Proceedings of the workshop on research projects on the safety of nanomaterials: reviewing the knowledge gaps

Brussels, 17-18 April 2008

Organised by Pilar Aguar
"Nano- and Converging Sciences and Technologies"

DG Research
EUROPEAN COMMISSION

Rapporteur: Dr. Juergen Hoeck, TEMAS AG

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More information on nanotechnology at the European Commission is available on
http://cordis.europa.eu/nanotechnology and http://ec.europa.eu/nanotechnology/index_en.html

Mr Ruggero Spalletta has been of great support for the organisation of this workshop and the organiser wishes to acknowledge his diligent and continuous efforts.

Table of Contents

Foreword

Nanotechnology related research must be translated into safe novel products and processes that improve the long term competitiveness of European industry and the quality of life of the citizens. However, concerns are rising regarding the potential of some nanoparticles to have a negative impact of the human health and the environment but to date data available are yet inconclusive.

In its opinion of 2006, the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) concluded that nanomaterials may have different toxicological and ecotoxicological properties than the substances in bulk form. A series of priorities for research in areas where there was insufficient knowledge where identified. Additional work of the Scientific Committees has further identified other areas where the lack of knowledge may represent a hurdle for adequate risk assessment.

The regulatory review performed by the European Commission services indicate that current legislation covers in principle the potential health and environmental risks while recognizing that adaptations or changes could be needed as scientific-based evidence becomes available.

In January 2005, the EC organised a workshop on research needs in which discussions were held around the knowledge gaps in the area of safety of nanomaterials and nanoparticles.

The Commission and the EU Member States have an extensive portfolio of research projects dedicated to investigate the toxicity of nanomaterials, available at EU nanotechnology R&D in the field of health and environmental impact of nanoparticles and research on the possible impact of nanotechnology on health and the environment is proposed to continue in next F7 calls for proposals.

It seemed, thus, timely to organize an event bringing together the researchers in the field so the preliminary findings of the ongoing projects are shared. Through this initiative, the main objectives were three:

- Facilitating the exchanges of information among the EU researchers as to enhancing the communication of research results, avoiding unwanted duplication and streamlining resources.

- Communicating the results of FP research to natural stakeholders improving the inputs needs for producing sound scientific advice to the policy makers.

- Reconsidering the current knowledge in the area to review, if need be, the priorities in safety of nanomaterials' research.

This report should help sharing the information exchanged at the meeting and finding the scientific support needed to ensure that nanotechnologies are developed and used in a safe and responsible way.

Herbert von Bose

Director

1 Executive Summary

The "workshop on research projects on the safety of nanomaterials: reviewing the knowledge gaps" has been organised by the European Commission with a focus on increasing the interactions between researchers, improving communication of the results of research to risk assessment bodies and other interested parties, and examining the current knowledge in the area.

The agenda of the event encompassed presentations of running 6th Framework Programme (FP6) EC funded projects, some 7th Framework Programme (FP7) proposals currently under negotiation, Member States and Associated Countries funded projects, as well as a session on "closing the knowledge gaps, what do we need (yet) to know and what are the most urgent questions to be answered?" Enough space was given between the presentations for substantial discussions on all presented results and possible future activities.

Participants were mainly researchers involved in FP projects, invited representatives of different risk assessment and risk management related bodies as well as policy makers from Commission services and from other governments, international bodies and industry.

The workshop has been a very successful event for exchanging information, networking, creating new synergies and partnerships, and a "think tank" for future actions.

The meeting has allowed to all participants to "know each other" and to receive a panorama of the activities in Europe, both financed by the EU Framework Programmes and by the Member and Associated States.

During the event, knowledge gaps which should be given a high priority have been detected by the participants in the areas of toxicology, ecotoxicology, exposure, and analytics.

Accordingly, recommendations made for the addressing of research priorities to be funded under the 7th Framework Programme include:

- Analytical tools
- Validation of test methods
- Standard operating procedures (SOPs)
- Life-cycle assessment of nanotechnology-based products
- Exposure scenarios
- Good practices
- Ecotoxicity and environmental fate of nanoparticles

2 Introduction and Objectives

R. Tomellini, Head of Unit Nano- and Converging Sciences and Technologies at the European Commission, opened the workshop and welcomed all participants, putting strong emphasis on the shared sense of importance of what should be achieved within the workshop. He stressed the pioneering role of the European Commission in the field of safety of nanomaterials. The Commission supported work on this topic since it first evolved, and immediately started to react when first evidence appeared that risks might be associated with some of the nanomaterials.

There are existing structural, methodological, organisational, metrological and knowledge gaps, only to name some. These gaps need to be addressed, using the means of research and intensified collaboration across the European Union, and internationally. In addition to the Commission's efforts also Member States and Associated Countries dedicate funds to focus on closing current knowledge gaps.

Three high-aiming objectives had been set for the workshop:

- Increasing interactions between researchers in order to enhance networking, sharing of outcomes of the research projects and boosting synergies between research groups across Europe and abroad
- Improving communication of the results of research to risk assessment bodies and other interested parties, providing sound science-based data that may be used for elaborating opinions and scientific advice to policy makers
- To examine the current knowledge in the area to review, if need be, the priorities in safety of nanomaterials' research

The workshop has thus been designed to take a picture of the state at the moment of where the most pressing gaps are, and how to address them with further actions and activities.

R. Tomellini directed three pleas to the participants:

- There is an existing compilation, done by the Commission, regarding current knowledge. This compilation always needs your input to be updated.
- As the Commission is always in a dynamic situation, information about latest developments in the field are vital, and topics regarding such information should be put on the agenda.
- The Commission continuously needs input regarding the question where to put funds in order to have the highest possible impact for the solution of the most important problems.

Richard Canady, a representative of the Food and Drug Administration (FDA) in the US, underlined the importance of a sense of cooperation across the Atlantic. He stated that the sense is there, and that direct bilateral interactions exist on all levels. He made clear that within nanotechnologies it is necessary to look at research first, because it may provide evidence needed to consider adjustments of the current regulatory frameworks. He stressed the need to look forward in order to early detect future needs for activities. He suggested

using all possible synergies between Europe and the USA, and also including international organisations like the Organisation for Economic Co-operation and Development (OECD).

P. Aguar, as the organiser of the meeting, and stressed the strong demand for such type of meeting. As specific objectives for the three communities present she named:

- Research community: learn from colleagues and increase collaboration
- European Commission: shape future needs for actions
- Regulatory arena: find required information, conclusions, and knowledge

3 Session 1: FP6 EC Funded Projects

Chaired by DG ENTR (A. Boenke)

The chairman opened the session by expressing expectations for the outcome of the workshop, namely clear conclusions, advice, and input suggestions on how to close knowledge gaps, to take home for all participants with regard to the main open questions in the fields

- Characterisation,
- Exposure,
- Toxicity,
- Qualitative and quantitative minimum toxicity criteria,
- Mitigation approaches,
- Test guidelines,

and others to be explored during the workshop.

3.1 IMPART

(Karl Hoehener, TEMAS AG, Switzerland)

http://www.impart-nanotox.org/

3.1.1 Presentation Summary and Main Statements

IMPART: Improving the understanding of the impact of nanoparticles on human health and the environment.

Project objectives: To prevent knowledge of the health and environmental implications of nanoparticles from lagging behind the technological advances.

Specific challenges: To foster communication between different initiatives, streamlining resources and facilitating cooperation.

Expected impact: Improvements in the understanding of the potential impact of nanoparticles on human health and the environment, and the dissemination to, and recommendations for, the respective stakeholder groups

IMPART (phase II) is now the successor of the former two projects IMPART and NANOTOX.

The workflow is based on 4 main steps:

- Inventory of ongoing projects and the state-of-the-art; a publically available report will be finished soon, a feedback on this report is required
- Assessment of existing and identification of missing data; recommendations for priorities to address the gaps will be given
- Elaboration of recommendations and guidelines for legislation policy makers, research policy makers and funding agencies, and industry, public and other stakeholders
- Dissemination and knowledge transfer via home page, nano safety and risks database, a main conference for policy makers (scheduled October 16, 2008 in Brussels), a conference with opinion leaders, and national workshops

The first knowledge gaps detected were shortly presented, and a recommendation for an approach for self-declaration (intended for its use by industry) explained. With regard to the database, possible collaborations are sought in order to keep it alive after the ending of the project.

3.1.2 Discussion

The main concern of the participants was the presented database as an output of the IMPART project, which was considered a helpful tool in the coordination of existing activities. The participants recommended to

- Make the database publically available
- Coordinate with forthcoming FP7 projects
- Strongly and early coordinate with the OECD, who are currently building a prototype database

3.2 CANAPE

(Dr. Arie Bruinink, EMPA, Switzerland)

http://www.canapeweb.com/

3.2.1 Presentation Summary and Main Statements

CANAPE: Carbon Nanotubes for Applications in Electronics, Catalysis, Composites and Nano-Biology (CANAPE).

Focus of the presentation: WP6 (Health), In vitro effects of nanosized particles.

Objective of the Workpackage: To gain data on possible toxicity of nanotubes by in-vitro tests.

Tasks:

- Determine in how far a test like biocompatibility ISO10993-5 test is valid to define nano particles (NP) toxicity
- Assess key aspects of in vitro NP toxicity (e.g. cell-type and carbon nanotube[CNT]-type specificity)

Results: By reducing the degree of dispersion the toxicity of single-walled carbon nanotubes (SWCNTs) on MSTO211H cells is increased. A test like biocompatibility ISO10993-5 test is not valid to define NP toxicity; the results are strongly dependent on cell type and measured parameter. For (sub) toxicity the parameters specificity, pathway, appearance, exposure, solubility, and stability are key; no simple test can give a correct result.

After uptake of nanoparticles, three stages, with specific parameters to define them, are relevant:

- Early; ROS and gene activity
- Middle: cell activity, adhesion, migration
- Late: proliferation, apoptosis/necrosis

The following results were obtained:

- SWCNT and multi-walled carbon nanotubes (MWCNT) induces reactive oxygen species (ROS) partly in a preparation dependent way
- SWCNTrm affects gene activity in a cell-type dependent way and different from asbestos
- SWCNTrm affects cell adhesion & migration
- SWCNT and MWCNT affect necrosis and early apoptosis to a similar degree, but MWCNT is more toxic

Regarding No-effect concentration (NoEC) the shift of the concentration – effect relationship depends on endpoint and SWCNT purity. The exact mechanism is still unknown.

It has thus been shown that nearly all endpoints are affected by SWCNT and MWCNT. It is important to classify which test is applicable to what material.

As there is a real gap between suppliers' data and real contents and purity of nanomaterials it was recommended to perform regular and extensive characterisation of incoming material's tests.

3.2.2 Discussion

It was pointed out by the participants that a critical point in testing procedures is always the extent of interference with the surrounding medium. It was made clear that in this project there is awareness of this, and a reference to current relevant literature was given: Belyanskaya L, Manser P, Spohn P, Bruinink A, Wick P (2007) The reliability and limits of the methylthiazol tetrazolium (MTT) reduction test for carbon nanotubes - cell interaction. *Carbon 45, 2643-2648.*

For a full understanding of results presented it was discussed and explained that the ISO test validated for the use with CNTs within the project is originally used for testing implant materials.

It was recommended to have a closer look at

- Mechanisms of actions of nanoparticles, and the affection of end points

- The classification of the type of tests which are valid for different materials, including extensive characterisation tests for testing materials

3.3 Particle-Risk

(Dr. C Lang Tran, Institute of Occupational Medicine, UK)

http://www.iom-world.org/research/particle_risk.php

3.3.1 Presentation Summary and Main Statements

Particle-Risk: Risk Assessment for Particle Exposure.

Background: Exposure to NP can lead to adverse health effects in the target systems: pulmonary, hepatic and cardio-vascular.

Objectives:

- acquire a bank of NP
- assess the health risk from exposure to these materials with a work programme integrating in vitro experiments, in vivo models of healthy/susceptible individuals and exposure/risk assessment.

Investigated were fullerenes, quantum dots, nano-gold, SWCNTs, and carbon black.

Results were achieved in the following fields:

- Characterisation: No individual technique can satisfy a meaningful characterization of NP. The optimum set of required techniques should be selected on the basis of the investigated NP type.
- NP Liver effects: QD621 (+ve) has great effects in glutathione (GSH) depletion, cytotoxicity and inhibition of bile production in vitro.
- NP biokinetics: after installation and iv injection – translocation to other organs beyond portal of entry. Translocation is dependent on NP size and NP surface charge.
- NP in vivo pulmonary effects: instillation of NP on ApoE mice
    - 3hr: IL-6, mip-2 and mcp-1 mRNA highly increased in lung tissue for +ve and –ve charged QD and to a lesser extent SWCNT and CB also induced the mRNAs
    - 24hr: the 3 mRNA markers were also significantly increased for SWCNT and CB
    - Gold and C_{60} elicit weak inflammatory response
- Genotoxicity: All NP showed increased genotoxicity measured by the comet assay on BAL cells 3hr after instillation
- Platelet aggregation: Ranking of potency: Gold 2nm >> Gold 20nm=Gold 100nm >> QD –ve >>>QD +ve =CB=C60=SWNT
- Exposure and Risk Assessment: a report will be available soon

3.3.2 Discussion

The participants stated that for an evaluation of the exposure with gold nanoparticles the oxidation state of the metal has to be taken into consideration (Au(0) or Au(I)). Another

important point is the verification of the size distribution of the nanoparticles under investigation. In Particle-Risk this is taken care of according to existing protocols.

It was recommended that the selection of testing methods and analytical techniques should be tailored to the selected particle types.

3.4 NANOTRANSPORT

(Dr. Qinglan Wu, Det Norske Veritas AS (DNV) Hoevik, Norway)

http://research.dnv.com/nanotransport/

3.4.1 Presentation Summary and Main Statements

NANOTRANSPORT: Behaviour of aerosols released to ambient air from nanoparticle manufacturing (a Pre-normative Study).

The project will be finished end of April 2008.

The NANOTRANSPORT project addresses the behaviour of aerosols released to ambient air from nanoparticle manufacturing. The aim of the project is to analyse and typify relevant exposure scenarios of workers to manufactured nanoparticle aerosols at industrial workplace and to develop recommendations to the Commission regarding realistic test conditions in terms of test aerosols characteristics for use within toxicology studies, but also for testing the efficiency of existing engineering control systems and personal protective equipment.

The approach includes:

- Determine typical scenarios of release and transport of airborne nanoparticles in an occupational setting;
- Select model parameters controlling physical change which airborne nanoparticles will undergo after release in workplace environment
- Identify methodologies for generating test aerosols and measuring the relevant parameters
- Carry out experiments to explore the relevance of model parameters of change and model exposure scenarios
- Develop recommendations based on experimental results

The experimental results allow drawing a number of key conclusions of significance in the context of the development of recommendations:

- The results show that nanoaerosols evolve considerably with time: the average particle size increases while the number concentration decreases due to homogeneous coagulation processes.
- Another important conclusion is that natural background aerosols are scavengers for NP by heterogeneous coagulation. This leads to the occurrence of the physical/chemical presence of nanoparticles in size classes other than the one in which they were originally emitted.

- Filtration of nanoparticles can be done with high efficiency using state of the art filters. However, the shift of nanoparticles to larger size classes by both homogeneous and heterogeneous coagulation may lead to the occurrence of nanoparticles in the size range where filters are least efficient, the most penetrating particle size (MPPS) in the range of about 80-200 nm.

Based on the experimental results, a set of recommendations to the Commission has been prepared and is now under discussions with experts and stakeholders:

- Test aerosols for nanotoxicology studies:
    - To describe nanoaerosol characteristics immediately after release, aerosol dynamic parameters and models are necessary.
    - Characteristics of "aged" nanoaerosols are typical size range for auto-agglomeration (20-200 nm), and typical size range for attachment to background aerosol (<1 µm).
    - Study the toxicity of aged nanoaerosols (homogeneous, heterogeneous agglomerates and agglomerate stability) in addition to primary NP needs to be considered.
    - The generation of test NP in aerosol phase to simulate the scenario of primary NP release in workplace air may be a helpful tool.
- Testing of filters and protective equipment in the workplace, consider the filter efficiency for the size range of agglomerates in addition to primary NP
- Research priorities:
    - Characterize release mechanisms and sources of hazardous NP types (source strength, size range, structure).
    - Develop model aerosol sources for relevant classes of release mechanisms and species for use in toxicological studies.

3.4.2 Discussion

It was discussed by the participants that primary particles tend to stick to agglomerates very fast, and that therefore the filters should be designed in a way to take out the agglomerates. One participant mentioned that a most penetrating particle size (MPPS) of around 100 nm is not a real challenge for existing filter systems. On the other hand participants stated that the effect of eliminating agglomerates on smaller primary particles might translate in a longer lifetime in the system, because they do not find larger particles to attach to.

Areas recommended for further investigations were

- Test filters and protective equipment also with focus on agglomerates
- Development of different test aerosols for toxicological studies
- Develop aerosol dynamic models for predicting nanoparticle evolution, validation and refinement of existing models

3.5 NanoCap

(Pieter Van Broekhuizen, IVAM UvA BV, Netherlands)

http://www.nanocap.eu/

3.5.1 Presentation Summary and Main Statements

NanoCap: Discuss and deepen the understanding of NGOs and trade unions on nanotechnology on

- Environmental issues
- Occupational health and safety issues
- Ethical issues
- Critical assessment of benefits

NanoCap aims for:

- Developing a balanced view on NT and related policies
- Identifying benefits of nano-applications
- Improving understanding of nanotechnology relating to use of products, health and environmental risks
- Discussing the uncertainties and risks of nanoparticles with NGOs, Trade Unions and General Public
- Discuss a safe NT approach with industry
- Developing a portfolio on ethical issues
- Developing preliminary recommendations for public authorities

NanoCap activities:

- Five internal Working Conferences:
  - Problem definition
  - Technical nanotechnology issues
  - Environmental and Occupational Health issues
  - Ethical issues
  - Critical assessment of benefits
- Meetings/discussions with industry, authorities, other stakeholders
- Website (www.nanocap.eu)
- Electronic newsletters
- Public final conference: Presentation and public debate on position statements, Brussels, 23 April 2009

A precautionary approach for provisional occupational exposure limits (OEL) has been derived, based on existing OEL for fine particles by US-NIOSH, on the example of nano-TiO_2. As the deposition behaviour of NPs in airway system differs significantly from behaviour of fine particles, a correction factor for nano-TiO_2 of 15 with respect to the NIOSH approach has been assigned.

An occupational hygiene strategy for a workplace adapted to NP characteristics has been developed:

- Elimination of the emission source (Full containment process, Substitution)
- Exhaust ventilation direct at the source
- Separation of worker and source
- Personal protective equipment:
    - Perfect fitting respirators with high efficiency particulate air (HEPA)-filters with fibrous filters
    - Non-woven fabrics against NP-penetration
    - Use 2 layers of gloves

A formalisation of the roles of workers' representatives in design and monitoring, and the involvement of the Labour Inspectorate in control and enforcement for compliance with the "voluntary" safety related codes has been suggested.

3.5.2 Discussion

Participants expressed that a definition of nanoparticles via only their size or size distribution is not adequate for a future regulation of those nanoparticles. Their surface area, and even more so their surface chemistry should be taken into consideration. NanoCap however cannot address this for the time being, here it is only sought to develop a tool for precautionary measures.

3.6 Wrap up discussion for session 1

In the wrap-up discussion for this session participants made a number of recommendations regarding points to be looked at in future:

- Correction factors for OEL cannot always be adequate because systemic effects for the translocation of small particles may not be the same as for larger particles.
- When looking at work place exposure, two scenarios have to be considered: a.) the accidental release of fresh nanoparticles on a short time scale in a small area, and b.) the dissemination of aged and agglomerated particles on a longer time scale in a larger area. For workers on the machine protection is sufficient against the fresh particles, but for all workers there is also a non-negligible exposure by aged and agglomerated particles in the ambient working atmosphere.
- Deagglomeration of larger particles into smaller ones needs to be considered under specific conditions (physiological and environmental) and the mechanisms for agglomeration / deagglomeration need to be investigated.

- Regarding REACH, in principle the same approach as for other chemicals applies. For the classification of nanomaterials specific data still need to be elucidated and a special group to address this topic has been set up.
- Stronger interactions with end-users, especially regulators in projects funded by FP7 is asked for, consumers' protection issues should not be forgotten.
- Research on nanotoxicology, nanoecotoxicology, and exposure may not alone deliver relevant input for regulation, synergies need to be found and established between those fields. A pragmatic approach is needed to define which scientific results are relevant.
- Risk communication strategies and risk perception by the public will need special attention, when communicating hard facts about nanosafety issues

Subsequently to this discussion, a summary of the relevant open questions and recommendations discussed during session 1 was made by the chairman:

- Agglomeration / deagglomeration behaviour of nanoparticles, interaction of primary particles with larger agglomerates
- Delocalisation of nanoparticles, and their mechanisms of action.
- The types of nanomaterials: which ones are representative, which ones are relevant? Can they be grouped?
- More data on (work place) exposure are necessary
- Effects of the aging of nanoparticles, also in consumers' products: a closer look into life cycles is recommended
- Effective doses that reach the cell
- Evaluation of risks should be done on a case-by-case basis
- Different types of research approaches may be needed for toxicology, ecotoxicology and regulation issues; synergies should be established
- Risk perception and communication will have increasing importance in the future

4 Session 2: FP6 EC Funded Projects

Chaired by DG RTD (P. Aguar)

4.1 Dipna

Dr Antonietta M. Gatti, Consorzio Nationale Interuniversitario Sviluppo Materiali - University of Modena and Reggio Emilia, Italia)

http://dipna.eu/

4.1.1 Presentation Summary and Main Statements

Dipna: Development of an integrated platform for nanoparticle analysis to verify their possible toxicity and eco-toxicity.

Project objectives:

- In vitro tests of interaction of engineered nanoparticles (NP) with cells
- Identification of the modes of NP-cell interaction
- Application of the laboratory-developed cellular models on the field investigations

Specific challenges: Development of

- in vitro nano-specific toxicological tests, and assays for assessment of single cell-to-NP interaction
- new sensors to detect specific NP-induced biological reactivities
- evaluation of NP pollution in industrial settings

Conclusions from the project results:

- The classical biological tests, including the biocompatibility tests of ISO/EN 10993 standards, cannot measure a biological effect by NPs at not toxic (very low) concentrations.
- The concept of individual cell exposure must be considered, i.e. evaluation of probabilistic effect on individual cells rather than the average effect on the cell population. NPs represent a discrete (quantum) stimulus.
- Detection of nano-effects on single cells needs detection tools of very high sensitivity at nanoscale dimension.
- Only with chronic and repeated exposures we can evaluate the possible effects of a single interaction.
- The toxicological tests for nanomaterials need more sophisticated sensors.

4.1.2 Discussion

In the discussion participants made clear that a lot is already known about biological effects of nanoparticles, but many results are still questionable. This is still mainly due to the fact that most of the available tests are not validated for testing nanomaterials, and that the variety of tests used leads to largely irreproducible results.

4.2 NanoInteract

(Prof. Kenneth Dawson, University College Dublin, Ireland)

http://www.nanointeract.net/

4.2.1 Presentation Summary and Main Statements

NanoInteract Work Plan, with a focus on understanding:

- Selected Nanoparticle synthesis or acquisition from industry?
- Protein corona and dispersion: which ones bind, and how strongly?
- Spatio-temporal aspects: where do the Nanoparticles go?
- Impacts on cellular signalling: what do the Nanoparticles do?
- Ecotoxicology and toxicology: the logic of ecotoxicology is similar to toxicology, but much more complex

Cells do not see clean Nanoparticle surfaces, but protein attached surfaces (they can attach rather selectively).

A significant entry of Nanoparticles into cells has been found. A remarkable result is that they also go back out.

Problems encountered in the project are:

- Irreproducibility of results due to different provenance and dispersion of Nanoparticles
- Biological irreproducibility
- Irreproducibility due to multi-partner collaboration

The following questions have been identified as future focus points:

- Near Term Challenges: Professional Standards
  - Supply of (relevant) 'reference' nanomaterials for European Projects
  - Nanoparticle reproducibility, batch to batch
  - Reproducibility of dispersions, without additives, and remaining 'relevant'
  - Reproducibility of biological materials
  - Characterization of nanoparticles in situ: 'dose', aggregation, in cell, etc.
  - Non comparability of 'tests'
  - Acceptance of community for need of these standards
- Longer Term Challenges: Making a Science
  - Acute impacts for several species now emerging (e.g. silica etc.)
  - Non-degradable nanoparticles reside within cell longer term impacts for known end points
  - As yet unknown end points; new methods?
  - Nanoparticle go new places; new target organs?

4.2.2 Discussion

Participants stated again that there is a strong need for validated test methods. It was explained that there are already some actions and projects which cover this topic. The first standardised reference samples are under discussion.

4.3 NANOSH

(Dr. Kai Savolainen, Finnish Institute of Occupational Health, Finland)

http://www.ttl.fi/Internet/partner/Nanosh/

4.3.1 Presentation Summary and Main Statements

NANOSH: Inflammatory and genotoxic effects of engineered nanomaterials.

Project objectives:

- Characterization and exposure: Characterization of nanoparticles and definition of exposure levels in laboratory conditions and in workplaces
- Health effects: Assessment of the genotoxic, inflammatory and microcirculatory effects of nanoparticles

Nanoparticles have been characterised in 3 different categories: the commercial nanoparticles (NP), NP collected from the workplaces and the NP used in health effect studies.

Main achievements of NANOSH in the first period:

- Characterization of nanoparticles, a sampling strategy as an advice guide for characterization of the test materials for toxicological tests.
- Exposure to nanoparticles, successful pilot studies in workplaces, report of outcome of pilot studies.
- Genotoxicity:
  - four different nanomaterials have been studied for genotoxicity in vitro
  - all materials increased DNA damage.
  - preliminary data from an inhalation experiment in mice showed no production of DNA damage in lung cells.
- Pulmonary inflammation, inhalation exposure to needle-like silicon coated TiO_2 lead on infiltration of inflammation cells in mouse lungs.
- Microvascular effects, and a mouse model suitable for the analysis has been established, and analyses of the effects have been started.

4.3.2 Discussion

One question was intensely discussed: how representative are laboratory conditions for work places, also regarding their use in simulations? It was stated that usually in real work places the concentrations are very low. In order to verify this, many real work places are now visited by the project partners. First data will be available in May, and then the question about the relevance and representative character of laboratory results can be answered.

It was recommended to

- Promote the consideration of health and safety aspects in all research related to nanomaterials
- Develop useful exposure settings both in-vitro and in-vivo

4.4 CellNanoTox

(Prof. Rafi Korenstein, Tel-Aviv University, Israel)

http://www.fp6-cellnanotox.net/index.html

4.4.1 Presentation Summary and Main Statements

CellNanoTox: Cellular Interaction and Toxicology with Engineered Nanoparticles.

CellNanoTox aims at:

- Development of multidisciplinary set of tests and indicators for toxicological profiling of NPs
- Interdisciplinary approach combining inorganic and organic chemistry, physiology, toxicology, cell biology and data analysis

Tasks within the project are

- Design and synthesis of coated and non-coated NPs
- Characterization of dispersion and modification of surface characteristics of the NPs following interaction with physiological media
- Elucidation of interaction and mechanisms of uptake and recycling of NPs by in-vitro cellular model systems of lung, intestine, liver
- Establishment of an in-vitro model of the lung and in-vitro evaluation of transport mechanisms through the lung-vascular and intestinal cells with respect to NPs characteristics
- Determination of the NP-induced toxicology and underlying mechanisms in in-vitro model systems of lung, intestine, kidney, liver and the immune system by conventional toxicology and toxicogenomics
- Elucidation of NP-induced metabolic changes in precision-cut slices of lung, liver and kidney by metabolomics
- Study of the activation and the inflammatory response of the immune system towards NPs. New approaches to study the interaction and induced effects of NPs with in-vitro cell/tissue models
- Development and adaptation of novel methodologies of Knowledge Discovery from Data (KDD) and Data Mining (DM) for toxicological studies

The project aims at the toxicologic profiling of different engineered NPs: Co, Au, Ferrites, CNTs, and quantum dots.

An explanation of data mining taxonomy was given, and the knowledge discovery from data (KDD) described.

4.4.2 Discussion

It was pointed out by the participants that oxidation and corrosion of particles has to be considered in dependence of the particles: they would be an issue for Co but not for Au.

It was recommended to take a deeper look into the physiological stability of nanoparticles and associated agglomerates.

4.5 NANOSAFE 2

(Dr. Frédéric Schuster, CEA-Grenoble, France)

http://www.nanosafe.org/

4.5.1 Presentation Summary and Main Statements

The NANOSAFE evolution: From NANOSAFE to NANOSAFE2 to SAPHIR (technological development).

Key modules of NANOSAFE:

- Approach based on the safety of the processes, demonstration at pilot scale – Nanosafe innovations implementation
- Characterization, detection, monitoring; especially the chemical composition of nanoparticles in air; benchmarking of methods
- Qualification of protections &measurements at workplace
- Tests at industrial workplace
- Design of safe processes
- The life cycle of nanoparticles: measurement of particle release & Nano-manufacturing process optimisation: Measurement of nanoparticle release from end-products, development of tracers nanoparticles
- Rapid in-vitro toxicity screening test
- Training tool for e-learning: Nano training and communication software under construction at CEA
- A continuous dissemination process: Preparation of Newsletter n°3
- Organization of the international conference nanosafe '08, 3-7 November 2008, Minatec, Grenoble.

4.5.2 Discussion

Participants mentioned that compared to the handling of really toxic substances the total protection scheme presented for the handling of nanoparticles might not be feasible and applicable in many of the cases, especially not for SMEs. It was made clear, however, that the focus of the project is the delivery of no-risk tools, irrespectively of the place to be applied at.

It was suggested on a long term to try to develop "nano-badges" in analogy to x-ray badges, in order to display a possible exposure at an early stage.

5 Session 2: FP7 EC Funded Projects

Chaired by DG RTD (P. Aguar)

The chairlady announced that there were two more projects being negotiated at the moment belonging to the last FP7 call, but that they were in a too early stage of negotiation to be presented, so their presentation had been cancelled for this workshop. Regarding the content of the projects, in one of them a database will be produced, and a comprehensive

evaluation of data available on toxicological properties of some nanoparticles elaborated in the other.

5.1 NanoImpactNet

(Dr. Michael Riediker, Institute for Work and Health, Switzerland)

http://www.nanoimpactnet.eu/

5.1.1 Presentation Summary and Main Statements

NanoImpactNet: The European Network on the Health and Environmental Impact of Nanomaterials.

Important question to be answered: What is the health, safety and environmental (HSE) impact of nanomaterials, seen from different viewpoints (companies, insurances, workers, consumers...). A life cycle analysis is necessary to cope with this question. Accordingly there is a need to bring together researchers to

- Identify knowledge gaps
- Define joint strategies and protocols; redundancy is good, duplication is not
- Facilitate collaboration between projects
- Network and Communicate
- Collaborate to achieve excellence in science (good for industry, workers, consumers and the environment)

This will be done by: Workshops, Collaborative work over Internet (virtual meeting room will soon go online), Reports and joint protocols, and Training schools.

Possible participation by:

- Researchers developing and testing methods to investigate the health or environmental impact of nanomaterials
- Stakeholders / Experts from industries, SMEs, governments, NGOs etc. that will have to deal in future with methods discussed in NanoImpactNet

Vision: Produce high quality science that contributes to the responsible and safe development and use of nanoproducts and -materials.

5.1.2 Discussion

With respect to the statements made about avoiding duplication of efforts and results it was stated that for the sake of reproducibility testing it might sometimes be beneficial to deliberately repeat investigations and measurements.

Participants agreed that all stakeholders must contribute from the beginning. In order to find and integrate them, it was recommended to:

- Use existing networks
- Use internet based tools
- Make contacts via the European Commission
- Speak to European Industry Platforms

5.2 NanoTEST

(Dr. Maria Dusinska, Norwegian Institute for Air Research - NILU, Norway)

5.2.1 Presentation Summary and Main Statements

NanoTEST: Development of methodology for alternative testing strategies for the assessment of the toxicological profile of nanoparticles used in medical diagnostics.

Overall aim: Develop alternative testing strategies and high-throughput toxicity testing protocols using in-vitro and in-silico methods essential for the risk assessment of NP used in medical diagnostics.

Specific aims:

- Define parameters describing properties of NP, and fully characterise NP to be used.
- Study specific and non-specific interactions of NP with molecules, cells and organs and to develop in-vitro methods which can identify the toxicological potential of NP.
- Validate in-vitro findings in short-term in-vivo models, to study manifestation of particle effects in animals of NP and ex-vivo in humans, and to assess individual susceptibility in the responses to NP.
- Develop in-silico models of NP interactions, to perform structure-activity modelling and physiologically based pharmacokinetic (PBPK) modelling of NP.
- Adapt the most advanced and promising assays for high-throughput automated systems and to prepare for validation by ECVAM (European Centre for the Validation of Alternative Methods).
- Ethically approved in-vivo models.

5.2.2 Discussion

It was proposed by Richard Canady to connect the project to existing actions in the US, where already a lot of data is available.

5.3 NANODEVICE (FP7 proposal under negotiation)

(Dr. Kai Savolainen, Finnish Institute of Occupational Health, Finland)

5.3.1 Presentation Summary and Main Statements

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

Overall objectives: New and innovative concepts and methods for measuring and characterizing airborne engineered nanoparticles (ENP) with novel portable and easy-to-use device(s) for workplaces.

General goals:

- Develop concepts and reference materials
- Justify chosen metrics
- Collaboration with other EU projects, elaboration and exchange of performance requirements
- Reliable testing and calibration protocols
- Explore impact in society

Specific objectives of the research project:

- To identify relevant physical and chemical properties for specific measurement of engineered airborne ENP, and to develop reference materials for ENP aerosols
- To investigate the relationships between physical and chemical properties of ENP and their potential toxicity or bioactivity
- To analyze the ENP emitted from industrial processes during the production and handling of ENP and to assess levels of ENP in workplaces in order to define performance requirements.
- To develop technologies that enable utilization of new concepts in miniaturized and field-worthy specific monitors for ENP
- To develop methods for calibration and testing of the newly developed concepts and methods and devices in simulated and real life exposure settings
- To effectively disseminate the results of the research, to promote the safety of ENP by guidance and standard development, to provide training and guidelines education, so that ENP can be safely produced and handled, and by promoting collaboration of all those concerned with the safety aspects of ENP

5.3.2 Discussion

Participants showed strong interest in the metrics to be used in the project. However, key metrics have not yet been selected but will only be selected during the project.

5.4 Wrap up discussion for session 2

In the wrap-up discussion for this session participants made a number of recommendations regarding points to be looked at in future:

- Published results are not always compatible for input into modelling activities, this should be harmonised. It was mentioned that this idea is already being taken up.
- Reproducibility of results is in many cases low, also to be considered are results in work places. It was suggested to conduct blind studies.
- Chronic and accidental risks should receive more attention.
- Epigenetic effects should be addressed.
- Ecotoxicology is largely unexplored, especially the exposure routes back from environment to humans should be considered more important (e.g. drinking water).

- Health and environment should not be too strictly considered separately, many basic findings should apply for both, and synergies should be developed by bridging the two of them.
- Communication of risks should only be based on hard facts.
- Researchers' safety in laboratories should not be neglected; a picture of existing good practices should be taken.
- In the field of skin absorption toxicology not enough data are available and the results of the NanoDerm project are not enough. It was suggested that missing data could be available in the US, interlinking would thus be beneficial.
- A discrimination of the effects of really engineered nanoparticles and the "background" produced by natural nanoparticles needs special attention.

The British Bureau of Standards has a document on risk assessment and practices for workers available: BS6699/PartII. Similar documents have been produced by governmental bodies and also by industry associations (i.e. NIOSH, VDI).

6 Session 3: FP7 proposals

Chaired by DG ENV (E. Hellsten)

Concluding from previous sessions the chairlady set out three issues which might need more attention in the future:

- The relationship between regulatory and basic research, and how to set priorities there. An important question will be how far it will be necessary to go
- The rapid development in the field of nanomaterials might make it necessary to already consider risks associated with 2nd or 3rd generation nanomaterials.
- New and adequate communication strategies.

6.1 NANOMMUNE (FP7 proposal under negotiation)

(Prof. Bengt Fadeel, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden)

6.1.1 Presentation Summary and Main Statements

NANOMMUNE: Comprehensive assessment of hazardous effects of engineered nanomaterials on the immune system.

Main objectives:

- Procurement, synthesis, and physico-chemical characterization of representative categories of nanomaterials
- Monitoring of potential toxicity with emphasis on immune effects using a panel of in vitro and in vivo model systems, as well as state-of-the-art in silico (transcriptomics) and lipidomics protocols
- Risk assessment of potential adverse effects of engineered nanomaterials on human health, and dissemination of consortium findings

A strong project focus lies on harmonising assessment criteria between EU and US

6.1.2 Discussion

Participants were interested in links of the project with other running activities. It was explained that those interactions will be developed during the project. For an effective collaboration and information exchange with other projects standard operating procedures (SOPs) for syntheses, analyses and protocols will be produced.

Answering to the remarks about the difficulties to clean industrial samples before use it was explained that therefore trust will only be put on materials synthesised in the laboratory. The materials to be investigated were specified as metals, oxides and CNTs.

6.2 NanoReTox (FP7 proposal under negotiation)

(Dr. Eva Valsami-Jones, Department of Mineralogy Natural History Museum)

6.2.1 Presentation Summary and Main Statements

NanoReTox: The Reactivity and Toxicity of Engineered Nanoparticles, Risks to the Environment and Human Health.

NanoReTox is composed of

- three primary strands:
    - Synthesis, characterisation, reactivity
    - Ecotoxicity
    - Human toxicity
- two secondary strands:
    - Risk assessment
    - Risk communication

Key concepts:

- Materials: Why synthesis, what particles, why characterisation, why reactivity
- Toxicity: Minimal use of animal models, in vitro uptake, mammalian/human cells, consider genotoxicity/carcinogenicity
- Ecotoxicology: Use of non-standard organisms, in vivo & in vitro uptake, risk models

Comprehensive links between ecotoxicology and toxicology are needed; an important issue to be tackled here is the influence of indirect exposure. Also of importance is the aging of surfaces and the resulting changes in properties.

6.2.2 Discussion

Participants were concerned with the question how account will be taken of indirect terrestrial exposure. It was explained that investigations on terrestrial organisms are planned for a later point in time, for the moment no standard species will be used. This is mainly due to the fact that regulatory approaches through standard species are already largely addressed by other researchers. Studies will be carried out at reasonably low concentrations with labelled particles used for tracing, also on a long-term basis.

6.3 NeuroNano (FP7 proposal under negotiation)
(Prof. Kenneth Dawson, University College Dublin, Ireland)

6.3.1 Presentation Summary and Main Statements

NeuroNano is a hypothesis driven project, assuming that there are other NP target organs at risk, about which we do not know enough. As an example the brain will be explored (like in the case of Alzheimer, Parkinson).

3 issues will be addressed:

- Can NPs get there? The preliminary answer is yes
- Can they induce oxidative stress there? The preliminary answer is also yes
- Is the rate of fibrillation in the brain affected by NPs? The preliminary answer is also yes

6.3.2 Discussion

In the discussion it was made clear that controlling the labelled nanoparticles is vital for the project, labelling will be achieved by inclusion of radioactive markers. The next plans in the project are the braking down of the hypotheses into smaller questions which all have a value and a valid statement on their own. It was explained that the blood brain barrier maybe penetrated in rather high amounts, and that the models replicate this fact rather well. Accordingly there is a high potential for in-vitro models.

7 Session 3: Member States and Associated Countries funded projects
Chaired by DG ENV (E. Hellsten)

The chairlady announced a change in the agenda: additional short presentations from Mexico and Russia had been included.

7.1 Germany's strategy on chances and risks in nanotechnology
(Dr. Martin Vogt, VDI GmbH)

7.1.1 Presentation Summary and Main Statements

As a part of the German High-Tech Strategy the Nano Initiative - Action Plan 2010 is a joint initiative of 8 ministries. Challenges addressed in the Action Plan 2010 are:

- Rapid transfer of nanotechnology research results to innovations; utilization of nanotechnology for the most important industrial sectors
- Elimination of innovation barriers and improvement of framework conditions by a joint approach of all involved federal ministries
- Evaluation of effects of nanomaterials on health and environment
- Communication with the public about chances and risks of nanotechnology
- Identifying future research needs

For the evaluation of effects of nanomaterials on health and environment 3 projects have been installed, which are described in the following.

NanoCare: Health Aspects of Synthetic Nanoparticles: Creation of an Information- and Knowledge-Base for Innovative Material Research

The project is composed of three key aspects: the generation, management and transfer of knowledge. The centre of the generation of knowledge are studies on primary particles, aggregates and agglomerates and their behaviour in biological media as well as their effects on biological systems. State-of-the-art analytical methods are used to characterise nanoparticles. Comparative studies on biological strengths as a function of particle characteristics and type will provide new findings concerning the effects of particles. Existing measuring technology will be developed further in order to enable different aerosols and nanoparticles to be screened directly at the work place. In addition to the production and characterisation of synthetic nanoparticles based on metal oxides (e.g. zirconium or zinc oxide); reference materials (titanium dioxide and industrial carbon black) will be established to allow comparison of the results from all project partners.

All data generated during the NanoCare project will be edited, interpreted and structured to form a web portal, and will thus be available to the general public. The portal will include a database that providing access to the research results via a user-friendly interface. Furthermore, the results generated by the project will be presented to the public at various events and put up for discussion. The aim is to provide an information platform for all interested societal groups on the opportunities and risks associated with nanomaterials.

Funding: The NanoCare research project will receive € 5.0 million in funding from the German Federal Ministry of Education and Research (BMBF). Industrial partners will contribute an additional € 2.6 million.

Project web site: www.nanopartikel.info

INOS: Evaluation of health risks of nanoparticles – A contribution to the sustainable development of nanotechnology

INOS is developing methods based on in-vitro testing for evaluating the hazard potential of engineered nanoparticles. The hazard analysis is based on a comprehensive investigation of the behaviour of nanoparticles and the changes they undergo in different cell culture media. Their reaction with cytosol components such as salts and proteins, changes in pH will also be included as well as the interaction of nanoparticles with cells as a function of their size, physicochemical nature and surface composition.
Research will concentrate on ceramic and metal particles such as diamond, tungsten carbide, titanium dioxide, titanium carbon nitride, cobalt, platinum, ceramic metal compounds, as well as CNT and carbon black. The materials differ in structure, chemical bonding and chemical stability, as well as in their dissolution and dispersion behaviour in aqueous media so that clear differences are expected in the reactions of cells with these particles. Scientists hope to be able to transfer and apply the knowledge they gain here to the testing of other materials.

Various human and animal cells, such as pulmonary and intestinal epithelial cells, epidermis cells, neurons and glial cells will be used as cell lines or primary cells for cytotoxicological investigations. Vitality, general stress response (e.g. changes in protein expression), oxidative stress, inflammatory and immune-modulating effects, gene toxicity,

cell death, etc. will be examined. The results will subsequently be made available to the public in a database.

Funding: The INOS project involves four research institutions together with a biotechnology company and has been granted € 1.1 million in funding by the BMBF.

Project web site: www.nanotox.de

TRACER: Toxicology and Health Risk Assessment of Carbon Nanomaterials

CNT and carbon nanofibres (CNF) will be key materials of the 21st century. A broad application is to be expected in central fields of technology such as chemistry, the automotive industry and aerospace engineering. The emerging industrial production of carbon nanotubes is likely to open up other fields of technology and thus pave the way for a variety of mass applications.

The project "TRACER" aims to assess the biocompatibility of PEEK (polyether-ether ketone) and PUR (polyurethane) composite materials along the exemplary value chain of production – processing – semi-finished products – functional models. It will focus on answering questions concerning the cytotoxicity of carbon nanofibres and carbon nanotubes.

A comprehensive physicochemical characterisation of the materials will allow referencing the cytotoxicity investigated in terms of standardised material properties. In parallel, several simulation tools will be adapted for the uptake and distribution pathways of carbon nanomaterials in human organisms, which will facilitate the prediction of the dose-response correlation.

Results obtained will be included in the evaluation of size-resolved determinations of the number of particles released along the value chain. They will be used as a basis for handling recommendations during production and processing, and for the use of possible end products.

Funding: The TRACER project has been awarded € 1.5 million in funding by the BMBF and another € 1.5 million will be provided by industrial partners.

Project web site: www.nano-tracer.de

Future activities:

- BMBF funding programme NanoNature:
  - Application of nanotechnology in environmental protection
  - Effects of nanomaterials on environment
- BMBF funding programme NanoCare II (working title): effects of nanomaterials on health

7.1.2 Discussion

With regard to the comparability of the data produced within NanoCare it was explained that SOPs for synthesis, characterisation and in-vitro systems have been developed. The advantage in the materials studied is that they are already being produce by the industry and in use and therefore give more realistic results.

7.2 UK Environment, Health and Safety Research Projects (Jaya Shah, Department for Environment, Food and Rural Affairs - Defra)

7.2.1 Presentation Summary and Main Statements

Public engagement activities highlight that there are concerns about the lack of knowledge about human health and environmental risks arising from nanotechnologies. Alongside this there is strong support for fundamental science to arrive at answers to these questions.

There is a strong need to develop standardised, well characterised reference nanoparticles which are recognised as being key to all areas of research

Exposure:

- A methodology for reproducing various nanoparticles has been created which might provide an alternative route for the production of reference materials.
- The inability to discriminate between engineered and environmental nanoparticles (e.g. particles produced from combustion of diesel) creates problems in measurement.

Human health hazard and risk assessment:

- There is concern over the differences in the measurement and understanding of nanoparticles before and after assimilation within the cell. Although the properties and parameters of certain nanoparticles had been characterised this could not provide a guarantee of their behaviour and effects once exposed to the environment and within the cell. It was important to understand the kinetics of nanoparticles within the cell. The application of in vivo tests could help in discerning the characteristics of nanoparticles responsible for causing potential damage within the cell. Because any of a multitude of characteristics could be responsible for any potential damage to the cell, it would take much time to test them all; hence it would be important for a prioritisation of the characteristics of nanoparticles. This would allow likely factors which could potentially cause damage to be identified and investigated first.

Environmental health hazard and risk assessment:

- Progress on the objective of capacity building to enable research to develop fundamental understanding on the environmental fate, behaviour and ecotoxicology of nanomaterials. To focus on one important subset of this, namely impacts on microbial communities, flora and fauna in soils and ground waters, reflecting the use of nanoparticles in remediation, where deliberate introduction into the environment can occur as well as other potential sources (e.g. sewage sludge). This requires a strongly interdisciplinary approach.

Current research priorities, which are based on the identification of problems needing urgent attention:

- Characterisation and metrology
- Properties important for biological interactions
- Testing plan for priority materials (REFNANO project)

- Fitness for purpose of testing methods
- Review of risk assessment methods
- Economic, social and ethical considerations

Measurement and characterisation are underpinning requirements for all EHS studies.

7.2.2 Discussion

The question was posed if the minimal set of characteristics defined in REFNANO could be used as a standard parameter set. The answer was that this set of characteristics had only been developed in order to have a minimum characterisation for useful reference materials, the question cannot be answered yet.

The current lack of knowledge regarding behaviour and interactions of the nanomaterials with the environment research was highlighted as a main concern area for future activities; participants suggested to the European Commission and the Member States to consider it as a priority when establishing the future calls for research proposals. .

7.3 Research on the safety of nanomaterials in Switzerland (Dr. Andreas Werthmueller, State Secretariat for Education and Research)

7.3.1 Presentation Summary and Main Statements

Public research funding in Switzerland is done by

- Federal Administration (Research in Swiss Government Departments, Swiss Federal Institutes of Technology)
- Swiss National Science Foundation (SNSF)
- Cantons
- European Framework Programmes

Efforts towards coordinated actions are made by

- National Research Programmes (SNSF)
- National Centres of Competences in Research (SNSF)
- Action Plan on Synthetic Nanomaterials (Federal Administration, for details see below)
- Target oriented research funded by the Administration
- Self-organising coordination on the national level
- Installation of several databases: www.aramis.admin.ch, www.projectdb.snf.ch, www.researchportal.ch

Coordination on the national level faces several constraints:

- Coordination versus competition
- Coordination versus diversification
- Coordination versus freedom of research

Selected projects from target oriented research funded by the Administration:

- FOEN, 2001.I.02, (from 06/2001 to 12/2003): Deposition and Behaviour of Ultrafine Particles in the Lung
- FOEN, 2002.I.07, (from 07/2002 to 12/2002): Particulate measurement program (PMP)
- FOEN, 2005.H.02, (from 09/2005 to 12/2007): Nanorisk: Safety and Risks of Carbon Nanotubes
- FOPH, 06.000943, from 05/2006 to 06/2008: Nanoinventory
- FOPH, 06.001691, from 07/2006 to 06/2009: Analysis of the human exposure to nanomaterials CH
- EMPA, (from 04/2008 to 03/2011): Ecotoxicology of Nanoparticles: Biota-Nanoparticle-Pollutant Interactions in aqueous systems - Comparison of Black Carbon and Carbon Nanotubes
- UniBE, (from 10/2007 to 09/2010): Interplay of lung cells and their cellular responses upon exposure to combustion-generated ultrafine particles and manufactured nanoparticles
- UniNE, (from 10/2007 to 09/2009): Nanopore Sensing

The Swiss Action Plan on Synthetic Nanomaterials has been approved by the federal council on April 9, 2008. The main objectives are:

- Generate framework for responsible/sustainable use
- Generate knowledge for informed decisions/use
- Promote dialogue on benefits and risks
- Funding schemes to promote development of sustainable applications

Conclusions from the current situation are:

- Major fields related to risk are covered by excellent work
- Coordination is sometimes lacking (which might be benefit or disadvantage)
- Knowledge gaps should lead to the implementation of precautionary principles
- First steps to create a framework for informed decision/use are done
- Common understanding that the definition of standards and rules should be done on a supranational level must be created

7.3.2 Discussion

The question "precautionary principle against economic development" was discussed. It was noted that the precautionary principle is not widely used internationally. It was mentioned that in Switzerland no legislation in this direction is planned.

7.4 Projects and Research Networks in Italy

(Dr. Sergio Iavicoli, National Institute of Occupational Prevention and Safety - ISPESL)

7.4.1 Presentation Summary and Main Statements

Italian partners are involved in the European projects NanoCap, CANAPE, Particle-Risk, CellNanoTox, and DIPNA. Two important national projects are currently running: NanOSH and PRIN.

NanOSH: a project for development of innovative methodologies and techniques for risk assessment of occupational exposure to nanomaterials (coordinator: Dr. S. Iavicoli). The main project goals are:

- Characterisation of nanomaterials
- Development of an experimental model for in-vitro research
- Estimating workers potentially exposed to risks related to nanomaterials in Italy
- Identification of main processes with high levels of occupational exposure
- Developing an innovative methodology for assessing and preventing risks related to nanomaterials, in an integrated approach for workers' health and environment

PRIN: Interaction of newly synthesised nanomaterials with biological systems: experimental models for human health risk assessment (coordinator: Prof. E. Bergamaschi). The main goals of this research project are:

- To clarify the mechanisms underlying the toxicity of different nanomaterials, such as CNTs or metal oxide nanoparticles, so as to yield a solid toxicologic rationale based on the relationships between structural features and biological responses in relevant systems
- To develop experimentally validated, reliable in-vitro methods to assess nanomaterial toxicity, so as to characterise a battery of tests suitable for the assessment of human health risks associated to newly synthesised nanomaterials

Further networking activities have been set up by AIRI (Italian Association for Industrial Research), namely the AIRI/Nanotec IT. It is acting as a bridging point connecting the Italian players in nanosciences and nanotechnology in industry, public research, and governmental institutions. Its mission is to promote nanotechnology and its applications. Since 2003, it collects and disseminates information on N&N related RTD, applications, facilities, programmes/initiatives, EHS issues, regulation and others. AIRI/Nanotec IT is presently involved in w FP7 projects: FramingNano and ObservatoryNano.

The National Institute of Occupational Prevention and Safety (ISPESL) is involved in international networking:

- PEROSH: Partnership for European Research in Occupational Safety and Health
- METROnet: Mediterranean Training and Research in Occupational Safety and Health Network
- WHO Global Network of Collaborating Centres in Occupational Health

At a national level, ISPESL has set up

- 7 research programmes on nanotechnology and OSH in the Work Plan 2008-2010
- A national steering group on prevention of exposure to nanomaterials at the workplace

- Information tools, courses, and publications on nanoparticles at work

The 4th International Conference on Nanotechnology - Occupational and Environmental Health NanOEH 2009 will be held in Helsinki, August 26-29, 2009.

Risks related to nanotechnology and exposure to nanomaterials have been rated top research priority in the emerging research priorities setting.

7.4.2 Discussion

With regard to the activities presented several participants expressed their appreciation of the efforts now being taken in Italy.

7.5 Projects funded in Mexico

(Velumani Subramaniam, CINVESTAV)

Dr. Velumani Subramaniam gave a short overview of the OECD-recognised Cinvestav (Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional) of which he is the coordinator for international cooperation. The institute has a number of running programmes; details can be found at www.cinvestav.mx.

There is a great interest to cooperate with ongoing activities in Europe in the field of nanotechnology safety and risks, for forthcoming workshops in Mexico all interested participants of the current Brussels workshop are invited.

7.6 Projects funded in Russia

(Marina Melkonyan, A.V. Shubnikov Institute)

Marina Melkonyan, representing the NCP in Russia stated that support from Government has basically started in 2007, now there is an increasing number of activities. Coordination and collaboration with other running EU initiatives / programmes would be highly welcome. She invited everybody to the "NanoBio'2008" Conference in St. Petersburg in June, which includes a workshop on nanosafety http://www.spbcas.ru/nanobio/.

7.7 Projects funded by EU industries

(Dr. Marc Willuhn, CEFIC)

7.7.1 Presentation Summary and Main Statements

CEFIC (European Chemical Industry Council) is funding projects on nanosafety issues. In this context CEFIC is initialising a "Long Range Research Initiative" (LRI), calls are in preparation:

- Tiered approach (testing of OECD guidelines for selected nanomaterials with specific characteristics and physicochemical properties)
- Environmental fate of nanomaterials
- Contribution of nanotechnology to improved safety

The following statements were made:

- The expression safety is too generic if applied to different nanomaterials. Exact systems have to be defined, and good characterisation methods applied.

- Currently, end-users are of the opinion that existing regulation (e.g. REACH) fully applies. Existing methods and guidelines as assessed by the OECD are relevant.
- The goal for RTD is a better understanding of existing test methods, not to be forgotten is the reporting of non-findings.
- In Germany BAuA/VCI have elaborated a recommendable guidance for workplace
- Road Maps for safety research should be developed, as Dechema in Germany has already done it.
- Testing requirements need a careful check.
- The large industry participation in CEFIC projects is beneficial.
- The conclusion drawn from the point of view of CEFIC is that current regulatory framework applies and that for safety testing a focus should be put on collaboration with OECD.

7.7.2 Discussion

As there is no data on funding figures no clear numbers could be given on the total amount of money spent for nanosafety research. But within LRI 2 Mio Euros will be reserved for the start in 2008. As two projects are foreseen this makes 1 Mio Euros per substance, which is in line with OECD practices.

Regarding the in house research and development areas covered, information should be taken from the website of each company.

The question of knowledge about exposure measurements available within the industry was discussed rather controversially.

8 Session 4: Closing the knowledge gaps, what do we need (yet) to know and what are the most urgent questions to be answered?

Chaired by DG RTD (R. Tomellini, P. Aguar)

8.1 Introduction Speech: Nanotechnology risk - an overview of research activity and gaps (Rob Aitken, IOM)

An outline of nanotechnologies was given, stating that they bring about a lot of issues to tackle because of the involvement of new materials, new properties, new products, and new processes.

Assessment of risks has to take account of exposure (level, intensity, duration, population, systems, measurement, forms; also to be looked at: workers, transport, storage, next users/processes, waste), and hazard (toxicity, dose, bioavailability, toxicokinetics), subsequent risk management and definition of limits are required.

Risk assessment is hampered by the need to take into account multiple scenarios, multiple materials, multiple properties on the basis of limited data and not validated methods.

In order to give an actual evaluation of existing knowledge gaps, the current situation was brought face to face with a scenario of five challenges to be addressed in order to make nanotechnologies safe, as defined in a publication by R. Aitken, A. Maynard and others in Nature, Vol. 444/16, November 2006, 267-269.

In the following, the 5 challenges are listed together with the respective remaining open issues as identified in the presentation:

Challenge	Open issues / questions / gaps
1. Instruments to assess exposure to engineered nanomaterials in air and water	• Issues of metrics remain unresolved • A universal sampler still some way off • Measurement methods and improved off-line analysis • Discrimination from background • Smart sensors still some way off
2. Effective and relevant nanotoxicity test methods	• Understanding of the underlying mechanisms of harm • Relevance of assays to all potential routes of exposure (inhalation, dermal, ingestion, and injection) • Scaling of assays to high throughput • Need for new assays • Reproducibility of assays • Availability of in-vivo data for validation • Relevance of the respective particles • Unique problems of fibre shaped particles
3. Systems that can predict the potential impact of new engineered nanomaterials	• Availability of validated models for exposure, translocation, dose responses • Prediction of harm based on physicochemical properties • Relevance, reproducibility, and scaling of the systems
4. Systems to evaluate the impact of nanomaterials from cradle to grave	• Lack of data for occupational and consumer exposure, and few systematic attempts to collect such data • Availability of underlying models • Comprehensive investigation of limits of current methods
5. Effective strategic research programmes involving collaboration, communication, and coordination	• No unified top down strategy • Need for bottom up coordination and sharing • Context of emerging research findings often insufficient

General observations:

- There is a move towards the application of risk assessment methodologies while there is still considerable uncertainty in the underlying mechanisms of harm
- Most of the effort is focussed on the toxicology, very little yet on understanding exposure
- In an EU context there is still not enough emphasis on ecotoxicology issues
- Very little focus is currently placed on consumers' exposure
- Nanoparticles in food are not well addressed
- Publication in peer-reviewed journals should be enhanced

8.2 Discussion

Following the presentation several topics were discussed by the participants related to gaps in current knowledge or practices:

- Many data on no-effect / negative studies are lost because they are usually not considered publishable. Here participants suggested that some type of platform is needed for publication of such results. Participants agreed that a high scientific quality of those publications is a prerequisite for a strong impact. No matter where the results are published, all of them should be available as fast as possible. It should not be forgotten that all results are valid results if the quality of the studies is high enough. Especially for the benchmarking of systems with the state of the art, such types of investigations are important.
- Ecotoxicology must be stressed more than has been done so far. In this area much less data are available, in addition the field is more complex with respect to different environmental conditions in different compartments
- Accidental risks during production, packaging, transport and use need to receive more focus. With increasing production this type of risk will play a more prominent role
- Along with the assessment of existing and new risks of nanomaterials the prevention of new nano-related risks by the search for relevant alternatives should be considered in future
- A combination of existing bottom-up and top down approaches for assessing and managing nano-risks is needed, in order to use resulting synergies. Attempts for global top down cooperation were encouraged by the participants who also recognised the international research initiatives launched by the EC and the work undertaken at OECD. Cooperation among FP7 member countries was also encouraged and the possible use of the ERANET scheme was mentioned.
- A benchmark of knowledge created in projects on nanotoxicology with regard to increased knowledge in this field should be done regularly.

9 Conclusions and Recommendations

9.1 Conclusions

Participants appreciated the workshop as a very successful event, with a critical mass of experts, in view of an achievement of the high aiming objectives:

- Increasing interactions between researchers
- Improving communication of the results of research to risk assessment bodies and other interested parties
- Examining the current knowledge in the area

Increasing interactions between researchers. The workshop has formed a very successful platform for all participants to get acquainted, and to know each others' expertise, qualification and activities. They were given the opportunity to receive a panorama view of current activities in Europe, both financed by the EU Framework Programmes and by Member and Associated States, as well as some international input. The workshop has lead to an intensification of existing, and development of new networking activities among the researchers, thus stimulating an efficient exchange of information, leading to the creation and exploitation of new synergies, and possibly the formation of new partnerships.

In that sense the workshop acted as a catalyst for the formation of a "think tank" for future action in the field of research on the safety of nanomaterials.

The main part of the discussions and debates were lead in a very open and informal manner, contributing substantial and detailed information as the basis for more in-depths interactions with the research community.

In order to further facilitate the interaction of the participants among each other it was decided to make the presentations as well as a participants list of the meeting available to all attendants.

Improving communication of the results of research to risk assessment bodies and other interested parties. It was perceived that the only acceptable basis for communication of results is the validation of existing data before their spreading. Only if sound and science-based data are provided, they can be used for the communication of opinions, the elaboration of recommendations and scientific advice for policy makers. By reviewing current knowledge gaps and fostering the discussion of how to address them, the workshop has largely contributed to reach this objective.

Examining the current knowledge in the area. There has been consensus in the fact that since the adoption of the nanotechnology strategy and action plan by the European Commission, research on the impact of nanotechnology and particularly of nanoparticles on health and the environment has substantially grown during the last 4 years. However, there has been also the shared perception that the scientific and technological challenges in that field still remain great. The participants specifically recognised challenges in the following fields:

- **Toxicology:**
    - Role of impurities, intrinsic defects, foreign substances, non-stoichiometry in multi-component oxides

- Potential long-term toxicity, degradation and metabolism of nanoparticles
- Influence of other primary effects apart from oxidative stress (inflammation, lung disease, immune reaction)
- Validated test methods
- pattern recognition of the main toxicological parameters
- Mechanisms of actions of NPs, affection of end points
- Elucidation of as yet unknown end points
- Influence of physical environment of nanoparticles
- Influence of aging of nanoparticles
- Different R&D strategies for toxicology, ecotoxicology and environment: finding of synergies
- Reproducibility of dispersions and biological materials
- Development of rapid, user-friendly, valid in vitro toxicity screening tests
- Relationship between structural features of nanomaterials and biological responses
- Understanding of the mechanisms of harm
- Models for dose response

- **Ecotoxicology:**
  - Dissemination and mechanism of action
  - Validated test methods
  - Definition of environmental conditions (as compared to physiological conditions)
  - Influence of de-agglomeration for environmental exposure
  - Influence of physical environment of nanoparticles
  - Influence of aging of nanoparticles
  - Different R&D strategies for toxicology, ecotoxicology and environment: finding of synergies
  - Reproducibility of dispersions and biological materials
  - Exposure routes from environment back to humans
- **Exposure:**
  - Validated test methods
  - Environmental exposure routes
  - Relevant particles for real exposure scenarios in workplace and environment, and their behaviour there
  - Influence of physical environment of nanoparticles

- Influence of aging of nanoparticles
- Implementation of exposure simulation
- Different RTD for toxicology, ecotoxicology and environment: finding of synergies
- More date on skin absorption of nanoparticles
- Forecasting of numbers of workers exposed
- Nanoparticles in food applications
- Accidental risks
- Selection of good and cost-effective practices in laboratory and industry

- **Analytics:**
  - Development of testing guidelines
  - In situ characterisation of nanoparticles, definition of suitable parameters
  - Interactions of particles with larger agglomerations
  - Aging of particles in consumer products, look into Life Cycle Analysis.
  - Which proteins attach to surface of nanoparticles under physiological conditions
  - Validation of test methods
  - Benchmarking of measurements, characterisations and detections
  - Developments of reference and test materials
  - Conductions of blind studies for reproducibility
  - Development of metrology
  - Natural particle “background”
  - Establishment of SOPs
  - “High throughput” screening methodology
  - Understanding of the mechanisms of interaction of nanoparticles in real matrices and conditions
  - Development of appropriate analytical and measuring devices and methods (including the preparation and handling of samples)

The picture taken within the workshop of the momentary state, and the challenges in the field of safety of nanomaterials also threw some light on complementary issues not covered by the scientific and technological fields as given above:

- Publication of no-effect / no-result studies: Publication of such results is of utmost importance and, results should be available as fast as possible, all results are valid results if the quality is high enough.
- Information exchange with industries: many data are assumed to be already known by the industries, but not being published because of constraints like confidentiality or lack of confidence in partners. An open and honest dialogue with the industries

should thus be fostered so the research community and the policymakers could use these data.

- Tool for self declaration: as long as nano-specific regulations are not available, tools are needed to facilitate self-evaluation and self-declaration of producers and merchants of nanoproducts. Such tools must follow pragmatic approaches
- Prevention of specific nano risks by careful evaluation of alternatives
- Early inclusion of regulators in projects in order to establish an efficient push-pull interaction, where the case.
- Development of suitable data mining and knowledge management tools.
- Communication strategies of results, and inclusion of mechanisms of risk perception in studies.

9.2 Recommendations

Participants highlighted the need that the EU 7th Framework Programme continue financing research in the field of nano-safety and risks, and encouraged the Commission also to pursue structural improvements such as for the realisation of databases, in collaboration with OECD, and the promotion of dedicated infrastructures.

Participants encouraged the European Commission to continue supporting networking activities, and if possible, to involve researchers from other countries, particularly from the USA, and this both for research, metrology and regulatory aspects.

Recommendations have been made by the participants on research priorities to be funded under the 7th Framework Programme, namely:

- Analytical tools
- Validation of test methods
- Standard operating procedures (SOPs)
- Life-cycle assessment of nanotechnology-based products
- Exposure scenarios
- Good practices
- Ecotoxicity and environmental fate of nanoparticles

Participants stated that in view of the rapid development in this field and the sometimes unpredictable demands, topics on which calls for proposals have been opened should receive funding in regular manner, in order not to leave knowledge gaps remaining unexplored.

The possibility of repeating this meeting in 2009 or 2010, with a possible structure of parallel and plenary sessions in order to allow more participants to take part, has been mentioned and strongly encouraged by the participants.

10 Annex I: List of participants

Downloadable separately

11 Annex II: Presentations

Downloadable separately