Workshop on Testing Strategies to Establish the Safety of Nanomaterials 7- 8 November 2005, Barcelona

Workshop Report No. 7

Brussels, August 2006

ECETOC WORKSHOP REPORT No. 7

European Centre for Ecotoxicology and Toxicology of Chemicals
4 Avenue E. Van Nieuwenhuyse (Bte 6), B-1160 Brussels, Belgium.

The workshop was co-sponsored by the

Cefic Long-range Research Initiative (LRI)

Workshop on Testing Strategies to Establish the Safety of Nanomaterials

CONTENTS

1. EXECUTIVE SUMMARY

Nanotechnology produces an increasing number of engineered nanoparticles. A better understanding of the tests available to assess exposure levels of nanoparticles in the occupational setting, to the consumer and to evaluate the potential health and environmental impact is necessary. It is also necessary to agree how thee tests should be applied and where new developments are needed.

A workshop was held to develop testing strategies to establish the safety of nanomaterials. It brought together about 70 scientific and clinical experts from industry, academia, governmental agencies and one environmental non-governmental organisation. The primary questions to be addressed were the following: What can we do today? And, what do we need for tomorrow? The three major themes of the workshop were: 1) the need for enhanced efforts in nanomaterial characterisation; 2) methodologies for the assessment of airborne and internal exposures to nanomaterials; and 3) evaluation of the hazard potential, primarily through pulmonary or dermal routes of exposures.

The major summary conclusions of the workshop included the following:

For the development of nanoparticle characterisation, the working definition of nanoparticles was agreed as < 100 nm in one dimension. In addition, it was suggested by some that the criteria be expanded to < 1000 nm to include aggregates and agglomerates. Moreover, it was concluded that although many physical factors can influence the functional, toxicological and environmental characteristics of nanoparticles, their impact is largely determined by:

- composition;
- dissolution;
- surface area and other surface characteristics;
- size;
- size distribution (including aggregation and agglomeration state); and
- shape.

Most of the information on potential systemic effects has thus far been derived from combustion-generated particles with a major focus on the cardiovascular system.

With respect to the assessment of external exposures and metrics appropriate for nanoparticles, the general view of the participants was that it is not currently possible to select one form of dose metric (i.e. mass, surface area or particle number) as the most appropriate. However, it was clear that the metric, namely surface area, was likely to be of interest and needed further development. Standardisation of methods for quantifying dose metrics will be necessary. In addition, there is a clear need to develop monitoring instruments which are smaller, more portable and less expensive than the state of the art instrumentation currently available.

Overall, few occupational exposure data are currently available. Since exposure and hazard data form the integral components of risk assessment processes, it will be necessary to develop the workplace exposure data in a systematic and reproducible fashion. Detailed characterisation of nanoparticle exposure methodologies should be documented and provided.

With regard to a general testing approach for human health hazard evaluation of nanoparticles the following was concluded:

- A first step would include a prioritisation-type *in vitro* screening strategy to assess the possible reactivity, biomarkers of inflammation and cellular uptake of nanoparticles. This strategy would determine likely potency but should ultimately be validated using *in vivo* techniques.
- A Tier 1 *in vivo* testing strategy would include a short-term inhalation or alternate route such as intratracheal instillation of nanoparticles as the route of exposure in the lungs of rats or mice. The effects that should be assessed include endpoints of lung inflammation, cytotoxicity, oxidative stress as well as cell proliferation and histopathology of the respiratory tract and the major extra-pulmonary organs.
- For Tier 2 *in vivo* testing for hazard identification, a longer term inhalation study is recommended, and this would include more substantive mechanistic endpoints such as determination of particle deposition, translocation and disposition.

At present, there is little evidence that nanoparticle aggregates and agglomerates at a size exceeding 100 nm penetrate through the skin barrier into the living tissue. The penetration of nanoparticles at a size less than 100 nm should be a topic of further investigation.

When analysing the dermal exposure and the hazard potential of nanoparticles, it must be taken into consideration that the dermal uptake of nanoparticles will be an order of magnitude, or more, smaller than the uptake by inhalation or the oral route. For the evaluation of the health risk of nanoparticles, it has to be determined whether they are harmful to living cells and whether, under realistic and practical conditions, they penetrate through the *stratum corneum* of the skin into the living tissue. Cell culture experiments are broadly used for toxicological assessments. Three methods were mentioned that are available for the evaluation of skin penetration.

Environmental safety testing, applications of nanoparticles for medical purposes and pathways of inhaled nanoparticles to the central nervous system were also briefly addressed during this workshop. It has become clear that these topics should be subject of separate workshops.

2. INTRODUCTION

Nanotechnology involves creating and using particles a few billionths of a metre in size. Evaluating the potential hazards of this technology and its products is an emerging area in toxicology and health risk assessment. The generation of a safety database and exposure assessments to nanoscale materials is evolving as new particles, materials and exposure methodologies are being researched and developed. Although similar in size, these engineered nanoscale materials may have different health impacts when compared to combustion-generated ultrafine particles. A related issue is the extent to which nanoparticle toxicity can be extrapolated from existing toxicology databases for macro- and micro-scale particle types and fibres. One of the aims of this workshop was to provide fundamental information to better understand the rapidly emerging field of testing strategies to establish the safety of nanomaterials. An appreciation of the chemistry and corresponding material science issues related to nanoscale particle composition as well as evolving airborne exposure assessment methodologies are absolute prerequisites to a better understanding of the health impacts of nanomaterials.

The workshop brought together approximately 70 scientific and clinical experts from industry, academia, government agencies and one non-governmental organisation, and focused on testing strategies to establish the safety of nanomaterials. What can we do today? What do we need for tomorrow?

This workshop was immediately followed by a one-day workshop when the majority of the participants were the same and discussed societal aspects of nanotechnology. This will be published as ECETOC Workshop Report No.8.

The workshop on testing strategies covered three major issues:

- nanomaterial characterisation;
- exposure, both airborne and internal (particle deposition in lungs and skin);
- assessment of hazard potential.

This report briefly presents the introductory lectures given by key researchers in this area of science. It summarises the plenary discussions and the outcome of the breakout groups and presents the conclusions and recommendations. In addition, for completeness and easy reference, summaries of earlier papers related to the subject have been included.

The outcomes of the discussions are general agreements reached during the workshop. They cannot be considered, though, as full consensus because the limited time available did not allow discussing every aspect in depth.

3. CHARACTERISATION OF NANOMATERIALS

3.1 Plenary lectures

Characterisation from a physico-chemical perspective

Dr. Haubold presented the possibilities that nanomaterials offer due to their reduced size. Most materials show a variation in chemical and physical behaviour when reduced to nanometre dimensions. Examples of semiconductors such as CdTe, gold and TiO_2 where shown. In the case of semiconductors, absorption and emission is strongly dependent on size. Gold nanoparticles change their colour from yellow to red and TiO_2 becomes photo-catalytically active. Dr. Haubold then presented different methods to produce nanoparticles, for example spray pyrolosis and wet chemical procedures. Following their production, a careful physico-chemical characterisation of the particles is needed. Among the methods used, X-ray diffraction, analytical ultra centrifugation, UV/VIS-spectroscopy and transmission electron microscopy are amongst the most important. It was also demonstrated that the applications to which the particles could be put to use strongly depended on their dispersibility in a resin-matrix. Therefore, it is crucial to design the particle surface such that it can fulfil its function for each specific application.

Characterisation from a toxicological perspective

Professor Borm addressed the sources of evidence for the toxicity of nanoparticles (NP), discriminating three classes of NP: bulk, combustion and engineered nanoparticles. The current discussion on engineered nanoparticles is mainly driven by data on combustion NP (diesel exhaust ultrafines) and a small set of bulk NP (carbon black, TiO_2). He stressed however that the current data-set on engineered NP is growing and that the qualitative effects (inflammation, atherosclerosis, oxidative stress, Ca-transport, etc.) are gradually being investigated with various products such as single wall nanotubes (SWNT). Some studies allow bridging of data, such as recent work (Radomksi *et al*, 2005)[1] on platelet aggregation with different nanomaterials, but also including ambient particulate matter (PM) samples. Before gaining a conceptual understanding of nanomaterials, the following issues need careful consideration with respect to research programmes and regulatory action, since there is a plethora of outstanding toxicological questions.

When compared to ambient-derived ultrafine particles, some effects of nanomaterials are probably similar to the effects of engineered NP. This is not a priority for further research, but current testing on effects requires validation. There is a need to identify effects that are novel for (engineered) NP and that may occur in populations other than under occupational conditions. Almost no data are available on ecotoxicity or absorption, distribution, metabolism, excretion

[1] Radomski A, Jurasz P, Alonso-Escolano D, Drews M, Morandi M, Malinski T, Radomski MW. 2005. Nanoparticle-induced platelet aggregation and vascular thrombosis. *Brit J Pharmacol* 146:882-893.

(ADME) of NP and this area should receive research priority. When choices are to be made in testing and research they should be driven by the application of the nanostructured materials.

3.2 Minimum characterisation of nanomaterials (plenary discussion)

Chairman: **Dr. Pridöhl.**

Dr. Pridöhl introduced this session by stressing that an adequate physical and chemical characterisation of nanomaterials was necessary. For many materials it should be carried out more rigorously than it has been to date. An appropriate characterisation should be based on the current knowledge of potential toxicity, since there was a strong likelihood that physico-chemical parameters affect nanomaterial toxicology. He proposed to keep three basic questions in mind when discussing which physico-chemical parameters were needed to investigate toxicity to specific target organs:

- *Are quantum properties themselves relevant for toxicity?*
- *What parameters are crucial for translocation?*
- *Is electrical conductivity, e.g. of carbon nanotubes (CNT), a relevant parameter for toxicity?*

Several small breakout groups were formed, generally organised by target organs and for ecotoxicity. Each group was asked to identify a maximum of five most important physico-chemical parameters with respect to a specific target organ. The following list was suggested for consideration:

- Specific surface area;
- particle size;
- particle size distribution;
- porosity;
- shape (top down, bottom up);
- state of aggregation (chemical covalent bonding);
- state of agglomeration (Van-der-Waals forces);
- chemical composition and defectivity;
- crystal phases and/or amorphous content;
- contaminations (like heavy metals in CNTs);
- surface modifications (chemistry and kind of attachment);
- hydrophobicity;
- surface charge;
- dissolution (rate) (remark: media of dissolution depend on the target organ considered);
- catalytic activity (e.g. attachment of proteins, oxidative activity);
- dustiness;
- magnetic properties.

The parameters suggested by the breakout groups are listed below:

Lung	Body distribution
1. Size and size distribution	1 Size
2. Specific surface area and surface modification, adsorption, interference with lung	2. Surface properties
3. Dissolution (most bulk materials have data available)	3 Dissolution rate
4. Chemical composition and defectivity (closely related to dissolution, only important if the particle is dissolved)	4. Chemical composition with emphasis on surface
5. Shape	5. Crystalline phase

Cardiovascular system	
Group 1	**Group 2**
1. Size	1. Surface area
2. Surface area	2. Size and size distribution
3. Solubility	3. Agglomeration/aggregation
4. Contaminations	4. Surface modification or chemistry (including contaminations)
5. Chemical composition	5. Dissolution rate

Skin	
Group 1	**Group 2**
1. Dissolution	1. Skin penetration and cytotoxicity/distribution
2. Size	2. Size and size distribution
3. Partition coefficient (but probably not specific for nanoparticles)	3. Surface properties (including surface area, chemical composition, hydrophobicity, surface modifications)
	4. Agglomeration
	5. Dissolution

Brain	Ecotoxicity
1. Hydrophobicity and surface charge (potential to cross barriers)	1. Particle size (distribution and uptake in organisms)
2. Size and shape	2. Agglomeration state
3. Chemical composition	3. Contaminations
	4. Hydrophobicity (waterborne/sediment/distribution in soil), dissolution rate, persistence/stability (linked to dissolution, biodegradation). On these parameters no ranking was suggested.

In summary, it was concluded that nanoparticle

- composition,
- dissolution,
- surface area and other surface characteristics,
- size,
- size distribution (including aggregation and agglomeration state),
- shape,

are parameters needed for any target organ toxicity assessment. Depending on the type of toxicological study undertaken, other physico-chemical parameters would also be required.

4. EXPOSURE ASSESSMENT

4.1 Plenary lectures

Measuring in the occupational setting

Dr. Maynard talked about current measuring techniques for workplace exposure of nanomaterials and potential development needs. Since the widespread adoption of mass-based aerosol exposure limits around half a century ago, occupational aerosol exposures have generally been characterised using relatively simple techniques such as filter sampling and gravimetric analysis. However, the size, shape and structure-related properties of engineered nanomaterials are challenging conventional approaches to exposure measurement. Given the vast range of current and potential engineered nanomaterials, the task of selecting appropriate measures of exposure is daunting. Despite the many possible biologically relevant attributes of nano-structured aerosols, these will most likely be associated with relatively few physical metrics, including number, surface area and/or mass concentration.

A number of studies have demonstrated an association between aerosol surface area and biological response, suggesting this to be an important exposure metric. Although surface area measurement methods are currently limited, methods such as diffusion charging are being developed that may lead to viable occupational exposure monitors. However, there is still uncertainty over the general applicability of surface area concentration measurements, suggesting that viable number and mass concentration measurement methods also need to be considered.

Whichever metric is more relevant, measurement methods will need to be specific to particles within specific size ranges, depending on which regions of the body they are more likely to impact. In some cases, it may be sufficient to rely on current size-selective aerosol sampling standards. However, current research suggests that more sophisticated standards will be required for some materials. This is perhaps one of the greatest immediate challenges to developing new methods of monitoring nano-aerosol exposure.

Experience from carbon black

Dr. Kuhlbusch presented data from work area measurements at several plants producing carbon black. They showed that no ultrafine particles were emitted by the process and bagging during normal conditions. The main sources of ultrafine particles were either related to other combustion sources inside (e.g. forklifts, heaters) or outside (e.g. traffic) of the plants. In the case of maintenance and repair work, emission of ultrafine particles, most likely organic carbon, was observed as it was in another case where there was a leak in the production line.

These measurements demonstrate that care has to be taken when measuring ultrafine particles at nanoparticle workplaces with regard to the source. Adequate measurement strategies are needed. These strategies should also include some detailed information on the nanoparticles since the hazard potential may vary with, for example, particle size, morphology, solubility or chemical composition.

Dermal exposure and hazard potential

Professor Lademann gave a presentation on a non-invasive method for the investigation of penetration kinetics and penetration pathways of topically applied substances. In the past, it was assumed that the intracellular penetration inside the lipid layers around the corneocytes was the only penetration pathway for topically applied substances. However, recently it has been determined that follicular penetration also has to be taken into consideration.

Analysing the penetration of commercial products of TiO_2 with a size ≥ 100 nm, usually used in sunscreens, it was found that these particles are located only on the skin surface or in the uppermost layers of the *stratum corneum*. No particles could be found in the deeper parts of the *stratum corneum*, even after long-term application. In regard to skin biopsies, it was found that the nanoparticles can penetrate into the hair follicles; however, not all hair follicles contain nanoparticles. Therefore, concerning penetration, a distinction must be made between open and closed hair follicles. It could be shown that the closed hair follicles were covered with a mixture of corneocyte elements and dry sebum. In contrast, hair follicles are open for penetration if sebum production or hair growth can be observed. In all cases, the nanoparticles were located only in the hair follicles, but not in the surrounding living tissue. With time, the hair follicles became depleted by sebum production and hair growth. It can be expected that all nanoparticles which had penetrated into the hair follicles were subsequently transported back to the skin surface.

Analysing the penetration of fluorescent dyes in the nanoparticle-form and in the non-particle-form, it was found that the nanoparticles penetrate much better into the hair follicles than the non-particle-form, if massaging action is applied. Additionally, the residence time of the nanoparticles in the hair follicles was up to ten days, whilst the non-particle containing formulation could be detected in the hair follicles only up to four days. The reason for the better penetration of the nanoparticles into the hair follicles was found in the action of the moving hair. It seems that the moving hair acts as a geared pump if the size of the nanoparticles corresponds to the surface structure of the hairs. The moving hair pushes the nanoparticles deep into the follicles, whilst sebum and hair growth, in time, move the nanoparticles out of the hair follicles.

It was stated that, up to now, there has been no evidence that nanoparticle aggregates and agglomerates with a size ≥ 100 nm penetrate into living tissue under normal conditions. The results of the diffusion experiments published in the literature, which demonstrated that

nanoparticles could pass a skin membrane, should be discussed taking into consideration that the skin samples had a thickness of 500 nm. This means that the thickness of the membranes was less than the length of the hair follicles in the tissue. In this way, the membranes contained open channels, which can act as an efficient pathway for nanoparticles.

Summarising the results, it was stated that in contrast to the *stratum corneum*, hair follicles represent an efficient long-term reservoir for topically applied nanoparticles. The optimum size for the penetration into the hair follicles was 300 to 700 nm; the nanoparticles were removed out of the hair follicles by sebum flow and hair growth. However, no real evidence has been presented, to date, that nanoparticle aggregates and agglomerates at a size larger than 100 nm penetrate through the skin barrier into living tissue.

4.2 Measuring exposure (plenary discussion)

Chairmen: **Dr. Aitken** and **Professor Fissan**.

Professor Fissan began the session with a short presentation in which he outlined the main metrics (size, number, surface area and mass) which may be used to quantify exposure in the workplace. He also provided information on methods used to measure these metrics and the difficulties associated with measuring and comparing them. Due to lack of sensitivity, it is necessary to rather use number or surface concentrations instead of mass concentration. It is also necessary to take account of background concentrations and to subtract these from any measured concentration associated with a task or process.

Mass distributions at high concentration can be measured using low pressure impactor devices such as the electrical low pressure impactor, which can provide information on particle sizes ranging from nanometres to several micrometres. Number distributions can be assessed by various devices including the scanning mobility particle sizer and the fast mobility particle sizer. These devices are commercially available.

Techniques for the direct measurement of a surface area distribution or measurement of biological effects as a function of particle size, both of which are of interest in relation to nanomaterials, are not yet widely available. However, a recent development has been the implementation of the surface area monitor by a leading manufacturer of devices. It uses charge on particles to develop an estimate of total lung deposited surface area in different compartments of the lung.

All these devices are primarily static devices which are well suited for measurement of concentrations within a room for example. They are not, however, particularly well suited to measurements of personal exposure concentrations and may need to be developed towards personal samplers.

Dr. Aitken then provided a series of questions for the group to consider. These were as follows:

- *What is the relative importance of inhalation, dermal, and ingestion exposure routes*?
- *What is the best choice of metric for each and why? Is this appropriate for all nanoparticles*?
- *What strategies (and instruments) are appropriate for demonstration of control for routine surveillance for collection of epidemiological data? What new methods and approaches might be developed*?
- *How can we better collect and share exposure information*?

The discussion broadly followed these questions.

What is the relative importance of inhalation, dermal, and ingestion exposure routes?

It was generally considered that, based on current knowledge, the most important route of exposure was inhalation. Although relatively few studies have been reported, there was not much evidence to suggest that dermal exposure is likely to provide a route which results in a systemic dose. This is not to exclude the possibility that dermal exposure could result in local effects, such as dermatitis. It was noted that there is almost no information regarding ingestion as a significant route of exposure.

Most of the remaining discussion therefore focused on inhalation as the principal route of exposure. It was noted in the discussion that exposure did not occur only in the manufacturing of these products but also in handling, packaging, downstream use, etc. Exposure to nanoparticles also occurs in other processes such as welding where the nanoparticles are incidental by-products of the process. These have been monitored in industrial scenarios for many years. However, although incidental nanoparticles are clearly relevant, the focus of the discussion was on engineered nanoparticles rather than on the former.

What is the best choice of metric and why, and is this appropriate for all NPs?

The earlier discussion had focused on some of the difficulties associated with the measurement of all these parameters. The general view of the meeting was that, at present, it was not possible, nor desirable, to select one single, preferred metric. Currently, the level of understanding of the toxicological issues does not provide clear guidance. In different circumstances, alternative measurements may be more appropriate. However, it was clear that the metric, namely surface area, was likely to be of interest, but was currently not well covered by existing approaches and needed further development.

A clear message from the meeting was the need for researchers and others to be very explicit about the way in which they have taken their measurements. Currently many people simply

quote mean and/or standard deviation data. This is insufficient and additional information is required. For example, records should include details of the instruments used, the size range of the instrument, what assumptions had been made, which summary statistic had been used, the duration of exposure, whether background information had or had not been subtracted, etc.. In addition, more clarity should be provided when comparing different studies.

What strategies (and instruments) are appropriate for demonstration of control, for routine surveillance, and for collection of epidemiological data? *What new methods and approaches might be developed*?

Many of the instruments currently available are large, expensive and not easily placed in industrial locations. There is a clear need to develop smaller, more portable and less expensive instruments. Ideally, instruments which were suitable for the measurement of personal exposure would be the most useful. Although there are a number of potential technologies which may be used to develop such instruments, none are yet commercially available.

The development of the new surface area monitor was seen as a welcome step forward. However, it is likely that much validation work will be needed before a greater understanding of the information obtained from this instrument can be fully integrated and/or utilised. This is especially in relation to its limits of detection, upper and lower size boundaries, and the range of capability. General guidance is available, for example in EN 689 (ANSI, 1995)[2].

In the meantime, it is important to work with the instruments which are currently available and develop the best understanding of the advantages and limitations of these for the purposes described.

Other issues to be considered include agglomeration. Some instruments such as particle surface area monitors are likely to be more applicable in situations where agglomerates are present. Relying simply on, for example, particle counting systems where there is significant agglomeration will lead to an erroneous view of the nature of the aerosol. An important question is the following: Do agglomerates consisting of primary nanoparticles survive mechanical stress and humidity effects during handling and in the surfactant in the lung? If they do, the transport behaviour of the big agglomerates is likely to be very different from that of single primary particles.

How can we better collect and share exposure information?

There are real difficulties in sharing exposure information given some of the discussion above. If the details of the methods used are not available, the simple count of particle concentration in two

[2] ANSI. 1995. EN 689. Guidance for the assessment of exposure by inhalation to chemical agents for comparison with limit values and measurement strategy. *American National Standards Institute.*

very different scenarios is likely to be of limited benefit, as would comparing an industrial situation with an environmental one.

There was a view at the meeting that there might be some data sets which relate to exposures that are either not yet available or not yet in the public domain. Clearly if this is the case, then there are some advantages in making such data more widely available. It was recognised however that there may be commercial difficulties involved. However, there was a strong view at the meeting that sharing these data should be encouraged, even in anonymous form.

Other comments and issues

One of the ways to help resolve the issue of the most appropriate metric is to investigate populations that are currently exposed to nanomaterials. An evaluation should be made of the various exposure metrics to see if it is possible, based on an epidemiological study, to associate exposure as assessed by the various metrics with some health effects.

In principle such a study would be possible, for example for a population of welders. Although welders have been studied for health effects, it would be important to attempt to utilise some of the emerging techniques for exposure assessment as well as the new instruments and the new approaches in these studies and thus to try to ascertain the validity of using these approaches.

5. HAZARD POTENTIAL

5.1 Plenary lectures

Tiered testing strategy for pulmonary exposure to nanomaterials

Dr. Warheit explained that lung bioassay models can be useful for evaluating the pulmonary hazards related to exposures to nanoparticulate materials. A short-term pulmonary bioassay has been developed to assess the lung toxicity of inhaled particulates. These studies have been designed as hazard screens to determine whether engineered nanoparticle test substances impart significant toxicity in the lungs of rats by assessing numerous biomarkers and comparing the results with other positive and negative reference particle types. The combination of utilising bronchoalveolar lavage and lung tissue studies concomitant with an experimental design consisting of dose response, time course evaluations and the inclusion of reference particle types provides a powerful tool for assessing the acute pulmonary toxicity of the nanoparticle test material.

Key elements of a pulmonary toxicity screening strategy for engineered nanomaterials were outlined in the presentation. The proposed methodology is similar to the lung bioassay models that have been utilised in previous studies, but two important additions or enhancements have been suggested. First, the assessment of the physico-chemical properties of the nanoparticle test material must become more robust. Thus, in addition to identifying the composition of the nanoparticle, it is important to provide additional characteristics, including the average particle size, shape, surface area, crystal structure, aggregation status and other defining features – preferably in both the bulk starting material as well as in the dosing preparation utilised in the exposure phase of the study. Secondly, given that nanomaterials may have a greater tendency (relative to fine-sized particles) to translocate from alveolar regions in the lung to the interstitium or vasculature (and enter the systemic circulation), it will be important to assess the potential adverse effects of nanoparticle exposures on extra-pulmonary organs. Thus, histopathological evaluation of the major organs is recommended.

A tiered approach in rats is suggested for the assessment of hazards to nanoscale test materials. Tier 1 is viewed as a screening study and would include shorter-term exposures (either inhalation or intratracheal instillation) along with several biomarker evaluations for post-exposure periods extending to 3 months. Tier 2 evaluations could include longer-term pulmonary exposures, (including regulatory guideline studies) as well as *in vivo* mechanistic studies such as particle deposition, translocation, clearance/biopersistence studies and animal models of susceptibility.

In summary, the primary features of a lung bioassay study include in the experimental design, the following: 1) dose response characteristics; 2) time course assessments to evaluate the transient nature or persistence of any measured effects; and 3) the inclusion of appropriate positive and negative control reference materials (particularly for the instillation studies). Accordingly, the

major endpoints of the study should include: 1) extensive physico-chemical characterisation of the test material; 2) pulmonary inflammation and cytotoxicity indices as measured in bronchoalveolar lavage fluids; 3) cell proliferation and histopathological endpoints in lung tissues; and 4) histopathology screening evaluations in major extra-pulmonary organs.

Complementary testing for mechanistic aspects

Professor Donaldson talked about a number of short-term testing systems that are available to enhance our understanding of the potential toxicity of nanoparticles. Although different particle types may produce different mechanisms which result in adverse cellular effects, there are some common pathways and properties related to the interactions of cells and particles. The dominant hypothesis for the mechanism of the toxic, pro-inflammatory and mutagenic effects of nanoparticles (NP) is oxidative stress. A variety of methods are available to assess the oxidative stress potential of particle samples, such as electron paramagnetic resonance. Particle-derived oxidative stress leads to a number of pro-inflammatory effects in target cells such as cellular oxidative stress, calcium flux and signalling pathway activation. These can all be assessed in cells in culture over a few days. In addition, we need to consider the portals of entry in the selection of cells, i.e. the skin, lungs and gut. Because of translocation issues, a number of other potential target tissues also exist. These include endothelium, vessel wall, blood cells, liver, spleen, brain and foetus. No validated assays exist to measure translocation but there are potential strategies to develop ways to measure the movement of NP across cell membranes and monolayers and also within cells. The effects of NP on elements of the cardiovascular system can be studied *in vitro* using target endothelial cells, monocytes, platelets, the complement system, etc.. Direct effects on the brain and heart could be studied by exposing neurons and heart muscle cells respectively to NP and assessing relevant endpoints. Genotoxicity of NP samples can be assessed using a number of target cells and endpoints such as comet formation and chromosome aberrations.

5.2 Key safety issues related to inhaling nanomaterials (plenary discussion)

Chairmen: **Professor Seaton** and **Dr. Mauderly.**

Professor Seaton and Dr. Mauderly co-chaired a session on inhalation toxicology of nanomaterials. In their introductory remarks they said that the range of relevant safety issues remains broad because, to date, individual research efforts form little more than an anecdotal database. An exception is the more systematic study of certain nanoparticles of pharmaceutical interest; however, much of that information is not broadly disseminated. Despite the title of the session, this summary makes no attempt to recite the litany of safety issues. There has been no shortage of meetings convened to explore safety questions and communicate current research

approaches and findings. Rather, this summary (and the actual tenor of the discussion that occurred) deals largely with over-arching issues that are thought to be key to significant progress.

The fundamental safety issues are:

- the extent and nature of adverse health effects that could be caused by plausible exposures to NP;
- the types of NP associated with these hazards;
- the dose and dosing pattern required to induce effects that are sufficiently important to guard against.

Because the known range of physico-chemical species of NP is large and will only grow, the range of potential health hazards and risks can be expected to be similarly broad. There is at present little history of confirmed health problems associated with NP from which to build the knowledge base and research approach. However, there is no shortage of speculation. This circumstance could become a fortunate opportunity to do the research necessary to prevent serious harm, were it not for the fact that the development and broad use of NP-based technologies will undoubtedly outdistance the pace of safety research.

A broadly agreed taxonomy of NP is a much needed over-arching facilitator of progress in the field. Such taxonomy does not presently exist in any systematic or widely used form. Indeed, clinicians, occupational hygienists, and laboratory researchers are largely left with only particle size as a unifying classifier of NP, despite recognition by all that this is a woefully inadequate (although important) metric. An adequate framework for communication and development of research strategies requires a mutually-intelligible taxonomy that includes not only size, but also shape, composition (notably of the surface) and solubility. Going beyond these minimal descriptors to also address surface and internal structures and chemical moieties will be necessary to fully understand the biological fates and hazards of NPs. No single research group, federal agency, or nation could conduct the full range of NP studies that will be needed in coming years. Establishing a systematic framework for communication and tracking progress among agencies and researchers is absolutely necessary.

Accompanying the need for a systematic NP taxonomy is the need for a co-ordinated, universally-accessible repository of information. For greater value, the repository should catalogue, according to the taxonomy of both ‘natural’ and ‘manufactured’ NP types, information on where and how exposures may occur, routes of exposure and likely doses (or exposure concentrations), the range of physico-chemical composition within categories, biological disposition (see below) and known effects (from molecular to clinical). Great advantage would accrue if the database was to also include indexed listings of ongoing research and publications. This, of course, is a huge undertaking, and would require not only that producers of NP and researchers provide data, but also that a substantial infrastructure be established for database management.

The former two needs point to a third general issue and that is the identification of an entity to take the lead in ensuring that the taxonomy and database efforts are carried through from mere visions to functional realities. It is easy enough to state the needs; it is another thing to implement the actions. The truism that 'something that is everybody's business is nobody's business' is familiar to all. That is, unless some organised entity assumes responsibility for taking the co-ordinating and financial steps necessary to develop a NP taxonomy and database. Information will continue to exist only in effete, fragmented forms. The discussion did not conclusively identify the most appropriate entity; however, all (who spoke) ratified the call for one to be identified.

The fourth overarching need is one that flows from, and is facilitated by, the first three. It is the development and tending of a systematic research strategy that takes into account the types and exposures to NP likely to have the greatest health impact, tracks findings and ongoing research, and marshals research resources (or tracks resources marshalled by others) to ensure that important knowledge gaps are filled. Admittedly, an international research 'management' framework is implausible. The point to be taken, therefore, is not that such centralised management could (or should) occur, but that resolving health questions about such a broad range of materials as NP requires systematic research and research management perspectives. If a universal taxonomy and co-ordinated database of cumulative research and findings were established, individual federal agencies and research groups could pursue better research strategies within their particular realm of interest.

Having dealt with some very broad issues, a more specific need was voiced in this discussion. It was mentioned, but was also evident throughout the meeting. There is a general need for better information on the disposition of NP having entered the body via inhalation, ingestion or dermal penetration. In particular it is necessary to gain an improved understanding of the:

- fractions of NP that are taken up;
- pathways by which they are distributed in the body;
- transfer rates;
- retention time and accumulation rates in different anatomical sites;
- processes, pathways and rates of dissolution and excretion.

There are certainly many other important facets of biological interactions and mechanisms of adverse responses that need investigating. However, a better understanding of the disposition of different types of NP is 'enabling knowledge' that will greatly facilitate the other investigations.

Studies of the disposition of NP could be greatly facilitated by two advances. One is a greater availability of tracer species for quantitative assessment of amounts in tissues and fluids, and (ideally) which can be viewed by some form of imaging. The other is a greater availability of standardised NP species that are representative of different physico-chemical types. The purpose of the latter, of course, is so that identical NP can be used in repeated studies and by different

researchers. These technical facilitators could greatly improve the systematic pursuit of research issues.

5.3 Testing strategies to establish dermal exposure and hazard potential (break-out group discussion)

Chairman: **Professor Lademann**, *Rapporteur*: **Professor Butz**.

The following questions were addressed:

- *Which tests should be conducted to establish exposure potential through skin barriers?*
- *When is dermal hazard testing necessary?*

For the evaluation of a potential health risk of nanoparticles, two aspects have to be considered:

- Potential hazard: are nanoparticles harmful to living cells?
- Exposure aspects: do nanoparticles penetrate under real conditions through the skin barrier into living tissue?

The first aspect may be evaluated using cell culture experiments, as wildly applied in toxicological assessments. However, such experiments should always include the appropriate microparticle control groups, in order to determine whether observed adverse effects are substance or nanoparticle related. In addition, the capacity of mammalian cells for phagocytosis/endocytosis of insoluble particles should be considered. It is well established that endocytosis of insoluble particles may cause toxicity in mammalian cells. For example, international guidelines of *in vitro* genotoxicity studies recommend that poorly soluble substances should not be tested in mammalian cells beyond concentrations producing precipitation (ICH, 1996)[3].

For the evaluation of the second aspect several methods are available for the evaluation of penetration of nanoparticles through the skin barrier. Using the method of differential stripping, the penetration kinetics of nanoparticles in the *stratum corneum* and the hair follicles can be estimated. The method of differential stripping is based on the well-known method of tape stripping, which is used to completely remove the *stratum corneum*. After analysis of these tapes, the amount of nanoparticles that remain on the skin surface or the upper layers of the *stratum corneum* as well as the amount of removed corneocytes can be determined. Based on these measurements, penetration profiles in the *stratum corneum* may be calculated. Using the method of cyanoacrylate surface biopsies after tape stripping, the amount of nanoparticles

[3] ICH. International Conference of Harmonisation of Technical Requirements of Pharmaceuticals for Human Use. 1996. Genotoxicity: specific aspects of regulatory genotoxicity tests for pharmaceuticals. ICH topic S2A, April 1, 1996. CPMP document reference CPMP/ICH/141/95.

penetrating into the hair follicles can be determined, given that cyanoacrylate stripping removes the hair follicles. By analysing the penetration and storage kinetics of nanoparticles in the *stratum corneum* and the hair follicles, information on the penetration of these substances through the skin barrier can be obtained. The determination of the amount of nanoparticles removed from the *stratum corneum* and the hair follicles after penetration permits the establishment of a mass balance. Using this calculation the amount of topically applied substances passing through the skin barrier can be evaluated. Nevertheless, a disadvantage of the method is that it is only the depletion of the reservoir which is evaluated. The amount of a test substance that has penetrated into or through the skin is not directly measured.

A second method is based on diffusion experiments using skin membranes. Usually, the thickness of these membranes is several hundred micrometres, which means that the thickness of the skin samples is less than the length of hair follicles in living tissue. Therefore, tissue membranes contain openings, whereby topically applied substances as well as nanoparticles may penetrate into the receptor fluid.

Under normal conditions these tissue openings, consisting of the hair follicles have little effect on the penetration process. One reason could be that a close network of elastin and collagen fibres surrounds the hair follicles. Usually, human tissue samples obtained from surgery are stretched for their application to the diffusion cells. During this procedure, the interfollicular space may be stretched more than the follicles themselves which may produce an opening of the follicular orifice resulting in microscopic holes in the membrane. Therefore, the ratio of intracellular versus follicular penetration could depend on the stretching procedure. Experiments on pig-ear skin are useful for the evaluation of this problem due to the large hair follicles of pig skin. Additionally, mechanical stimulation of hair may affect the penetration of nanoparticles. Usually, in diffusion cell experiments no massage or mechanical force is applied which may move the hair and may push nanoparticles into the tissue. Overall, given these limitations, diffusion experiments appear to be less suited for penetration measurements of nanoparticles.

A highly prospective method for the determination of the penetration of topically applied substances, including that of nanoparticles, into and through the skin barrier is the use of fluorescent dyes in combination with laser scanning microscopy. These non-invasive methods allow the determination of the position of topically applied substances in different layers and depths of the *stratum corneum*, i.e. up to 250 µm deep. The disadvantage of this method is that laser scanning microscopy cannot be applied to all types of topically applied substances, but only to those with efficient fluorescent properties. The development of 2-photon systems, which allow the analysis of the penetration of nanoparticles into deep tissue layers with a high sensitivity, opens new perspectives for the determination of penetration processes in the future.

Another method for the determination of the penetration of topically applied substances, including nanoparticles into and through the skin barrier, is the microscopic analysis of histological sections removed from the skin after topical application of nanoparticles and

subsequent biopsy. Thin (approx. 10 µm) and ultra-thin (approx. 50 nm) cross-sections can be analysed by high resolution transmission electron microscopy and ion beam techniques such as particle induced X-ray emission mapping. High resolution transmission electron microscopy allows visualising individual nanoparticles with diameters around 10 nm and above; in addition, the chemical composition of individual particles is obtained. The disadvantage is that the field of view is limited and substantial sample preparation is required with the risk of introducing preparation artefacts. Particle induced X-ray emission mapping with a lateral resolution of about 300 nm, on the other hand, does not visualise individual nanoparticles below this size. The advantages are that a large field of view can be scanned with the option to zoom into regions of interest, quantitative elemental maps are obtained, and the risk of introducing preparation artefacts is minimised.

The most sensitive technique uses nanoparticles radiolabelled with positron emitters. This requires skin explants from surgery to which formulations are applied topically. Thin cross-sections are subsequently dipped into nuclear micro-emulsions and, after exposure, individual positron tracks can be detected. Again, there is a risk of contamination during sample preparation.

Summarising the results of the discussion, it can be established that, up to now, there is no evidence that nanoparticle aggregates and agglomerates at a size exceeding 100 nm penetrate through the barrier in healthy skin. Today's knowledge about penetration of nanoparticles is based on the available methods, which have their limitations, particularly concerning detection limits. The potential skin penetration of much smaller nanoparticles (<<10 nm) will be the subject of further investigations. It must be taken into account that nano-emulsions, which are applied for cosmetics and medical treatment, also form structures with a size of several nanometres. These are comparable to the size of extremely small nanoparticles. On the other hand, solutions contain distinct molecules, which are even smaller than nanoparticles. Therefore, it may be assumed that the skin penetration rate of substances in the form of microemulsions is between that of a particulate form and that of a solution of the same substance. During the discussion of dermal exposure and its hazard potential it must be kept in mind that the dermal uptake of nanoparticles will, in any case, be orders of magnitude smaller than the uptake by inhalation or oral routes.

5.4 Testing strategies concerning systemic exposure (break-out group discussion)

Chairman: **Professor Oberdörster**, *Rapporteur*: **Dr. Haltner**.

The following question was addressed:

- *Which tests should be conducted to determine whether systemic exposure occurs, and if so, what is the impact on human health?*

The responses were subdivided into the three categories listed below:

- *Exposure measurements at workplaces*
- *Physico-chemical characterisation – for exposure*
- *Tiered approach for testing*

Exposure measurements at workplaces

Given that exposure assessors utilise different procedures, the first step that needs to be taken is a full and detailed description of the measurement methodology that was used. This would include specific descriptions of the conditions for operation, including details of the instruments utilised, the specific sampling techniques and descriptions of standard operating procedures. Ultimately, it will be important to develop a standardisation of methodologies with other organisations such as ISO; however, this process is a lengthy one. In the meantime, it will be important to improve and adjust measurement techniques as existing systems are not always suitable for the assessment of nanoparticle exposures (e.g. appropriate dose metrics: mass vs. surface area vs. number, portability and appropriate equipment to conduct the required studies). Ultimately, the goal will be to assess personal exposures to nanoparticles using the appropriate dose metrics.

Physico-chemical characterisation – for exposure

The preferred characteristics should include particle size, particle size distribution, surface properties including surface area, chemistry and particle mass. The ultimate goal will be to measure exposure using state of the art methods and equipment.

Tiered approach for testing

The first tier would include important physico-chemical parameters for characterising nanoparticles. These were determined to be particle size, particle size distribution, chemical composition, crystallinity, surface properties and stability in physiological systems.

In vitro assays were considered to be important components of a tiered testing system, including non-cellular (e.g. reactivity) and cellular assays. The following characteristics were viewed as desirable outcomes for cellular *in vitro* testing: nanoparticle dose levels, particle uptake within cells, possible absorption through cells and oxidative stress endpoints.

Ultimately, it will be important to generate *in vitro* and *in vivo* databases on a particular nanoparticle type in order to compare the *in vitro* with *in vivo* results. In addition, the conditions under which particulate samples were stored prior to testing (e.g. humidity, temperature, inert gas-filled containers) will be important to document for comparison with later investigations. Finally, the breakout group suggested testing of typical or generic nanoparticle types, particularly with the inclusion of carefully selected positive and negative control reference particle types.

6. EMERGING TOPICS

6.1 Plenary lectures

Impact of particles on the brain

Dr. Dorman talked about the olfactory system being the only part of the central nervous system in direct contact with the external environment. Interestingly, a number of environmental agents including metals and solvents may enter the brain via the olfactory nerve (reviewed in Arvidson, 1994[4]; Tjälve and Henriksson, 1999[5]). One of the more thoroughly studied metals is manganese. Olfactory transport of manganese has been demonstrated to occur in the rat, mouse, and freshwater pike following intranasal instillation (Gianutsos *et al*, 1997[6]; Tjälve and Henriksson, 1999[5]; Tjälve *et al*, 1995[7]). Studies by Dorman and co-workers have demonstrated that in rats, inhaled manganese is absorbed by the olfactory epithelium and subsequently undergoes transport via the olfactory nerve to the olfactory bulb (Brenneman *et al*, 2000[8]; Dorman *et al*, 2002[9]).

More recently, Dorman and co-workers have demonstrated that manganese sulphate-exposed monkeys develop markedly increased olfactory epithelial and olfactory bulb manganese concentrations (Dorman *et al*, 2006a[10], 2006b[11]). Absolute manganese concentrations in the manganese-exposed monkeys demonstrated a decreasing peripheral-central concentration gradient within the olfactory system: olfactory epithelium >> olfactory bulb > olfactory tract > olfactory cortex. The increase in olfactory bulb manganese concentration measured in the manganese-exposed monkeys was qualitatively similar to that seen in young male rats exposed subchronically to comparable concentrations of manganese sulphate.

Brain magnetic resonance image studies of those manganese-exposed monkeys demonstrated marked signal hyperintensities within the olfactory bulb, a finding consistent with increased manganese concentrations at that site. There is a critical need to determine whether nanoparticles undergo olfactory transport. Studies with commercially available polystyrene nanoparticles will examine the initial uptake of these materials by the olfactory epithelium. This phase of the

[4] Arvidson B. 1994. A review of axonal transport of metals. *Toxicology* 88(1-3):1-14.
[5] Tjälve H, Henriksson J. 1999. Uptake of metals in the brain via olfactory pathways. *Neurotoxicology* 20(2-3):181-195.
[6] Gianutsos G, Morrow GR, Morris JB. 1997. Accumulation of manganese in rat brain following intranasal administration. *Toxicol Sci* 37(2):102-105.
[7] Tjälve H, Mejare C, Borg-Neczak K. 1995. Uptake and transport of manganese in primary and secondary olfactory neurones in pike. *Pharmacol Toxicol* 77(1):23-31.
[8] Brenneman KA, Wong BA, Buccellato MA, Costa ER, Gross EA, Dorman DC. 2000. Direct olfactory transport of inhaled manganese ($^{54}MnCl_2$) to the rat brain: Toxicokinetic investigations in a unilateral nasal occlusion model. *Toxicol Appl Pharmacol* 169(3):238-248.
[9] Dorman DC, Brenneman, KA, McElveen AM, Lynch SE, Roberts KC, Wong BA. 2002. Olfactory transport: A direct route of delivery of inhaled manganese phosphate ($^{54}MnHPO_4$) to the rat brain. *J Toxicol Environ Health* 65(20):1493-1511.
[10] Dorman DC, Struve MF, Marshall MW, Parkinson CU, James RA, Wong BA. 2006a. Tissue manganese concentrations in young male rhesus monkeys following subchronic manganese sulfate inhalation. *Toxicol Sci* Apr 19 [Epub ahead of print].
[11] Dorman DC, Struve MF, Wong BA, Dye JA, Robertson ID. 2006b. Correlation of brain magnetic resonance imaging changes with pallidal manganese concentration in rhesus monkeys following subchronic manganese inhalation. *Toxicol Sci* Apr 19 [Epub ahead of print].

project will rely upon the use of rat olfactory explant cultures. The second set of experiments will rely on nasal instillation studies to explore the potential of polystyrene nanoparticles to undergo olfactory transport. Nasal instillation studies have proven to be a cost effective way to examine these transport processes. One significant disadvantage of this approach is that the data derived from nasal instillation studies cannot readily be applied to inhalation risk assessments. Thus, the last set of experiments will characterise the olfactory transport of inhaled nanoparticles in rats. One advantage of these studies is that additional knowledge concerning lung and systemic delivery and clearance of these materials can also be developed coincidentally. Data derived from these studies can be used by CIIT scientists that have developed dosimetry models that describe nasal and lung deposition of particles as well as other models developed to describe the olfactory transport of inhaled materials by laboratory animals. The research conducted by CIIT will improve our understanding of the dosimetry and toxic potential of nanoparticles.

Environmental testing

Dr. Stone explained that applications intended for newly engineered nanoparticles are diverse and include a number of applications that are likely to result in release into the environment, e.g. bio detectors, remediation, algal control, water filters, self-cleaning glass and catalysts. The ecosystem is a complex interaction of organisms living in air, aquatic and terrestrial environments, and these organisms range from simple single celled organisms to complex vertebrates. An impact by toxins on one part of the ecosystem leads to impacts on other species within the environment, for example via the various food chains. For this reason nanoparticles being released into the environment need to be tested in terms of their impacts on micro-organisms, invertebrates, vertebrates and plants.

Several studies demonstrate antimicrobial effects of nanoparticles. For example, composites of nanoparticulate TiO_2 and carbon nanotubes have been reported to have greater photocatalytic activity than TiO_2 alone, leading to increased toxicity to *Bacillus cereus* endospores. However, some of the endospores survived the exposure possibly due to endospore aggregation. Endospore survival was not observed with nano TiO_2 alone. Alumina nanoparticles have been shown to inhibit root growth in several plant species via a mechanism involving reactive oxygen species, suggesting a similar mechanism to that observed in rodent and cellular toxicology models.

Many nanoparticles tend to aggregate; therefore some studies have used a variety of protocols to aid disaggregation. The method of nanoparticle preparation seems to have an impact on the toxicity to invertebrates (and probably other species). The 'carbon sixty atom molecule' (C60) prepared in the solvent tetrahydrofuran (THF) was more potent than C60 prepared by sonication, at inducing lethality in the aquatic invertebrate *Daphnia magna*. In this study, THF prepared C60 was more potent than THF prepared TiO_2. Another study in fish (largemouth bass) found that THF prepared C60 induced lipid peroxidation. However, it is worth noting that even though THF was apparently removed in these studies by rotor evaporation, some THF remained trapped

between clusters of C60 particles, resulting in a mixed exposure. Further studies are required to ensure that the results obtained in the *Daphnia magna* and largemouth bass are specific to the nanoparticle types being tested rather than the solvent employed.

Since relatively more is known about the toxicology of nanoparticles, this information should be used to drive the strategy for ecotoxicology studies. For example, the sublethal studies conducted with nanoparticles should concentrate on the ability to induce oxidative stress and the impact of this stress. Impacts on the immune function of aquatic and terrestrial organisms are also essential as such effects could increase susceptibility to infection and disease. Ecotoxicology studies will require the use of a wide range of organisms, preferably using standardised protocols adapted for nanoparticles. These studies should consider the entire life cycle of the nanoparticle products, including the pure nanoparticles, but also nanoparticles contaminated with other components of the retailed product, as well as environmental contaminants (e.g. detergents and metals). Such studies should investigate the biopersistence/biodegradability of the nanoparticles in order to determine the long term risk associated with their release into the environment, as well as their fate and transport in the environment. It would be preferable to approach this problem with a multidisciplinary team in order to maximise the information gained and speed progress. Finally, it would be preferable to generate a standardised testing strategy that could be applied across the nanotechnology industry when developing new products.

Nanomedicine

Professor Duncan started her presentation by stressing that nanomedicine was not only important to Europe from the social and welfare aspects, but also for its economic potential. It includes all products that can be defined as 'systems and technologies for healthcare, aimed at prevention, diagnosis or therapy'. Few market data are published specifically about nanomedicine at present. However, an analysis of the market segments for medical devices, drugs and pharmaceuticals gives an idea about the leverage of nanomedicine on the markets. These two market segments represented in 2003 an end-user value of €535 billion, of which the drugs segment is the most important, with a value of €390 billion. Globally, this market has been growing at an annual rate of 7-9%, with variations according to country, technology and market segment. Drug delivery and related pharmaceutical development in the context of nanomedicine should be viewed as science and technology of nanometre size scale complex systems (10-1000 nm), consisting of at least two components, one of which is an active ingredient. The whole system leads to a special function related to treating, preventing or diagnosing diseases sometimes called smart-drugs or theragnostics. Depending on the origin, the materials employed include synthetic or semi-synthetic polymers, and natural materials such as lipids, polymers, and proteins. The primary goals for research of nano-bio-technologies in drug delivery include:

- faster development of new safe medicines;
- more specific drug delivery and targeting;
- greater safety and biocompatibility.

The main issues in the search for appropriate carriers as drug delivery systems pertain to the following topics that are basic prerequisites for design of new materials. They comprise (i) biocompatibility, (ii) biodistribution, (iii) functionality, (iv) targeting and (v) drug incorporation and release ability. A number of nanobased drug delivery systems (based on liposomes or polymer-carriers) are already on the market, while a larger number are in stage 2 or 3 clinical trials. Certainly none of the carriers developed so far fulfil all the above parameters to the full extent. The progress made in nanotechnology *inter alia* emerging from the progress in the polymer-chemistry, however, can provide an intriguing basis on which to tackle this issue in a promising way. To use the potential of nanotechnology in nanomedicine, full attention is needed to be paid to safety and toxicological issues. A recent working group on nanomedicine of the European Science Foundation has addressed this issue and seeks to improve communication and collaboration between disciplines such as materials science, polymer chemistry, pharmacokinetics, pharmacology and medicine.

7. SUMMARY AND CONCLUSION

Chairman: **Professor Greim.**

The workshop was concluded by Professor Greim who summarised the discussion on the various parts of the programme.

Background

For nanoparticle characterisation the working definition has been agreed as < 100 nm in one dimension. In addition, it was suggested by some that the criteria be expanded to < 1000 nm to include aggregates and agglomerates.

Although many physical factors can influence the functional, toxicological and environmental characteristics of nanoparticles, their impact is largely determined by:

- composition;
- dissolution;
- surface area and other surface characteristics;
- size;
- size distribution (including aggregation and agglomeration state);
- shape.

Most of the hazard information on nanoparticle types has been derived from studies with carbon black and ultrafine TiO_2 particles. Available data suggest that the nanoparticle induced toxicity is qualitatively similar to that of traditional, fine-sized particles. The effects in the lung are primarily related to inflammation and, possibly, secondary genotoxicity. Systemic effects may impact the cardiovascular and central nervous systems. Most of the information on potential systemic effects is derived from combustion-generated particles with a major focus on the cardiovascular system.

Toxicokinetics are generally determined by size and surface characteristics of particles. The decrease in particle diameter could lead to increased passage through cellular and/or intracellular membranes, along with possible transport through nerve axons. In addition, particle deposition patterns are influenced by particle size.

A variety of methods for assessing nanoparticle exposures currently exist. However, there is a significant need to develop, improve and adjust measurement methodologies to obtain accurate information on the most suitable parameters such as particle size, particle number and particle surface area. To this end, the development of standardised procedures is required.

General testing approach upon inhalation exposure

A first step would include a prioritisation-type *in vitro* screening strategy to assess the possible reactivity of nanoparticle types (including biomarkers of inflammation) along with cellular uptake.

This would be followed by repeated dose inhalation studies with evaluation of the major organs including biochemistry, haematology, and histopathology. These tests could be supplemented by specific tests to determine subtle effects including inflammation, transcription factors and corresponding gene expression, oxidative stress related to formation of free radicals, and possible secondary genotoxic effects.

Specific recommendations for a tiered testing strategy upon inhalation exposure

(i) Preliminary testing and characterisation of reactivity

In vitro systems should be developed to determine the reactivity and potency of nanoparticle types in comparison to reference materials, such as carbon black. At present there are no recommended specific test systems. As a consequence, it is suggested that five to ten currently available cellular or acellular systems be selected to develop a data base and that this is used to compare the results on specific nanoparticle types with results from *in vivo* studies.

(ii) Tier I - *in vivo* testing for hazard identification

A short-term inhalation (or alternate route such as intratracheal instillation) study is recommended with an evaluation of pulmonary effects in rats or mice. The dosimetric considerations should include particle size, size distribution, surface properties, chemical composition, shape and aggregation status. The exposure duration should be a minimum of 2 weeks for the inhalation or 1-2 doses for the instillation study. The effects that should be evaluated include lung inflammation and cytotoxicity, cell proliferation, histopathology of the respiratory tract and the major extra-pulmonary organs. Suggested post-exposure observations for the instillation study should be made at 24 hours, 1 week, 1 month, and 3 months. For the inhalation study, the post-exposure observations should include at least one recovery time period.

(iii) Tier II - *in vivo* testing for hazard identification

A longer term inhalation study is recommended in rats. In addition to the Tier I parameters described above (e.g. substantial physico-chemical characterisation, histopathology and bronchoalveolar lavage endpoints), some suggested mechanistic-based studies could include the determination of particle deposition, translocation and disposition. It is recommended that particle overload concentrations should be avoided in such long-term exposure studies.

General testing approach upon dermal exposure

Up to now, there is little evidence that nanoparticle aggregates and agglomerates at a size exceeding 100 nm penetrate through the skin barrier into the living tissue. The penetration of nanoparticles at a size less than 100 nm should be a topic of further investigation.

When analysing the dermal exposure and the hazard potential of nanoparticles, it must be taken into consideration that the dermal uptake of nanoparticles will be an order of magnitude, or more, smaller than the uptake by inhalation or oral uptake. For the evaluation of the health risk of nanoparticles, it has to be determined whether they are harmful to living cells and whether, under normal conditions, they penetrate through the *stratum corneum* of the skin into the living tissue.

Cell culture experiments are broadly used for toxicological assessments. In principle, three methods are available for the evaluation of the penetration processes:

- Using the method of differential stripping, the penetration kinetics of nanoparticles in the *stratum corneum* and the hair follicles can be evaluated. This analysis can be carried out *in vivo*.
- Diffusion cell experiments are an efficient method for *in vitro* penetration studies.
- Laser scanning microscopy is well suited to test penetration kinetics although requiring fluorescent labelled nanoparticles.

Emerging topics

Environmental safety testing, applications of nanoparticles for medical purposes and pathways of inhaled nanoparticles to the central nervous system were also briefly addressed during this workshop. It has become clear that these topics should be subject of separate workshops.

BIBLIOGRAPHY

The following publication contains an extensive list of publications related to the human health and environmental impact of nanomaterials:

Borm JA, Robbins D, Haubold S, Kuhlbusch T, Fissan H, Donaldson K, Schins RPF, Stone V, Kreyling W, Lademann J, Krutmann J, Warheit D, Oberdörster E. 2006. The potential risks of nanomaterials: a review carried out for ECETOC. *Particle and Fibre Toxicology* 3:11.

ABBREVIATIONS

ADME	Absorption, distribution, metabolism, excretion
C60	Sixty atom carbon molecule
CdTe	Cadmium telluride
CIIT	Formerly: Chemical Industry Institute of Toxicology Now: Centers for Health Research
CNS	Central nervous system
CNT	Carbon nanotubes
Dechema	Gesellschaft für Chemische Technik und Biotechnologie (Germany)
DNA	Deoxyribonucleic acid
EN	European norm
GSH	Gluthathione
HSE	Health and Safety Executive (UK)
HSL	Health and Safety Laboratory (UK)
ILSI	International Life Sciences Institute
ISO	International Standards Organization
MnO_2	Manganese dioxide
mRNA	Messenger ribonucleic acid
NIOSH	National Institute for Occupational Safety and Health (USA)
NP	Nanoparticles
OECD	Organisation for Economic Co-operation and Development
PM	Particulate matter
PM_{10}	Particulate matter of less than 10 μm
SiO_2	Silicium dioxide
SWNT	Single wall nanotubes
THF	Tetrahydrofuran
TiO_2	Titanium dioxide
UFP	Ultrafine particles
UV/VIS	Ultraviolet and visible (spectroscopy)
VCI	Verband der Chemischen Industrie (Germany)

APPENDIX 1: WORKSHOP PROGRAMME

Testing Strategies to Establish the Safety of Nanomaterials

Day 1: Monday 7 November 2005

08.30-09.00	Registration	
09.00-09.30	**Welcome**	Dr. Mike Gribble Secretary General ECETOC
	Introduction	Dr. David Warheit DuPont de Nemours
	1. CHARACTERISATION	
	Lectures:	
09.30-10.00	**Characterisation of Nanomaterials from a Physico-chemical Perspective**	Dr. Stephan Haubold Nanogate Coating Systems
10.00-10.30	**Characterisation of Nanomaterials from a Toxicological Perspective**	Prof. Paul Borm Zuyd University, Heerlen
10.30-10.45	Coffee Break	
	Plenary Discussion:	
10.45-11.45	**What should be the minimum characterisation of nanomaterials?**	Chair: Dr. Markus Pridöhl Degussa
	2. EXPOSURE ASSESSMENT	
	Lectures:	
11.45-12.15	**Measuring in the Occupational Setting**	Dr. Andrew Maynard Woodrow Wilson International Center for Scholars, Washington
12.15-12.45	**Experience from Carbon Black**	Dr. Thomas Kuhlbuch Universität Duisburg-Essen
12.45-13.00	**Dermal Exposure and Hazard Potential**	Prof. Jürgen Lademann Universitätsmedizin Charité, Berlin
13.00-14.00	Lunch	
	Plenary Discussion:	
14.00-15.00	**How should exposure be measured?**	Chairs: Dr. Robert Aitken, IOM, Edinburgh and Prof. Dr.-Ing. Heinz Fissan, IUTA, Duisburg
15.00-15.15	Coffee Break	

Testing Strategies to Establish the Safety of Nanomaterials (cont'd)

3. HAZARD POTENTIAL

Lectures:

15.15-15.45	**Tier Testing Strategy for Pulmonary Exposure to Nanomaterials**	Dr. David Warheit
15.45-16.15	**Complementary Testing for Mechanistic Aspects**	Prof. Ken Donaldson MRC/University of Edinburgh

Plenary Discussion:

16.15-17.45	**What are the key safety issues relating to inhaling nanomaterials?**	Chairs: Prof. Anthony Seaton, IOM, Edinburgh and Dr. Joe Mauderly, Lovelace Respiratory Research Institute
17.45-18.00	**Planning for Breakout Groups on Day 2**	[Meeting of the Organising Committee only]
19.30-22.00	Dinner	

Day 2: Tuesday 8 November 2005

08.30-10.30 **Breakout Groups:**

I Testing Strategies to establish dermal exposure and hazard potential:
Which tests should be conducted establishing exposure potential through skin barriers?
When is dermal hazard testing necessary? Chair: Prof. Jürgen Lademann
Rapporteur: Prof. Dr. Tilman Butz, Universität Leipzig

II Which tests should be conducted determining whether systemic exposure occurs, and if so, what is the impact on human health?
Chair: Prof. Günter Oberdörster, University of Rochester
Rapporteur: Dr. Eleonore Haltner, Across Barriers

Report of Breakout Groups and Plenary Discussion:

10.30-11.15	**Breakout Group I**
11.15-12.00	**Breakout Group II**
12.00-13.00	Lunch

Testing Strategies to Establish the Safety of Nanomaterials (cont'd)

4. EMERGING TOPICS

Lectures:

13.00-13.30	**Impact of Particles on the Brain**	Dr. David Dorman CIIT Centers for Health Research, USA
13.30-14.00	**Environmental Testing**	Dr. Vicki Stone Napier University
14.00-14.30	**Nanomedicine**	Prof. Ruth Duncan Cardiff University
14.30-15.00	Coffee Break	
15.00-16.00	**Plenary Discussion and Summary of First Part of Workshop**	Chair: Prof. Helmut Greim Technische Universität München
19.30-22.00	Dinner	

APPENDIX 2: PRESENTATION ABSTRACTS

Characterisation of Nanomaterials: a Physico-chemical Perspective

Stephan Haubold
Nanogate Coating Systems
Saarbrücken
Germany

Chemical nanotechnology enables scientists to design particles to their wish. All materials change their behaviour when turned into nanoparticles. In principle they show two basic effects:

- Size effects, e.g. the same material shows different colours depending on its size;
- Surface effects, e.g. the same material shows different behaviours in different solvents depending on its surface modification.

The talk will show an overview of synthetic methods and some possibilities of surface manipulation of nanoparticles.

Characterisation of Nanomaterials: a Toxicological Perspective

Paul J. A. Borm
Centre of Expertise in Life Sciences
Zuyd University
Heerlen, The Netherlands

Nanoscience and its emerging technologies are expected to bring a fundamental change in manufacturing in the next few years. Engineered nanoparticles (< 100 nm) are an important tool to realise these new applications and products. The reason why these nanoparticles (NPs) 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. NPs on the one hand have a large (functional) surface that is able to bind, adsorb and carry other compounds such as drugs, probes and proteins. On the other hand, NPs have a surface that might be chemically more reactive as compared to their fine (> 100 nm) analogues. Many of these special purpose-engineered NPs are produced in small quantities.

In addition to these specifically engineered nanomaterials, nano-sized particles are also being produced non-intentionally in diesel exhaust and other combustion processes. These combustion derived NPs are included in particulate matter (PM) which is measured by mass and related to adverse effects in patients with lung and cardiovascular disease. Combustion NPs have also been denominated as ultrafine particles, and are primary particles or agglomerates with a diameter smaller than 100 nm. Numerous toxicological studies have now suggested that these ultrafine particles might be responsible for adverse effects, but so far few human studies have been able to investigate this.

Interestingly most of the toxicological work on NPs has been generated with a small set of bulk nanoparticles, which have been used in industry for some decades and are produced in quantities that currently exceed many tons per year. According to several market surveys, the largest production volume in 2004 was for colloidal silica, titanium dioxide, and various iron-oxides. All these bulk NPs were considered to be so-called nuisance dusts until it was observed that upon prolonged exposure in rats, inflammation and lung tumours could occur.

The current uncertainty about toxicological properties of nanomaterials and their environmental impact is an important issue for manufacturers, insurance industry and regulators. For hazard characterisation and classification of newly engineered nanomaterials, several crucial questions need to be answered:

Which effects are specific for nanomaterials, and which effects are merely stronger?

Nanoparticles may cause the same effects as 'traditional' particles (e.g. inflammation) but they may be more potent because of their greater surface area. Nanoparticles could also cause new types of effects not previously seen with larger particles. With regard to the latter, it has recently been reported that carbonaceous NPs and gold can translocate from the nasal cavity through the olfactory epithelium (2 cm^2) along the olfactory nerves to the central nervous system (CNS). Such a mechanism was first reported for the poliovirus (30 nm) and the colloidal gold particles (50 nm) moving into the olfactory bulb of various primates. This is a mechanism specific to NPs and it remains to be established whether this uptake is also associated with a specific effect.

Can we extrapolate available data and concepts?

The epidemiological evidence on ultrafine particles has revealed several effects, mechanisms of action and susceptible groups upon inhalation of ultrafine particles. It is crucial to explore whether these concepts can be used for nanoparticles released from manufactured nanomaterials. For this, communication is needed between those creating new materials with specific properties and applications, and those that convert these thoughts into potential interactions with biological targets.

Are current testing procedures specific enough to detect the effects of nanomaterials?

Nanoscience and nanotechnologies produce a growing set of materials whose properties are largely unknown and for which current testing procedures and legislation may produce many false negatives and/or false positives. The central question here is whether current testing and classification protocols are appropriate or sufficient. A range of *in vitro* and *in vivo* tests should provide information that can contribute to hazard assessment. Both classical tests and newer models reflecting current insights into the mechanisms of NPs should be employed. The key questions for these tests are whether they are suitable to detect the qualitative and quantitative differences that are posed by nanomaterials in comparison to their fine equivalents. Currently, both the International Life Sciences Institute (ILSI) and OECD are starting up explorations and procedures to cope with the emerging industrial need in this matter. Questions that need to be answered include the handling, interpretation and registration of surface modifications, particle size variation and other physicochemical properties.

Measuring Airborne Nanomaterial Exposure in the Workplace

Andrew D. Maynard
The Woodrow Wilson International Center for Scholars
Project on Emerging Nanotechnology
Washington DC
USA

Since the widespread adoption of mass-based aerosol exposure limits around half a century ago, occupational aerosol exposures have generally been characterised using relatively simple techniques such as filter sampling and gravimetric analysis. However, the size, shape and structure-related properties of engineered nanomaterials are challenging conventional approaches to exposure measurement, leading to a re-examination of how occupational exposures to aerosols of nano-scale and nano-structured particles (nano-aerosols) should be characterised. Given the vast range of current and potential engineered nanomaterials, the task of selecting appropriate measures of exposure is daunting. One approach grounded in conventional occupational hygiene is to consider separately particle attributes potentially associated with biological response, and measurable quantities that are related to these attributes. The former may include attributes such as size and surface chemistry, while the latter 'metrics' may include measurable quantities such as number and surface area concentration. Despite the many possible biologically relevant attributes of nano-structured aerosols, these will be associated with relatively few physical metrics, including number, surface area and/or mass concentration.

A number of studies have demonstrated an association between aerosol surface area and biological response, suggesting this to be an important exposure metric. Although surface area measurement methods are currently limited, methods such as diffusion charging are being developed that may lead to viable occupational exposure monitors. However, there is still uncertainty over the general applicability of surface area concentration measurements, suggesting that viable number and mass concentration measurement methods also need to be considered.

Apart from constraints on sensitivity, size and cost, nano-aerosol exposure monitoring methods need to be specific to particles of concern where heterogeneous or varying aerosols are encountered. This may entail specificity to attributes such as particle chemistry, shape or even agglomeration state. However, a critical requirement will be specificity to particles within significant size ranges. In some cases, it may be sufficient to rely on current size-selective aerosol sampling standards. However, the size-related activity of nanoparticles and potential significance of size-dependent particle translocation from the respiratory system, suggest that more sophisticated standards will be required for some materials. This is perhaps one of the greatest immediate challenges to developing new methods of monitoring nano-aerosol exposure.

Experience from Carbon Black

Thomas A. J. Kuhlbusch[a], Heinz Fissan[a,b]
[a] Institute of Energy and Environmental Technology e.V.
47229 Duisburg, Germany
[b] Process and Aerosol Measurement Technology
University Duisburg-Essen, Germany

Nanoparticles[12] and their agglomerates are commonly produced in industry as e.g. carbon black, TiO_2, or are unintentional by-products of e.g. combustion processes. Effects of particles below 100 nm (ultrafine particles) on human health are currently heavily discussed since first investigations showed high potential effects mostly related to the human cardio-vascular system. Ultrafine particles in the ambient environment, their concentration, composition, morphology and effects on humans, are nowadays a strong area of research in epidemiology and toxicology. On the other hand, only very few data on nanoparticle and ultrafine particle exposure, including their physico-chemical characterisation at working places are available. The latter information is necessary to assess their potential hazard by toxicological studies. The hazard potential of nanoparticles may vary similar to different chemical compounds due to their changing properties dependent on particle size and chemical composition.

Results from work area measurements at several carbon black producing plants are presented and used to give examples on difficulties in work area data interpretation and possible measurement strategies. Future needs related to necessary information for exposure and risk assessments to achieve a sustainable nanoparticle production and safe working areas will be discussed.

[12] The term nanoparticle is used for a single intentionally produced particle with a diameter <100 nm whereas the term ultrafine particles is used for all particles (single or agglomerates) with diameters <100 nm.

Dermal Exposure and Hazard Potential

Jürgen Lademann, Sabine Schanzer, Heike Richter, Alexa Teichmann, Nina Otberg, Ulrike Blume-Peytavi, Wolfram Sterry
Center of Experimental and Applied Cutaneous Physiology (CCP)
Department of Dermatology
Universitätsmedizin Charité
Berlin
Germany

When analysing the penetration of TiO_2 nanoparticles used in sunscreens into the skin, it was found that the nanoparticles were located only in the upper corneocyte layers of the corneocytes in the *stratum corneum* and were penetrated deep into the hair follicles. Currently, it has been established that not every hair follicle is open for penetration. For the first time, the effect of 'open' and 'closed' follicles was observed. Penetration of the TiO_2 nanoparticles into the viable dermis could not be detected. It was found that the follicles are open for penetration if they show sebum production and/or hair growth. These observations support the hypothesis that hair follicles are closed by a covering, which has to be removed from inside by sebum production or hair growth to give the topically applied substances the opportunity to penetrate into the hair follicles. If substances penetrate into the hair follicles, the hair follicles represent a long-term reservoir for these substances. Analysing the penetration of particle and non-particle containing formulations, it was found that particles with a size between 300 and 700 nm penetrate much more efficiently into the hair follicles than smaller or larger particles. Using the method of differential stripping, it is possible to determine the amount of nanoparticles that are stored in the hair follicles.

From the structure analysis of hair surface and hair follicles, it is known that the cuticle produced by keratinocyte desquamation forms a structured surface, which can be approximated by a zigzag relief. This relief is determined by the thickness of the keratin cells, which is between 500 and 800 nm. If the hairs are set into motion by massage, the cuticle cells may act as a geared pump. Particles, comparable in size to the surface structure of the hairs and hair follicles, are probably pushed into the follicles by means of the pump movement of the hairs.

Tiered Hazard Testing for Pulmonary Exposure to Nanomaterials

David B. Warheit
DuPont Haskell Laboratory
Newark, Delaware
USA

The objective of this presentation is to propose some key elements of a pulmonary toxicity screening strategy for nanomaterials (defined herein as a particulate with a single dimension < 100 nm). The methodologies for screening the hazard potential of engineered nanomaterials are similar to fine particles, but there are at least two important differences. First, it is critical to conduct an extensive physico-chemical characterisation of the nano test material. Second, there appears to be a greater likelihood that following deposition in the lung, inhaled or instilled nanoparticles (relative to fine-sized particles) may have a greater tendency to translocate from alveolar regions to the lung interstitium or vasculature and, as a consequence, enter the general systemic circulation with potential exposures to extra-pulmonary sites. Therefore, it will also be important to screen the major organs of the body post-exposure for adverse effects related to nanoparticle exposures via transit from the respiratory tract.

A tiered approach in rats is suggested for the evaluation of nano test materials. Tier 1 can be viewed as a screening study and would include shorter-term exposures and evaluations (up to 3 months, either via inhalation exposure or intratracheal instillation exposure, depending on the circumstances).

The primary features of a pulmonary bioassay are 1) dose response assessments, 2) time course evaluations to gauge the persistence of any observed effect and 3) (particularly for intratracheal instillation studies) the inclusion of appropriate positive and negative reference-type particulates in the experimental design of the study. Thus, the major endpoints of this study would be the following: time course and dose/response intensity of 1) pulmonary inflammation; 2) cytotoxicity; 3) cell proliferation and 4) histopathological effects of particle-exposed lung tissues. In addition, histopathology-screening evaluations of major organs of the body are recommended.

Tier 2 evaluations could include longer-term pulmonary exposures as well as regulatory guideline studies; particle deposition, translocation and biopersistence/clearance investigations; as well as *in vitro* or *in vivo* mechanistic studies.

What Do We Know of the Mechanisms of Adverse Effects of Nanoparticles and How Can We Utilise this Knowledge in Additional Short-term Testing Strategy?

Ken Donaldson
MRC/University of Edinburgh
Centre for Inflammation Research
ELEGI Colt Laboratory
Queen's Medical Research Institute
Edinburgh, Scotland, UK

Faced with a large number of diverse nanoparticle types that require testing we may recommend a uniform, limited, tiered testing system. However, we may also select from a number of short-term tests that will provide additional useful information. Such additional short-term tests should be based on the known mechanisms of the harmful effects of particles and nanoparticles. Different particles have diverse mechanisms of causing adverse effects but there are some common pathways and properties of harmful particles. The general model for the harmful effects of low solubility particles is total surface area × surface 'reactivity'; in the case of particles with a soluble component, a factor due to the toxicity of that soluble material may be added. Certain pulmonary endpoints are linked with particles in general and with combustion-derived nanoparticles, the best studied nanoparticles in toxicological terms. Testing of new engineered nanoparticles in these tests could be expected to yield important information regarding their potential pulmonary toxicity.

1. *Inflammation*. Inflammation is caused by all pathogenic particles and can be studied in short-term tests by testing for points in the known molecular biology of inflammation e.g. transcription activation, gene expression for cytokines and chemokines.
2. *Genotoxicity*. Many particle types cause genotoxic effects *in vitro* using tests such as DNA strand breaks or cell cycle analysis e.g. quartz, PM_{10}.
3. *Oxidative stress*. Oxidative stress is a common pathway for pro-inflammatory and genotoxic effects of particles and can be used to detect direct effects of the particles themselves or oxidative stress in cells treated with the particles. Oxidative stress can be detected in a number of ways such as GSH depletion or with redox-sensitive dyes.

With these new materials, there may arise as-yet unidentified pathways for adverse effects and, as these crop up, they should be incorporated into short-term testing methodology. This situation, I believe, already arises for nanotubes which show an unusual proclivity to cause rapid fibrosis in rat models. For that reason, nanotubes should be tested in short-term tests of their ability to stimulate lung cells to release growth factor cytokines or stimulate fibroblast growth directly.

In addition, the PM_{10} epidemiology literature and limited studies with some model nanoparticles in toxicological studies suggest that there may be extra-pulmonary effects of nanoparticles. Some of these extra-pulmonary adverse effects might arise as a consequence of pulmonary

inflammation but others may be a consequence of translocation of particles from the lungs to these target organs such as the blood, liver, spleen, CNS etc.. It should be noted that no complete toxicokinetic data are available for any nanoparticle to determine the dosage (if any) that a target organ might experience after inhalation. It may be possible to develop tests to detect the propensity of a nanoparticle sample to cross epithelial barriers. Once particles penetrate to the pulmonary interstitium, they may interact with endothelial cells and blood cells such as monocytes and platelets, and so tests of the impact of nanoparticles on these cells may be informative with regard to cardiovascular effects. Target organ cells can also be tested to determine potential effects of nanoparticles on them, assuming that they reach these organs e.g. hepatocytes, Kupfer cells, immunocompetent cells from the spleen and lymph nodes, nerve cells, brain cells etc..

In addition, the skin and gut are also potential portals of entry for nanoparticle exposure. Short-term tests could be developed that examine the effects of nanoparticles on gut and skin cells and organ cultures to address specific hypotheses relating to adverse effects.

Delivery of Inhaled Particles to the Central Nervous System

David Dorman
CIIT Centers for Health Research
Research Triangle Park, NC
USA

Particle deposition within the respiratory tract is influenced by particle size. Inhaled materials can be deposited in the pulmonary region and some undergo subsequent systemic delivery to the central nervous system (CNS). A large percentage of inhaled nanoparticles (~ 1 nm) deposits in the nasopharyngeal region. There is growing evidence that xenobiotics deposited within the nasal cavity can be absorbed at this site and then undergo transport along either the olfactory or trigeminal nerve. Xenobiotics that undergo olfactory or trigeminal nerve transport may gain access to the CNS. Studies conducted in rodents have shown that inhaled ultrafine graphite carbon particles can undergo direct nose-to-brain transport. Another particle of special concern is manganese, a neurotoxic metal shown to be able to cross synapses in the olfactory bulb and migrate via secondary olfactory neurons to more distant nuclei of the brain. Studies conducted by our laboratory have shown that inhaled manganese undergoes olfactory translocation to the rodent brain. Monkeys likewise develop markedly increased olfactory epithelial and olfactory bulb manganese concentrations following manganese inhalation. These studies have clearly demonstrated that direct olfactory transport is a major pathway by which inhaled manganese reaches the olfactory bulb. However, the toxicological significance of olfactory transport of manganese remains poorly understood.

Environmental Testing of Nanoparticles

Vicki Stone, Alex Ford, Nick Christofi and Teresa Fernandes
Centre for Health and Environment
Napier University
Edinburgh, UK

Very few studies have been conducted in relation to nanoparticles in the environment, their fate, behaviour or ecotoxicology. Knowledge of the fate and behaviour of nanoparticles in the environment is required in order to assess which species are likely to be exposed in significant quantities. In terms of ecotoxicology it is essential to consider a broad spectrum of species ranging from micro-organisms to complex organisms such as fish and birds.

A number of studies already suggest that a variety of nanoparticles including metal oxides, C60 and silver exhibit antimicrobial effects. In fact a number of these materials are being commercially developed to exploit such properties, including the remediation of contaminated land or water. The problem with such applications is that the impact on essential non-target bacteria required for ecosystem balance is, at present, unknown.

A few studies have investigated the impact of C60 on aquatic invertebrates such as *Daphnia magna* (water flea) and vertebrates (largemouth bass). Such studies have encountered protocol problems relating to the hydrophobicity of C60 and the inability to generate a solution or suspension that is stable. Some studies have employed the use of an organic solvent, but it is unclear as to whether the observed effects are due to the C60 particles or the residual contaminating solvent. Effects in *Daphnia* include altered swimming behaviour, while effects in the fish include lipid peroxidation in the brain.

The toxicology of low solubility nanoparticles such as carbon black, TiO_2 and polystyrene black have been studied in rodent models in relation the lung and cardiovascular toxicity. The results of these studies are useful in that they provide evidence of mechanisms by which nanoparticles can induce toxicity. These studies suggest that particle size, surface area, dimensions, composition and level of contamination are all important physical parameters that must be considered. In terms of toxicity, it is also clear that the ability to generate oxidative stress and inflammation are key factors in driving biological effects. Hence, the knowledge gained from toxicity studies will be useful in informing the design of ecotoxicity studies.

Finally, in addition to providing essential information regarding the ecotoxicology of nanoparticles, such studies may also provide beneficial alternative models to the use of animals. For example, bacteria can be engineered to generate light in response to specific signals such as oxidative stress or genotoxicity and hence provide a useful tool for screening varieties of nanoparticles.

Nanomedicines Transfer from Laboratory to Clinic: Current Status

Ruth Duncan
Centre for Polymer Therapeutics
Welsh School of Pharmacy
Cardiff University
Cardiff, Wales, UK

Statistics tell us that someone dies of cancer every four seconds, of AIDS every 11 seconds and of Alzheimer's disease every 86 seconds. The average UK pharmacy has about four patients with multiple sclerosis. The pressing need for improved therapies for these and other life-threatening, debilitating and chemotherapy-resistant diseases is obvious. There is increasing anticipation that nanotechnology, as applied to medicine, will bring significant advances in the diagnosis and treatment of disease. This has prompted many governmental and funding agencies to strategically review the field[13]. The primary objectives have been to ascertain current status, to establish a common terminology, to assess potential benefits and risks and to establish priorities for future funding initiatives. When a field suddenly becomes fashionable, it is important to keep perspective and, most importantly, distinguish the science fact from science fiction. Although not widely appreciated, progress in the development of nano-sized hybrid therapeutics and nano-sized drug delivery systems over the past decade has been remarkable. A growing number of products including liposomes[14], antibodies and their conjugates[15], nanoparticles[16] and polymer therapeutics[17,] have already secured regulatory authority approval. In turn, these products are supported by a healthy clinical development pipeline. They can rightly be viewed as the first 'nanomedicines' and they are already bringing clinical benefit to thousands of patients.

Even agreement on the basic definitions is proving a challenge to those working in the multidisciplinary field of nanotechnology[18]. Within the confines of the nanoscale size range, the discipline of 'nanomedicine' can be best defined as the science and technology of diagnosing, treating and preventing disease. On one hand, device miniaturisation is providing exciting opportunities, whilst on the other progress in synthetic and supramolecular chemistry is generating ever more sophisticated, multi-component nanosized technologies. Recently the European Science Foundation's Forward Look on Nanomedicine identified five distinct, but overlapping sub-themes contributing to the field of nanomedicine. These are:

[13] NIH/NCI Cancer Nanotechnology Plan July 2004 http://nano.cancer.gov; European Science Foundation Forward Look on Nanomedicine Policy Briefing 23 (2005) www.esf.org.
[14] Milenic DE, Brady ED, Brechbiel MW. 2004. Antibody-targeted radiation cancer therapy. *Nat Rev Drug Discov* 3:488-499.
[15] Torchilin VP. 2005. Recent advances with liposomes as pharmaceutical carriers. *Nat Rev Drug Discov* 4(2):145-160.
[16] Brigger I, Dubernet C, Couvreur P. 2002. Nanoparticles in cancer therapy and diagnosis. *Adv Drug Deliv Rev* 54(5):631-651.
[17] Duncan R. 2003. The Dawning Era of Polymer Therapeutics. *Nat Rev Drug Discov* 2:347-360.
[18] Ferrari M. 2005. Cancer nanotechnology: Opportunities and challenges. *Nat Rev Cancer* 5(3):161-171.

(i) analytical techniques and diagnostic tools,
(ii) nano-imaging and manipulations,
(iii) nanomaterials and nanodevices,
(iv) nanomedicines designed either as biologically active therapeutics or drug delivery systems,
(v) all the issues relating to their pharmaceutical development, and clinical use with particular regard to potential toxicity.

This presentation will review the current status of the field and the current approach to preclinical and clinical toxicological evaluation of the first polymer therapeutics.

APPENDIX 3: THOUGHT STARTER

Nanomaterials: what do we need to know?

For hazard characterisation, risk assessment and future regulation of nanomaterials, several crucial issues need to be considered:

1. *Effects measured may not be specific or unique for nanoparticles (NP) per se,* but also present for the same or other materials of larger size or aggregates. In this case, effects of NP are quantitatively different but not qualitatively different, and regulation may be adapted by changing values and/or metrics of respective standards.

   Example: Exposures to overload concentrations of nanoparticle TiO_2 or carbon black can induce lung tumours in rats at considerably lower gravimetric lung burdens when compared to their larger sized analogues and actually the retained particle surface metric has been used to describe the lung tumour rate in chronic animal studies. 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 low toxicity particles due to its pro-mutagenic effects and actions on cell proliferation. Still this surface dose concept is probably an over-simplification, and careful evaluation is needed.

2. *Effects may be qualitatively different* based on size, surface chemistry or another specific interaction. In this case normal standard setting could be used, since the critical effect is simply different from the fine-sized analogues. This however implies that the same material, based on size differences may have different standards, also based on different effects.

   Example: 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. Such a mechanism was first reported for poliovirus (30 nm) and colloidal gold particles (50 nm) moving into the olfactory bulb of various primates. This is a mechanism specific for NP and observed for different materials (carbon, gold, MnO_2). Similarly, 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 the food industry to increase bioavailability of compounds that normally have a low bioavailability (vitamins, proteins). To do so, pure chemical substances are synthesised into nanoparticles with crystalline structure and in this way may be taken up through the immune system in the gut.

3. *Current regulation of chemicals is driven by area of application.* We deal with a growing set of materials of which some properties are largely unknown and current testing procedures and legislation may produce many false negatives and/or false positives. The second issue already illustrated that the same material dependent on size may exert different (qualitative) effects. The central question here is whether current testing and classification protocols are appropriate and sufficient. Nanotechnology also promotes convergence of technologies, and similar materials may be applied in automotive and life sciences sectors. To stimulate production and marketing of safe nanomaterials, exchange of data between sectors is recommended.

Nanomaterial testing: how to fill the gaps?

There is a limited amount of data on the toxicity of NP. Moreover, these data are mainly based on a limited number of NP types (combustion derived NP, TiO_2, carbon black) and the assumption that many effects of PM are driven by the ultrafine particles in it. Due to this background of the data and the specificity of most preparations of engineered nanoparticles, much work needs to be conducted with regard to characterisation and biological testing of engineered NP. In this regard, it is recommended to perform testing driven by the anticipated application and classification by risk and not by hazard. From the above, it is clear that a range of endpoints should be considered for the testing of NP for potential hazards. Some engineered NP, which become airborne, will pose inhalation hazards, while cosmetics with NP provide dermal exposures. Each should be tested in the requisite ways focusing on their portal of entry. Other engineered NP are being used as devices to target drugs to specific tissues, to increase their biological half time, or for imaging/sensor purposes. In developing testing procedures and protocols, a number of basic questions need to be addressed:

1. *Which components should be tested?*
   The following should all be considered: native particle with surface modification, stability of the surface coating, effects of the NP + surface coating, materials used for synthesis of NP.

2. *What type of tests should be used?*
   A range of *in vitro* and *in vivo* tests should provide information that can contribute to hazard assessment, although *in vitro* hazard studies must be first validated using *in vivo* methods. Both classical tests and newer models reflecting current insights into the mechanisms of NP should be employed. The key questions for these tests, is whether they are suitable to detect the qualitative and quantitative differences that are posed by nanomaterials in comparison to their fine-sized equivalents. Currently Dechema/VCI, the HSE, ILSI and ECETOC, and are starting up explorations and procedures to cope with the emerging industrial need in this matter.

3. *Surface modifications included in testing?*
   Whatever tests will be used, it needs to be realised 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 in order 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. Apart from modifying the surface, the compounds used in post-synthetic routes such as 4-dimethylaminopyridine, various thiols, fluoroalkanes, alkoxysilanes or phosphorous may be released and need to be included in testing protocols.

4. *Particle dissolution: good or bad?*
   Analogous to the conceptual understanding of fibre-induced malignant effects, during initial discussions particle dissolution has been mentioned as a potential screening property to prevent chronic effects. Current EU legislation for new fibres has incorporated *in vitro* dissolution of fibres based on the body of evidence connecting high *in vivo* durability (low dissolution) to lung tumours. Although the testing strategies for nanomaterials require further exploration, one should be aware of two major complications. First, some nanoparticles (quantum dots) contain highly reactive or toxic components that may cause effects when dissolved. The second problem is provided by definitions of how to assess nanoparticle dissolution.

The potential risks of nanomaterials: a review carried out for ECETOC. [19]

Borm PJA, Robbins D, Haubold S, Kuhlbusch T, Fissan H, Donaldson K, Schins RPF, Stone V, Kreyling W, Lademann J, Krutmann J, Warheit D, Oberdörster E. 2006. *Particle and Fibre Toxicology* 3:11 (Abstract).

During the last few years, research on toxicologically relevant properties of nanoparticles has increased tremendously. A number of international research projects and additional activities are ongoing in the EU and the US, nourishing the expectation that more relevant technical and toxicological data will be published. Their widespread use allows for potential exposure to engineered nanoparticles during the whole lifecycle of a variety of products. When looking at possible exposure routes for manufactured nanoparticles, inhalation, dermal and oral exposure are the most obvious, depending on the type of product in which nanoparticles are used. This review shows that:

1. Nanoparticles can deposit in the respiratory tract after *inhalation*. For a number of nanoparticles, oxidative stress-related inflammatory reactions have been observed. Tumour-related effects have only been observed in rats, and might be related to overload conditions. There are also a few reports that indicate uptake of nanoparticles in the brain via the olfactory epithelium. Nanoparticle translocation into the systemic circulation may occur after inhalation but conflicting evidence is present on the extent of translocation. These findings urge the need for additional studies to further elucidate these findings and to characterise the physiological impact.
2. There is currently little evidence from skin penetration studies that *dermal applications* of metal oxide nanoparticles used in sunscreens lead to systemic exposure. However, the question has been raised whether the usual testing with healthy, intact skin will be sufficient.
3. Uptake of nanoparticles in the gastrointestinal tract after *oral uptake* is a known phenomenon, of which use is intentionally made in the design of food and pharmacological components.
4. Finally, this review indicates that only few specific nanoparticles have been investigated in a limited number of test systems and extrapolation of these data to other materials is not possible.

Air pollution studies have generated indirect evidence for the role of combustion-derived nanoparticles in driving adverse health effects in susceptible groups. Experimental studies with some bulk nanoparticles (carbon black, titanium dioxide, iron oxides) that have been used for decades suggest various adverse effects. However, engineered nanomaterials with new chemical and physical properties are being produced constantly and the toxicity of these is unknown. Therefore, despite the existing database on nanoparticles, no blanket statements about human toxicity can be given at this time. In addition, limited ecotoxicological data for nanomaterials preclude a systematic assessment of the impact of nanoparticles on ecosystems.

[19] Funded by ECETOC, without a formal peer-review by the ECETOC Scientific Committee.

Principles for Characterizing the Potential Human Health Effects from Exposure to Nanomaterials: Elements of a Screening Strategy

Oberdörster G, Maynard A, Donaldson K, Castranova V, Fitzpatrick J, Ausman K, Carter J, Karn B, Kreyling W, Lai D, Olin S, Monteiro-Riviere N, Warheit D, Yang H. 2005. *Particle and Fibre Toxicology* 2:8 (Abstract).

The rapid proliferation of many different engineered nanomaterials (defined as materials designed and produced to have structural features with at least one dimension of 100 nanometres or less) presents a dilemma to regulators regarding hazard identification. The International Life Sciences Institute Research Foundation/Risk Science Institute convened an expert working group to develop a screening strategy for the hazard identification of engineered nanomaterials. The working group report presents the elements of a screening strategy rather than a detailed testing protocol. Based on an evaluation of the limited data currently available, the report presents a broad data gathering strategy applicable to this early stage in the development of a risk assessment process for nanomaterials. Oral, dermal, inhalation, and injection routes of exposure are included recognising that, depending on use patterns, exposure to nanomaterials may occur by any of these routes. The three key elements of the toxicity screening strategy are: physico-chemical characteristics, *in vitro* assays (cellular and non-cellular), and *in vivo* assays.

There is a strong likelihood that biological activity of nanoparticles will depend on physico-chemical parameters not routinely considered in toxicity screening studies. Physico-chemical properties that may be important in understanding the toxic effects of test materials include particle size and size distribution, agglomeration state, shape, crystal structure, chemical composition, surface area, surface chemistry, surface charge, and porosity.

In vitro techniques allow specific biological and mechanistic pathways to be isolated and tested under controlled conditions, in ways that are not feasible in *in vivo* tests. Tests are suggested for portal-of-entry toxicity for lungs, skin, and the mucosal membranes, and target organ toxicity for endothelium, blood, spleen, liver, nervous system, heart, and kidney. Non-cellular assessment of nanoparticle durability, protein interactions, complement activation, and pro-oxidant activity is also considered.

Tier 1 *in vivo* assays are proposed for pulmonary, oral, skin and injection exposures, and Tier 2 evaluations for pulmonary exposures are also proposed. Tier 1 evaluations include markers of inflammation, oxidant stress, and cell proliferation in portal-of-entry and selected remote organs and tissues. Tier 2 evaluations for pulmonary exposures could include deposition, translocation, and toxicokinetics and biopersistence studies; effects of multiple exposures; potential effects on the reproductive system, placenta, and fetus; alternative animal models; and mechanistic studies.

Developing Experimental Approaches for the Evaluation of Toxicological Interactions of Nanoscale Materials

A workshop addressing the challenges of conducting and interpreting studies of potential toxic effects of nanoscale materials.

On November 3-4, 2004 a group of international experts met in Gainesville, Florida to identify and discuss issues associated with the proper conduct of studies to characterise the potential toxicities of manufactured nanoscale materials. The 75 invited participants represented expertise in biology, medicine, toxicology, physics, chemistry, and materials science drawn from government, industry, academic and public interest sectors. The participants heard presentations and addressed in breakout sessions; a) characterisation and dosimetry of nanoscale materials, b) delivery of nanoscale materials to test systems, c) toxicology study protocols appropriate for nanoscale materials, d) detection and quantification of nanoscale materials in test systems/organisms and the environment, e) laboratory safety and disposal issues, and f) specific issues related to uptake and toxicity to the respiratory, skin, and immune systems.

Over the course of the two-day workshop, several central themes emerged from the presentations and discussions:

- It is essential that the physical and chemical characterisation of nanoscale materials be much more complete than has been the case in the sparse toxicology literature appearing to date. State of the art analytical characterisation techniques were described and their application to all phases of toxicology studies was considered. The use of currently available analytical techniques to detect and quantify nanoscale structures in biological systems was considered critical for both guiding the selection of the specific toxic endpoints of interest, and for following the movement of nanoscale materials in biological systems. The group recommended that scientific journal editors be urged to require proper physical and chemical characterisation of nanoscale materials for all publications in the newly emerging field of 'nanotoxicology'.
- Most participants agreed that 'nanotoxicology' need not be a new scientific discipline. Based on our current understanding, the traditional approaches and study protocols now used for routine toxicological characterisations of chemicals or larger particles are sufficiently robust to provide meaningful toxicological characterisations of nanoscale materials. While nanoscale materials clearly have unique physical and chemical properties that may lead to unpredictable distribution and effects within biological systems, there was general agreement that the manifestation of biological interactions of nanoscale materials will likely be the same as for any potentially hazardous agent. The participants recognised that more suitable approaches for nanoscale material characterisation, detection and/or toxicological evaluations may emerge with time and experience.

- Participants stressed the need to approach nanotoxicology studies from a multidisciplinary approach and recommended that government agencies explore ways to create and promote linkages between toxicologists and experts in materials science, physics, chemistry and other appropriate disciplines. Government agencies were also asked to provide assistance with the creation of standard reference materials, and in the development of accreditation programmes for analytical laboratories engaged in the analysis and characterisation of nanoscale materials.

The workshop was organised by the University of Florida and the US Department of Health and Human Services' National Toxicology Program. Workshop funding was provided by the Air Force Office of Scientific Research, the US Environmental Protection Agency, the National Institute of Environmental Health Sciences, the National Science Foundation, and the University of Florida.

International Symposium on Occupational Health Implications of Nanomaterials
Sponsored by the Health and Safety Executive (HSE), the Health and Safety Laboratory (HSL) (UK) and by the National Institute for Occupational Safety and Health (NIOSH) (US), held on October 12-14, 2004 in Buxton, United Kingdom.

HSE/HSL and NIOSH convened the research summit to examine occupational health issues related to the production and use of nanomaterials:
What is currently known about potential exposures to nanoparticles in such processes? What more do scientists and policy makers need to know, in order to understand the potential occupational health impacts of this 21st century technology?
From three days of scientific presentations and workshop deliberations, several consistent themes emerged:

- In themselves, studies to date do not provide all the information needed for determining, with confidence, whether nanomaterials have occupational health effects. However, they provide a good springboard for designing new research that will move scientific understanding significantly forward. "I came here thinking there were really major gaps in our knowledge," Dr. Fullam remarked. "I go home thinking, 'That's true, but it's not true.' "

- To fill existing gaps, collaborative research is needed across different scientific disciplines. For example, studies are needed to better define the properties and behaviour of nanoparticles; to develop a 'metric' for measuring exposure to nanoparticles in ways that correlate with potential health factors; to assess the adequacy of personal protective equipment; and to better assess the relevance of data from laboratory animal studies for predicting potential human effects.

- While further research is planned and conducted, makers and users of nanomaterials can take precautionary steps to control exposures, using the traditional risk assessment/risk management approach and instituting controls as appropriate.

- Tools to measure, assess, and control exposures need to be standardised internationally, to avoid confusion and to promote scientific collaboration.

- Scientists and policy makers should maintain open communication with the public as research, development, and application of nanotechnology advances. It is important to engage discussion "not only among you as experts in the field," Mr. Ewins of the HSE told the participants, "but [also to secure] the involvement and the confidence of the public. If we lose the public's trust about anything, then we are in an uphill battle to recover it."

Consensus language reached by the participants at the Buxton workshop

"Considering the large amount of research on effects and mechanisms of NP it is surprising to note, that little of this work can be used to screen NP to prevent adverse biological effects in susceptible targets. There is a need to develop and validate new test models as well as to evaluate and validate existing methods for testing of new NP. For this it seems a good approach to assemble a panel of old and new NP and perform a number of existing tests with these NP. In this case the 'old' NP would function as a qualitative standard for the detection and comparison of effects. Oxidative stress seems a mechanism to generate a number of simple, pragmatic tests (plasmid DNA unwinding, OH-generation) for further study and validation but the interpretation of test results (qualitative) is crucial.

There are different opinions on the statement whether existing tests may not pick up all of the hazards. Existing tests may pick up the risks of NP but not be sensitive enough, or hazards are not seen at all, because insensitive models are being used. The latter is underscored by the negative outcomes of animal research trying to reproduce the effects of particulate matter seen in epidemiological studies. A consensus is reached on the fact that currently there is nothing better than approaches used in pharmaceutical industry, i.e. a case-by-case approach. There is definitely a need to develop concepts of testing, which can be done by bridging studies with the right dosimetry. The dosimetry is related to the anticipated application of the nanostructured materials and to the metric, which is chosen or investigated. Another reason for the right dosimetry is that often only small amounts of nanomaterials are available, and for instance (chronic) inhalation studies are virtually impossible.

There is considerable consensus on the fact that screening of mutagenicity may become a crucial issue in the registration of new nanomaterials. Several pieces of evidence indicate that NP can translocate into the nucleus and mitochondria and interact with endogenous machinery such as DNA, DNA-repair enzymes, mRNA and proteins. Both high and active surface NP may cause damage to DNA and/or absorption or (in)activation of crucial factors in cell growth and proliferation. However, for a good interpretation of this hazard, one realises again that the test model and dosimetry should be valid. In fact there is a need for good models to test *in vivo* mutagenicity."

APPENDIX 4: LIST OF PARTICIPANTS

Name	*E-mail*	*Affiliation*
R. Aitken	rob.aitken@iomhq.org.uk	Institute of Occupational Medicine Edinburgh, UK
K. Andrieux	karine.andrieux@cep.u-psud.fr	UMR CNRS 8612, Laboratory of Pharmaceutical Technology, France
A. Bassan	arianna.bassan@jrc.it	ECB -JRC - IHSCP, EU
N. Bergman	nicklas@intergalactic.se	Nanofactory Instruments, Sweden
P. Borm	p.borm@hszuyd.nl	Zuyd University, Heerlen, The Netherlands
J. Bruch	joachim.bruch@ibe-marl.de	IBE Marl, Germany
	j.bruch@uni-essen.de	University Clinics Essen, Germany
T. Burns	tom.burns@soc.uu.se	Uppsala University, Sweden
		Norwegian University of Life Sciences
T. Butz	butz@physik.uni-leipzig.de	Universität Leipzig, Germany
P. Carthew	philip.carthew@unilever.com	Unilever, SEAC, UK
G. Cartlidge	george.cartlidge@hse.gsi.gov.uk	HSE, Industrial Chemicals Unit, UK
H. Certa	hans.certa@de.sasol.com	Sasol, Germany
C. Cudicini	corinne.cudicini@eu.rhodia.com	Rhodia Services, France
D. Dahmann	dahmann@igf-bbg.de	Ruhr Universität Bochum, Germany
R. Denison	rdenison@environmentaldefense.org	Environmental Defense, USA
M. Dominguez Estevez	manuel.dominguezestevez@rdls.nestle.com	Nestlé, Switzerland
K. Donaldson	ken.donaldson@ed.ac.uk	University of Edinburgh, UK
D. Dorman	dorman@ciit.org	CIIT Centers for Health Research, USA
R. Duncan	duncanr@cardiff.ac.uk	Cardiff University, UK
T. Epprecht	thomas_epprecht@swissre.com	Swiss Re (Swiss Reinsurance Company), Switzerland
H. Fissan	h.fissan@uni-duisburg.de	Universität Duisburg-Essen, Germany
H. Fogelberg	hans.fogelberg@sts.gu.se	Göteborg University, Sweden
V. Froment-Louia	valerie.froment@arkemagroup.com	Arkema, France
J. Garrod	john.garrod@defra.gsi.gov.k	Dept. for Environment, Food & Rural Affairs, UK
R. Gibson	rosemary.gibson@hsl.gov.uk	Health & Safety Laboratory, UK
H. Glimell	hans.glimell@sts.gu.se	Göteborg University, Sweden
M. Gonzalez	mar.gonzalez@oecd.org	OECD, France
H. Greim	helmut.greim@lrz.tum.de	Technische Universität München, Germany
M. Gribble	michael.gribble@ecetoc.org	ECETOC, Belgium
A. Grinbaum	alexei.grinbaum@polytechnique.edu	GRISE-CREA, Ecole Polytechnique, France
M. Haag-Grönlund	marie.haaggroenlund@astrazeneca.com	AstraZeneca, Sweden
E. Haltner-Ukomadu	dr.haltner@acrossbarriers.de	Across Barriers, Germany
S. Haubold	stephan.haubold@nanogate.com	Nanogate Coating Systems, Germany
M. Hengstberger	manfred.hengstberger@itcf-denkendorf.de	ITCF Denkendorf, Germany
C. Hennes	christa.hennes@ecetoc.org	ECETOC, Belgium
T. Hesterberg	tom.hesterberg@nav-international.com	International Truck & Engine Corporation, USA
P. Hoet	peter.hoet@med.kuleuven.be	Katholieke Universiteit Leuven, Belgium
R. Hollander	rhollandnsf@aol.com	National Science Foundation, USA
D. King	kingddj@bp.com	BP, UK
	duncan.king@uk.bp.com	
T. Koch	koch.t@pg.com	Procter & Gamble Eurocor, Belgium

Name	*E-mail*	*Affiliation*
N. Krüger	nils.krueger@degussa.com	Degussa, Germany
T. Kuhlbusch	tky@iuta.de	IUTA, Germany
J. Lademann	juergen.lademann@charite.de	Charité Universitätsmedizin Berlin, Germany
M. Marrec-Fairley	mfa@cefic.be	Cefic, Belgium
P. Martin	philippe.martin@cec.eu.int	DG SANCO, EU
J.-P. Marty	jean-paul.marty@wanadoo.fr	Paris University, Faculté de Pharmacie, France
J. Mauderly	jmauderl@lrri.org	Lovelace Respiratory Research Institute, USA
A. Maynard	andrew.maynard@wilsoncenter.org	Woodrow Wilson International Center for Scholars, USA
M. Mehta	michael.mehta@usask.ca	University of Saskatchewan, Canada
C. Miller	miller@lafollette.wisc.edu	University of Wisconsin-Madison, USA
J. Morris	morris.jeff@epamail.epa.gov	US EPA, USA
G. Nohynek	gnohynec@rd.loreal.com	L'Oréal, France
A. Nordmann	nordmann@phil.tu-darmstadt.de	Technische Universität Darmstadt, Germany
G. Oberdörster	gunter_oberdorster@urmc.rochester.edu	University of Rochester, USA
R. Oliver	raymond.oliver@cenamps.com	Cenamps, UK
M. Puolamaa	maila.puolamaa@cec.eu.int	DG SANCO SCENIHR, EU
M. Pridöhl	markus.pridoehl@degussa.com	Degussa, Germany
P. Priem	peter.priem@solvay.com	Solvay, Belgium
G. Randall	geoff.randall@freeuk.com	ECETOC, Belgium
H. Rauscher	hubert.rauscher@jrc.it	JRC IHCP - BMS, EU
G. Rowe	gene.rowe@bbsrc.ac.uk	Institute of Food Research Norwich, UK
F. Saykowski	franz.saykowski.fs@bayer-ag.de	Bayer, Germany
S. Schanzer	sabine.schanzer@charite.de	Charité Universitätsmedizin Berlin, Germany
J. Scheel	julia.scheel@henkel.com	Henkel, Germany
A. Seaton	a.seaton@abdn.ac.uk	University of Aberdeen, United Kingdom
V. Stone	v.stone@napier.ac.uk	Napier University, Edinburgh, UK
K. Thomas	kthomas@ilsi.org	ILSI, USA
R. Turner	robert.turner@hse.gov.uk	HSE, UK
T. Vanhaecke	tamaravh@vub.ac.be	Vrije Universiteit Brussel, Belgium
H. van Lente	h.vanlente@geo.uu.nl	Universiteit Utrecht, The Netherlands
G. Visser	germ.visser@dsm.com	DSM, The Netherlands
D. Warheit	david.b.warheit@usa.dupont.com	DuPont de Nemours, USA
U. Weyand	weyand@vci.de	VCI, Germany
K. Wiench	karin.wiench@basf.com	BASF AG, Germany
B. Wynne	b.wynne@lancaster.ac.uk	Lancaster University, UK
R. Zellner	reinhard.zellner@uni-essen.de	Universität Duisburg-Essen, Germany

APPENDIX 5: ORGANISING COMMITTEE

From an ECETOC Task Force on Nanomaterials, the following members participated in the Organising Committee of the Testing Strategies to Establish the Safety of Nanomaterials Workshop:

Dr. David Warheit
DuPont Haskell Laboratory
Newark, DE 19174-0050
United States of America

Prof. Paul Borm
Hogeschool Zuyd
6400 AN Heerlen
The Netherlands

Dr. Christa Hennes
ECETOC
1160 - Brussels
Belgium

Prof. Jürgen Lademann
Pneumology Section
Universitätsklinikum Charité
10117 - Berlin
Germany

ECETOC WORKSHOP REPORTS

No.	Title
No. 1	Workshop on Availability, Interpretation and Use of Environmental Monitoring Data. 20-21 March 2003, Brussels
No. 2	Strategy Report on Challenges, Opportunities and Research Needs Arising from the Definition, Assessment and Management of Ecological Quality Status as Required by the EU Water Framework Directive Based on the Workshop EQS and WFD versus PNEC and REACH - Are They Doing the Job? 27-28 November 2003, Budapest
No. 3	Workshop on Use of Human Data in Risk Assessment. 23-24 February 2004, Cardiff
No. 4	Influence of Maternal Toxicity in Studies on Developmental Toxicity. 2 March 2004, Berlin
No. 5	Workshop on Alternative Testing Approaches in Environmental Risk Assessment. 7-9 July 2004, Crécy-la-Chapelle
No. 6	Workshop on Chemical Pollution, Respiratory Allergy and Asthma, 16-17 June 2005, Leuven