JRC Technical Notes

# Report on Early Harvest of Research Results on Nanosafety

Summary of the 1st Joint CASG Nano and ENPRA Workshop held on
14-15 april 2010 at the Institute for Health and Consumer Protection, IHCP (Ispra, Italy)

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Cover: Chairs and Rapporteurs of the workshop

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# Report on Early Harvest of Research Results on Nanosafety, a Joint CASG Nano –ENPRA Workshop

## 1. INTRODUCTION

The Joint CASG-Nano and ENPRA Workshop on Early Harvest of Research Results on Nanosafety, held in Ispra the 14-15th of April 2010, intended to present and share among participants the state-of-the-art of key areas of nanosafety from finalised and ongoing research projects, to support the initiatives to implement European chemical legislation REACH for nanomaterials (NM). Three cornerstones of the current international work about NM were discussed within breakout sessions, that grouped experts participating in the meeting: 1) measurement, characterisation and categorisation; 2) exposure measurement and mitigation; 3) toxicity and ecotoxicity. The Workshop was organized by the Nanobiosciences Unit of the European Commission's Institute for Health and Consumer Protection at the Joint Research Centre (IHCP/JRC) together with the Unit Chemicals-REACH of Directorate General Enterprise and Industry (DG ENTR). Invited participants included: CASG Nano (Competent Authorities Sub Group on Nanomaterials) members and observers, specifically nominated experts to follow the REACH Implementation Projects on Nanomaterials (RIP-oNs) 2 and 3, the Institute of Occupational Medicine (IOM) led consortium for developing the RIPoN2-3, ENPRA (Engineered Nanoparticles Risk Assessment, FP7 Project) partners, NanoTEST (FP 7 Project) partners, and other key research projects as well as members of the OECD Secretariat and its Working Party of manufactured Nanomaterials (WPMN). The final agenda and the full participants list are included as annexes to this document. A link to the presentations can be found at: (http://ihcp.jrc.ec.europa.eu/docs/nbs_enpra/presentations_nano_workshop.pdf).
In the following pages, a summary of the presentations, the associated discussions, and the discussions and recommendations of the breakout groups is presented.

## 2. THE WORKSHOP OBJECTIVES

In the introductory session of the workshop, Hermann Stamm, Head of the Nanobiosciences Unit of the Institute for Health and Consumer Protection (JRC) welcomed the participants and expressed his appreciation to the large interest of researchers, regulators and stakeholders for the workshop. He presented the activities of the JRC on Nanomaterials and Nanotechnologies that are serving various regulatory policy fields of the Commission and are performed in active cooperation with numerous European research projects. The ENPRA project (Engineered Nanoparticles Risk Assessment, FP7) was presented by Lang Tran (IOM), to provide a framework for the subsequent discussions in the workshop sessions and in the breakout groups. The ENPRA project aims to develop an integrated approach to risk assessment of nanoparticles, by using in vitro toxicity data (validated by small in vivo experiments) to construct mathematical models to extrapolate the exposure-dose-response relationship from in vitro to in vivo and to humans. Within this project, collaboration and communication plays an important role. During the ENPRA project presentation, the links to the NanoTEST project (Development of methodology for alternative testing strategies for the assessment of the toxicological profile of nanoparticles used in medical diagnostics) were underlined, since a strong collaboration and information exchange between the two projects has already been established. It was highlighted that the bridging to various user communities and stakeholders such as the ones attending the workshop would strive to maximise the impact of the project findings. Subsequently, the actual situation of NM in REACH and the ongoing REACH Implementation Projects on Nanomaterials managed by JRC were presented by Maila Puolamaa (DG ENT) and

Frans Christensen (JRC). Finally, Juan Riego-Sintes from JRC presented the workshop objectives and wished the participants two days of intensive and productive work.

## 3. HIGHLIGHTS FROM RESEARCH PROJECTS

### 3.1. Other related research projects

The session on the recently started FP7 projects was chaired by Christoph Klein (JRC). To stimulate the networking in the workshop, the work programmes of the recently started FP7 projects HINAMOX[1], InLiveTox[2], ENNSATOX[3], NEPHH[4], NeuroNano[5] and NANORETOX[6] were briefly presented.

### 3.2. Measurement and Characterization of Nanomaterials

In this session, the chair (Hubert Rauscher from JRC) introduced the issues related to measurements, characterization and categorization of nanomaterials (NM). In particular, the need to establish, validate and harmonise standard operational protocols (SOP) for varying conditions of hazard and exposure assessments was emphasized by the chair. It was also stressed the importance to ensure inter-laboratory comparability.

The presentations in this session focused on the need to determine the intrinsic properties of NM, taking into account the modifications of NM characteristics that may occur in exposure media, i.e. in dispersions used for toxicity testing, as well as in the environment. A key message was the need to describe homogeneity and stability of NM in dispersions, taking into account parameters such as reactivity and biopersistence. This issue was considered essential to improve understanding of the links between NM properties and hazard. Among all NM, the characterization of nanofibers and nanotubes was recognized as a special challenge. In a wider perspective, it was considered important to consider a full life cycle analysis (LCA) approach for NM, in the measurement and characterization. This would especially consider physico-chemical changes in NM taking place in the environment (e.g. aging) such as the degradation/modification of the coating and nanoresidues released from commercial nanoproducts during their use.

The subsequent discussion summarised the findings and highlighted some specific issues. In particular, it was indicated that the physico-chemical parameters listed in the OECD-WPMN guidance for the Sponsorship Programme are adequate for NM, but to ensure full comparability of the results, the existing SOP for the characterization of NM in different media need to be harmonised and validated. Furthermore, some of the available analytical methods for characterization were mentioned.

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1 Health Impact of Engineered Metal and Metal Oxide Nanoparticles: Response, Bioimaging and Distribution at Cellular and Body Level.

2 Developing complex in vitro models for nanotoxicity screening.

3 A study on the structure and biological activity of engineered nanoparticles in their transport through the aquatic environment.

4 Nanomaterials related environmental pollution and health hazards throughout their life cycle

5 Towards new generation of neuro-implantable devices: engineering NEUROn/carbon NANOtubes integrated functional units

6 The Reactivity and Toxicity of Engineered Nanoparticles: Risks to the Environment and Human Health

## 3.3. Exposure measurement and assessment

The chair of this session (Achim Boenke, DG ENT) introduced the challenges of exposure assessment and mitigation. He addressed issues like the selectivity of measurement techniques, measurement strategies to handle backgrounds issues, methodologies of exposure assessment (metrics, area concentrations/ personal exposure, fibre counting) and mitigation, as well as quality control and reference materials.

Topics addressed in this session included the need to develop novel, portable and easy-to-use devices suitable for NM monitoring in workplaces, and measure occupational exposure (workplace and professional uses) and consumer exposure as well as recent and foreseen activities to develop exposure scenarios.

In particular, the FP7 NanoDevice[7] project will develop portable tools to enable continuous on-line measurements of mass, number and surface area concentrations of nanoparticles. Concurrently issues related to calibration methods, metrics and physico-chemical properties, reference materials, relationships of physico-chemical and toxicological properties of NM, and emission of NM from industrial processes are investigated. An ongoing research initiative (Nanex[8] project) to build an exposure database and to develop exposure scenarios according to REACH regulation was also presented. The goal of this project is to identify knowledge gaps and further research needs. Apart from ongoing research, results from existing experiences with occupational exposure measurement in various operational tasks for a variety of exposure sources were discussed. In particular, an evaluation of the NANOSH[9] dataset indicated that activities related to manipulation of nano-objects[10] last in general a short time, the concentrations of nano-objects are generally low and close to 'background' levels and only in 30% of cases the exposure to nano-objects was found 'likely' (in tasks such as handling/powder transfer/blending/mixing or with higher production and use during manufacturing of nano-based products). A specific worst case scenario on environmental exposure from coatings indicated that grinding of surface treated with nanocoatings can cause NP concentrations comparable to heavy traffic road. However, it was shown that nanoparticle releases did not depend on the nanomaterial presence in the original matrix, but on the grinding process and hardness of the polymer matrix. In particular, the nanomaterial was not released as such (i.e. free particles), but embedded into larger particles composed by coating product and substrate.

Overall, the presentations and the discussion indicated continued difficulties in distinguishing background nanoparticles from process specific ones by routine monitoring equipment. A methodology was suggested to overcome the problem that involves deducting NM levels generated in the non-production phase from the ones generated during the production phase. About metrics, it was recognized by the experts that the recommendations from the SCENIHR and OECD-WPMN/SG8 to measure mass, number concentration, surface area and particle size distribution are appropriate to assess NM exposure. It was also stressed that the respective devices should also indicate the optimal size ranges for the specific measurement conditions. It was concluded that exposure measurements should be complemented by simulation models/systems and exposure scenarios, which thus need to be further developed. Suitable simulation tests exist for surface coatings (e.g. abrasion, grinding, leaking), and are now going into standardization, but more of such simulation tests are needed also for other potential consumer and environmental exposure.

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7 New and innovative concepts and methods for measuring and characterizing airborne ENP with novel portable and easy-to-use device(s) for workplaces

8 Development of Exposure Scenarios for Manufactured Nanomaterials

9 Inflammatory and genotoxic effects of engineered nanomaterials

10 See ISO/TS 2768:2008

## 3.4. Human toxicity

### *3.4.1. In vitro and in vivo methods*

The session was chaired by Lang Tran, (IOM, UK), and Bengt Fadeel (Karolinska Institutet, SE).

The first part of the human toxicity session was mainly focussing on *in vitro* and *in vivo* tests for NM. The speakers presented and discussed NM genotoxicity, the links between *in silico*, *in vitro* and *in vivo* methods, and the methodology to develop dose-response curves by combining *in vitro* and *in vivo* tests. No general conclusions can be drawn yet of NM's genotoxicity and its role in carcinogenicity. The studies carried out so far indicated that most small particles can induce DNA damage *in vitro*, however the mechanisms are still unclear. It was stated that the significance of results from *in vitro* genotoxicity studies can only be fully validated once enough *in vivo* data is available. Furthermore, there is no consensus yet regarding which genotoxicity assays should be used and which characteristics are important for determining the genotoxic and carcinogenic potential of NMs.

The integration of *in silico*/*in vitro* and *in vivo* methods was considered important because it can allow the validation of *in vitro* findings and tests, and the development of high throughput screening. Moreover, while *in vitro* can facilitate the understanding of the mechanism of toxic action in relation to NM characteristics, *in vivo* testing provides information of potential systemic effects of NM. The key challenges identified in the toxicity testing of NM were: the selection of NM and the availability of reference material and standards, the identification of positive and negative controls, characterization (with standard protocols), NM treatment in conditions comparable to real exposure and dispersion, choice of assays that do not introduce confounding factors (e.g. interference of exposure media), the use of representative cell types, the identification of relevant endpoints and understanding the cellular uptake and across barriers transport.

The availability of results from *in vivo* tests is essential to understand and validate *in vitro* tests, and this is one of the main goals of both projects NanoTEST and ENPRA, which will focus especially on oxidative stress, inflammation, genotoxicity, fibrogenicity and developmental toxicity. The main hypothesis of these projects is that oxidative stress is the likely mechanism for lung toxicity, and that inflammation indicated by pro- and anti-inflammatory markers is involved in the generation of oxidative stress and other pathogenic changes.

In ENPRA, *in vitro* and *in vivo* models to investigate dose-response relationships will be developed for pulmonary, hepatic, renal, cardio-vascular (including blood) and developmental endpoints. The expected result will provide further information on the predictive value of *in vitro* tests, on the biological mechanisms of NM toxicity and on the sensitivity of target organs. All this information will be used to perform an integrated risk assessment.

NanoTEST integrates the investigation of toxicological properties and effects of NPs in several target systems by developing a battery of *in vitro* assays using cell cultures derived from different biological systems, such as blood, vascular system, liver, kidney, lung, placenta, digestive, renal and central nervous systems. It focuses especially on the cross-cutting areas of NP uptake and transport through biological barriers and on studying oxidative stress, inflammation, cellular toxicity, immunotoxicity, genotoxicity and related endpoints in cells of the above mentioned organs and tissues. NanoTEST is developing SOPs and generates a common database of protocols of *in vitro* and *in vivo* toxicological results. *In vitro* findings will be verified in an *in vivo* rat model using the same endpoints and *in silico* assays (SAR, PBPK modelling) are carried out in parallel.

The discussion following the first part of the session indicated that a precondition for all toxicity studies is an appropriate characterization of NM as manufactured, as administered and as absorbed by cells and/or organisms. Much effort has been devoted to develop and harmonies SOPs for the *in vitro* tests, and the experts agreed that this will allow a better comparison between results from *in vitro* studies as well as between *in vitro* and *in vivo* studies. The results of the ongoing projects will also provide further insight on the most promising areas for high throughput *in vitro* toxicity testing. Specific ring testing of developed *in vitro* tests will be necessary for their validation and inclusion in relevant guidance documents. It was emphasized that the NapiraHub database (based on the OECD-WPMN reporting templates) is used for data sharing by NanoTEST and some of the other projects presented in the workshop. This will facilitate data comparability and modelling of NM toxicity.

### *3.4.2. Toxicity of nanomaterials*

The second part of the session addressed experimental results of specific NM toxicity, especially carbon nanotubes (CNT) and metal oxides.

Two presentations were focussing on studies investigating the respiratory toxicity of carbon nanotubes (CNTs).

The first speaker presented studies on the influence of metal impurities and structural defects in CNT on endpoints like inflammation, granuloma formation and genotoxicity. No such effects were observed, when these factors were removed, and a key message from this presentation was that toxicologists should work together with developers of NM in order to make nano applications safe by design. A two years bioassay of intraperitoneally injected MWCNT did not reveal any carcinogenic effects, irrespective of structural defects. The CNTs used in this study were however much shorter than those used in other similar assays, where the induction of mesotheliomas by long MWCNTs could be observed.

The second speaker presented the results of the toxicity tests with Baytubes® (a special type of short, tangled and thin MWCNTs). The authors determined a NOAEL of 0.1 $mg/m^3$ for the Baytubes® on basis of a subchronic inhalation study and calculated an occupational exposure level (OEL) of 0.05 $mg/m^3$. The other toxicity studies (acute inhalation, acute oral and dermal toxicity, skin and eye irritation, sensitization and genotoxicity) showed no relevant adverse effects at realistic exposure levels (all studies were performed under OECD GLP rules). According to the authors of this study, the currently available toxicity test methods were considered applicable also for NM and there was no indication for NM-specific toxic effects that would not be detected by the currently available methods. However, they recommended extended post exposure observation and bronchoalveolar lavage (BAL) analysis for inhalation studies and they also highlighted that, due to physico-chemical interference, confounding factors needed to be considered especially for *in vitro* testing.

The results presented for metal-based NM were focusing on the identification of the relationships between the physical-chemical properties of NM and the toxicity and mechanism of action. In general, it was stressed that it is important to pay attention to the chemistry and forms of nanoparticles to understand the results of (eco)toxicological tests, by linking physico-chemical and (eco)toxicological properties of NM. For instance, oxidizing NM showed higher toxicity than reducing NM, while shape of nano silver oxide affected considerably the toxic power (triangle> spherical > rod). This was considered to be due to reactivity of different crystalline phases. Finally, the ageing of nanoparticles (such as $TiO_2$ included in sunscreen) may also change the toxicity. A specific *in vitro* study on micro and nano sized metal oxides indicated that the toxicity (described in terms of cell viability and mitochondrial toxicity) was related mainly to the chemical composition. Taking into account various copper and copper oxide particles, results

showed that copper oxide nanoparticles displayed high *in vitro* toxicity, mainly due to particles and not to ionic species, while membrane damage by copper particles depends on surface area. On the contrary, the damage of erythrocytes measured with a haemolytic assay is masked by interaction with haemoglobin of copper and Cu/Zn particles, and the protein interactions are nanospecific (showed specifically in nano-sized particles) and related to ionic species (type of metal).

The discussion after the session concluded that the existing test guidelines with some modification are applicable for the testing of NM. This is in line with the preliminary results from the OECD-WPMN assessment (Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials (http://www.olis.oecd.org/olis/2009doc.nsf/linkto/env-jm-mono(2009)21). The results also suggested that not all CNTs are causing asbestos like symptoms, which probably depend mainly on the length, while impurities and surface defects seem to affect other toxicological properties of CNTs. However, it was agreed that further work is necessary to investigate the impact of various dispersion protocols on the toxicity testing, as agglomerates and aggregates do commonly appear in actual nano applications. Important to investigate is also the mechanisms of action as well as the cellular transfer of NM. Future studies should also focus on whether NM can be grouped according to some specific properties.

## 3.5. Ecotoxicology and environmental fate

Key issues were presented by Teresa Fernandes (Edinburgh Napier University, UK) in her introduction of the session. The audience was invited to consider the challenges of environmental fate/behaviour and hazards of pristine NM vs. NM released from consumer products, assessment methods of environmental exposure in various conditions, and the methodologies of ecotoxicity measurements such as sample preparation, characterization, metrics, potential contamination, and the constrains of modelling the environmental fate/behaviour. To date there is still much lack of information in regard to interactions of NM with other contaminants, as well as possible trophic transfers of NM.

The presentations covered the state-of-the-art of several general topics: modelling, sample preparation, and toxicity to a range of species living in the aquatic (primary producers, invertebrates, fish) and in soil (oligochaete) environments. Due to the current practical difficulties to measure the NM levels in environmental media, and due to the lack of knowledge about fate and behaviour in the environment, modelling was suggested as a tool to estimate potential doses of NM on the basis of production volumes, product life cycles and environmental behaviour of NM. On the basis of the estimation model of EMPA (Swiss Federal Laboratories for Materials Science and Technology), the current levels of CNTs and fullerenes in the environment seemed to be not problematic, but further studies are needed on nanoforms of $TiO_2$, Ag and ZnO. However, the current estimation may change as new data are generated in the future.

The identification of a best parameter to quantify the exposure in toxicological testing was discussed, but a definitive answer was not provided, since the parameters used up to now (surface area, 10% of the sample size frequency distribution - d10 -) seem not to be fully suitable due to NM instability during the exposure period. It is also important to consider the influence of exposure conditions on toxicity results, with the example of photocatalytic activity of $TiO_2$. Finally, the application method of NM to soil was evaluated, and a methodology combining direct application via soil and indirect via dispersion with overlapping concentrations was suggested. An example for silver was presented, concluding that the method is reproducible, and that bioavailability of silver is highest from the dispersion but is reduced in soil, especially in silica sand.

The current status in testing and assessment of nanoparticles in soil on the basis of earth worm studies was presented. A literature survey showed scattered studies, with a wide range of species tested, in very different conditions (e.g. sample preparation, particle form used, soil composition), and often not using existing guidelines. The exposure is such studies was not well characterised. However, according to the speaker, in general the standard population measures such as mortality, growth, reproduction and internal responses are suitable for NM hazard studies. Other endpoints, such as enzymatic changes at individual level, can be used to identify early toxic effects. Therefore, there is the need of a rapid knowledge expansion in this field. Finally, further modelling of the relationships between characteristics, effects on human health and environment studies to use all available information (*in vitro* human studies, ecotoxicological studies, *in silico*, etc.) in an integrated manner is needed.

Finally, the various limitations in the current knowledge of NM toxicity in fish were presented. The behaviour of NM in the environment, including speciation, uptake as well as ageing, was particularly stressed and therefore the following parameters were suggested as crucial in fish toxicity testing: source, environmental fate, transport and bioavailability, route of exposure, organism effects on NM and effect of NM on organism. Since it currently seems that NM are different from traditional chemicals, and that NM toxicity and exposure are heavily influenced by the environment, it is important to be careful about toxicity assessment. So far the evidence of 'nano-specific' effects is still a question mark.

On the basis of the presentations and discussions after the session, experts agreed that it seems important that the environmental exposure addresses direct and indirect exposure to NM and NM composites. At the present level of knowledge, modelling and simulation of environmental exposure on the basis of information on production, environmental fate and behaviour can provide valuable indications about potential areas of concern and guide further studies on pristine NM and residues of nano applications in environmental media. Considerable methodological uncertainties remain in the ecotoxicological testing and behaviour of NM in environmental media (e.g. Trojan horse effects). Finally, it was stressed that further studies are necessary on the relationships between NM characteristics, human and environmental toxicity and on their fate in the environment.

## 4. RECOMMENDATIONS FROM THE BREAKOUT SESSIONS

The breakout sessions goal was to discuss the issues raised during the previous sessions, to formulate recommendations for risk assessment and implementation of REACH related to NM. The session was initiated by a presentation of the results of ENHRES project which provides a comprehensive overview on the scientific literature on the risk assessment of fullerenes, carbon nanotubes (CNTs), metals and metal oxides NM up to the end of 2008. These findings allow a good overview of the situation in science and will be also used for the RIPoN2-3 projects. The following paragraphs describe the results of breakout groups' discussion.

### 4.1. Breakout Group I - Measurement, characterisation and categorisation

The breakout group considered a common list of endpoints such as the ones of the OECD-WPMN and ISO/ TC 229 very useful for the characterization of NM for risk assessment purposes. However, the group recommended that the list could be made more user friendly by further grouping of physico-chemical parameters in two groups: general parameters (e.g. surface area, TEM picture, size distribution) and application-specific parameters (e.g. zeta potential, aggregation/agglomeration). It would also be possible to group the parameters according to NM types, which would be useful for the purposes of the NM repository and would further enable studies on categorization according to properties and NM applications. The third possibility could be to group parameters according to SOP that would be useful for the standard toxicity testing and for addressing specific cases. The group considered also important to evaluate at the early stages of the risk assessment process whether an association between the above mentioned properties and risk does exist.

The group considered as well the availability of analytical methods for REACH reporting purposes. For instance the size determination and agglomeration state *in situ* seems not possible, since it changes in different conditions. Furthermore, it is still uncertain what information on coatings would be needed for risk assessment purposes. Information on surface chemistry was considered essential as well as electrical charge, while it was recognized that they may change under changing environmental conditions. Comparison to bulk material was considered also important.

It was recognized that dispersion protocols from OECD-WPMN, NanoTEST, ENPRA and other research projects are available and should be compiled and harmonised. The same applies to specific SOP for both *in vitro* and *in vivo* studies. However, there are shortcomings in methods for NM concentration measurements in liquids. In addition, optical contrasts in microscopic methods may vary between NM (e.g. metals and $TiO_2$ seem to have higher contrast).

The typical ‘nano’ challenges of particle characterization relate to the disperse systems, changing dispersion state, need to use complex metrics, varying sensitivity of different analytical methods and noise caused by larger particles, non-availability of on-line methods as well as artefacts caused by high dilutions in most laboratory methods. These challenges are addressed by an Engineered Nanoparticles Action Group within the European coordination project Co-Nanomet. A revised scoping document and a strategy paper will be published before the end of 2010, based on the European workshop on "Instruments, standard methods and reference materials for traceable nanoparticle characterization" on 28 & 29 April 2010 (www.co-nanomet.eu).

## 4.2. Breakout Group II - Exposure measurement and mitigation

*4.2.1. Introduction*

The exposure measurement and mitigation session included different presentations that addressed occupational, environmental and consumer exposure. These presentations formed the basis for a more in depth discussions during a panel discussion and a final breakout group discussion. Given the time constrains for a detailed in depth discussion on both exposure measurement and mitigation, it was decided to focus the discussions on exposure measurements by including occupational, environmental and consumer exposure and focussing further on the following fields:

- General aspects of exposure measurements;
- Selectivity of exposure measurement techniques;
- Metrics of exposure;
- Source of exposure;
- Validation, harmonisation of methodologies.

A range of different questions were prepared and discussed. The results are summarised and grouped under the above bullet points.

*4.2.2. Results and Conclusions*

*4.2.2.1 General aspects of exposure measurements*

Most information in this important field is available for occupational exposure, while environmental and consumer exposure measurement approaches are still lagging behind. In the area of occupational exposure available information allowed for the generation of initial acceptance criteria for a preliminary exposure decision logic that needs more confirmatory work. Bayer AG is the first company that set occupational exposure levels (for Baytubes, the Bayer AG set a specific occupational exposure value of 0.05 mg/m$^3$ that is not an occupational exposure limit). However, some simulation, end-of-life testing techniques and rough environmental probabilistic modelling approaches do exist that allow first insights and are aimed at helping to assess different products and identify priority environmental sampling sites. Also, certain simulation and especially end-of-life product testing approaches are currently being standardised, which will help in the further development of new and updating of existing guidance documents.

It was concluded that, in comparison with hazard assessment activities, more efforts are required in the exposure field in order to perform proper risk assessments. This applies in particular to the areas of environmental, consumer, occupational exposure. Especially, in the occupational exposure field, validation and harmonisation of existing methodologies for spherical particles is rapidly required in order to ensure comparability and acceptance of results. Moreover and in relation to exposure measurements in general and environmental exposure, in particular, it is essential to:

- Use overlapping concentrations to allow for re-calculation of data afterwards when new knowledge becomes available;
- Address the residue of NM composites/coatings during their use and ageing (i.e. larger (lower µm-range) particles) and develop respective techniques including simulation approaches;
- Address NM and NM composites direct and indirect exposure;
- More exposure case studies are required and their subsequent data should be combined in databanks to boost needed evaluation work and the development of well targeted risk management and mitigation approaches, where necessary.

*4.2.2.2 Selectivity of exposure measurement techniques*

Information on background and artefact production form essential aspects of exposure measurements for NM. In this respect, it is also essential to note that air, being one of the major exposure route to NM, in a normal room can contain 10,000 to 20,000 (natural or human activities derived) nanoparticles/$cm^3$, whilst these figures can reach 50,000 nanoparticles/$cm^3$ in a forest and 100,000 nanoparticles/$cm^3$ in urban streets. Although the mass concentration of nanoparticles is low, it still amounts to substantial numbers. These concentrations imply that every hour, individuals breath millions of nanoparticles, and it is estimated that at least half of these reach the alveoli.[11]

At present, background particles cannot be distinguished from manufactured NM using routine monitoring equipment. This needed task will still require some years of research to succeed. Nevertheless, background levels can be obtained by measuring during non-production hours. Artefacts in the measurements (i.e. due to measurement conditions and instruments properties) will be considered by applying other measure that include contamination testing, and appropriate calibration approaches that are fine-tuned to the relevant matrix and drift testing. A final answer on what practical, time efficient and cost effective measurement campaigns, designs, systems and/or approaches do exist to handle simultaneously different metrics and distinguishing various backgrounds at the same time could not be obtained during this breakout session - this, however, is needed. The use of simulations and scenarios will be complementary to exposure measurements. With respect to the exposure measurement of Carbon Nanotubes (CNTs), the instrument manufacturer NANEUM has recently developed a promising small device based on a catalytic method that relies on the impurities of CNTs to detect certain CNTs independently from the background and the width and length of CNTs - similar devices could be developed for other NM in the future. Finally it was concluded that, when information on NM exposure measurement devices is provided, it is essential that it includes information on the maximum size range that such a device can measure.

*4.2.2.3 Metrics of exposure*

The selection of what is the metric for NM exposure measurements was found to be a very difficult question for the participants and will certainly require much more discussion. It should also be taken into account that the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) concluded that, for the determination of dose – response relationships, special attention should be given to the expression of the metrics of the NM dose since mass concentration is not necessarily the best description of dose for these materials and number concentration and surface area are likely to be more appropriate[12]. Nevertheless and based on the presented information, the participants arrived at the conclusion that there is currently no 'most appropriate exposure metric(s)' yet; experts on toxicology and exposure would have to work closely together to characterise and associate exposure with toxic effects. However, a multiple metric may therefore be kept in the meantime, including: mass, surface area and particle number concentration. These metrics are the least ones that should be measured, taking as well into account the following aspects (assuming that particles are poorly or non-soluble):

[11] Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) (2006), modified Opinion (after public consultation) on "The appropriateness of existing methodologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies", Adopted by the SCENIHR during the 10th plenary meeting of 10 March 2006 after public consultation, pp. 34, see: http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_023.pdf.

[12] Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) (2006), Opinion on the Appropriateness of the Risk Assessment Methodology in Accordance with the technical Guidance Documents for New and Existing Substances for Assessing the Risks of Nanomaterials, pp. 4, see: http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_010.pdf.

- Stage of agglomeration/aggregation;
- Non-spherical particles such as nanotube bundles, if, e.g., length/diameter will be critical;
- Impurities; and
- Measuring a health relevant indicator that can integrate all the physical properties, e.g., the redox-potential of production of the reactive oxygen species (ROS).

*4.2.2.4 Source of exposure*

Based on the information available to participants, it was concluded that there is a strong emphasis on occupational exposure given the fact that the highest probability of exposure is expected in occupational areas. These are especially relevant in the downstream industry and finalised products industry and there in the packing and handling (including abrasion) processing steps. Although work on environmental and consumer exposure has started, much more needs to be done in these areas. Specific work is required, for example, to ascertain whether simulations can also be used for skin penetration.
Related specifically to environmental exposure, the following conclusions and recommendations were drawn:

- Need to measure nominal concentration and size distribution for mass of particles;
- Modelling will be a major tool to estimate exposure including fate and behaviour of nanomaterials in different environmental compartments (air, water, soil & biota);
- Defined conditions simulating environmental fate and distributions together with production and potential use pattern of products containing nanomaterials are required to further improve modelling;
- Exposure measurements for NM and for NM in association with other substances from various environmental compartments (air, water, soil & biota) are also required based on the current results obtained from simulation tests;
- Learn more from the findings and results obtained in various published remediation projects about fate and behaviour of NM in soil and water compartments as well as on forming and distribution behaviour of aggregates/agglomerates from NM under various conditions.

As far as consumer exposure is concerned, the following conclusions and recommendations were drawn:

- Simulating exposure using release rates in the 'laboratory' seem useful in combination with 'use patterns';
- Using models for skin penetration to estimate internal exposure would be helpful;
- Obtain more information on use of consumer products containing NM as this will allow for a more accurate and representative modelling of consumer exposure;
- Based on that use accelerating ageing tests to control the stability or release of nano by-products from commercial nanomaterials/products incorporating nanoparticles.
- It would be more cost effective to use release rates that are often determined for contaminants and update this information as soon as more details for representative nanomaterials become available;
- Learn more from coatings and other products about forming and distribution behaviour of aggregates/agglomerates from nanomaterials under various conditions.

Finally, the need to identify more details on what are the extra or nanomaterials special exposure relevant effects was perceived as very important by participants.

#### *4.2.2.5 Validation, harmonisation of methodologies*

Given the fact that different groups use different approaches, procedures and methods, it was concluded that harmonisation (here: all using same techniques) is critical for a good comparison of results. Moreover, analytical capacities, limit of detection and determination need to be clearly stated as this will determine the scope (here: area & mode of application) of methodologies. Moreover, not only the measured value but also the conditions and setting of the measurement have to be specified to be able to replicate the findings. For example, a BET surface area value can vary based on the temperature and pressure during the measurement.

### *4.2.3 Open questions*

The following questions could unfortunately not be discussed in detail because of the time constrains, and it was hoped that they could be discussed at the next workshop of this kind that is planned for Spring 2011:

- Related to qualitative indicators of exposure:
  - What are qualitative indicators of exposure?
  - How can personal exposures be addressed measured?
- Can surface area measurements be used to obtain personal exposures?
- How can fibre counting be realised?
- What is the state of the art for estimating number concentration of fibres with nanoscale width in air and how should quality control be operationalised?
- What new reference materials are available for quality control, standard test methods and what are the future directions in this activity area?
- What is the mechanism to establish and maintain a database for exposure measurements?
- What are means originating from exposure measurements that form inputs for possible mitigation activities?

## 4.3. Breakout Group III - Toxicity and ecotoxicity of nanomaterials

The breakout group considered a large number of common issues between ordinary chemicals and NM, while a further adjustment to NM would be important for the assessment of potential hazards. These adjustments relate especially to endpoints and test conditions, exposure verification, positive and negative controls and their validity, similar to the OECD principles of Performance Based Test Methods. Further work is especially needed in regard to validation and regulatory acceptance of *in vitro* methods that are widely used for screening and mechanism studies for a large variety of endpoints and where SOP have also been developed. The same applies to exposure verification both in terms of characterization of NM in various media and the need of online measurements. For controls and reference materials, interlaboratory comparisons would be necessary. As regards ecotoxicity and fate studies, it is important that relevant types of exposure (e.g. dietary exposure, or medium exposure) as well as the relevant taxa (e.g. fish, daphnia, algae) should be considered.

Nanospecific issues relate especially to detailed characterisation of the material as manufactured, administered and in the test media. This is particularly important in environmental studies where forms of NM may be affected by changes in environmental conditions (pH, humic acids, salinity etc.). Dosimetry forms another area which has been addressed in the recent guidance from OECD-WPMN and where several metrics will be needed in addition to mass for NM. Furthermore, very high doses used in *in-vitro* studies should be further evaluated in relation to *in vivo* dose-response studies. Further clarification will also be needed for the differentiation of toxic effects deriving either from ions or particles of metals. For widely used non-validated *in vitro* methods, their relevance as individual methods or in combinations of methods in regard to *in vivo* methods has to be urgently verified and their regulatory acceptance ensured. The need for determining additional parameters or effects (e.g. BAL or oxidative stress) for NM should be further considered, as well as the possible carrier effects of NM and contaminants. For ecotoxicity studies, characterisation in sediments and soils forms a special challenge as well as the exposure routes.

The recommendation to RIP-oN 2-3 relate to the above mentioned issues and their regulatory aspects where the list of questions indicates key question areas. Special issues relate to data requirements, validation and regulatory acceptance of currently used in vitro methods and test batteries, dosimetry, long-term toxicity studies and timescales. For ecotoxicological studies, further consideration of ageing of NM, characterization, exposure routes and terrestrial exposure, as well as the use of human toxicity data, are needed. As regards additional information requirements, some pleaded to wait for the results from the forthcoming 1$^{st}$ phase of REACH registration as that will provide better insights on the risk assessment practices. Due to time constrains, these could not be further elaborated in the breakout group.

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**Abstract**
This report informs on the workshop 'Early Harvest of Research Results on Nanosafety'. The workshop was aimed at providing an overview and a forum for discussion to the participants, in particular to regulators and the responsible of the RIP-oN (REACH implementation Project on Nanomaterials) projects, on recent developments in the field of Environmental Health and Safety Assessment of Nanomaterials. Summaries of the contents of the oral presentations as well as the main points discussed subsequently are presented. In addition, a summary of the discussion, conclusions and recommendations of the three breakout sessions (1. Measurement, characterisation and categorisation; 2. Exposure measurement and mitigation; 3. Toxicity and ecotoxicity of nanomaterials) are presented as well.

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