictor of pulmonary response to inhaled particles. Several statistical models were used to estimate the doses associated with specified excess risks of lung tumours. Approaches for synthesizing the risk estimates from these different models included Bayesian model averaging. Regression models were also fit to rat pulmonary inflammation data (measured as polymorphonuclear leukocyte, PMN, cell count in bronchoalveolar lavage fluid); and doses associated with the upper 5% of the distribution of PMNs in unexposed rats were estimated. Human lung dosimetry models were used to estimate the 45-year working lifetime mean exposure concentrations that would result in mass lung burdens equivalent to the particle surface area doses identified in the rat model (adjusting for species differences in lung tissue mass). Under these assumptions and modeling strategies, the maximum likelihood estimated working lifetime mean concentrations associated with a 1/1000 excess risk of lung tumors ranged from 0.9 to 11 mg/m^3 for fine TiO_2 and 0.07 to 1.0 mg/m^3 for ultrafine TiO_2. For pulmonary inflammation, these working lifetime concentration estimates varied from 1.4 to 2.6 for fine TiO_2 and 0.12 to 0.22 mg/m^3 for ultrafine TiO_2. These results reflect the greater toxicity observed in rodent studies of ultrafine particles compared to an equal mass of larger particles of similar composition.

Aldehydes as Biomarkers of Lipoperoxydation: Development of an Analytical Method for Assessing the Oxidative Stress of Diesel Exhaust Exposed Workers. Preliminary Results

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A central hypothetical mechanism to explain the adverse effects of particulate matter (and particularly the fine/ultrafine size fraction) is their ability to generate reactive oxygen species (ROS). Such a process has been postulated for particles like PM_{10}, asbestos and man-made mineral fibers or ultrafine TiO_2 or diesel exhaust particulates (DEP) [1, 2].

One way these ROS are acting at the cellular level is through the oxidation of the cell lipidic membrane, leading to lipoperoxidation. This oxidative stress triggers the formation of a wide variety of degradation products, including carbonyl compounds including mainly n-alkanals (such as hexanal), 2-alkenals (such as trans-2 hexenal) and hydroxyalkenals (such as 4-hydroxynonenal). They are relatively stable and able to diffuse out of the cell. They could therefore be appropriate biomarkers of lipoperoxidation and oxidative stress.

An analytical method for aldehydes determination in urine and serum has been developed and very preliminary results for these biological matrices are presented here. Sample preparation is consisting of a derivatization step with pentafluorobenzylhydroxylamine followed by an isolation on reverse phase C18 SPE cartridge. For hydroxylated aldehydes, like 4-hydroxynonenal (4-HNE), a second derivatization step is achieved with BSTFA+TMS. Quantification is done by GC-MS ion trap in SIS mode.

Method applied to a healthy volunteer's urine and to a smoker's serum gave the following preliminary results:

	Urine		Serum		
	Hexanal	Octanal	Hexanal	Nonanal	4-HNE
Conc [nmol/ml] ± std dev	0.34±0.09	0.21±0.02	16.4	6.0	1.7
LOD[a] [nmol/ml]	0.10	0.12	4.4	4.1	0.3
Recovery [%] (sem)[b]	107 (2)	101 (1.5)	84 (3)	107 (10)	99 (4)

[a] Limit of detection
[b] sem: standard error of the mean

This method seems to be sensitive enough to be applied for aldehydes determination in human urine or serum. Next steps will be to assess the robustness of this method and to determine if such compounds could be useful biomarkers for oxidative stress, considering DEP as the causative agent.

[1] Donaldson K et al. *Toxicol Letters*, 1996, 88:293-298
[2] Hiura T.S., et al. *J.Immunol*, 1999, 163:5582-5591

Mixed Exposure Issues for Nanotechnology Safety

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The National Occupational Research Agenda (NORA) was initiated in April 1996 by the National Institute for Occupational Safety and Health (NIOSH) and its business, labor, academic, and government partners. NORA is directing and focusing occupational safety and health research in 21 areas to produce improvements in worker safety and health. Information about NORA is available at www.cdc.gov/niosh/. The NORA Mixed Exposures Team is considering mixed exposure scenarios relevant to nanotechnology safety and health, including health concerns for nanoparticles and nanomaterials in a range of particle sizes; mixed dermal and inhalation exposure pathways; multiple simultaneous exposures to individual agents; exposures to different agents at different times in workers' lives; generic classes of nanoparticles or nanomaterials with multiple components; and the combined effect of other stressors such as heat on nanoparticle exposures and behavior. Insights into health and safety issues for nanotechnology may come from lessons learned in studies of complex mixtures such as asphalt fumes, welding fumes, fossil fuels, and combustion products. The NORA program is also interested in the possibility that advances in nanotechnology may create tools to better enable research on mixed exposures; e.g. development of nanosensors that can be used to study health effects from exposure to mixtures (not necessarily involving nanoparticles). The NORA Mixed Exposures Team invites collaboration to improve the anticipation, measuring, modeling, and mitigation of potential health effects from nanotechnology activities.

Respiratory Toxicity of Carbon Nanotubes

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Carbon nanotubes (CNTs) are a new type of crystalline carbon material focusing the attention of many scientists because of their huge potential of industrial applications. However, there is still a lack of information on the toxicological properties of these particles. The aim of this experimental study was to characterize the potential pulmonary toxicity of multi-wall CNTs (MWNTs) in a rat model (i) by assessing the biopersistence of CNTs in the lung and (ii) by characterizing the pulmonary response after exposure to CNTs in comparison with particles such as asbestos fibers and carbon black. We administer MWNTs intra-tracheally in single doses (0.5, 2 and 5 mg) to Sprague-Dawley rats and assessed lung inflammation and fibrosis biochemically and histologically. We observed that CNTs were biopersistent in the lung and induced inflammatory and fibrotic reactions of the same intensity as asbestos fibers. Pulmonary lesions induced by CNTs are characterized by the formation of collagen-rich granulomas in the bronchi as well as interstitial inflammation (alveolitis). These results suggest that CNTs are potentially pneumotoxic particles.

Potential Environmental Impacts of Nanomaterials

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A recent Royal Society report highlighted the lack of information on the environmental safety or distribution of nanoparticles in soil, water and air or through food chains. From an environmental perspective the greatest concerns are:

- The use of nanoparticles in remediation where deliberate release is likely to result in locally high levels;
- The disposal, destruction or recycling of materials containing nanoparticles;
- Their use in consumer products, e.g. sunscreens, which may result in their being released directly into the environment, e.g. through bathing in the sea, or through waste water.

There is an extensive literature on environmental risk assessment methodology for chemicals, such as pesticides. However, there is a need to assess the applicability of such approaches to nanomaterials due to their distinctive characteristics. Currently the use of a chemical in nanoparticulate form does not require additional testing under UK chemicals regulation, or under the European REACH proposals. It is likely that nanomaterials behave very differently in the environment to their 'standard' forms. There have been only a handful of studies so far into the likely impact of nanoparticles on living organisms. Some of these preliminary studies have shown that nanomaterials may cause extensive, non-specific damage to cellular structure and function. In determining the environmental risks from nanomaterials, the impact of both the absorbed materials on individual organisms, and on ecosystem function, e.g. soil nitrification and respiration, need to be considered. In addition, the impact of possible accumulation of nanomaterials in wildlife food items, such as earthworms, or uptake in plants that may result in transfer to higher vertebrates, should also be considered. Adventitious contamination by deposition of nanomaterials via air pollution may also have impacts, e.g. on pollinators, through collection of deposited particles with pollen from flowering crops.

Novel Techniques for Characterization of Ultrafine Metal Aerosol Formation: Lessons from Diesel Combustion

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Many nanoparticles and nanomaterials involve carbon matrices in conjunction with metallic constituents. Insights into possible acute and chronic health effects from exposures to such nanomaterials may come from studies of diesel particulate matter (DPM). One theory holds that the potentially large number of ultrafine particles and their characteristic high lung-penetration efficiency may play a role in toxicity and that the presence of metals may be a contributor. This poster will discuss research approaches, insights, and findings to date from an investigation to characterize the metal content of diesel nanoparticles. NIOSH conducted this work in collaboration with the University of Minnesota. For the study, DPM was generated by a 1.5-liter diesel engine and ferrocene was added to the fuel in varying amounts to enhance the level of metal (in this case iron) in the system. The exhaust particles were analysed in real time using a recently developed single particle mass spectrometer (SPMS). In parallel, size selected samples were taken for subsequent analysis using transmission electron microscopy/energy dispersive x-ray spectroscopy (TEM/EDS). Results show that at a threshold Fe/C value of 0.013 (for this engine), self-nucleated metallic nanoparticles are formed and their number and size increase with level of doping. Iron-bearing particles that span a larger size range are also formed and it is observed that the metal to carbon ratios are greater for smaller particle sizes. Hydrogen to carbon ratios were measured and those ratios also increase for smaller sized particles. Agglomeration of metallic and carbon particles is observed in two distinct modes: attachment of iron primary particles (5-10 nm in diameter) to carbon agglomerates, and coagulation of iron agglomerates (20-200 nm in diameter) with carbon agglomerates. Results of this work imply that the generation of metallic nanoparticles could be fostered as new engine technologies reduce carbon levels in the engine, which could, in turn, potentially create a new health concern. The characterization approaches and insights from this work may guide the focus and design of studies to evaluate possible acute and chronic health risks of exposures to metal-containing nanomaterials.

NIOSH Nanotechnology Safety and Health Research Program

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A number of active research programs within the National Institute for Occupational Safety and Health are investigating ultrafine and nanoparticle behavior and the health risks associated with nanomaterials. A NIOSH Nanotechnology Research Center is being developed to coordinate institute-wide nanotechnology-related activities. The Institute is also working with other agencies to address health issues associated with nanotechnology, including participation in the National Nanotechnology Initiative (NNI) and the Nanoscale Science, Engineering and Technology Subcommittee (NSET) of the National Science and Technology Council committee on technology. Building on these initiatives, NIOSH is developing a strategic plan to address immediate and long-term issues associated with nanotechnology and occupational health in partnership with other federal agencies, research centers, and industry. The NIOSH Nanotechnology Health and Safety Research Program is a five-year multidisciplinary study into the toxicity and health risks associated with occupational nanoparticle exposure. The program currently includes projects on Nanotechnology Safety and Health Research Coordination, Generation and Characterization of Occupationally Relevant Airborne Nanoparticles, Pulmonary Toxicity of Carbon Nanotube Particles, the Role of Carbon Nanotubes in Cardio-Pulmonary Inflammation and COPD-Related Diseases, Particle Surface Area as a Dose Metric, and Ultrafine Aerosols from Diesel-Powered Equipment. Other projects are monitoring nanoparticle exposures with respect to aerosol surface area concentration, assessing risk for nanoparticle exposure, assessing filter bypass leakage and nanoparticle recirculation in the workplace, evaluating the surface activity of inhaled particles, evaluating occupational nanoparticle exposures, and characterizing metallic nanoparticles from diesel combustion. Additional information about the NIOSH nanotechnology safety and health research program can be found at http://www.cdc.gov/niosh/topics/nanotech/.

Comprehensive Characterization Strategies for Ultrafine Particles: Lessons from Beryllium Health and Safety Studies.

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Information on the physicochemical and bioavailability properties of nanoparticles is needed to help identify appropriate metrics of exposure (e.g., airborne mass, particle size, surface area, and/or chemical composition) for protecting worker health. Knowledge of appropriate exposure metrics would aid in developing exposure limits based on bioavailability through the inhalation and dermal routes of exposure. Thus, a suite of standard analytical techniques for characterization of properties of nanoparticles would be of value. Experience from studies of ultrafine beryllium aerosols demonstrate the capabilities and limitations of physicochemical and bioavailability characterization techniques as well as practical considerations for analysis.

Traditional analytical techniques and methodologies for ultrafine particles (e.g., microscopy, electron and x-ray analyses, surface area, and spectroscopy) are applicable for nanoparticles. Care must be taken to ensure that particle properties measured in the laboratory are representative of the particle properties in their native environment. These considerations would include the type of test (destructive or nondestructive), and influence of sample preparation (heating, drying, etc.) and analysis (e.g., beam probe-particle interaction) on the particle sample.

Appropriate cell-free in vitro models are needed to study bioavailability of a material in a target biological compartment. Standard dissolution solvents include serum ultrafiltrate (SUF), a simulant of extracellular lung fluid having neutral pH; artificial sweat, a simulant of skin surface fluid having pH 6.5; and phagolysosomal simulant fluid (PSF), a simulant of the macrophage phagolysosome having pH 4.5. Traditional dissolution techniques, e.g., static, flow-through, etc. are applicable for nanoparticles. Note that use of membrane filters having pore sizes of 0.025 μm or greater to separate the particle sample from the dissolution solvent in these techniques may not always be feasible for nanoparticles. Thus, alternative approaches to preventing solid material from migrating into the solvent to bias dissolved mass measurements may be needed, e.g., isolating via centrifugation, ultrafiltration, or using density gradient separation.

Welding Fume Number Concentrations – Laboratory Measurements

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Welding fume consist of various metallic compounds, and adverse health effects are related to inhaled fume. Although the HTP-values of the fume compounds are typically defined as mass concentrations the median particulate size of the fume is measured to be rather in the fine particulate size fraction than in the coarse size fraction. Number–weighted particulate size distribution and total number concentrations describe fine and ultra fine size fractions more precisely.

Welding fume number concentrations were measured by using Scanning Mobility Particle Sizer (SMPS). The SMPS consisted of DMA and CPC (Type 3010, Tsi Inc.). The studied welding processes were Gas Metal Arc Welding (GMAW), Manual Metal Arc Welding (MMAW) and Flux Cored Arc Welding (FCAW).

The welding was conducted manually or by robot in a chamber. The samples were taken from the discharge air channel of the welding chamber. The chamber supply air was filtered through HEPA filters.

There were high number concentrations in the fine particulate size fraction, and most of the processes generated bimodal size–distributions. The median diameter[1] for the most frequent mode was about 90 - 200 nm and for the other, not so frequent mode over 200 nm. The measured particle size fraction was from about 10 nm to about 450 nm.

FCAW measurements were carried out with combination of different values of welding parameters: wire feed speed (*wfs*), current (I) and voltage (U). Different parameter combinations produced different total number concentrations [cm^{-3}], and different size distributions. Total number concentrations varied within 10^5–$10^7 cm^{-3}$, and the median diameter for the most frequent mode within 90–200 nm for all studied welding processes.

[1] electrical mobility equivalent

Cellular and Molecular Interactions between Nanoparticles and Macrophages

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The expansion of nanotechnology and hence the potential for human exposure requires that the toxicology of nanoparticles is investigated. In previously studies we have reported that carbon (14 nm diameter) or polystyrene (54 nm diameter) nanoparticles generate reactive oxygen species (ROS) that induce oxidative stress macrophage cell lines leading to the induction of cytosolic calcium signalling pathways. The carbon nanoparticles stimulated the macrophages to make the pro-inflammatory cytokine tumour necrosis factor alpha (TNF) and addition of a variety of antagonists of calcium signalling all prevented nanoparticle induced TNF production. The ability of TiO_2 and carbon nanoparticles to deplete the antioxidant glutathione and to induce TNF protein production was related to the surface area dose of the particles administered.

Our previous studies have also indicated that nanoparticles and iron salts can interact to potentiate the production of ROS *in vitro* and inflammation *in vivo*. However, when this was investigated further using macrophages and epithelial cells treated with 14 nm carbon (125 g/ml) and/or iron chloride (100 M), no potentiation or synergism was observed. More recent studies, using zinc chloride (100 M, 4h) and 14 nm carbon black (31 g/ml) indicate that the zinc potentiates the production of TNF protein production by the nanoparticle treated macrophages. These studies suggest that interactions may only occur at lower particle doses, and/or they may be specific to certain metal salts.

In conclusion, the relatively small size of nanoparticles enhances their potency in a number of *in vitro* systems resulting in the production of oxidants and the generation of pro-inflammatory mediators. These effects are relevant to relatively low toxicity materials, and may be enhanced by metals and for nanoparticles constructed of materials of greater toxicity.

Effects of Nanoparticles on Cytokine Release and Cytokine Gene expression in Human Peripheral Blood Monocytes and Lung Epithelial Cells.

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Ultrafine or nanoparticles are a component of particulate air pollution which have been suggested to be responsible for the health effects associated with elevations of this pollutant. We have previously suggested that nanoparticles, through the induction of oxidative stress may induce inflammation in the lung, thus exacerbating pre-existing illness in susceptible individuals. Alveolar macrophages and lung epithelial cells are considered to play a key role in particle-mediated inflammation and lung disease. The effect of nanoparticles on human blood monocytes and treatment of A549 epithelial cells with conditioned medium from treated monocytes was investigated with reference to cytokine release and cytokine gene expression. The calcium channel blocker Verapamil, the intracellular calcium chelator BAPTA-AM, the calmodulin inhibitor W-7 and the antioxidants Trolox and Nacystelin were included in the treatments. Monocytes were treated with nanoparticles and the calcium antagonists, RNA was extracted and PCR for IL-1 alpha gene expression. Results indicated that IL-1 alpha was up-regulated by nanoparticles and this effect could be modulated with calcium antagonists. Treatment of A549 lung epithelial cells with supernatants from treated monocytes resulted in increased IL-8 release which was reduced when calcium antagonists were present. Incubation of supernatants with anti-TNF antibody prior to A549 treatment reduced IL-8 release. These findings suggest that nanoparticles may exert their pro-inflammatory effects by modulating cytokine production through a calcium and reactive oxygen species-mediated mechanism.

Project funded by the Colt Foundation.

The Relationship between Mass, Number and Surface Area Concentrations of Nanoparticles - Test Aerosols in the Laboratory

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It is currently unclear as to which property or metric of nanoparticles best relates exposure to the material to health risk. Whilst mass is the current metric for measuring exposure to the 'coarse' aerosol fractions, there is evidence to suggest that it may not be the right choice for nanoparticles. In a number of different studies carried out over the last 10 years, the possibility of correlations between the number or surface area concentrations of nanoparticles and toxicity have been demonstrated.

Whilst there are portable instruments for measuring number concentrations and light scattering instruments can be calibrated to give mass concentrations, there is no portable instrument for measuring surface area concentrations of nanoparticles in workplaces. The work reported here is the early stage of the laboratory phase of a project to establish the relationships between mass, number and surface area concentrations of airborne nanoparticles.

Test aerosols of sodium chloride, generated from solution using a Collison nebulliser, were dried and passed through a large parallel plate electrostatic elutriator set at 50 kV to remove particles larger than 900 nm. The resultant aerosol was fed (and spread uniformly with a small fan) into a large exposure chamber in which a number of continuously monitoring aerosol instruments were sited. A Scanning Mobility Particle Sizer (SMPS) was used to measure number weighted size distributions and total number concentrations of particles in the range 14 to 875 nm. Active surface area concentrations were measured using a diffusion charger monitor DC2 LQ, whilst a Tapered Element Oscillating Microbalance (TEOM) was used to measure mass concentrations. Samples of the aerosol particles for morphological analysis using electron microscopy were collected using a thermal precipitator. An Aerodynamic Particle Sizer (APS) was used to ensure that there were no large particles in the test aerosol.

The relationships between mass number and surface area concentration of three different sizes of aerosol were determined. The results obtained so far will be presented in the poster.

5. WORKSHOPS

5.1 INTRODUCTION

The aim of the workshops was to provide a means by which the assembled international experts and delegates could discuss the important factors related to the potential health effects of the production and use of nanomaterials in the workplace. Section 5.2 summarises presentations on current health-related programmes, while section 5.3 summarises workshop discussions on research gaps and section 5.4 summarises discussions on regulatory implications. . HSE and NIOSH did not seek the collective or consensus advice, opinions, or recommendations of the participants.

There were three main sessions:

1) Current health-related research programmes
Representatives from HSE (UK), NIOSH (USA), INRS (France), BIA (Germany) and TNO (Netherlands) gave brief overviews of their laboratory and national programmes for addressing the potential health problems associated with nanomaterials.

2) Research gaps
Four separate workshop groups were convened to discuss gaps in knowledge in the areas of a) measurement of nanoparticles , b) control of nanoparticles, c) mechanisms of toxicity of nanoparticles, and d) human experience in exposure to nanoparticles. Delegates were free to choose which group they attended. A set of draft questions were provided to the chairs of each group to use as a guide for discussion and the conclusions and main points from the discussions were presented to the symposium at a feedback session.

3) Regulatory implications
For this session the delegates were separated into five groups of equal numbers, chosen by the organising committee on the basis of their attendance at the research gaps workshop groups. The aim was to achieve a balance of interest and experience in each group so that a balanced discussion could be obtained. Nine questions were drafted with each group being asked to concentrate on two selected questions but to also consider the other questions if they had time. Each group reported back the main points and conclusions from their discussions to the symposium at a second feedback session.

Finally, the chair, Dr Brian Fullam of HSE summarised the broad outcome of the workshops and the symposium and encouraged international collaboration fill the knowledge gaps and provide agreed guidance on a risk based approach to the prevention of ill health from exposure to nanomaterials in the workplace.

5.2 CURRENT HEALTH-RELATED RESEARCH PROGRAMMES

5.2.1 HSE Activities on the Occupational Health Aspects of Nanotechnologies

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The UK Health & Safety Executive (HSE) first considered the regulatory aspects of workplace exposure to nanoparticles in the late 1990s and early 2000s, in the context of revisiting its regulatory approach for poorly soluble particles of low cytotoxicity. Reviews were undertaken of the toxicity of such particles, including those in the nanometre range (at that time, termed 'ultrafine' particles), and the UK exposure situation for nanometre particles was also addressed. One issue that emerged from this work was that of measurement parameters; it became clear that mass is an unsuitable dose metric for ultrafine particles, and that perhaps particle surface area is a more meaningful parameter.

More recently, HSE has addressed the regulatory implications of nanotechnologies as part of its horizon scanning activities. The aim of this was to identify any emerging workplace health risks associated with nanotechnologies; to consider whether or not the existing health and safety framework is sufficient to control any risks; and to identify important information gaps and consider how these might be filled.
At around the same time as beginning our horizon scanning work on nanotechnologies, the UK Government commissioned a wide-ranging review of nanotechnologies. This review, conducted by the Royal Society (RS) and the Royal Academy of Engineering (RAEng), was to include consideration of whether emerging nanotechnologies present health and safety issues not covered by current regulation. HSE therefore took the opportunity to feed into this review, which was published in Summer 2004.
HSE's horizon-scanning activity led to the production of three literature-based reviews, on explosion hazards, occupational hygiene aspects and the human health hazards associated with exposures arising from nanotechnologies. HSE also produced a short information note, aimed at those groups currently most likely to be exposed to nanoparticles – researchers and developers – to give generic advice on control.

These reviews, and that conducted by the RS/RAEng identified the emergence of nanotechnologies as a significant new scientific development. However, they also highlighted the gaps in our understanding of occupational health issues related to exposures arising from nanotechnologies. Consequently, there is a need to consider the robustness and adequacy of the existing regulatory position for the assessment and management of any risks arising from workplace exposures to nanoparticles. In this context, issues relating to the appropriate measurement and control of exposure to nanoparticles are a primary consideration.

To this end, HSE is funding a research project which aims to determine the inter-relationships between particle mass, number and surface area. It is anticipated that the research findings could underpin the development of a suitable measurement

technique. In addition, HSE also has a very minor role in Nanosafe2, a project within the EU 6th Framework Programme, looking at the safe production of nanomaterials.

5.2.2 Nanotechnology and occupational health – the US/NIOSH perspective

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'Nanotechnology' is a broad term adopted in recent years to describe the manipulation of matter at near-atomic length scales to form new materials, structures and devices. The technology isn't confined to a narrow range of disciplines, but is finding application in many diverse areas of research and development. This ability is opening up incredible opportunities, including efficient energy generation, usage and storage, high performance materials, innovative sensors and targeted medical diagnostics and therapeutics. At its most basic, nanotechnology encompasses the formation of structures between approximately 1 nm – 100 nm that allow unique quantum effects associated with this transition region to be exploited. The other end of the spectrum, which may be on the borderline between possibility and fantasy and certainly won't be realized for many years, is the construction of nanometer-scale machines that are capable of working directly with atoms, molecules or other nanoscale structures. In between these limits lies a reality that is poised to revolutionize society over the coming decades.

In the USA, the Federal Government has placed a strong emphasis on the need to address the societal implications of nanotechnology as it develops, including health impact (US Congress 2003). US Federal Research and Development in nanotechnology is overseen by the Nanoscale Science Engineering and Technology (NSET) subcommittee of the National Science and Technology Council Committee on Technology (www.nano.gov). Federal Agencies participating in NSET are currently funding extensive research into the toxicity, characterization, risk management and application of nanotechnology with respect to human health and the environment. Within NSET, a working group has been assembled specifically to address the environmental and health impact of nanotechnology from the Federal perspective. Formed from representatives of key regulatory and research agencies including the National Institute for Occupational Safety and Health (NIOSH), the Environmental Protection Agency (EPA), The Occupational Safety and Health Administration (OSHA) and the Food and Drug Administration (FDA), this working group is focused on coordinating environmental and health activities between relevant agencies, and facilitating appropriate activities to ensure adverse impact is minimized. The group is also supporting the development of guidelines for the safe handling of nanomaterials – a task that NIOSH is currently undertaking.

NIOSH is the US agency charged with ensuring worker safety and health through research, information, education and training (www.cdc.gov/niosh). The Institute has had a long history of cross-cutting research into the impact of exposure to nanometer-diameter particles from processes such as welding and combustion. More recently,

NIOSH has responded to the need to address exposure to engineered nanomaterials, structures and devices by forming an institute-wide nanotechnology initiative. This involves a coordinated approach to research, partnership and outreach across NIOSH addressing not only the implications of nanotechnology in the workplace, but also the application of the technology to ensure good health. Current research spans studies into the toxicity of carbon nanotubes and other nanomaterials, measurement and characterization of nanomaterials and exposure control. In addition, a joint Request for Applications (RFA) on the environmental and human health effects of manufactured nanomaterials has recently been issued in conjunction with EPA and the National Science Foundation (NSF).

To address immediate occupational safety and health needs within nanotechnology research, production and application communities, the Institute is in the process of developing a series of documents to support the development of good working practice guidelines. The first of these – a brief informational fact sheet – was published in October 2004 (NIOSH 2004). Frequently asked questions and answers on nanotechnology and occupational health will be published on the NIOSH nanotechnology web pages (www.cdc.gov/NIOSH/topics/nanotech) in 2005. These will be followed by a NIOSH Current Intelligence Bulletin on working with engineered nanomaterials in late 2005.

Through these and a number of other initiatives, NIOSH is working with national and international partners towards proactively reducing the potential for nanotechnology to lead to adverse health impacts in the workplace, while seeking ways of applying the technology in beneficial ways.

References

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5.2.3 French Activity on the Ultra-fine Particles in the Workplaces.

The INRS Research Project: Occupational Exposure to Ultra-fine Aerosols in the Workplace Atmospheres (EXPAU)

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INRS – Vandoeuvre Cedex, France

A new health-safety worldwide problem related to aerosols has recently emerged, that of ultra-fine particles. This is the subject of the research program entitled EXPAU, which follows on from the preparatory study of project launched in January 2003.

The information yielded by the epidemiological, environmental and toxicological studies shows that the question of ultra-fine particles is a very complex reality that is still insufficiently understood. In the work setting, the ultra-fine particles emitted by conventional processes and technologies employing a high-speed thermal or mechanical action can already be distinguished from those stemming from the emerging nanotechnology sector. However, very few data have been published and are available regarding the concentration levels and particle-size distribution of ultra-fine particles, and therefore on exposure assessment.

Concerns about the lack of knowledge and possible risks arising from exposure to ultra-fine aerosols (including nanoparticles), led the French Research Institute of Occupational Health and Safety (Institut National de Recherche et de Sécurité – INRS) to launch in January 2005 the EXPAU program (French acronym for « Occupational Exposure to Ultra-fine Aerosols »).

The aim of the EXPAU project is to contribute elements to answering the question: do the ultra-fine particles dispersed in the air of workplaces constitute an occupational risk?
The research project is a three-year program structured around four areas, the objectives of which are: (1) to have available in due course an ad-hoc metrology and a know-how adapted to the constraints of measuring ultra-fine aerosols in workplaces, (2) to establish a matrix describing ultra-fine particle pollution by industrial sector (and activity), (3) to study the near-field emission of ultra-fine aerosols during the handling of nanopowders and (4) to ensure a documentary follow-up, inform, and create a network of specialists.
The work will be performed mostly by the Aerosol Metrology Laboratory team from INRS and through collaborations with the CNRS and IRSN.

The exploitation foreseen consists in scientific and technical reports, publications in scientific or specialised journals of interest to prevention, and oral presentations at national or international symposia. The research engineer in charge of the EXPAU project at INRS is:

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5.2.4 German Activity on the Ultra-fine Particles in the Workplaces

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BGIA (the institute for occupational safety and health of the institutions for statutory accident insurance and prevention, old abbreviation: BIA), in conjunction with the German institutions for statutory accident insurance and prevention (Berufsgenossenschaften), carried out a measurement programme at selected workplaces. The aim was to gather and catalogue technical measurement information on ultra-fine particles occurring at different work processes. Since 1998 approximately 40 different industries had been examined and data on about 100 different workplaces had been gathered. The measurements were done at those places where ultrafine particles had been released occasionally as by-products of technical processes. Typical examples include welding fumes, metal fumes, soldering fumes, plasma cutting fumes, plasma spraying emissions, polymer fumes, vulcanisation fumes, amorphous silicic acids, powder coating emissions, oil mists, aircraft engine emissions, bakery oven emissions, meat smokery fumes, and particulate diesel motor emissions.

Ultrafine aerosol particles are for the most part the product of condensation in thermal and chemical reactions. The primary particles that are thus created have a size of only a few nanometres (nm). Often they also agglomerate directly after formation and form even larger particles.

The particle size distribution between approximately 10 nm and 700 nm and the number concentration of these particles were determined. BIA was equipped with a suitable measurement device (scanning mobility particle sizer – SMPS, TSI and Grimm) for these particular measurements. Additionally the mass distribution over the particle size and the concentrations of respirable and inhalable dust were measured.

Some typical results from workplace measurements of ultrafine aerosols were reported. As an example the fumes originating from manual metal arc welding of highly alloyed CoCrNi steel (Inconel 617) were measured and the size distribution is shown in the figure. The most occurring particle size is between 160 and 300 nm. The total concentration of all particles in the measurement range 14 to 673 nm is between 500 000 and 2 500 000 particles per cm^3. The yellow curve (12:48) represents an influence from nearby grinding activities and reveals more particles around 30 nm, which is probably due to pyrolysis of the organic binding matrix of the grinding disc.

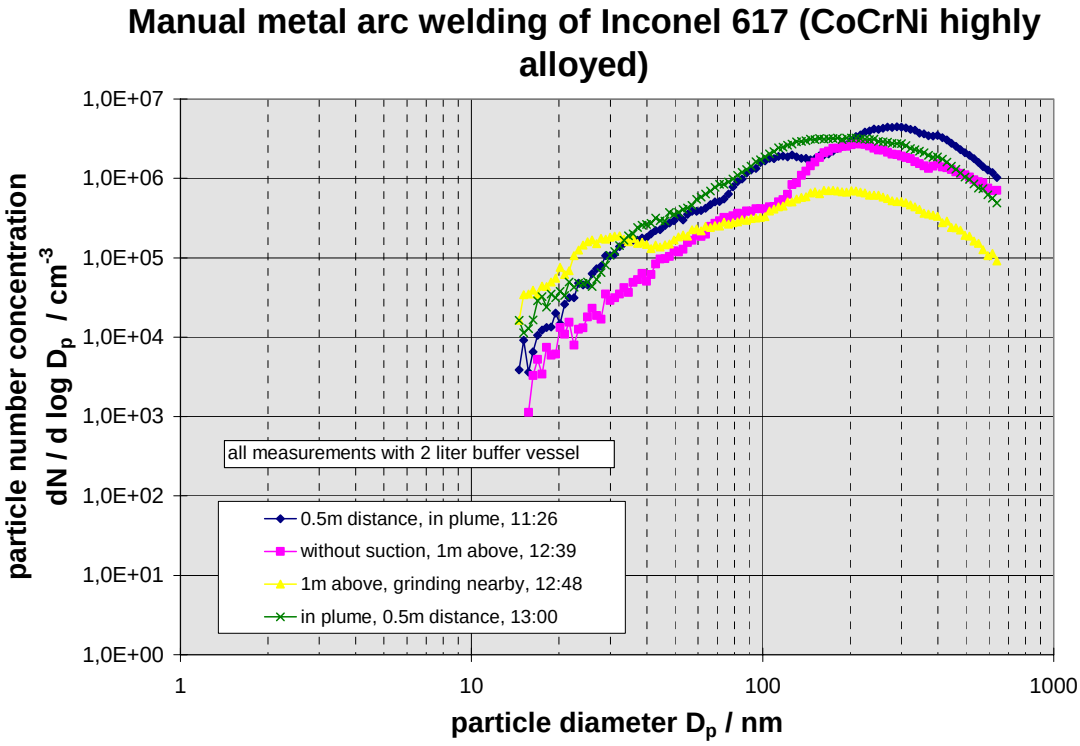

Figure: Ultrafine particles at welding of highly alloyed CoCrNi steel

A comparison of the occurrence of ultrafine particles in different workplace atmospheres is given in the table.

Process	Total concentration in measurement range 14-673nm particles/cm³	Maximum of number concentration nm
Outdoor, office	up to 10 000	
Silicon melt	100 000	280-520
Metal grinding	up to 130 000	17-170
Soldering	up to 400 000	36-64
Plasma cutting	up to 500 000	120-180
Bakery	up to 640 000	32-109
Airport field	up to 700 000	<45
Hard soldering	54 000 up to 3 500 000	33-126
Welding	100 000 up to 40 000 000	40-600

Table: Comparison of ultrafine particles in workplace air

A basic concentration outdoor or in clean indoor areas will usually be below 10 000 particles/cm³. Depending on the sources of fumes and additional ventilation measures, medium concentrations range between 100 000 and 1 000 000 particles/cm³, measured in the range 14 to 673 nm with the same instrument (SMPS) parameters. Depending on the amount of energy in the creation process of the fumes and the age of the aerosol or the stage of agglomeration, respectively, the maximum of the size distribution varies from a few ten nanometres to a few hundred nanometres. The highest concentration was measured in a plume created from laser beam welding.

Using the resulting data sets on ultrafine aerosols at workplaces, the German institutions for statutory accident insurance and prevention hope to contribute to a helpful discussion on this topic, and improve the possible methods of prevention.

5.2.5 Dutch Activity on the Ultra-fine Particles in the Workplaces

Derk Brouwer
TNO, Netherlands

Summary not ~~yet~~ received

5.3 RESEARCH GAPS

5.3.1 Workshop group A - Measurement of nanoparticles

Chair: Andrew Maynard (NIOSH, USA)
Rapporteur: Carsten Möhlmann (BIA, Germany)

Given the time available, the first few minutes in this workshop were spent identifying key measurement issues, and agreeing on three high priority areas for further discussion. The three top areas for discussion chosen were:

1. definitions of nanoparticles, nanoaerosols and ultrafine particles/aerosols
2. measurement strategies
3. release of airborne material from nanomaterials ('Dustiness')

Definitions

It was agreed that measurement methods could not be adequately addressed without agreeing on basic definitions of what we should be measuring. Key definitions discussed were for the terms ultrafine particle, nanoparticle, ultrafine aerosol and nanoaerosol.

Discussions initially addressed whether the terms ultrafine and nano- should be used interchangeably. There was a lot of support for this. However, as the discussion developed, it was clear that there were a number of issues that made the interchangeability of the terms difficult. These included the definitions of 'nanoparticle' used in materials science and other disciplines that are more specific than 'ultrafine particle'. [An additional observation added by Andrew Maynard when writing these notes up – ultrafine tends to be used in the context of airborne particles, where as nanoparticle is a generic term for nanometer-diameter particles in any media].

Consensus was reached on the following, although it was acknowledged that these are working definitions at this point to aid discussions on measurement and characterization:

Nanoparticle: A nanometer-diameter particle that exhibits size-dependent properties. 100 nm provides a nominal upper diameter limit. Functional upper diameter limit could be when quantum effects start to dominate over bulk effects

Ultrafine particle*:* A particles nominally smaller than 100 nm in diameter and stable in a gas (i.e. larger than the diameter necessary for condensation growth). It was acknowledged that this is not a biologically relevant definition, and useful only as far as it defines an approximate size regime of interest. The definition also implicitly refers to air/gas-borne particles.

Nanostructured particle: Particles with a biologically accessible or biologically relevant nanostructure. The upper size limit is dependent on the route of exposure – thus, if particles with a biologically relevant nanostructure as large as 100 µm can be inhaled, these would be considered as nanostructured particles. It was generally agreed that this term is more useful in the short term than ultrafine particle when considering

exposure to nanotechnology-related materials. It was also generally agreed that the term should apply to particles presented to the body in solid or liquid phases (i.e. suspended in droplets) wherever the nanostructure is subsequently biologically available. However, further focus is needed on particles where overall particle size influences particle behavior such as translocation.

Nanoaerosol: An aerosol of nanostructured particles

Ultrafine aerosol: An aerosol of ultrafine particles, present either as discrete particles or agglomerates where the ultrafine particles contribute to particle nanostructure.

Measurement Strategy

It was generally agreed that further research is needed to define biologically relevant particle parameters that should be measured. The process of designing and testing measurement methods and instruments will be an iterative one, as information becomes available on toxicity, health effects, production processes, exposure routes and control approaches [progress in these areas is dependent on measurement methods, which in turn require feedback from these areas on what is relevant in the measurement process – hence the iterative cycle and the need for interdisciplinary R&D].

At present, the following parameters seem to be most important:

- surface area
- surface chemistry
- number concentration
- size distribution [particle diameter]

It was stressed by a number of participants that if measurements are to be interpreted correctly and compared between studies, full documentation of measurement techniques and strategies is needed. This will also allow re-interpretation of measurements in the future, as new information on biologically relevant parameters becomes available. In particular, the need to document instrument response (such as the integration range in CPC's, and the precise physical/chemical parameters an instrument measures) was stressed.

Until more is known about what parameters should be measured, there was general support for measuring multiple parameters in parallel where possible (i.e. integrating different measurement methods to achieve as clear a characterization of exposure if possible).

Dustiness

It was agreed that much more information is needed on the propensity for powdered nanomaterials to release nanostructured particles into the air that can be inhaled or come into contact with the skin. Information is also needed on the nature of the particles

released. This was seen as a critical step toward understanding and controlling exposures.

The CEN pre-standard on dustiness (prEN 15051) and its relevance to nanomaterials was discussed. prEN 15051 covers evaluation of micrometer-diameter particle release from powders. Much of the discussion centered around whether this standard can be adopted and extended to evaluating release of nanostructured particles from nano-powders. It was acknowledged that there would be a number of technical hurdles to cross (current methods use filter collection, where a nanomaterial approach would probably have to use continuous monitoring for instance). However, it was generally agreed that current dustiness measurement methods would be a good place to start.

When developing dustiness testing methods for nanomaterials, it was suggested that close attention needs to be paid to the methods used to measure particle release, and in particular, the energy levels that are put into the aerosolization process, and how that energy is imparted to the bulk material. There were suggestions that characterizations need to be carried out over a range of energy input levels.

5.3.2 Workshop Group B – Control of nanoparticles

Chair: Gordon Peters (P + G, USA),
Rapporteur: David Mark (HSL, UK)

Several aspects of control were discussed. The general consensus of the group, based on current knowledge, is summarized below.

Engineering Control of Manufacturing Equipment

Nanotechnology is unlikely to require major new inventions in the area of engineering controls of the production process:

1. in terms of capture, nanoparticles are expected to behave somewhere nearer to a gas than a solid and are expected to be easier to capture than larger particles.

2. a simple visual way to ensure that a hood is capturing normal size particles is to position a smoke tube at various positions around the source and observe if the smoke is captured by the hood. This approach should still work for nanoparticles.

The above is not to say that there are no potential risks that need to be controlled. Some examples the group identified included:

- need for unplanned intervention with equipment (e.g. a sudden break down).
- planned maintenance.
- role of static charge – a process safety risk but also it may make nanomaterials harder to handle and harder to contain when doing work tasks such as dispensing.
- exposure from off-line work tasks (e.g. weighing or dispensing).

- re-circulation of ambient air

Risk Assessment

The group concluded that, like any other chemical operation, work involving nano technology needed to be preceded by a thorough risk assessment. This should identify risks, counter measures and means to verify the effectiveness of the counter measures. The risk assessment should cover every aspect of the operation from receiving raw material through to shipping out finished product. It should also include handling of any scrap/waste. Transportation of raw material and bulk finished product should also be subjected to risk assessment – whether it is by the supplier of the raw material or the manufacturer of the products containing nanotechnology.

Some key points were made:

1. current methodologies of risk assessment (e.g. what if? Fault tree Process hazard analysis) are likely to be applicable to nanotechnology. The group did not envisage the need to invent new approaches to risk assessment. However, there may be a need to develop new supporting tools to enable the risk assessment process e.g. improved means of measuring concentrations of nanoparticles in air may be needed.

2. it would be ideal to share learnings between organizations, especially for potentially high exposure situations. At first sight it appears that re-application potential is limited because of operational and scale differences across industries e.g. the electronics industry is very different from say the ceramics industry. However maximization of sharing and re-application can be achieved by thinking in terms of unit operations (e.g. weighing, pouring, mixing etc).

3. the main risk seems less in operation of process equipment and more in planned / unplanned events associated with daily operation (e.g. breakdowns, maintenance). Rather than focus on engineering controls for routine operations it may be more appropriate to focus on technologies for cleaning in place with respect to spills or build up.

Administrative Controls

Systematic risk assessments will identify and prioritize exposure sources. Engineering controls, whilst always the first to be considered, may not always be feasible or may not in themselves be adequate (e.g.emergency response to an unplanned event such as a spill).

Two broad applications for administrative controls were discussed:

1. some industries handling moderate to high hazard materials adhere to “Operational Guidelines”. e.g. no visible dust, no recurring spills. These

Operational Guidelines can influence work place culture significantly leading to improved hygiene standards. As such an Operational Guidelines approach could be extremely valuable in deployment of nano technology.

2. industry already makes extensive use of safe operating procedures. These are a form of administrative control generally based on hazard analysis – breaking a work task into steps and identifying possible hazards and countermeasures for each step.

Personal Protective Equipment

High Efficiency Particulate (HEPA) filters are expected to capture nanoparticles. There is at least one literature report showing very efficient capture of 10nm diameter sodium chloride particles. The main risk is that nanoparticles can pass into the breathing zone via a poor face seal. Thus full face piece or positive pressure respirators equipped with a HEPA filter are preferred.

The above said, a concern with HEPA filters was identified. HEPA filters do not protect against gases and vapours. At small enough particle size nanoparticles may behave like a gas/vapour and pass through a HEPA filter.

It was agreed to search for experimental data for HEPA filter capture of very small $\leq$ 10 nm diameter particles.

Similarly some nanoparticles may be able to penetrate the skin. In these cases (and in any case as good industrial hygiene practice) protective clothing may be recommended as a counter measure. For example "Wear protective suit X while carrying out maintenance work on a filling machine used to dispense nano material Y." A means to measure the penetration of the protective clothing was required.

Exposure Limits

A key question that was identified is what control limit should be used for assessing the adequacy of control measures? The main points brought out by the group were;

- what units best predict health effects – mass? Surface area? particle number? This is an important practical question when setting airborne limits. As the diameter is halved for any given mass, the surface area is doubled and the number concentration increased by 8X. Thus controlling against number limit will require more rigor than against a mass limit.
- will an 8 hour time weighted average be appropriate for airborne exposure or will there need to be a peak exposure limit as well as or instead of a time weighted average?
- can generic limits based on particle size be set or will there need to be individual limits set material by material based on toxicity testing?

- if/how can we use a control banding approach to nanoparticle applications be used?

Other Discussion Points

During the course of the workshop there were some other discussions, not strictly related to control but nevertheless important points in helping prepare for safe deployment in a manufacturing environment:

- safe on site storage is an area that needs to be considered e.g. how would storage vessels be spill protected?;
- waste disposal, whilst not a subject of the workshop, is a key area where capability needs to be developed. How will environmentally safe disposal of liquid and solid wastes be ensured? How can air emissions be prevented?;
- there are probably relevant learnings from industries handling fine powders or hazardous materials, Ceramics, pharmaceuticals etc.;
- what sort of hazards and guidance should be documented on MSDS's?

Main Conclusions (with respect to Control)

1. safe deployment of nanotechnology is unlikely to need major breakthrough in engineering control technology.
2. the main exposure risks will come, not from emissions from the manufacturing process but from daily operations – e.g. unplanned breakdowns or routine maintenance. As such breakthroughs in technologies of cleaning in place could be a useful engineering opportunity to support deployment of nanotechnology.
3. an Operational Guidelines approach could be used to support a hygiene culture within a manufacturing operation.
4. new approaches to risk assessment are unlikely to be needed but new supporting tools such as improved air monitoring capability may be needed.
5. sharing information in terms of unit operations can help re-apply learnings across industry groups.
6. there is potential for learning from existing practice in powder handling industries e.g. pharmaceuticals, ceramics.

Needs Assessment (with respect to control)

1. decide the basis for exposure limits – mass, surface area or number concentration? Also decide if time weighted average air borne exposure limits and/or peak exposure values are needed.
2. additional airborne monitoring capability.
3. studies of HEPA filter penetration by very small particle sizes ($\leq$ 10 nm).
4. a test to measure penetration of protective clothing.

5.3.3 Workshop Group C: Mechanisms underlying toxicity of nanoparticles

Chair: Ken Donaldson (University of Edinburgh),
Rapporteur: Paul Borm (Hogeschool Zuyd, Netherlands)

NP are small, and therefore can pass barriers that fine particles cannot. This implies that after inhalation particles do not remain in the lung, but may reach other systemic compartments. Understanding of mechanisms of translocation and properties is not yet complete, although know-how on uptake and distribution of viruses may serve as an example for understanding of penetration through olfactory epithelium as well as the intracellular uptake. The physiological condition of the organ may be critical since conditions such as inflammation are well known to affect volume of distribution as well as permeability. However, this has not been evaluated with regard to nanoparticles. This is true for both lung (inhalation) and the skin (permeability), where only reports in healthy organs are available although acute effects of NP are usually seen in compromised or diseases subjects.

The above raises another question, whether NP induce new effects or are the effects simply due to different distribution and kinetics? The answer is not simple and true for all nanoparticles. It is stated that some NP have unique material properties (which are often the reason of their use or application) that may translate into effects related to these properties. Quantum effects of metals in nanoparticle form are mentioned as one of properties that may give rise to biological responses. The same holds for reactive oxygen species that are generated by carbon surfaces such as fullerenes, which can be associated to cellular toxicity.
It is also stated that engineered particles may act quite differently from nanoparticles that were studied so far, need for further understanding at molecular level and genetic material.

Due to their large surface, NP can act as carriers of other exogenous or endogenous agents. They can adsorb PAHs, metals and other components and carry them to sites (intra-cellular) normally not reached by such components. Whether the absorbed components are then released or leached is considered unlikely due to high van der Waals forces or the formation of aggregates. On the other hand, soluble NP may release components simply by dissolution in environments that are different from that reached by fine particles. The formation of aggregates itself and their potential dissociation of such complexes posed problems to hazard and risk assessment because of semantics, the exact metric of exposure and/or the cause of adverse effects. Of course, we know that deposition of primary particles and larger aggregates can be very different, although both can be classified under nanoparticles (< 100 nm GMD). For nanotechnological applications, however, primary particles are often essential because the anticipated properties are only observed in primary, single particles. On the other hand, although we know that materials at nanosize can have distinctly different properties from macroscale materials, hazard assessment is based on chemical identity (and not size).

It was therefore recommended that the discrimination between primary particles and aggregates is to be continued throughout the different stages of exposure, uptake and distribution. This also important with regard to material testing protocols. To investigate the importance of this parameter there is a need for a comparative study with a set of well-defined materials of similar primary particle size, and test conditions that modify aggregate size at different phases of the test.

Among the mechanisms that have been forwarded to explain the effects of NP, oxidative stress by inflammation (mostly reported in lung research) or by particle surface have been studied most intensively. The ability of some NP to impair macrophage function and cause an inflammatory response is well documented, but the understanding how this occurs and which particle properties are most relevant to this effects is still lacking. In addition, most of these studies have been conducted with inhalation or intratracheal instillation models, and little studies are available on extra-pulmonary inflammation by NP after intravenous, oral or intraperitoneal administration. Studies on the sensitisation and adjuvant effects of NP (skin, lung) are forwarded by effects reported in human and animal studies with diesel exhaust particles and organic extracts, and intentional administration of ultrafine gels to stimulate antibody formation. Special attention needs to spent to compounds that can be released from NP by dissociation or dissolution.

A general issue during the workshop is the anticipated potential of many NP to be genotoxic or even mutagenic in a number of in vitro test-systems at relatively low-mass doses. It is noted that this may be a serious pitfall for many nanomaterials to reach the market. Based on know-how with fine particles (quartz, asbestos) and the difficulties of in vitro findings with these particles, it is recommended that relevant test-models will be developed to investigate the genotoxic hazards and risks of NP in its various applications.

It is interesting to note that in every single case that is discussed we cannot discriminate between a generic particle effect and the role of (surface) chemistry. The take home message is that both these properties play in concert, especially when regarding special purpose engineered NP.

Can we say now what is the appropriate metric for exposure assessment (from a toxicology point of view)?

Surface properties are crucial but hard to measure routinely. Surface area and surface reactivity are also relevant parameters, since many engineered NP are made to be reactive (switches, sensors). In addition, the relevant metric may vary between NP depending on the mechanism of toxicity. In the case of fibres and inhalation short, less-durable fibres will cause less harm than, bio-persistent fibres since these are known to cause more chronic effects upon inhalation. On the other hand, solubility may also give rise to the elution of elements from NP that can cause adverse effects. Such a scenario can be thought of with various quantum dots, which are used for highly sensitive imaging purposes but often made of heavy metals.

In summary, mass and numbers (size-distribution) are still considered the most pragmatic to assess exposure at the workplace. One could imagine that current TLVs for particles are revisited and readjusted to current toxicological views on the role of particle number and surface. This would mean that TLVs are still set on a mass basis but set on data including data on surface and particle number.

Are there any screening methods that we could employ for novel NP?

- screening should be based on biochemical mechanisms and susceptible targets. Relevant models should be developed.

- regulations should be pragmatic (flexible, sensible?) due to small amounts of material. Prove that NP are not worse than other particles?

- existing tests may not pick up all of the hazards. Pharma approach may be helpful in a case-by case approach. Need for bridging studies with good dosimetry (link to metric).

- use existing data for comparison to other particles. Oxidative stress seems a mechanism to generate a number of tests (plasmid DNA unwinding, OH-generation). Interpretation of test result (qualitative) is crucial.

- mutagenicity screening may become a crucial issue and there is need for \more relevant test models (e.g. in vivo).

Relative role/merits of mechanisms for toxicity evaluation

- data on low toxicity PSP may be used as a benchmark.
- case-by-case testing for materials versus generic understanding of mechanisms and properties that are crucial. In this aspect, surface coatings are equally important.
- unpredictability is a typical characteristic of NP. Materials change properties below 100 nm, which is often the reason behind their application.
- stimulate industry to\manufacture non-toxic nanomaterials.

5.3.4 Workshop Group D: Human experience in exposure to nanoparticles

Chair: Irene Bruske-Hohlfeld (GSF, Germany)
Rapporteur: George Cartlidge (HSE, UK)

Questions to be addressed:

1) What workplace experience do we have?
2) Can the epidemiological data in relation to environmental exposure to particles be used to inform us about hazards and risks of workplace exposure to nanoparticles?

3) Can we use these existing data to predict the human health hazards and risks of novel nanoparticles? If not, why not – what do we need?

The route of occupational exposure thought to be of highest concern was inhalation. The group thought that there were very few studies of workplace inhalation exposures to nanometre sized particles arising from existing compounds. No-one was aware of any such data on novel nanomaterials. However, several examples of workplace exposures to particles (including fibres) in the micrometre size range were discussed as to their relevance to potential nanoparticle exposures in the workplace.

It was agreed that occupational experience of exposures to some respirable dusts (with particles in the micrometre size range) can be associated with a spectrum of adverse effects in the lung. Most studies investigating this phenomenon have been performed in workplaces where potentially, there may be relatively high levels of exposure.

With regard to health effects, very minor lung changes without adverse functional consequences (e.g. siderosis) are seen in iron foundry workers after many years of exposure. In other scenarios, epidemiological studies of stone workers, exposures for a few years leads to lung fibrosis which can lead to potentially fatal decrements in gas exchange and lung function. Following longer exposures, such effects may be seen in coal miners exposed to coal dust. Lung cancer was not associated with such exposures. However, other respirable micrometre sized particles have been seen to exert toxic effects much sooner and with greater severity. It is widely accepted that respirable crystalline silica and asbestos, when compared (by dose) to other respirable poorly soluble respirable particulates, are particularly toxic to the lung. Furthermore, in some exposure scenarios (chronic and relatively high levels of exposure) lung cancer has also been observed in these workers exposed to crystalline silica and asbestos. Also, exposure to asbestos in such workers is causally associated with the development of malignant mesothelioma. Hence, it was agreed that each substance had to be treated on a case-by-case basis with regards to hazard and risk.

In considering these studies of existing chemicals, there may be some scenarios where exposure to the compound in the nanometre size range may have occurred, e.g. carbon black production. However, because workers are exposed to a spectrum of particle sizes, the contribution to health effects of the nanoparticle fraction cannot be determined. Such data tell us little about the role of nanoparticles. Hence, for the future, studies of workers exposed to nanoparticles will need the development of an agreed definition of nanomaterials, suitable exposure measurement techniques and a standardised exposure metric. In order to develop appropriate exposure metric(s), it may be necessary to understand further the mechanisms of toxicity involved (e.g. studies in experimental systems suggest that the surface area of the particulate may be a determinant of toxicity)

Epidemiological studies of exposures of the wider human population to micrometre sized particulates in air pollution episodes tell us a little more about the hazards and dose-response characteristics of exposure to respirable particles. It was put to the group that

long term exposure to particulate matter was associated with elevated cardiovascular mortality and respiratory symptoms. Studies of shorter term effects showed associations of daily exposure to particulate matter and mortality on the same day and subsequent days and that patients with pre-existing respiratory diseases and cardiovascular diseases were especially affected. Whilst stronger associations to such effects are seen with particulate matter of diameter 2.5 micrometres (PM2.5)compared to PM10, there are very few data on the contribution of nanometre sized particles. Again the necessary agreed definitions of nanomaterials measurement techniques and exposure metrics are not yet in place in order to further investigate the relationship between exposure to nanometre sized particles and health outcomes in this exposure scenario.

The group also discussed the possible role of health surveillance of workers exposed to nanoparticles. It was agreed that there was insufficient information available on which to establish a generic health surveillance approach.

Overall, it was agreed that:

- a multidisciplinary approach was necessary to investigate the health effects of nanoparticulates, including toxicological mechanisms of action;
- an agreed definition of nanomaterials and nanoparticles is required;
- an agreed exposure metric is required;
- development of practical devices which, with development of agreed exposure measurement techniques, would enable reliable measurement of workplace exposures to nanoparticles is required;
- no agreed health surveillance approaches were identified.

5.4 REGULATORY IMPLICATIONS

5.4.1 Workshop Group G

Chair: Eileen Kuempel (NIOSH, USA),
Rapporteur: Rob Aitken (IOM, UK)

Questions

1) What issues need to be considered when occupational health and safety regulatory authorities review the adequacy of their regulations with respect to nanomaterials?

2) Are specific regulations needed for nanomaterials or can the current regulatory regime be adapted?

In this workshop, participants were asked to consider two questions. These questions and the points raised in response to each are as a follows:

What issues need to be considered when occupational health and safety regulatory authorities review the adequacy of their regulations with respect to nanomaterials?

The workshop participants noted that all available data and information pertaining to the assessment of the hazard, exposure, and risk should be considered. Issues relevant to these assessments include:

- determine the number of people exposed and at what levels.
    - *More information needs to be collected on the number of people exposed or potentially exposed, what they are exposed to, and at what levels. Exposure by inhalation, ingestion or through the skin may all be relevant;*
- evaluate whether mass-based exposure limits are adequate.
    - *Mass-based exposure limits for respirable particles may not be adequate for nanoparticles. Current scientific evidence indicates that surface area is a better predictor of toxicity than is mass. Particle number may also be a better metric than mass in some cases;*
- investigate what measurement methods are available.
    - *There are no convenient methods currently available by which particle surface area can be measured in workplaces. Methods for measurement of particle number are available, and these data may be useful for estimating surface area if size distribution data are also available;*
- review the adequacy of personal protective equipment (PPE) (e.g., respirators, gloves, clothing) against nanomaterials.
    - *Few studies to date have specifically evaluated the effectiveness of PPE when working with nanomaterials, and more studies are needed;*
- determine if there are susceptible groups in the workforce.
    - *Air pollution studies indicate that older people or those with pre-existing conditions are more susceptible. Such relationships may also apply to*

deliberately engineered nanomaterials. If so, regulations need to recognize this;

- evaluate whether a "skin" notation is needed.
    - *Some studies suggest that dermal exposure may be an important route of exposure to particles in the workplace (e.g., beryllium), but more study on specific nanomaterials is needed;*
- consider the adequacy of labeling and of material safety data sheets (MSDS).
    - *An evaluation should be performed of the toxicity data applicable to the nanomaterial under consideration. In particular, attention should be given as to whether data on particles of larger size or different form or composition is relevant to the nanomaterial;*
- evaluate whether nano-forms of a material should be considered a new substance.
    - *Currently they are not. If they were, this would have important implications for registration. Whether nanomaterials should be given new CAS numbers, or a sub-category of CAS number, needs to be considered;*
- develop a framework to categorize or group nanomaterials for hazard classification and exposure limits.
    - *Given the almost limitless variety of nanomaterials possible through engineering, a systematic approach is needed to classify nanomaterials with regard to key characteristics that influence toxicity. Further research is needed to determine these key characteristics;*
- recommend interim measures and generic approaches until more specific information is available on nanomaterials.
    - *The working group participants thought that given the limited available data, it would be prudent to minimize workplace exposures to nanomaterials and to establish appropriate work practices.*

Are specific regulations needed for nanomaterials or can the current regulatory regime be adapted?

Two main issues were identified by the group:

- the current regime(s) appear to provide an adequate framework for regulations. However adjustments may be necessary, including the following:
    Determine if current testing (toxicology) protocols are adequate.
    - *Evaluate production mass or volume that triggers regulation (e.g., testing, reporting) for new substances. These triggers may not be adequate (i.e., too high) for nanomaterials.*
    - *Consider whether new size-based fractions may be appropriate for measuring and regulating nanomaterials (e.g., an extension of the current size-selective sampling definitions to add an ultrafine fraction);*
- international harmonization is important.
    - *The need for international harmonization in approaches and methodology (e.g., measurement methods, nomenclature) and information dissemination was identified as critical for ensuring safe and healthful workplaces given the global implications of the nanotechnology.*

5.4.2 Workshop I
Chair: David Warheit (Dupont, USA)
Rapporteur: Alex Stefaniak (NIOSH, USA)

Questions

3) With the current state of knowledge is it practicable for regulatory authorities to set lower OELs, in terms of mass, for manufactured nanomaterials?

4) Looking forward, how should regulatory authorities set OELs for nanomaterials?

Practical to set lower OELs?

There was general consensus among the group that:

- hazard information on bulk materials or macroscale/microscale materials could not be substituted for nanoscale particulate-types – in the absence of adequate safety data demonstrating that the toxicity of the nanoparticulate-type was not different from the bulk material;
- that under current circumstances, few if any (see below) safety data-sets existed on nanomaterial-types to provide a basis for setting an adequate occupational exposure limit;
- the one exception to this conclusion is likely to be Nano titanium dioxide (TiO_2) particles. There exists a reasonable pulmonary and dermal toxicity data-set for Nano TiO_2 particles . What is missing is a subchronic inhalation study in rats to gauge extrapulmonary effects. Subsequently, the hazard results of the various pulmonary and dermal toxicity studies should be extrapolated to humans in order to conduct a risk assessment.

Future studies on developing nanomaterials should be prioritized by production rates (which clearly can be correlated to exposure rates for future products).

Given that occupation exposure limits do not exist for nanomaterials, and the prospects for having a complete toxicology data-set to formulate risk assessments (other than TiO_2 – see above) will not be available for several years, the Workshop Committee concluded that, in the interim period, Best Practices for Exposure Mitigation should be utilized, and this should include a Hierarchy of Controls, based upon presumed exposures (e.g., dustiness) and the material in question (e.g. if the toxicity of the bulk material is known to be low, concerns about health risks related to exposures would not be as great relative to a material for which there is no safety information.

With regard to the question on the most appropriate metric for exposure indices, the Workshop Committee felt that more information was needed on the size distribution of the nanomaterial (singlets or aggregated particulates), the number concentration, the shape, as well as the surface area of the nanoparticulate-type.

How to set future OELs for nanomaterials?

There was general consensus among the group that:

- substantially more research funding is needed to conduct relevant exposure and toxicity studies on new and existing nanomaterials;

- additional considerations for studies may include
  - co-exposures
  - synergisms
  - exposure modifiers
  - smokers vs. non-smokers
  - sensitive populations;

- animal toxicity studies (should include multi-generational studies- are potential genetic effects passed to subsequent generations?)
  - Other considerations include Ethics (social implications) strategies (in vitro screening versus in vivo studies – provided the in vitro assays are validated);

- the planning and execution of future exposure and hazard studies would be strengthened by inclusion of multi-disciplinary efforts – including, but not limited to toxicologists, exposure assessors, epidemiologists, pathologists, chemists, material scientists, engineers, and social scientists

5.4.3 Workshop Group F

Chair: Christine Northage (HSE, UK)
Rapporteur: Mark Hoover (NIOSH, USA)

Questions

Q5. Are current methods adequate to assess risk and control exposure of people in the workplace to nanomaterials?

Q6. What interim measures are needed until we have adequate information to make informed decisions about the risk assessment and control?

Are current methods adequate to assess risk and control exposure of people in the workplace to nanomaterials?

There was a general consensus among the group that at the moment there is insufficient knowledge to allow us to answer this question properly. We need a better understanding of the hazards, exposure metrics, patterns of exposure, and the adequacy of currently available control measures for nanosized materials. Until such understanding is achieved, it is very difficult to say whether or not current methodologies are adequate, or what methodologies would be needed if they are not.

Having said this, the group felt that the process of risk assessment for nanomaterials should begin with the approaches used for "traditional" workplace exposures, but that nanomaterials should be treated as a distinct topic. It was further felt that manufacturers of engineered nanoparticles need immediate guidance on how to control exposure to these particles, including specific recommendations for "good control practice". Therefore it would be important to carry out research on the adequacy of controls as soon as possible.

There was some discussion about precautionary or pragmatic approaches to control. There was general agreement that a hierarchy of controls, specifically targeted at nanomaterials, would be useful. Wherever possible, initial containment measures should be established at a conservative level until more is known about the hazards that would trigger decreases or increases in control. At this stage, particularly for engineered nanomaterials, substitution is not applicable

What interim measures are needed until we have adequate information to make informed decisions about the risk assessment and control?

The group identified a series of 10 actions that should be taken:

1. examine and build on our knowledge about "known" categories of ultrafine particles such as diesel exhaust and welding fumes.
2. assemble specific examples of when nanoparticle risks or nanotechnology process risks are less than or greater than risks for materials or processes involving "traditional" materials. For example, what are the weights of evidence for or against lower limits of exposure for ultrafine titanium dioxide particles or ultrafine carbon particles as compared to allowable exposure limits for fine particle or coarse particle formulations of these materials?
3. take advantage of opportunities to limit occupational exposures to nanomaterials to levels that are as low a reasonably achievable.
4. develop methods to identify manufactured nanoparticles in the presence of background particles.
5. identify suitable nanomaterial surrogates for use in studies to improve instrumentation, control technology, and toxicology for nanomaterials.
6. develop and disseminate nanoparticle assessment and control strategies for small and medium enterprises (e.g., "risk management" or "control banding" toolboxes). In doing so, take industry-specific and task-specific factors into account; keep recommendations and methods simple to understand, accept, and apply; and foster a user-friendly, guided-question format for on-line interactions. Develop a system that is flexible enough to be able to be updated when new scientific information is available.
7. seek global harmonization of approaches.
8. establish easily accessible databases and information sources.
9. improve the content of Material Safety Data Sheets (MSDS) and other communications.

10. maintain our sense of urgency to answer practical questions now and to establish partnerships and approaches needed to address underlying questions of risk assessment, control, and toxicity mechanisms for nanomaterials.

5.4.4 Workshop Group E:

Chair: Anthony Seaton (University of Aberdeen, UK)
Rapporteur: Alex Tsavalos (HSE, UK)

Questions

7) Should chemicals in the form of nanomaterials be treated as new chemicals under the Notification of New Substances Regulations (NONS) – the UK implementation of the EU Dangerous substances Directive?

8) Do nanomaterials need any special consideration under the Registration Evaluation and Registration, Evaluation and Authorisation of Chemicals Directive (REACH); this is the proposed replacement to NONS and is currently under negotiation in Europe?

These questions were considered together.

Following an introduction of the main features of both the current NONS and proposed REACH systems (see websites http://www.hse.gov.uk/nons/index.htm and http://europa.eu.int/comm/environment/chemicals/reach.htm for details of these regulatory systems) the discussion dealt with these two questions together, often using the group's views of how traditional powdered TiO_2 and nanoparticle size TiO_2 should be dealt with. This was used because it exists and there is general agreement that animal studies have indicated that ultrafine or nanoparticle sized TiO_2 is more toxic than the traditional powder. It was acknowledged however that there are likely to be substantial variations in toxicity between different nanoparticles and that we regularly inhale many such particles in everyday life already – some 2-3 million per ml when you stir fry food and several 100,000/ml during an air pollution episode.

Under current UK/EU regulation, 'nano' versions of existing chemicals, which still have the same chemical formulation and the same crystalline state, etc, are not new substances. (Although it was stated by one of the workshop members that in Canada they would be new). However, the group agreed that nanomaterials are made because they exhibit different chemical or physical characteristics from those of the traditional material.

Thus 'nano' versions of previously existing chemicals can be supplied to the market as the traditional form, although the Chemicals (Hazard Information and Packaging for Supply) Regulations (CHIP) and the Control of Substances Hazardous to Health (COSHH) Regulations still apply and are able to take account of particle size. Dependent on the biological and physical characteristics of the material:

- CHIP could require different labelling information and packaging to be used

- COSHH could require different risk control measures to be taken.

It was agreed that there was a need for a new nomenclature to allow nanomaterials to be clearly identified and described. It was felt that current nomenclatures and means of describing complex materials (such as the way in which NONS handles polymers) are not sufficient and do not set any precedents that help with nanomaterials.

Should products containing nanomaterials be labelled as such? There was an overall view that such products should be clearly labelled to allow the public to choose whether or not to use them. Sunscreen was specifically mentioned in this respect.

As NONS helps generate the data that allows risk assessments to be done, some felt that for any material that had a different physical or biological property there should be a separate registration and a new data set developed, and that a lower level should be required to trigger the need for testing/registration, the proposed 1 tonne level set within REACH being felt by some to be too high. There was also, however, an acknowledgment of the need to avoid any large increase animal testing that such a change might cause.

There was some concern that nanomaterials are being unfairly singled out, as physical characteristics (such as fibrous shape) have been known to alter the biological effects of materials for some time. The 'new substance' approach would conflict with current practice for finely divided metal powders e.g. Aluminium solid is a non-hazardous material but as a finely divided powder it has explosive potential. Currently this is dealt with via the information on labels and material safety data sheets.

The problem of defining a size cut off was raised; when did TiO_2 become new? E.g. are 40nm particles sufficiently different from 41nm particles to be considered a 'new substance'. It was felt that there was no answer at this time and more research would be needed to be able to agree where exactly to set the boundaries, but there is at least a working definition from the RS/RAEng of 100nm in one dimension.

Following this discussion the workshop were asked to vote on the following questions; the chairman acknowledged that the group could not be thought of as representative of the population at large, but did represent a broad range of interests and that this would be a suitable way to close the discussion.

The workshop voted:

- 13 to 1 that materials in particles of less than ~100nm behave differently that traditional sized dusts.
- 10 to 4 that they did not have confidence in the current regulatory system to adequately control nanomaterials.
- 9 to 7 that all materials under an agreed size (possibly 100nm) should be considered new materials for the purpose of chemical regulation.

5.4.5 Workshop Group H
Chair: Bob Maynard (DOH, UK),
Rapporteur: Lang Tran (IOM, UK)

Question
9) Does the nature of nanotechnology require new risk management approaches to be developed and implemented? In particular, are there approaches that can be adopted that will account for incomplete information on nanomaterial hazard, exposure and control when process/handling strategies are developed?

Discussion
The current risk management paradigm is:

- hazard identification;
- exposure assessment;
- risk assessment;
- risk management.

The panel's view is that there is no need for a new paradigm. However, there is a need for new tools to increase our understanding of each part of the current paradigm. Specifically, for risk management, there is a need to:

- adopt the precautionary principle;
- reduce uncertainty by increasing knowledge through science.

The panel is aware that there are factors affecting the perception of risk, such as:

- external risk, e.g. litigation issues (product liability, waste disposal);
- subjective human experience of risk;
- quality of information.

The panel is also aware of the paucity of information. Specifically, on:

- the possible long-term effects
    - carcinogenicity
    - foetal exposure
    - neuro-toxicity
    - cardio-vascular effects;
- the population at risk
    - workforce
    - susceptible groups (elderly, children).

Finally, with respect to nanoparticles, the panel believed that there may be some unexpected adverse effects but no effect is inexplicable.

6 DISCUSSION AND SYMPOSIUM RECOMMENDATIONS

6.1 SYMPOSIUM FORMAT

The format of this first international symposium on the potential health implications of the production and use of nanomaterials was chosen to provide the maximum opportunity for discussion between delegates with a range of different disciplines and from a range of different perspectives. Invited speakers provided up-to-date plenary reviews of the various aspects of the topic and these were followed by well-attended workshops considering gaps in knowledge and regulatory implications. With the venue for the symposium being a large hotel in which most delegates stayed and with the seats in the main plenary room being arranged around large circular tables, there was ideal opportunity for discussions between delegates.

Over 150 delegates attended the symposium, with considerable numbers being turned away because of lack of space in the plenary room. There was a very useful mix of interested parties with 49 from government, 40 from industrial companies, 28 from academia and 20 from independent research laboratories. This was complemented by 5 delegates from the press, 4 from insurance companies, 8 from trade associations and 2 from instrument suppliers (who exhibited their wares). The symposium was truly international with delegates from 16 countries including: UK, USA, Germany, France, Netherlands, Belgium, Switzerland, Singapore, Poland, Denmark, Japan, Finland, Hong Kong, Norway, Sweden and Canada. HSE and NIOSH did not seek the collective or consensus advice, opinions, or recommendations of the participants.

6.2 RECOMMENDATIONS FOR FILLING GAPS IN KNOWLEDGE

The following recommendations were made within the workshops addressing current knowledge gaps in understanding the potential health implications of nanotechnology in the workplace. Inclusion in this report does not constitute endorsement by NIOSH and HSE

6.2.1 Measurement of exposure to nanoparticles

1. there needs to be internationally agreed definitions of the particles that we should be measuring to assess exposure.

2. the health-related importance of agglomerated nanoparticles as opposed to single discrete nanoparticles should be addressed to ensure that measurements include all particles that may have health effects.

3. further research is needed to define the biologically relevant parameters that should be measured.

4. until more is known about which parameters should be measured, it is recommended that multiple parameters should be measured in parallel, if possible. Information is then gained about relationships between parameters to allow links with past exposure data.

5. simple, relatively cheap personal monitors for measuring exposure to nanoparticles should be developed. These should be for particle surface area or number as they are likely to be the most biologically relevant parameters.

6. as a crude identification of nanoaerosol emissions, the measurement of particle number concentrations using a hand-held CPC is considered to be useful as a process control tool.

7. strategies for differentiating between new engineered nanoparticles and ambient combustion-derived nanoparticles should be developed.

6.2.2 Control of exposure to nanoparticles

1. the effectiveness of engineering control methods, such as containment, local exhaust ventilation (LEV) systems, etc., in controlling exposure to nanoparticles should be assessed.

2. the efficiency of HEPA filtration systems used in extraction equipment fitted to LEV system (especially where the air is recirculated) and vacuum cleaners should be assessed for nanoparticles. The integrity of seals is particularly important.

3. research and development should be carried out to improve the control of exposure to nanoparticles during breakdown, maintenance and clean up procedures.

4. the propensity for powdered nanomaterials to release nanostructured particles into the air should be assessed. The usefulness of current methods of dustiness testing for nanomaterials should be investigated.

5. mechanisms should be put in place to enable good control practice for nanoparticles to be shared between companies and industry sectors.

6. the efficiency of respiratory protection equipment for minimising exposure to nanoparticles should be investigated, especially in terms of face-seal leakage and for very small particles (< 5nm).

7. the penetration of nanoparticles through skin protection equipment (gloves, boiler suits, etc) should be investigated.

6.2.3 Mechanisms underlying toxicity of nanoparticles

1. the possible mechanisms by which engineered nanoparticles have the potential to translocate through the body and to affect cells in host organs should be further investigated at the molecular level.

2. the effect of the state of aggregation on the toxicity of nanoparticles should be investigated.

3. work should be carried out to determine the relative contributions to adverse health effects of the generic size of the nanoparticle and the role of surface chemistry including any agent that it may carry.

4. relevant test methods should be developed to investigate the genotoxic hazards and risks of nanoparticles in their various applications.

5. it was suggested that ~~OELs~~ exposure limits are still set on a mass basis as well as on data including surface area and particle number.

6. screening methods should be developed for new nanoparticles that are based on biochemical mechanisms and susceptible targets.

7. existing data on toxicity of other particulate materials should be used for comparison of hazards.

8. more relevant (in-vivo) screening methods should be used to assess potential mutagenicity of nanoparticles.

6.2.4 Human experience in exposure to nanoparticles

1. a multidisciplinary approach was necessary to investigate the health effects of nanoparticles, including toxicological mechanisms of action.

2. an agreed definition of nanomaterials and nanoparticles is required.

3. an agreed exposure metric is required.

4. development of practical devices which, with development of agreed exposure measurement techniques, would enable reliable measurement of workplace exposures to nanoparticles is required.

5. no agreed health surveillance approaches were identified.

6.3 RECOMMENDATIONS FOR REGULATORY ACTION ON THE CONTROL OF EXPOSURE TO NANOMATERIALS

. The views and recommendations expressed in this section are solely those of the workshop participants in the First International Symposium on Nanotechnology and Occupational Health. Inclusion in this document does not constitute endorsement by NIOSH or HSE.

6.3.1 Regulations for nanomaterials

When occupational health and safety regulatory authorities review the adequacy of their regulations with respect to nanomaterials the following issues should be considered:

1. determine the number of people exposed and at what levels;

2. evaluate whether mass-based exposure limits are adequate;

3. investigate what measurement methods are available;

4. review adequacy of personal protection equipment for nanoparticles;

5. determine if there are any susceptible groups in the workforce;

6. evaluate whether a “skin” notation is needed;

7. consider the adequacy of labelling and of the material safety data sheets;

8. evaluate whether nano-forms of a material should be considered to be a new substance (as in Notification of New Substances [NONS] regulations);

9. develop a framework to categorise or group nanomaterials for hazard classification and exposure limits;

10. recommend interim measures and generic approaches until more specific information is available on risk from nanomaterials.

The current regime was considered to provide an adequate framework for regulations with the following suggestions for improvement:

1. determine if current toxicological protocols are adequate;

2. evaluate (as an EU.-specific issue) whether current production triggers (in NONS) are suitable for nanomaterials;

3. consider establishment of new ultrafine sampling convention;

4. ensure that regulations are internationally harmonised.

6.3.2 Occupational exposure limits

1. it was concluded that there was currently insufficient data upon which to set any occupational exposure limits (OELs) for nanoparticles.

2. the one exception was nano titanium dioxide particles for which there is a reasonable data on pulmonary and dermal toxicity.

3. as a way forward to enable safe production of nanomaterials, it was recommended that best practice in controlling exposure be deployed.

In order for regulatory authorities to set OELs for nanomaterials, the following was recommended:

1. substantial research funding should be available to conduct exposure and toxicity studies on new and existing nanomaterials;

2. exposure and toxicity studies should be carried out by multidisciplinary teams;

3. consideration should be given to co-exposures, synergisms, exposure modifiers, smokers, sensitive populations;

4. animal toxicity studies should include multi-generational studies.

6.3.3 Risk assessment and exposure control

1. it was concluded that there is insufficient information to determine whether current methods to assess risk and control exposure are adequate.

2. the process of risk assessment for nanomaterials should begin with approaches used for traditional workplace exposures, treating nanomaterials as a distinct topic.

3. research on the adequacy of current methods of control should carried out as soon as possible.

4. specific recommendations for good control practice should be developed.

5. it was recommended that a hierarchy of controls specifically targeted at nanomaterials should be produced.

In order to manage the production and use of nanomaterials in a safe way the following interim measures were proposed:

1. examine and build on our knowledge about “known” categories of ultrafine particles such as diesel exhaust and welding fumes.

2. assemble specific examples of when nanoparticle risks or nanotechnology process risks are less than or greater than risks for materials or processes involving “traditional” materials.

3. take advantage of opportunities to limit occupational exposures to nanomaterials to levels that are as low a reasonably achievable.

4. develop methods to identify manufactured nanoparticles in the presence of background particles.

5. identify suitable nanomaterial surrogates for use in studies to improve instrumentation, control technology, and toxicology for nanomaterials.

6. develop and disseminate nanoparticle assessment and control strategies for small and medium enterprises (e.g., “risk management” or “control banding” toolboxes).

7. seek global harmonisation of approaches.

8. establish easily accessible databases and information sources.

9. improve the content of Material Safety Data Sheets (MSDS) and other communications.

10. maintain a sense of urgency to answer practical questions now and to establish partnerships and approaches needed to address underlying questions of risk assessment, control, and toxicity mechanisms for nanomaterials.

6.3.4 Classification of nanomaterials

1. there is a need for new nomenclature to allow nanomaterials to be clearly identified and described.

2. current nomenclature and means of describing complex materials in NONS are not sufficient for nanomaterials.

3. materials in particles less than ~100 nm were considered to behave differently than micrometer-sized particles.

4. the current regulatory system was considered to be inadequate to control exposure to nanomaterials.

5. it was recommended that in the EU, all materials under an agreed particle size (possibly < 100 nm) should be considered new materials for NONS and REACH. (delegates were split on this recommendation).

6.3.5 Risk management

1. there is no need for a new risk management paradigm when considering the production and use of nanomaterials.

but there is a need for new tools to increase understanding of each part of the current risk management paradigm. They are:

- consider adopting the precautionary principle;
- reduce uncertainty by increasing knowledge through science;
- consider perception or risk issues such as external risk, subjective human experience of risk and quality of information;
- understand the paucity of information on the possible long-term effects such as carcinogencity, foetal exposure, neurotoxicity and cardiovascular effects and on the population at risk including the workforce and susceptible groups such as children and the elderly.

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APPENDIX A – DELEGATE LIST

Delegate Name	Organisation
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Major Michael R Bell	US Army Center for Health Promotion & Preventative Medicine, USA
Ian Bennett	TSI Instruments Ltd, UK
Dr. Markus Berges	BIA, Germany
Dr Daniel Bloch	CEA, France
Fred Blosser	NIOSH, USA
Frederic Bois	INERIS, France
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Paul J A Borm	CEL, Netherlands
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Christopher Carroll	U.S. Army Center for Health Promotion & Preventive Medicine, USA
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Karl Curtis	Chubb Insurance, Europe SA,
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Dr Brian Fullam	Health & Safety Executive, UK

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Teruo Yagishita	NBCI, Japan
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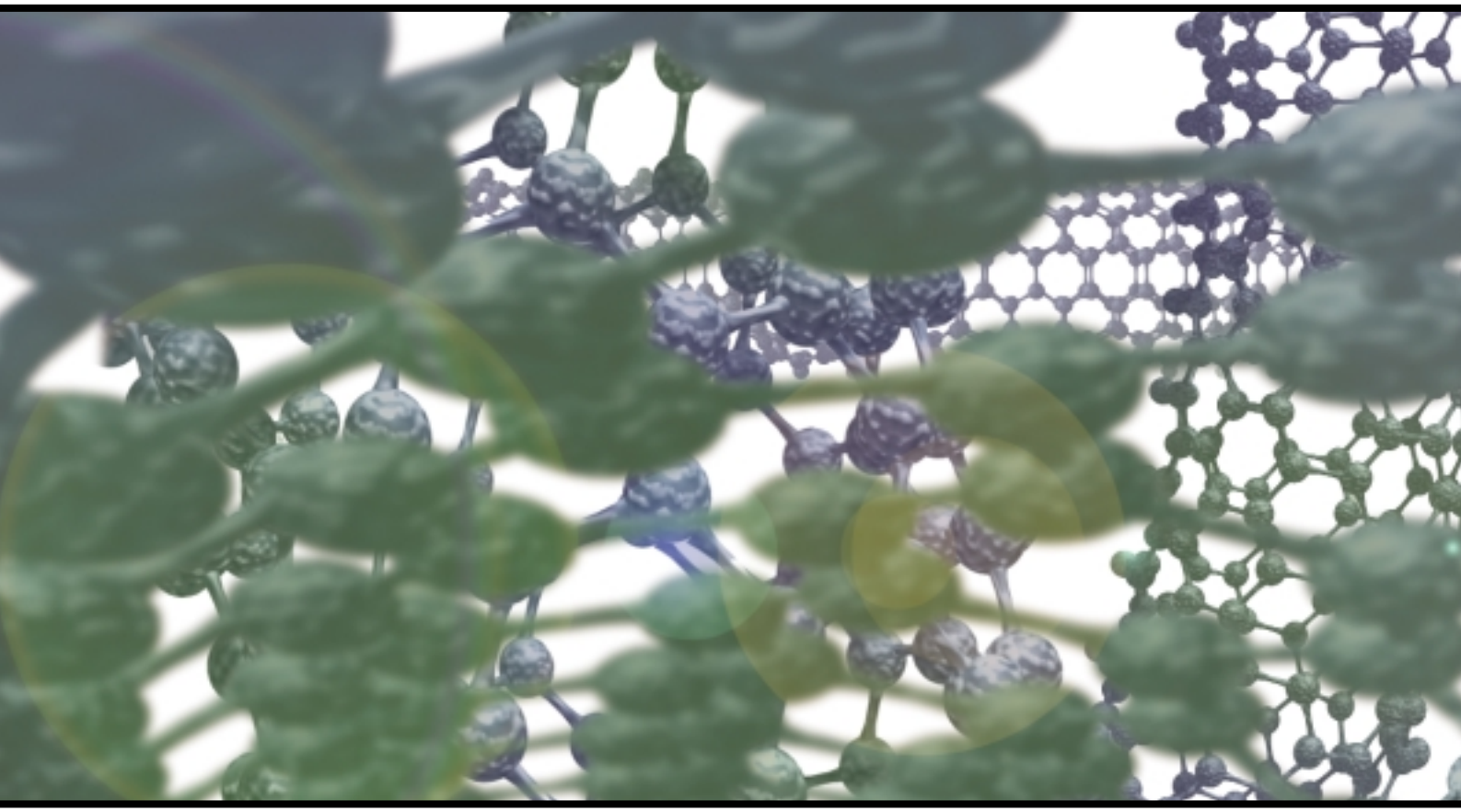

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