JRC Technical Notes

Challenges of Regulation and Risk Assessment of Nanomaterials

Summary of the Joint JRC Nano event and 2nd ENPRA Stakeholders Workshop held on 10-12 May 2011 at the Hotel Hilton Garden Inn, Somma Lombardo, Varese (Italy), organized in the frame of the JRC Enlargement and Integration Action

Editors: Christian Micheletti, Juan Riego Sintes, Stefania Vegro

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The mission of the JRC-IHCP is to protect the interests and health of the consumer in the framework of EU legislation on chemicals, food, and consumer products by providing scientific and technical support including risk-benefit assessment and analysis of traceability.

European Commission
Joint Research Centre
Institute for Health and Consumer Protection

Contact information
IHCP Communication Office
Address: Via E. Fermi 2749 – 21027 Ispra (VA) - Italy
E-mail: jrc-ihcp-communication@ec.europa.eu

http://ihcp.jrc.ec.europa.eu/
http://www.jrc.ec.europa.eu/

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1. INTRODUCTION

The Joint JRC Nano event and 2nd ENPRA Workshop on Challenges of Regulation and Risk Assessment of Nanomaterials, held in Somma Lombardo the 10-12 May 2011, intended to present the last findings of European research projects in the nanosafety field, with particular emphasis on the ENPRA project results. The main goal was to discuss these results having in mind the regulatory needs and experiences in Europe, US, and China. This discussion intended to contribute to building a bridge between researchers, industry, and regulators. Another main goal of the workshop was to promote collaboration among JRC, European Union research groups and researchers in enlargement countries. The early involvement of researchers and regulators of candidate and potential candidate countries into the European nanosafety network is important to foster a fruitful future collaboration and to smooth the implementation of nanosafety-related regulations.

The Workshop was organized by the Nanobiosciences Unit of the European Commission's Institute for Health and Consumer Protection of the Joint Research Centre (IHCP/JRC). There were about 80 invited participants, from national (e.g. national chemical agencies, health ministries, environmental agencies, US-EPA, European Commission DG, EU agencies) regulatory bodies, and researchers/regulators participating to international initiatives such as those led by OECD and ISO. Single industries as well as industrial associations (e.g. NIA, pigments associations, cosmetics association, etc.) were also represented, as well as researchers from universities and national institutes (e.g. China, Netherlands, UK). 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/events_workshops/joint-jrc-nano-enpra-2011/program/presentations
The workshop was arranged in sessions. The first day was devoted to regulatory development and implementation. It was followed by one and a half days for the presentation and discussion of scientific results on characterization, exposure, and effects. To allow enough time for discussion, a 'podium discussion' was organized at the end of each session, where the speakers and the audience were involved in an open dialogue about the session topic.

The presentations content and the discussion results are reported in the next chapters, followed by the abstracts of the presentations.

2. REGULATORY DEVELOPMENTS

2.1. Summary record

One objective of the ENPRA project is to provide results which contribute to nanomaterials' safety within the EU (as well as globally). In order to focus and maximize the impact of their work, ENPRA partners, as well as other scientists and stakeholders attending the annual workshops, need to have a clear view of the regulatory landscape in which their work will be considered, and need to be acquainted of recent developments in the regulatory framework. Thus, the first session of this 2nd ENPRA workshop was devoted to present and update on the state of the art and developments in the legislation and regulatory context in the EU and the world in relation to nanomaterials.

A. Kobe from DG Environment set the scene by presenting an EU perspective of nanomaterials in the regulatory framework. He made a review of the past and recent EU developments starting from the Commission Communication 'Towards a European Strategy for Nanotechnology' (2004) with some emphasis on the Communication on 'Regulatory Aspects' (2008). He also explained the conclusions from the Competent Authorities Sub-group on Nanomaterials regarding REACH and the Classification and Labelling regulation. He summarised the Resolution of the European Parliament on Regulatory aspects of Nanomaterials and explained the status of expected key deliverables by the Commission during 2011, i.e. Finalisation of the REACH Implementation Projects on Nanomaterials (RIP-oN) concerning Substance Identity, Information Requirements and Chemical Safety Assessment; the 2nd regulatory review of nanomaterials; the review concerning 'Nanomaterials in REACH registration dossiers and adequacy of available information'; a report on nanomaterial types and uses on the market and the definition of the term 'nanomaterial'. He also touched on the very important work going on in parallel in the research sphere and in international organisations such as OECD Working party on Manufactured Nanomaterials (WPMN).

B. Quinn from ECHA presented work following a request by the Commission with regard to the compilation of an inventory based on nanomaterials in REACH registrations & CLP notifications. She explained that in the context of REACH, nanomaterials can be considered as substances on their own or as forms of substances. The results will be a part of the Commission response to the European Parliament on Regulatory Aspects of Nanomaterials. After explaining the approach for screening the large database of registrations and notifications currently held at ECHA, the presenter explained that this work will give an indication of how registrants and notifiers have identified nanomaterials in their dossiers and consequently applied REACH and CLP. Furthermore, it will show how registrations or notifications of nanomaterials can be found in the database. In addition this screening will also be used to identify 50-60 registration dossiers that include information on nanomaterials for a JRC-ENV project for assessing the adequacy of information included on nanomaterials in a selected number of REACH registration dossiers. Detailed results of these activities should be available towards the end of 2011.

There is concern regarding nanomaterials in food and feed that depends on their particular intrinsic characteristics, the lack of in vivo toxicity data and in vitro validated tests and the limited practical experience on risk assessment in this area. Furthermore, there are many uncertainties are associated with measurement of nanomaterials in various matrices, limited experience with testing nanomaterials, a lack of information on long term oral exposure, and there is the possibility that bioaccumulating and persistent nanomaterials end up in the food/feed chain. D. Carlander presented the Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain prepared by the EFSA scientific committee, following a 2009 request by the

Commission. After long elaboration and wide consultation the guidance document was published, as announced, on 10 May 2011. The document describes the conventional risk assessment paradigm as it is considered appropriate for nanomaterials too, but it provides guidance on nano specific considerations that need to be assessed in addition to conventional aspects. Further to comprehensive information on the method of production, intended use, batch to batch variation, stability/shelf life etc, the guidance requires a comprehensive physical chemical characterisation of the nanomaterial (e.g. size, size distribution, morphology, surface chemistry, catalytic activity, stability/shelf life, specific surface area etc) and the description of its concentration, the dispersion medium, agglomeration-aggregation state etc in the test system. As determination under the intended use conditions is the main objective, the characterisation is required for the material as manufactured (pristine state), prior to use in food/feed (as delivered to producer), as used in food/feed, as used for toxicological testing, and in biological fluids and tissues. Consideration of likely exposure scenarios and properties that might give rise to increased or decreased concern (e.g. no persistence of ENM in preparations, no migration from food contact materials, transformation before or during digestion…) is necessary. A testing strategy considering this is developed that includes in vivo tests (ADME, repeat oral toxicity, considering extended endpoints (e.g. endocrine activity and immuno- and reproductive toxicity) and, as screening tools, not yet validated In vitro tests which may trigger additional testing. Regarding exposure assessment, the determination of the amount of material present in food/feed is vital. In any case, the guidance assumes that all added nanomaterial is present, ingested and adsorbed in the nanoform unless information suggest otherwise (worst case scenario).

J. Alwood presented the US approach for 'Regulation and Assessment of Nanoscale Materials under the Toxic Substances Control Act (TSCA)'. TSCA provides the US Environmental Protection Agency (EPA) with the authority to gather information on, require testing of, and screen and control unreasonable risks from new and existing chemicals. Depending on whether a chemical is listed in TSCA (based on molecular identity) it is considered *new* (fullerenes, CNT, quantum dots) or *existing* (some metal/metal oxides) and different regulatory tools exist to allow assessing them accordingly. The system applied to new chemicals, based on pre-manufacture notifications (PMN) submitted by producers, was illustrated (using mostly CNT as an example) referring to the more than 110 PMNs received (carbon, silica, and titania derivatives, fullerenes …) for which information requirements, risk management measures and Significant New Uses Rules (SNUR) have been issued. Each of the over thirty CNTs received is considered as a distinct chemical substance. Based on the assessment framework for other new chemicals, EPA would generally conclude low environmental hazard and risk for CNT, as manufactured. However, due to the transformation potential of CNT and the uncertainty of the relevance of existing data, EPA considers the ecological hazard and risk of CNT to be inconclusive. Similarly, due to the uncertainty of the relevance of both hazard and exposure data, EPA considers the human health hazard and risk of CNT to be inconclusive. Generation of additional data would be required if a company wanted to release CNT to the environment or to manufacture CNT without the current restrictions found in consent orders and SNURs (e.g. each CNT Consent Order contains a testing trigger for a 90-day inhalation study). A 'Nanoscale Materials Stewardship Programme' was launched 2008 and an Interim Report was issued in January 2009. The program was a limited success, as the results show that two thirds of the chemical substances from which commercially available nanomaterials are based were not reported, ninety per cent of the different nanomaterials that are likely to be commercially available were not reported, and the completeness of the reporting is uncertain. For nanomaterials falling under the existing chemicals group, EPA is developing a SNUR as well as related specific rules to require reporting of nanoscale materials data. In the future, EPA, while continuing current activities, intends to focus on the development of chemical categories for nanomaterials, to develop and improve methodologies for integration of data

into risk assessments and risk management, and to identify nanomaterials which may require actions for risk management or not.

The panel discussion that followed the presentations showed that issues associated with the risk assessment of nanomaterials as nomenclature, characterisation, detection and measurement of nanomaterials - particularly in the test systems and complex biological matrices - appropriate metrics, availability and relevance of hazard and exposure data, and applicability of toxicity and exposure methods and models are still outstanding. This points towards the need to continuously update regulation/guidance for nanomaterials assessment as scientific knowledge evolves.

2.2. Abstracts

Nanomaterials in the Regulatory Development: an EU Perspective

Kobe A.[1]

[1]*Unit D.3 Chemicals, Pesticides and Nanomaterials, DG Environment, European Commission*

When identifying the EU perspective, one needs to be aware that there is no overarching 'documented' EU perspective on nanomaterials shared by all principal EU actors: the European Commission (the Commission), the European Parliament (EP) and the Member States. The overall Commission Policy for Nanotechnology was expressed in the Commission Communication *'Towards a European strategy for nanotechnology'* and in the Action Plan 2005-2009 on safe, integrated and responsible development of nanotechnologies. The Commission is currently preparing a plan for the next five year period. Its current draft importantly builds on the last Action Plan, outlining three major strands that need to be further addressed through a coherent approach: science & innovation, safety & regulation, and societal aspects.

The Commission ambition for the safety & regulation strand continues to be set by the EU Treaty that explicitly requires that the EU policies shall aim at the high level of protection (of human health and the environment). It also strives to ensure that the design of its legislative proposals as well as the implementation of adopted legislation achieve this goal in a most cost-efficient manner, fostering competitiveness and innovation. In 2008 the Commission adopted a Communication concluding that the existing legislation in principle already covers the safety aspects of nanomaterials, and that the protection of health, safety and the environment needs mostly to be enhanced by improving implementation of current legislation as well as further research. For more general reasons, the Commission also commenced the work on the definition of nanomaterials that could be used across the EU regulatory framework.

Some Member states are presently developing or considering national nano-specific regulation. These may influence the EU perspective that always takes into account subsidiarity, regulating at EU level only when the objectives cannot be achieved effectively by national approaches.
In 2009, the EP adopted a resolution expressing scepticism towards the Commission's 2008 conclusions, requesting a more solid and in-depth review of legislation (specifically mentioning environment - REACH, waste) and "*an inventory of the different types and uses of nanomaterials on the European market*". In its role as the legislator, the EP in the recent years consistently pushed (and in some cases succeeded) to introduce nano-specific provisions in the EU legislation.
A number of nano-related regulatory reviews and activities launched by the Commission are currently ongoing, and some will be discussed at the Workshop. The Commission is planning to issue by the end of 2011 the second Communication on the regulatory aspects of nanomaterials, together with an annex on the information on nanomaterial types and uses, including safety aspects. The Communication will most probably also include needs on further research and science-based implementation guidance. Further important regulatory reviews are scheduled in 2012 (e.g. REACH).

Nanomaterials in REACH registrations and C&L notifications

Quinn B.[1]

[1]*European Chemicals Agency (ECHA), Annankatu 18, P.O. Box 400, 00121 Helsinki Finland*

ECHA is assisting the European Commission prepare the REACH and CLP aspects mentioned in the 2nd Commission communication on the Regulatory aspects of nanomaterials. This will involve the compilation of information on nanomaterial types and uses, including safety aspects, which has been reported by the chemical companies either in their registration dossiers submitted under the REACH Regulation or in notifications to the Classification and Labelling Inventory submitted under the CLP Regulation.

Since 1 June 2008, ECHA has received more than 26,000 REACH registrations for approximately 4700 distinct substances (7 March 2011) and more than 3.2 million CLP notifications for approximately 109,000 distinct substances (1 April 2011). The tiered REACH registration deadlines for existing (or phase-in) chemicals mean that the registrations received by the 1 December 2010 deadline refer to those that are manufactured or imported per legal entity at > 1000 tons per year and those that are CMR or have a classification R50/53 that are manufactured per legal entity at greater than 1 and 100 tons/year respectively. New substances manufactured or imported after REACH came into force 1 June 2007 are required to be registered when their tonnage exceeds 1 ton/year. The requirement to make a CLP notification by the 3 January 2011 deadline refers to substances placed on the market at > 1 ton per year and substances classified as hazardous under CLP and present in a mixture above the concentration limits specified in Annex I to CLP or in Directive 1999/45/EC which results in the classification of the mixture as hazardous irrespective of tonnage. ECHA therefore has a very large repository of REACH registration and CLP notification dossiers that will provide information on what nanomaterials have been registered or notified and are therefore on the market.

ECHA will screen these received registration and notification dossiers for those that refer to nanomaterials and compile an inventory of nanomaterials based on the information included in registration dossiers and the C&L notifications. For each substance on the inventory, the criteria used for its inclusion will be given.

Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain

Carlander D.[1]

[1]*European Food Safety Authority (EFSA), Parma, Italy*

The guidance document adopted by the Scientific Committee of the European Food Safety Authority presents a practical approach for assessing potential risks arising from applications of nanoscience and nanotechnologies in the food and feed chain. The risk assessment paradigm (hazard identification and hazard characterisation followed by exposure assessment and risk characterisation) is appropriate for these applications, and consequently relevant data and information for the various steps should be made available to the risk assessor to carry out a risk assessment. Guidance is provided on the physico-chemical characterisation requirements of engineered nanomaterials used e.g. as food additives, enzymes, flavourings, food contact materials, novel foods, feed additives and pesticides. Information is provided on testing approaches to identify and characterise potential hazards arising from the nano properties, which when exposure to an engineered nanomaterial is demonstrated, should include information from in vitro genotoxicity, absorption, distribution, metabolism and excretion and repeated-dose 90-day oral toxicity study in rodents. The guidance allows for reduced information to be provided when no exposure to the engineered nanomaterial is verified by data indicating no migration or when complete degradation/dissolution is demonstrated. Based on information from the initial testing, additional in vitro and in vivo studies may be required. The guidance document indicates uncertainties that should be considered when performing a risk assessment. As the area of risk assessment of engineered nanomaterials is under fast development, consequently the guidance document will be revised as appropriate.

Regulation and Assessment of Nanoscale Materials Under the Toxic Substances Control Act

Alwood J.[1]

[1]*United States Environmental Protection Agency, Office of Pollution Prevention and Toxics*

The United States Environmental Protection Agency (EPA) is already assessing and taking measures to prevent risks from exposures to nanoscale materials that are new chemicals under the Toxic Substances Control Act (TSCA). EPA is also taking other steps to develop data and adapt its regulatory framework for chemicals to include nanoscale materials. After a brief introduction to TSCA the presentation will discuss how EPA has applied TSCA authority to nanoscale materials. There will be a description of some of the types of nanoscale materials assessed. The issues associated with the risk assessment of nanoscale materials to date will be identified including nomenclature, material characterization, metrics, available hazard and exposure data, and applicability of toxicity and exposure models. The types of risk management actions EPA is taking to control exposures and potential risks will be described. How those actions are sometimes made with minimal risk assessment data will also be discussed. Some of these issues with conducting risk assessments and implementing risk management actions will be illustrated from specific examples pertaining to carbon nanotubes. There will also be a summary of actions EPA has taken or will take for nanoscale materials already in commerce. This will include a brief discussion of a voluntary approach. The presentation will also outline other key issues regulators must consider such as potential benefits from new technologies such as nanotechnology, interactions with stakeholders, and collaboration with other United States government agencies and international organizations and governments. In addition to summarizing risk assessment and risk management actions, there will also be a summary of the types of environmental health and safety data being developed to make better assessments of nanoscale materials, how EPA expects to obtain this data, and how it will integrate that data into futures assessments and actions.

Current Progress of activity and efforts on nanomaterials Environmental health and safety in China

Chunying Chen[1]

[1]*National Center for Nanoscience and Technology, China*

With the rapid development of nanosciences and nanotechnology, diverse types of manufactured nanomaterials have been currently utilized in thousands consume products to improve their performance of pristine functions, or completely create novel functions, such as industrial products, semiconductors, electronics, stain-resistant clothing, ski wax, catalysts, other commodity products such as food, sunscreens, cosmetics, automobile parts, etc. There is an urgent and growing need for a thorough and detailed examination into the effects of nanoparticles on biological systems.

With the increasing potential for workers and consumers to encounter nanoparticle-containing products, there is a limited and lagging understanding of how these materials interact with cellular systems and what the health and environmental consequences may be. To address these challenging issues, i.e., toxicity of manufactured nanomaterials; their Fate, transport and transformation in human body; human exposure and risk assessment; Transport and transformation of nanoparticles in the environment, the Chinese government, the Ministry of Science and Technology (MOST), the National Natural Science Foundation of China (NSFC), the Ministry of Education (MOE) and Chinese Academy of Sciences (CAS), has put forward on the issues of ‘‘Nanosafety: Biological, Environmental and Toxicological Effects of Nanoscale Materials/Particles’’ since 2004.

As in many countries, programs and initiatives on nanotechnology in China were started in the 1980s. To date, quite a number of centres for research and development of nanoscience and nanotechnology have been established in the CAS and universities. Acting as a technology platform for domestic researchers from research institutions and industries, and a communication window for international linkage or collaboration on nanotechnology, the National Center for Nanoscience and Technology (NCNST) of China is co-founded by CAS and MOE. Within NCNST, China has established Key Laboratory for Biological Effects of Nanomaterials and Nanosafety, mainly including research on the mutual interactions between nanostructures and biological entities, and the use of nanoscience/technology to explore fundamental problems in life sciences. The main goals of the Nanosafety Lab are to form a research network in China to address the issues concerning the occupational and environmental health and safety of nanomaterials and find a solution to reduce the potential environmental, health and social risks.

3. REGULATORY IMPLEMENTATION

3.1. Summary record

K. Rasmussen presented the work of the Working Party on Manufactured Nanomaterials (WPMN) was established in September 2006 to promote international co-operation in human health and environmental safety aspects of manufactured nanomaterials. Its aim is to assist countries in their efforts to assess the safety implications of nanomaterials. The WPMN brings together more than 100 experts from governments and other stakeholders (OECD Countries; non-member economies such as China, Israel, the Russian Federation, Singapore, South Africa, and Thailand; and observers and invited experts from UNITAR, FAO, WHO, ISO, BIAC2, TUAC3, and environmental NGOs) with corresponding networks behind

The WPMN has launched 9 projects, each managed through a steering group (SG). The presentation focused on SG 3: Safety Testing of a Representative Set of Manufactured Nanomaterials: SG 4: Manufactured Nanomaterials and Test Guidelines nd SG 7: The role of Alternative Methods in Nanotoxicology.

SG 3 manages the Sponsorship programme in which 59 end-points are tested or addressed for 13 nanomaterials. The aim of the Sponsorship programme is to understand the applicability of the OECD test guidelines (and other) to nanomaterials, as well as to obtain a dossier describing the intrinsic properties of the selected nanomaterials. After a period of project definition the actual implementation of the first phase the Sponsorship programme takes place from 2009 to 2012, where dossiers mainly containing information on physicochemical properties and short term (eco)toxicity tests are prepared for each material. A second phase testing additional endpoints may start in 2012 after evaluation of first phase results.

SG 4 was established to review existing OECD and others Test Guidelines (TG), for adequacy in addressing manufactured nanomaterials, and based on this, identify the need for development of new or revision of existing test guidelines. A review of current TG has shown the applicability of some and the drawbacks of other. A document on Preliminary Guidance on Sample Preparation and Dosimetry has been produced and is currently in revision, as these were considered crucial issues for the assessment of nanomaterials.

SG 7 was established to support SG 3 on the use of alternative methods and testing strategies, e.g. to identify missing endpoints that could be addressed by in vitro methods as well as needs for development of new or adaptation of existing test methods by using in vitro methods. An additional goal was to develop integrated testing strategies. This project has outlined a framework strategy for testing, a compilation of in vitro methods and assessment of promising candidates and case studies are under development

Results from other projects were also mentioned as the development of a global resource (a database), detailing research projects and identifying research needs, a report on regulatory regimes, a workshop on risk assessment of MN in a regulatory context, a report on risk assessment critical issues, documents on identification of sources and release of airborne MN in the workplace as well as comparison of guidance on selection of skin protective equipment and respirators for use in the workplace, etc.

The international effort of OECD WPMN program takes time but ensures wider acceptance. There is currently a large amount of work in progress whose success relies on proactive information exchange as many gaps in knowledge are still outstanding both with regard to effects and the exposure. Results

of the Sponsorship programme and proposals for new or updated TGs are expected in 2012. This will be a crucial year to show the first worthwhile results.

One challenge for the implementation of the current chemicals regulations for nanomaterials is the uncertainty over the adequacy of the regulation itself and the accompanying guidance for such materials. Accordingly, in 2010, the European Commission launched two REACH Implementation Projects on Nanomaterials with the purpose of advising how the guidance on Information Requirements (the RIP-oN 2 project) and Chemical Safety Assessment (the RIP-oN 3 project) could be updated. S. Hankin and R. Aitken respectively presented the current status and preliminary findings of these projects.

Both projects, while having different objectives, contain common overlapping tasks, reflecting the intrinsic interdependence of the several aspects of Chemicals Risk Assessment. Actually, both projects co-ordinately commenced with the identification and review of all relevant information sources for carrying out the subsequent tasks. Similarly both of them produced a common document that carefully analyses and outlines needs and options for metrics/parameters in the hazard assessment as well as exposure assessment parameters/metrics in order to prepare a meaningful risk characterisation.

RIP-oN 2 objectives were to develop specific advice on how REACH information requirements on intrinsic properties of nanomaterials can be fulfilled, and on the information that is needed for safety evaluation and risk management of nanomaterials, in particular whether information is needed beyond or in addition to the current information requirements listed in REACH Annexes VI-X. Accordingly, a number of task have been undertaken as an analysis of the current REACH information requirements and testing and whether these requirements are appropriate for nanomaterials; identification of additional relevant specific intrinsic properties for which an adaptation of the information requirements and testing and other information generation methods/strategies might be needed for nanomaterials; identifying needs for further research and development of test methods and other information generation methods /strategies in regard to nanomaterials. Where relevant, additional information requirements beyond current REACH requirements were identified when it was considered they are needed to address adequately the properties of nanomaterials. Based on the combined results of these tasks a complete final report is being developed with concrete proposals and options to update the current REACH guidance to specifically address the issues identified for nanomaterials. Among these, some outstanding ones are the use of appropriate metrics and possibly to use several metrics simultaneously, the extent and effect of (de-)agglomeration and aggregation ?, the need of thorough characterisation of the nanomaterial at several stages of assessment – in particular regarding granulometry- read-across and categorisation and relation with the "bulk" material properties, all the issues related to sample preparation/design/monitoring of the test systems and the relevance and integration of information on some additional properties

RIP-oN 3 objectives were, on the one hand, to develop specific advice on how to do exposure assessment for nanomaterials within the REACH context including, development of Exposure Scenarios, evaluation of operational conditions and risk management/mitigation measures and exposure estimation. On the other hand develop ideas on how to conduct hazard and risk characterisation for nanomaterials. Accordingly an evaluation was carried out of the evidence base to identify the key scientific issues arising which had the possibility of implications for the REACH guidance. This included, in relation to exposure and exposure scenarios, examination of industry case studies and harvesting results from on-going activities in relation to operational conditions and risk management measures and exposure estimation. In relation to hazards, similar harvesting and analysis considered how no effect levels could be established and hazard and risk characterisation.

Subsequently, a section by section analysis of the existing REACH guidance was performed in order to identify the optimum set of changes which could be made to the guidance and develop detailed proposals for possible guidance changes. In addition to the common issues with RIP-oN 2 (Metrics, (de-)agglomeration and aggregation and characterisation), some issues have been identified as relevant for guidance improvement of further research as the need of more information on (downstream) use of nanomaterials, the fact that current exposure models are not validated for nanomaterials, the need of new guidance on exposure measurements, the need of further work to validate current risk management measures, how to establish adequate assessment factors and to ensure that data pertain to relevant uses and forms of the material.

At the time of the workshop, findings from both projects (expected to conclude mid-2011) were being collated to form advice on specific issues related to nanomaterials for further consideration and integration into the REACH guidance documents, and to identify further research and development on relevant issues.

The Development of Exposure Scenarios for Manufactured Nanomaterials by the project NANEX and subsequent conclusions was presented by M. van Tongeren. Exposure scenarios are crucial to implement current regulations for risk management. In this project hypothetical occupational and consumer exposure scenarios for carbon nanotubes, nano-TiO2 and nano-silver were developed, either based on models (for consumers) or using exposure data from literature and two measurement campaigns (occupational). After revising the concept of exposure scenario, the presenter illustrated knowledge gaps using four hypothetical case studies as examples.

The key findings indicated that for occupational exposure scenarios mainly data from primary manufacture process are available, while little or no data available from downstream users and often studies refer to pilot plant facilities, rather than fully operational facilities, that contextual information is lacking or is not described properly and that concentration is measured over short periods rather than intending to determine long-term exposure. Regarding consumer exposure, there are indeed little or no empirical data, accordingly the derived exposure scenarios were based on assumptions and models which were not developed or validated for nanomaterials, thus leading to leading to over-conservative or unrealistic estimates. The outcome of NANEX clearly demonstrated that the currently available exposure data is of insufficient quality to develop robust and reliable exposure scenarios.

The presenter made some recommendations in order to improve the quality of the exposure descriptions and potential for data sharing, as a minimum data set that would include information specific for the nanomaterials (physico-chemical information on nanomaterial at source, including chemical composition and size distribution; physico-chemical information on any matrix; amount of nanomaterial used in the matrix; description of released/detected particles (either embedded in a matrix, agglomerated or as single particle and elemental composition); other sources of ultrafine and other particles and multi-metric reporting (mass, number, and size distribution)), as well non nano-specific information (information on process; description of site; risk management measures and sampling and data analysis strategy).

Van Tongeren summarised the short term research needs (test the effectiveness of RMM and control banding approaches; there is a need for harmonization of the characterisation and the exposure metrics, increase data collection for assessing manufactured nanomaterials exposure in all life stages and sharing of results and develop selective instrumentation for nanomaterials and measurements of personal exposure). For long term research, under an overarching effort to define health-relevant exposures to manufactured nanomaterials, there is a need to develop new metrics of exposure or methods to express multi-metric exposures, identify and understand the key determinants of

exposure using new metrics and to develop, calibrate and validate quantitative models for occupational and consumer exposure.

S. Friedrichs, as Director of the Nanotechnology Industries Association (NIA), presented the current Industry experience with conducting nanomaterial safety assessments. In order to enable the highest possible impact of the considerable financial investment that is necessary for reliable and reproducible nanomaterials safety assessments, the NIA approach is that data generated in measurement/testing (research projects) on manufactured nanomaterials needs to be useable for broad policy information, (potential) regulatory compliance and prioritisation of standardisation. It is a requisite that research concentrates on endpoints agreed by (global) policy makers, that it makes any measurement/testing under OECD TGs or harmonised / policy-agreed adaptations in order to eventually conform with the Mutual Acceptance of Data (MAD) agreement and data are collected using the OECD Harmonised Templates which provide a comparable format which is (potentially) regulatory relevant (IUCLID).

Accordingly, NIA is involved in research into the likelihood and possible pathway of exposure via inhalation arising throughout the lifecycle of a selection of commercially available articles containing carbon nanotubes. Industry has also been involved in development of techniques of detection of engineered nanomaterials in the environment and the potential techniques for Nanomedicine and nanotoxicolgy, the RIP-oNs projects, the NANEX project and the PROSPECT initiative.

PROSPECT is a public-private partnership dedicated to supporting the safe and responsible exploitation of nanomaterials, and developing a better understanding of their impact on humans and the environment. This initiative has published literature reviews on Cerium Oxide and Zinc Oxide, a Protocol for Nanoparticle Dispersion, Guidelines and Protocol for Sampling, an Evaluation and Assignment of Nanoparticle Dispersion/Characterisation Methodologies, to be developed under PROSPECT and a Video on Dispersion Protocol. NIA has actively contribution to the implementation of the JRC repository of reference nanomaterials, helping in the characterisation of some of the NM series materials (zinc oxide, silver).

NIA is a enthusiastic supporter of the JRC NANOhub as it provides a tool allowing to many scientific projects (across the globe) contributing to the OECD Sponsorship Programme to store their data and generate results and individual reports. Reporting in harmonised templates necessary to enable read-across, and 'interpolation' and 'extrapolation' of results, this contributes to the value of the OECD Sponsorship Programme, that lies in the agreed, harmonised conduct of tests in many laboratories in many countries on many same representative NMs.

S. Vázquez Campos presented the Nanopolytox approach for the Life Cycle Assessment of Nanomaterials in Polymer Nanocomposites. Life Cycle Assessment (LCA) is a comprehensive analysis tool that can be used to evaluate how a product or material affects ecosystems and human health from its production to its end-of-life. It is currently accepted that it provides an appropriate perspective both for producers, regulators and uses to assess products and materials.

Nanopolytox will use the LCA tool to establish a full understanding of the environmental benefits and drawbacks of nanotechnology and nanomaterials compared with those of conventional technologies and products over their complete life cycles, in particular, polymeric nanocomposites of different nanomaterials (Carbon nanotubes, nanoclays and metal oxide nanoparticles) will be addressed. These nanocomposites will be studied in all their life cycle stages as: i) manufactured nanocomposites, ii) processed nanocomposites, iii) aged nanocomposites and iv) recycled nanocomposites.

Although the ISO framework for LCA is not specific for nanomaterials, it can be adapted to them. The main problem with LCA of nanomaterials and nanoproducts is the lack of data and understanding in certain areas. Major efforts are needed to fully assess potential risks and environmental impacts of nanoproducts and materials (not just those related to LCA). There is a need for protocols and practical methodologies for toxicology studies, fate and transport studies and scaling approaches. Further research is needed to gather missing relevant data and to develop user-friendly eco-design screening tools.

Nanopolytox will address the following issues for nanomaterials over their life cycle: physical and chemical characterization at the different stages; hazard characterization (human toxicity and ecotoxicity) at the different stages; transformation, migration and release of nanomaterials included in products; environmental and biological fate of released nanomaterials; LCA analysis of nanomaterials included in polymeric products; technological solutions for recycling and final treatment of polymer nanocomposites. S. Vázquez Campos, summarised the Nanopolytox workplan and timelines as well as its methodological aspects. The project will finalise in 2013.

The presentation on Health, Safety, and Environment: Assessment Methods was made by D. Hristozov, including the comprehensive weight of evidence approach adopted within the ENPRA project. According to him, substantial limitations and uncertainties hinder the risk assessment (RA) and the lifecycle assessment (LCA) of engineered nanomaterials (ENMs), thus making difficult the implementation of appropriate risk management measures.

Limitations of conventional RA lie mainly in the fields of hazard identification, dose-response relationship assessment and exposure assessment. Accordingly, there is an Increasing interest in development of complementary methodologies methodologies/models (as the XL Insurance Database Methodology, the precautionary matrix for synthetic nanomaterials, the ANSES System, the Stoffenmanager Nano 1.0 and the NANOSAFER) and tools (weight of evidence (WoE), multi criteria decision analysis (MCDA), control banding, expert elicitation (EE)) for RA of ENMs. After making a review of them, the presenter concluded that none of the methodologies/models are intended to facilitate regulatory decision making, but instead mostly to serve as preliminary risk screening tools for use by SMEs and industry. There is indeed a need for quantitative risk assessment approach supporting near-term regulatory decisions.

On the other side, although LCA is usually applied to matured technologies, there is a general trend toward an earlier adoption of LCA, indeed LCA of nanotechnology and nanoproducts can provide an opportunity for precautionary action in order to prevent or minimize potential impact to human health and the environment and can add supplementary environmental information to support decisions on the regulation of certain nanomaterials or nanoproducts. However few LCA studies on nanotechnologies have been published and whereas the ISO 14040 framework is appropriate, a number of issues need to be addressed as the lack of inventory data: (i.e. data about nanomaterial releases during manufacturing, use and disposal stages are not available) and how to correlate the nanomaterials releases with the characterization factors/ environmental indicators used within the LCA framework.

Actually RA and LCA procedures are complementary. Indeed, although LCA is readily applicable to nanoproducts, the methodology faces limitations when addressing the toxicity of ENMs released throughout their life cycle, which is a strength of the RA. Both LCA and risk assessment are costly exercises, integration of RA and LCA (i.e. a pragmatic screening approach combining the use of LCA, risk assessment and scenario analysis) holds promise to support a more holistic nanotechnological assessment that will allow better understanding of the environmental and health impacts and the economic and social benefits of the nanoproducts. Clearly understood and communicated benefits

and risks would increase the probability for social acceptance and economic success of nanomaterials/nanotechnologies

In this context a novel approach for risk assessment and prioritization of ENMs has been developed within the ENPRA project, aimed to quantitatively assess the occupational and consumer risks from ENMs. The ENPRA approach is meant to complement the REACH CSA in cases when nanomaterials are concerned and provide sound and robust RA results for regulatory purposes. The ENPRA approach uses all tools (WoE, MCDA, EE) at appropriate stages to Weigh and integrate all available effects and exposure information into a risk index, Estimate uncertainty related to different aspects and data input, Design a flexible methodology, that allows handling different types of data and scenarios. It allows inclusion of expert judgment into the risk assessment process, ensures compliance with the basic REACH requirements and support regulators in prioritizing ENMs.

H. Rauscher presented the JRC Repository of Representative Nanomaterials and the NANOhub information platform. The current situation in Nanosafety assessment is characterized by an inflation of scientific projects, initiatives and results produced without co-ordination, with (frequently bad characterized) diverse and non-comparable materials, without harmonized methodologies and heterogeneous presentation schemes that make comparative studies almost impossible. This hinders implementation or improvement of regulations, as it makes very difficult to conclude on the potential hazards and risks of nanomaterials. This points out to the need to make readily available representative test nanomaterials provided in identical samples from a single, controlled source. A second need refers to a platform for managing information on nanomaterials that uses harmonised templates for reporting, provides easy data availability, exchange and storage in a way that is relevant, not only for basic research, but for risk assessment and regulation.

The JRC Repository of Representative Nanomaterials intends to answer the first need by making readily available to all stakeholders the NM series of reference nanomaterials. These have been selected among the range of the most important NMs assumed to be used in significant volumes in consumer products as stated in the OECD list of representative NMs. The samples have been prepared under Good Laboratory Practice (GLP), stored under controlled conditions and provided free for EC funded projects. Currently the repository contains more than twenty materials including nanomaterials of TiO2, SiO2, ZnO, CeO2, Silver, MWCNT and nanoclays.

The NANOhub database is the tool developed by JRC to address the second need above. It is a comprehensive web based IT platform for hosting and managing information on nanomaterials in a harmonized/standardized structure and provides full compatibility with existing regulatory framework because is based on the OECD Harmonised Templates and IUCLID (database used for REACH registration). Its uses minimizes time delays for cooperation and information search and exchange, includes extensive search capabilities, provides features for quality control and data exchange and consists of independent, consortium-specific installations hosted by the JRC with options to protect confidentiality but also to share results between different consortia. It is supplied free-of-charge by JRC, with support. H. Rauscher explained the access, structure and templates of NANOhub, as well as its main features and functionalities for storing and managing data, reporting and quality control and data exchange and administration. He also explained that JRC acts only as provider, host and eventually help provider of the individual installations, whose ownership correspond to the consortium that requested the installation. The consortium decides on the terms of use and access of the data and information contained in its own installation.

Finally the concept of a future NanoPortal that would integrate functions of the NANOHub, the Repository and an eventual Inventory of nanomaterials and/or products containing nanomaterials was outlined.

3.2. Abstracts

OECD Working Party on Manufactured Nanomaterials

Rasmussen K.[1], Riego-Sintes J.[1]

[1] *European Commission, Joint Research Centre, Institute for Health and Consumer Protection, Ispra, Italy*

The OECD Chemical Committee established the Working Party on Manufactured Nanomaterials (WPMN) in September 2006 to promote international co-operation in human health and environmental safety aspects of manufactured nanomaterials. Its aim is to assist countries in their efforts to assess the safety implications of nanomaterials.

The WPMN has launched 9 projects, each managed through a steering group (SG) and the project areas are:

SG 1& 2: An OECD Database on Manufactured Nanomaterials to Inform and Analyse EHS Research Activities
SG 3: Safety Testing of a Representative Set of Manufactured Nanomaterials
SG 4: Manufactured Nanomaterials and Test Guidelines
SG 5: Co-operation on Voluntary Schemes and Regulatory Programmes
SG 6: Co-operation on Risk Assessment
SG 7: The role of Alternative Methods in Nanotoxicology (added Nov. 2007)
SG 8: Exposure Measurement and Exposure Mitigation (added Nov. 2007)
SG 9: Co-operation on Environmentally Sustainable Use of Nanotechnology (added March 2009)

The presentation will focus on SGs 3, 4 and 7. SG 3 manages the Sponsorship programme in which 59 end-points are tested or addressed for 13 nanomaterials. The aim of the Sponsorship programme is to understand the applicability of the OECD test guidelines (and other) to nanomaterials, as well as to obtain a dossier describing the intrinsic properties of the selected nanomaterials. SG 4 was established to review existing OECD and others Test Guidelines, for adequacy in addressing MNs, and based on this identify the need for development of new or revision of existing test guidelines. SG 7 was established to support SG 3 on the use of alternative methods and testing strategies, e.g. to identify missing endpoints that could be addressed by in vitro methods as well as needs for development of new or adaptation of existing test methods by using in vitro methods. An additional goal was to develop integrated testing strategies.

REACH Implementation Project on Nanomaterials - RIP-oN2: Information Requirements

Hankin S.M.[1], Peters S.A.K.[1], Poland C.A.[1], Ross B.L.[1], Varet J.[1], Aitken R.J.[1]
Foss Hansen S.[2], and Holmqvist J.[3]

[1]Institute of Occupational Medicine; [2]DTU; [3]Cefic

In the European Union, the REACH chemicals regulation effectively shifts responsibility from authorities to industry to gather information on chemical substances and assess their safety. The regulation does not currently distinguish between nanoscale substances and their bulk equivalent at the micro or macro scale. There is also uncertainty over the adequacy of the regulation and the accompanying guidance for the emerging and rapidly developing nanotechnology industry. As a result, the means of regulating nanomaterials is under an intense spotlight in the EU.

In 2010, the European Commission launched two REACH Implementation Projects on Nanomaterials with the purpose of advising how the Information Requirements (IR) and Chemical Safety Assessment guidance could be updated to better reflect the registration requirements for nanomaterials.

The objectives of the RIP-oN 2 project were to develop specific advice on how REACH information requirements on intrinsic properties of nanomaterials can be fulfilled, and the information that is needed for safety evaluation and risk management of nanomaterials, in particular if information is needed beyond or in addition to the current information requirements listed in REACH Annexes VI-X.

The project commenced with the identification and review of all relevant information sources (Task A) for carrying out the subsequent Tasks (B1-B5 and C), which included: an analysis of the current REACH information requirements and testing and whether these requirements are appropriate for nanomaterials (Task B1); identification of additional relevant specific intrinsic properties for which an adaptation of the information requirements and testing and other information generation methods/strategies might be needed for nanomaterials (Tasks B2 & B3); identifying needs for further research and development of test methods and other information generation methods /strategies in regard to nanomaterials (Tasks B4 & B5); an outline of the needs and options for metrics/parameters in the hazard assessment compatible with the exposure assessment parameters/metrics in order to prepare a meaningful risk characterisation (Task C). Where relevant, additional information requirements beyond current REACH requirements have been identified when it is considered they are needed to address adequately the properties of nanomaterials.

Findings from the project, concluding in mid-2011, are being collated to form advice on specific issues related to nanomaterials for further consideration and integration into the REACH guidance documents, and to identify further research and development on relevant issues.

REACH Implementation Project on Nanomaterials - RIP-oN3: Specific Advice on Exposure Assessment and Hazard/Risk Characterisation for Nanomaterials under REACH

Aitken R.J.[1], Hankin S.M.[1], Peters S.A.K.[1], Poland C.A.[1], Tran C.L.[1], Foss Hansen S.[2], Friedrichs S.[3], and Holmqvist J.[4]

[1]Institute of Occupational Medicine; [2]DTU; [3]NIA; [4]Cefic

In 2010, the European Commission launched two REACH Implementation Projects on Nanomaterials with the purpose of advising how the Information Requirements (IR) and Chemical Safety Assessment guidance could be updated to better reflect the registration requirements for nanomaterials.

The objectives of the RIP-oN 3 project were twofold. The first was to develop specific advice on how to do exposure assessment for nanomaterials within the REACH context including, development of Exposure Scenarios, evaluation of operational conditions and risk management/mitigation measures and exposure estimation. The second was to develop ideas on how to conduct hazard and risk characterisation for nanomaterials.

The initial activity was the collection and review of information from a wide range of sources (Task A). This was followed by an evaluation of the evidence base to identify the key scientific issues arising which had the possibility of implications for the REACH guidance. This included, in relation to exposure and exposure scenarios, examination of industry case studies (Task B1), and harvesting results from on-going activities in relation to operational conditions and risk management measures (Task B2) and exposure estimation (Task B3). In relation to hazards, similar harvesting and analysis considered how no effect levels could be established (Task C1) and hazard and risk characterisation (Task C2). The issue of metrics was carefully considered (Task D).

The Final stage of the project was a section by section analysis of the existing REACH guidance. The assessment considered in detail the optimum set of changes which could be made to the guidance. Based on this analysis, detailed guidance changes were developed along with recommendations for research where this was indicated.

Findings from the project, concluding in mid-2011, are being finalised to form advice on specific issues related to nanomaterials for further consideration and integration into the REACH guidance documents, and to identify further research and development on relevant issues.

NANEX: Development of Exposure Scenarios for Manufactured Nanomaterials

van Tongeren M.[1], Clark K.[2], Brouwer D.[3], Christensen C.[4], Micheletti C.[4], Friedrichs S.[5], Nowack B.[6], Gottschalk F.[6], Schmid K.[1], Aitken R.[1], Vaquero C.[7], Gkanis V.[8], Uzu G.[9], Golanski L.[9], Gerritsen R.[3], Riediker M.[2]

[1]IOM, Edinburgh UK; [2]IST, Lausanne, Switzerland.; [3]TNO, Zeist, The Netherlands; [4]JRC-IHCP, Ispra, Italy; [5]NIA, Brussels, Belgium; [6]EMPA, St. Gallen, Switzerland; [7]LEIA, Miñano, Spain; [8]Demokritos, Athens, Greece; [9]CEA, Grenoble, France

Exposure scenarios are important tools in risk management and generally include information on substance, process and activities, presence of any risk management measures, and estimates of exposure. NANEX aimed to develop occupational and consumer exposure scenarios for carbon nanotubes, nano-TiO_2 and nano-silver based on currently available information. The consumer exposure scenarios were based on models, while the occupational scenarios were developed using exposure data from literature and two measurement campaigns. In total, 57 occupational and 5 consumer exposure scenarios were developed. A number of exposure scenarios were developed in partnership with companies using or manufacturing nanomaterials. Many scenarios lacked contextual information, such as presence of risk management measures, duration of activities, operational conditions, etc. In addition, measurements were often carried out to characterize emission rather than exposure and several studies were carried out in laboratory or pilot studies, rather than real-life conditions. More detailed information on operating conditions and risk management measures were available for the development of the case study scenarios. However, the case studies did not explore all downstream use exposure scenarios. The outcome of NANEX clearly demonstrates that the currently available exposure data is of insufficient quality to develop robust and reliable exposure scenarios. To improve the quality of the exposure descriptions and potential for data sharing a minimum data set is proposed, including nano-specific and generic information. In addition, research needs were identified. In the short-term there is a need for harmonization of exposure metrics, for verification of effectiveness of risk management measures and for development of risk management strategies to be applied while waiting for development of more detailed exposure and risk assessment methodologies. Longer term research needs to focus on collection of high quality exposure and contextual data over the life cycle of nanomaterials, the advancement of our understanding of multi-metric exposure and the key exposure determinants, as well as the development, calibration and validation of nano-specific exposure estimation models.

Industry experience with conducting nanomaterial safety assessments

Friedrichs S.[1]

[1]*Nanotechnology Industries Association, 101 Avenue Louise, 1050 Ixelles (Brussels), Belgium*

Companies active in the manufacture and use of nanomaterials have conducted many nano-specific safety assessment approaches over the last few years; in some cases, companies developed the necessary approaches in house, in others, companies joined initiatives that had been developed by policy-making groups (such as the OECD WPMN) or public R&D projects (such as FP7 / FP8 projects) concerned with nano-hazard and/or –exposure assessments.

Some of the most successful approaches to the safety assessment on nanomaterials were conducted on a pre-competitive basis, and involved companies of different sizes and at different stages of their establishment, representing the manufacture or use of a specific nanomaterial at different stages of its value chains. Most approaches were developed and conducted in collaboration with policy-making or –informing bodies and standardisation organisations, in order to enable the highest possible impact of the considerable financial investment that is necessary for reliable and reproducible nanomaterials safety assessments.

Life Cycle Assessment of Nanomaterials in polymer Nanocomposites (Nanopolytox approach)

Vázquez-Campos S.[1], Escamilla M.[1], Janer G.[1], Aubouy L.[1]

[1]*LEITAT Technological Center, C/ de la Innovació 2, 08225 Terrassa, Barcelona, Spain*

The increase in the production and use of engineered nanoparticles (NP) makes exposure of the natural environment to these compounds more and more likely (1). The risks posed by NP are determined by their potential hazards (such as toxicity), as well as by the extent the material will come into contact with an organism (2, 3). One approach that can improve the understanding of the possible impacts of nanotechnology is Life Cycle Assessment (LCA). This comprehensive analysis tool can be used to evaluate how a product or material affects ecosystems and human health from its production to its end-of-life. It is now universally accepted that the product life cycle is the proper perspective for thinking about materials (4), including nanoparticles. Currently, knowledge of the exposure routes and of the potential environmental impacts of nanoparticles is limited. In addition, potential resource and environmental advantages of nanomaterials and products using nanomaterials over conventional products are, in most of the cases, under investigation. Therefore, a clear need exists to establish a full understanding of the environmental benefits and drawbacks of nanotechnology and nanomaterials compared with those of conventional technologies and products over their complete life cycles. LCA is the essential tool to achieve this.

Nanopolytox will use the LCA tool to evaluate the environmental impact of polymeric nanocomposites of different nanomaterials (Carbon nanotubes, nanoclays and metal oxide nanoparticles) over their life cycle. The application areas consider for the LCA study will be those where the products are fabricated for external uses (automotive, aeronautics, construction…). These nanocomposites will be studied in all their life cycle stages as: i) manufactured nanocomposites, ii) processed nanocomposites, iii) aged nanocomposites and iv) recycled nanocomposites. The general considerations proposed in Nanopolytox for the LCA methodology will be explained in this presentation.

[1] Nowack B *et al, Environ. Pollut.* **2007**, 150, 5-12.
[2] Wiesner, MR *et al*, *Environ. Sci. Technol.* **2006,** 40, 4336-4345.
[3] Colvin VL, *Nat. Biotechnol.* **2003**, 21, 1166-1170.
[4] Davis JM, *J. Nanosci. Nanotechnol.* **2007**, 7, 1-8.

Health, Safety, and Environment: Assessment Methods

Hristozov D.[1], Gottardo S.[1], Critto A.[1], Zuin S.[2], Marcomini A.[1]

[1] *Ca' Foscari University of Venice, Dorsoduro 2137, 30123 Venice, Italy*
[2] *Venice Research Consortium, Via della Liberta 12, 30175 Venice, Italy*

It has been largely recognized that substantial limitations hinder the risk assessment (RA) and the lifecycle assessment (LCA) of engineered nanomaterials (ENMs). The present deficit of nano environmental, health and safety (EHS) data would lead in the near term to uncertain and ambiguous, largely qualitative estimations, based on expert judgments, which may fail to support adequate regulatory decisions.

Currently most scientific debates are focused on the generation of new experimental data, relevant for the hazard and exposure assessment of ENMs in the context of the REACH Chemical Safety Assessment (CSA). Minor attention has been paid to the application of novel approaches and tools, facilitating the near-term estimation of risks and impacts of nanomaterials from a lifecycle perspective by integrating the existing data and information. A number of nano risk tools have been recently proposed (e.g., the Swiss Precautionary Matrix, the Dutch Stoffenmanager Nano, the French Anses system) and some are still under development (e.g., the Danish NANOSAFER). However, most of them are not intended to facilitate regulatory decision making, but instead to serve as preliminary risk screening tools for use by SMEs and industry. In this context a novel approach for risk assessment and prioritization of ENMs has been developed within the EU FP7-funded ENPRA project, aimed to quantitatively assess the occupational and consumer risks from ENMs. The ENPRA approach is meant to complement the REACH CSA in cases when nanomaterials are concerned and provide sound and robust RA results for regulatory purposes.

Moreover, the proposed RA approach could be supportive and complementary to LCA procedures. Indeed, although LCA is readily applicable to nanoproducts, the methodology faces limitations when addressing the toxicity of ENMs released throughout their life cycle, which is a strength of the RA. The integration of these two different but complementary approaches holds promise to support a more holistic nanotechnological assessment that will allow better understanding of the environmental and health impacts and the economic and social benefits of the nanoproducts. This is a significant added value for industry because the social acceptance of the risks posed by new technologies is easier if their benefits for the consumer are clearly assessed and communicated.

The JRC Repository of Representative Nanomaterials and the NANOhub information platform

Rauscher H.[1], Rasmussen K.[1], Klein C.[1], Stamm H.[1]

[1]*European Commission, Joint Research Centre, Institute for Health and Consumer Protection, Ispra, Italy*

Today many studies on nanomaterials are performed, including synthesis, characterisation, in vitro and in vivo studies, and complete life-cycle analyses. The generated knowledge is however typically collected in individual reports and islands of knowledge are created. These islands often lack connections through harmonized methodologies (comparability of materials, study design, standard operating procedures, etc.), timely sharing of results and similar reporting schemes (e.g. common information collection templates). Hence the potentials of existing collaborations and even completed studies are often exploited in a suboptimal manner, resulting in delayed or insufficient dissemination of knowledge and increased total cost. Two instruments aiming at improving this situation will be presented. (i) The JRC Repository of Representative Nanomaterials was created to foster harmonisation in safety assessment by creating a common and same nanomaterial source for all stakeholders, including European national authorities and EU funded research projects. The repository contains most types of nanomaterials currently assumed to be used in significant volumes in consumer products as stated by the OECD Working Party on Manufactured Nanomaterials (OECD WPMN). (ii) The NANOhub with its web-based functionality is a comprehensive IT platform intended to boost interconnections and to facilitate synergies between collaborations. It is designed for hosting and managing information on nanomaterials in a harmonized/standardized structure, and provides compatibility with existing regulatory framework on chemicals. It minimizes time delays for cooperation and information search and exchange and provides features for quality control and data exchange. NANOhub consists of independent, consortium-specific installations hosted by the JRC with options to protect confidentiality but also to share results between different consortia.

4. CHARACTERISATION AND DETECTION OF NANOMATERIALS

4.1. Summary record

A comprehensive characterisation of nanomaterials is the starting point of most efforts aimed at a risk assessment of such materials. The characterisation should include the basic physical and chemical properties of the nanomaterial, because they can be very different from those of the bulk form (if the latter exists). An extrapolation of the bulk properties to the nanoform is often not straightforward and therefore the properties of each nanomaterial must be carefully and individually characterised. This is a considerable challenge, as nanomaterials are dynamic systems which have properties that depend on their history or on the method of preparation for measurements, which can be a challenge in itself.

For risk assessment and life cycle analysis it is not sufficient to characterise the nanomaterial as such but it is also necessary to understand its interaction with the environment where it will be found during its life cycle, i.e., in biological systems, food, feed or in the ecosystems where it may be released after use, such as soil, air or runoff water. This poses a huge challenge because the methods and methodologies for detection and characterisation of nanomaterials in such complex matrices are still in the development phase.

Standard operating protocols (SOPs) for the preparation of the test items and harmonised protocols for the measurements necessary for characterisation of the nanomaterials are hence indispensable if comparability of characterisation studies on nanomaterials between different laboratories is desired. A protocol database, which was established within the FP7 project NanoImpactNet to enable the comparison of methods used in different laboratories for the testing of nanomaterials was presented. The database is also intended as platform for developing common protocols and strategies for the testing of nanomaterials regarding their interaction with biological systems and their physicochemical properties. Another aim is to share its protocols with the entire nanoscience field.

Characterisation of nanomaterials is becoming the most important point: without a thorough characterization, hazard results are not interpretable and/or comparable. An overview of the state of the ENPRA work package on characterisation of test materials selected for the ENPRA project was showed. Materials were dispersed using a single protocol and characterised with a variety of methods in batch dispersion as well as in *in-vitro* exposure mediums with the aim to arrive at a comprehensive overview of properties thought to be important for potential hazard identification. Encountered challenges include for example method-dependent results on particle size distribution and quantitative analysis of unknown particle surface functionalisation. The need of improving the preparation of test items and to understand the behaviour of nanomaterials in exposure mediums was pointed out.

Another main issue is the detection and characterisation of nanomaterials in the environment. In detail, characterisation and detection of nanoparticles in environmental media was addressed in a presentation. Monitoring an extremely low concentration of manufactured nanoparticles within a large and complex background of natural and unintentionally produced nanoparticles is a great challenge. Single particle ICP-MS was presented as a new method to tackle this problem and it was demonstrated with an example that the method used in combination with field flow fractionation was able to detect metal nanoparticles in road runoff waters.

In the discussion of the session the importance of addressing and understanding the biological activity of nanomaterials by characterising and quantifying its active sites was pointed out. Furthermore, if nanomaterials are to be characterised in a complex environment (e. g. biological

exposure media) it is important to understand if and how the measurement or the sample preparation themselves may change the objects to be measured.

Developing special protocols aimed at the characterisation of specific properties and finding the appropriate dose metrics in toxicological studies were identified as additional challenges in risk assessment approaches for nanomaterials.

4.2. Abstracts

An example of the advantages and disadvantages related to producing a protocol database for nanoscience

Clift M.J.D. [1], **Gosens I.**[2], Boschung N.[3], Stone V.[4], Kuhlbusch T.[5], Gehr P.[6], Lynch I.[7], Cassee F.R.[2], Riediker M.[3], Rothen-Rutishauser B.[1]

[1]Department of Clinical Research, University of Bern, Switzerland; [2]National Institute for Public Health and the Environment, Bilthoven, The Netherlands; [3]Institute for Work and Health, Lausanne, Switzerland; [4]Heriot-Watt University, Edinburgh, United Kingdom; [5]Institute of Energy and Environmental Technology, Duisburg, Germany; [6]Institute of Anatomy, University of Bern, Switzerland; [7]University College Dublin, Dublin, Ireland

Over the past 3 years, the FP7 EU project 'NanoImpactNet' (NIN) (Grant Agreement 218539) has constructed an online-space (http://www.nanoimpactnet.eu/) for sharing research protocols with other members within NIN. The aim of this exercise is so that laboratories may easily compare their methods and subsequently develop common protocols and strategies for the testing of nanomaterials in regards to their interaction with biological systems and their numerous physical characteristics. This is achieved by:

Members of NIN submitting their protocol to the NIN protocol database using the agreed protocol template.

NIN members are then able to view these submitted protocols, test them within their own laboratories and then comment upon the protocol itself. These comments are then available to all NIN members for an open discussion.

After NIN members decide to apply a specific protocol, either in its original format or slightly modified, the protocol is then upgraded to a 'protocol recommended by NIN'.

The protocol is then further scrutinized by NIN members, and then if adequate becomes an 'officially recommended protocol by NIN' and highlighted throughout the field and international bodies as a 'recommended protocol' for nanoscience.

Initially, only protocols that had been published in peer-reviewed journals were considered. This was ineffective however, with only 8 protocols being submitted and transferred into the NIN protocol template in the first 2 years of NIN. In an attempt therefore, to increase the number of protocols submitted to this protocol database, collaboration between previous and existing EU projects (namely ENPRA, NanoInteract and NanoCare) enabled a significant increase in the number of protocols published on the NIN protocol database website. Additionally, interaction with institutions in the United States of America (i.e. NIST), also facilitated a further increase in the protocols submitted to NIN. Via these collaborations, the secondary aim of the protocol database has been achieved in regards to sharing protocols with the entire nanoscience field, and not solely within NIN itself. Currently, 21 protocols are published on the database, with another 16 in preparation. The presentation will highlight both the advantages to this database and the problems that have been encountered during the past 3 years.

Primary and secondary characterization of the ENPRA ENP

Jensen K.A.[1], Bilanikova D.[2], Brunelli A.[2], Pojana G.[2], Birkedal R.[1], Levin M.[1], Koponen I.K.[1], Kofoed-Sørensen V.[1], Clausen P.A.[1], Marcomini A.[2], Jacobsen N.R.[1], Wallin H.[1]

[1]*National Research Centre for the Working Environment, Copenhagen, Denmark*
[2]*University Ca' Foscari Venice, Italy*

The nanomaterials in ENPRA comprise six materials from the OECD WPMN sponsorship programme [NM101 (TiO_2), NM110 (ZnO), NM111 (silane-coated ZnO), NM300 (dispersed silver particles: Ag), NM400 (carbon coated multi-walled carbon nanotube) and NM402 (multi-walled carbon nanotube)]. Additional four TiO_2 samples were selected: A 10 nm- (NRCWE-001) and a 100 nm-size rutile TiO_2 (NRCWE-004) of which a subsample of NRCWE-001 was modified to achieve a chemical positive (NRCWE-002) and negative charge (NRCWE-003). All test materials were selected to obtain a "dynamic range" in physico-chemical properties and toxicity.

In this work, we characterize the selected test materials and the particle characteristics in the in batch dispersion and in vitro exposure mediums. We have also established a common serum-based method for particle batch dispersion for the ENPRA toxicity testing. The primary characterization is important to document the test materials (Figure 1), but the data are also highly warranted as many of the end-points (especially primary size and surface area) are thought to play a mechanistic role in particle toxicity. In addition, we also describe the hydrochemical reactivity (hydroxyl radical formation capacity and redox potential in test mediums) of the NMs and their emission potential by rotating drum dustiness testing.

Some of the major challenges in the characterization of primary physico-chemical characteristics, are inappropriate methods for bulk characterization of CNT, method-dependent results on size distributions, variation in metal catalyst extraction efficiencies from CNT for ICP elemental analysis, quantitative analysis of unknown organic ENP surface functionalisations, applicability of the non-catalyzed benzoic acid test method for quantification of intrinsic hydroxyl radical formation capacity. Methods and results will be discussed in the lecture and special focus will be set on the need to improve our understanding of test item preparation and the behavior and reactivity of NM in both exposure mediums and specific biological compartments.

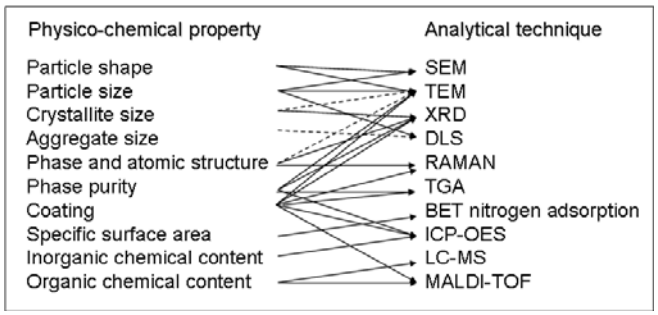

Figure 1. Overview of the main primary physico-chemical characteristics and analytical methods applied in ENPRA. As indicated by the arrows, several methods can give supporting information to improve the understanding of the materials.

Characterization and detection in environmental media

Hassellöv M.[1], Tuoriniemi J.[1], Cornelis G.[1], Gustafsson S.[2], Olsson E.[2]

[1]*Environmental Nanochemistry, Dept. of Chemistry, University of Gothenburg, Gothenburg, Sweden;*
[2]*Microscopy and Microanalysis group, Applied Physics, Chalmers Univ. of Technology, Gothenburg, Sweden*

The same properties of nanomaterials that make them so interesting from application point-of-view can also give them toxic effects. It has therefore been emphasized that one of the grand challenges for safe implementation of nanotechnology is the development of methods to monitor nanoparticles (NPs) in workplaces, products and in the environment. The relevant physicochemical characteristics and potentially useful methods will be briefly discussed, and then the remaining talk will be dedicated to analytical challenges for manufactured NP (MNP) in environmental samples. The main challenge is that there are extremely few MNPs but plenty of natural or unintentionally produced NP. It is difficult to detect particles present in extremely low concentrations among a large number of background particles with microscopic techniques such as TEM, and SEM. However, there are certain types of EM methods that can provide stronger chemical contrast to enable screening of the samples to find certain types of MNP, and then characterize those in detail.

We have addressed this problem of complex background by further developing single particle ICP-MS (spICP-MS) for detection and sizing of individual nanoparticles. spICP-MS is a real-time ultrafast scanning mode that allows capture of the ion burst events occurring when individual NPs are vaporized, atomized and ionized in the plasma. The frequency of these ion bursts are proportional to the NP (of the specific element) number concentration in the sample. The spike height is proportional to the number of atoms in each particle. Therefore in principle it is possible to obtain data on particle concentration and size (element mass) by the method.

In addition to use of the spICP-MS as a stand-alone method for screening MNP in environmental samples (e.g. Ag and Ti in waste water), examples of using spICP-MS as an online detector following Field-Flow Fractionation will also be demonstrated for Pt and W in road runoff waters.

5. EXPOSURE ASSESSMENT

5.1. Summary record

Exposure is a central concept in risk assessment: if there is no exposure, there is no risk. Therefore, exposure assessment studies for nanomaterials (NM) are a cornerstone to carry out reliable risk assessment. However, there are very few studies so far and mostly concentrated in the occupational area. The goal of this session was to present some examples of exposure measurement and estimation, hoping to have more of these studies available in near future.

Among other exposure pathways, the consumer exposure via spray products is considered a priority, since it could lead to significant acute exposure to NM. In the market there are several products to treat surfaces obtaining a nanofilm inducing the loto effect (increased hydrophobic properties of the surfaces). In general the goal is to simplify cleaning and to reduce the amount of cleaning substances used. Not all of these products contain actual NM, but they were considered because of the generation of a nanofilm. This case shows how it is somewhat difficult to define and identify 'nanoproducts' that are on the market: for example sometimes the content of a NM is not declared, and sometimes the use of a NM in the product is not necessary to decide that we are dealing with a nanoproduct. A case study presented during the session showed the amount and form of NM released during the use of various spray products commercialized in Denmark. Exposure was measured in laboratory, simulating the actual use. According to these results, nanoparticles are formed in air, but after short time (few minutes) the number of particles decreased. During the discussion it was suggested that decrease of particle number could be related to adsorption of particles to the walls of the chamber used in the test, but this hypothesis was not checked. Anyway, size development during and after exposure was monitored. Using the spray can, a high nucleation was observed, with formation of bigger particles in short time, maybe due to the presence of O3. For two of the products an inhalation toxicity test on mice was performed, and for one product it was found an unacceptable risk at concentrations very near the concentrations found during normal use, but related to a chemical compound which was not a NM. During the discussion it was highlighted that besides inhalation also oral exposure could play a big role in consumer exposure. According to 1 study, oral intake is 1000 to 10000 higher than inhalation, and the translocation from gut to blood is significant. Therefore there is the need to have more data about this route of exposure, especially about transit time and translocation. However, if it is important to collect more experimental exposure data measured in realistic conditions, it is not practical to expect that exposure will be measured in all possible conditions and for all products. As it happened for conventional chemicals, also for NM it is essential that modelling tools are made available to risk assessors and regulatory bodies in order to screen the possible workers, consumers and environmental exposure. Current models, e.g. ECETOC TRA, ConsExpo, Stoffenmanager, are still based on conventional chemical parameters, and the identification of properties of NM that could influence the exposure and their parameterization are lacking. To try to understand the applicability of the human exposure models to NM, available models where applied to some exposure scenarios for workers, and brief considerations where made for consumers. In this last case, both ECETOC TRA and ConsExpo are in principle applicable to estimate the inhalation of NM, but there are some limitations due to the fact that values for some parameters are defaults not addressing NM specificities, and that agglomeration is not taken into account. For workers, a thorough assessment of suitability and performance check was carried out. According to the results, the models' basic concepts are suitable, but there are issues related to the variable categories (e.g. dustiness) that should be refined and adapted to NM, to exposure metrics that probably are not always optimal. Finally, there is no calibration for NM. Models were applied in few exposure scenarios using real conditions, and the results were compared to experimental exposure data measured in the same conditions to check the model performance. The

final result was that for inhalation no correlation could be observed between model outcome and particle number concentration, suggesting that model resolution was not fully exploited due to not suitable dataset, and that mass concentration as exposure metric is probably not optimal. A work is going on to fill these gaps and to develop a nano-specific model for inhalation in occupational settings. These models can be used as screening tool for risk prioritization and control banding for risk management in work places. In order to have a quantitative exposure models, there is the need to pool future exposure data, collected according to standardized methods and using common and suitable metrics.

5.2. Abstracts

Sources and Release: Exposure to nanofilm sprays

Jensen K.A.[1], Nørgaard A.[1], Larsen S.T.[1], Nielsen G.D.[1], Wolkoff P.[1]

[1]*National Research Centre for the Working Environment, Copenhagen, Denmark*

Nanofilm sprays are popular products, which are designed to induce non-stick and/or "self-cleaning" properties on different surfaces such as bathroom tiles, floors, textiles and windows. Photocatalytic properties may also be added using e.g. nanocrystalline anatase (TiO_2).

When applied, the product is sprayed onto a surface, and a thin solid film is formed, during evaporation of the solvent, involving condensation reactions between organo-functionalized silanes and in some cases engineered nano particles (ENP). These processes may also occur in aerosols potentially released during application potentially resulting in inhalation exposure to both reactive surface-functional chemicals and ENP.

Four different nanofilm products (NFPs) were tested: NFP1 coating for non-absorbing floor materials, NFP2: coating of ceramic tiles, NFP3: window coating with photo catalytic titanium dioxide and NFP4 a multipurpose coating product. NFP1, 2 and 3 are delivered in hand pump spray bottles and NFP4 in a pressurized can.

The results showed that, independent of presence ENP or not in the formulation, use of all products resulted in particle release [1]. The particle phase were dominated by nano-size particles and ranged up to ca. 3 to 7 µm depending in the product. The particles may be a mixture of solid particles, condensed semi-volatile compounds, and gas-phase reaction products, depending on the product. Presence of solid particles was verified by TEM. Formation of gas-phase reaction products causing a nucleation event was specifically evident after use of the NFP4, which contained numerous VOCs including chlorinated acetones, limonene, and a perfluorinated silane.

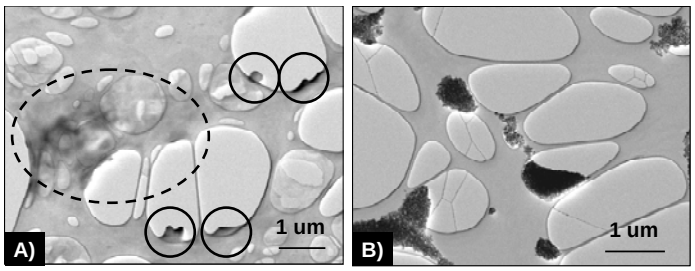

Figure 1. TEM-image of dried "coarse" spray drops collected onto a holey carbon TEM Cu-grid. A) Si-rich condensates (encircled) in spray from NFP1. B) Patches of anatase nanocrystals in spray from NFP2.

NFP1 and NFP2 were subsequently tested for potential toxicological effects. NFP1 showed acute lung toxicity (atelectasis, emphysema, and hemorrhages) in mice between 16 and 18 mg/m^3 due to a hydrolysed perfluorosilane [2]. Due to estimated exposure levels in small poorly ventilated rooms, the Danish EPA found the user risk critical and requested NFP1 removed from the market due to erroneous hazard labeling.

References

[1] Nørgaard et al. Environmental Science and Technology 43(20), 7824-7830 (2009) ; [2] Nørgaard et al. Toxicological Sciences 116(1), 216–224 (2010)

Application and adaptation of exposure models

Brouwer D.[1], van Tongeren M.[2], Jensen K.A.[3]

[1]TNO, Zeist,The Netherlands; [2]IOM, Edinburgh, UK; [3]NRCWE, Copenhagen, Denmark

Models to predict either inhalation or dermal exposure of workers and consumers to chemical substances have been developed and are currently used for (initial) risk evaluation purposes, e.g. REACH. For inhalation most exposure models are based on a source- receptor approach, distinguishing emission, transport, immission, and personal exposure. The applicability for estimating exposure to nanoparticles has been evaluated both conceptually (Schneider et al., 2011) and empirically by comparison of model outputs with actual data collected in workplace measurement campaigns as part of the NANEX project.

Conceptually the models should be appropriate; however, modules that address the potential for coagulation, and adjust for deposition should be included. Further, it was concluded that the model variables, or factors that modify the transport of an aerosol from a source to the receptor, should be refined. In addition, the model output should be able to estimate other relevant exposure parameters and exposure metric. The latter may be illustrated by lack of correlation between model outputs (mass concentration) and actual data (particle-number concentration) collected in workplace measurement campaigns. In addition to the challenges in correlation between e.g. mass and number concentrations, lack of correlation will also be due to the variability of the exposure data combined with the relatively narrow range in possible model exposure estimates. It was concluded that refinement of parameter categories is needed, in view of typical activities for nanomaterial handling, amount of handling categories, time-resolution etc.

Currently, the first refinements of models have been developed and are introduced in risk banding tools, e.g. Stoffenmanager Nano® (NL) and NanoSafer® (DK). Consequently, the exposure bands part of the latter models provide a more comprehensive exposure assessment compared to other Control Banding tools where only the potential for emission is used as a proxy for exposure.

6. ECOTOXICITY AND ENVIRONMENTAL FATE

6.1. Summary record

The objectives of the ecotoxicology session were to discuss the links between ecological risk assessment and regulatory issues and needs, and to present an update of the research in aquatic and terrestrial toxicity and environmental fate and behaviour of nanomaterials. In addition, communication with a wide range of stakeholders was an important goal of the session as for the whole workshop. The first part of the session included two presentations about methodological issues and concepts, which was followed by a dedicated podium discussion to allow a focused dialogue on these topics. The second part of the session included four presentations about environmental fate ecotoxicological research, also followed by a general discussion. This summary reports the main message from the presentations and the discussion.

Given the wide use of nanomaterials and nanotechnologies, and the increasing importance of this field in the economy, there is a need to act to regulate nanotechnologies and to assure the safety of nanoproducts. In the area of environmental protection, there are real challenges related to lack of information, uncertainties, and complexity of ecological systems. In order to achieve the management of risk and address any issues in regards to public perception, each stakeholder group should deal with its own challenges. Regulators have to establish a regulatory strategy, and indicate if and how nano-specific regulations are needed; Industry on the other side, should keep in mind that as a general rule, products placed on the market have to be safe and they should collect data to demonstrate that; finally, the research area should be very clear about actual and future knowledge requirements, and where uncertainties may not be reduced. The best way forward to achieve the goals of sustainable nanotechnology is to do a tiered, integrated assessment, i.e. by including socio-economic issues besides hazard and exposure assessment, using different tools at different stages of the product life cycle (e.g. from conceptualization to market placement, and finally to the end of the product's life), and expressing risk as magnitude and probability. In particular, it was stated in this session that the use of NOEC is not appropriate for risk assessment of nanomaterials, and that the use of non-linear dose-response curves as suggested by Environment Canada would allow the use of models which would include all relevant available data and thus the estimation of probability of risk. This would allow assessment of different scenarios and the effectiveness of risk management measures. In a tiered integrated assessment framework, integrating testing strategies are essential to identify the best ecotoxicological tests for each assessment tier. Standardized schemes are already available, however modifications to deal with nanomaterials peculiarities are deemed necessary. In addition, given the large diversity of nanomaterials, it is impossible to consider a case-by-case assessment in the long term. A revised scheme should allow grouping of materials with comparable properties, including additional ecotoxicity endpoints relevant for nanomaterials and for all the environmental compartments (e.g. soil, water, sediments). Grouping of nanomaterials allows a reduced testing effort. Only a specific number of materials belonging to a derived specific group, will be studied in detail to provide comprehensive information on properties and effects of the group. Some modifications of the existing OECD guidelines have already been proposed concerning e.g. sample preparation, exposure conditions, and effects measurement techniques. During the discussion links between available data and regulators needs were addressed. In particular, it was stated that no effect data are important and therefore should be published, since toxicity of some nanomaterials could be overstated; it was also highlighted that the actual dataset is skewed toward sensitive organisms. However, evidence indicates that the range of concentrations tested in ecotoxicological tests reported in the literature, even if perceived high and unrealistic, are not far from the actual concentrations found for example in sewage treatment plants and possible in landfill

areas. The final remark was that there are enough data to allow regulators to start the regulatory process of nanomaterials in the environment.

The second part of the sessions included focused presentations on environmental behaviour in water, and assessment of ecotoxicological effects of nanomaterials in water and soil. The first issue to be considered was exposure. ENM entering wastewater will end up in the treatment plants. Since the main pollutants removal process is happening due to particles settling, it is expected that ENM will end up mainly in sewage sludge. In fact, experimental measures show that a large fraction (around 80%) of colloidal Ti will end up in sludge, and it is safe to assume that some of it is in nanoform. Therefore, ENM contamination is a soil issue, and soil should be considered a primary target for ecotoxicological studies. Once in the environment, engineered nanomaterials (ENM) undergo a degradation process. To study degradation, it is important to focus on ENM forms that are used in commercial products and thus most likely to be released into the environment. Degradation (or ageing) of nanomaterials is an important process which modifies the physical-chemical properties of ENM and thus their potential behaviour and toxicity. Even if powder released from polymers is in the nano-range, it has been demonstrated that ENM are part of the matrix and not free ENM. However, a study carried out on SiO_2 in composites showed that powder containing nano SiO_2 was more toxic toward Escherichia coli than powder from composites without nano. Another study concerned nano TiO_2 in sunscreens. The particles have a TiO_2 core, and a shell composed by two layers, the internal one consisted of Al-OOH which was used to block ROS production, and the external one aimed to improve dispersion of particles in the matrix. The study revealed that particles in water undergo a complete degradation of the outer shell layer in a very short time (48h), making particles hydrophilic since the inner shell layer was still in place. In addition, in toxicity tests carried out so far no toxicity to Daphnia of these ENM by-products was observed. However, long term studies are needed in more complex systems (e.g. mesocosms) to assess any further degradation, behaviour and toxicity of nanoTiO_2 by-products. It is especially important to study degradation of ENM at different pH conditions, such as in mammalian GI tract: different degradation stages or partial degradation of the shell may lead to different levels of toxicity caused by the core of the ENM. Both pristine and degraded by-products of ENM once in the environment can exert toxic effects on soil and aquatic species.

Earthworms are representative species of soil, and it was shown that they are a good indicator of the level of pollution of soils. Results of ZnO nanoparticles toxicity and accumulation into earthworms, compared to soluble Zn, were presented, discussing especially the relevance of soil conditions for the test results. It was stated that the way used to mix particles to soil does not cause significant changes in ENM uptake. It was also highlighted that standard conditions used in soil tests are not so common in nature, where the variability is very high. For example, taking into account only the pH, while standard pH is around 6, the natural soils pH range is from 3 to 9. Therefore, it is important to test a range of soils in ecotoxicological assays, since pH may influence the stability of nanomaterials, as well as degradation (and as such would also affect ENM bioavailability), and their toxicity. It was also demonstrated that nano ZnO can be internalized in tissues by worms via different routes (passive diffusion, ingestion, air). The clearance kinetic should be further investigated, especially for essential metals in nano form.

Toxicity of ENM to fish was also presented, in relation to characterization, exposure route, absorption, and effect type (e.g. nano-effect or not). The main issues concerned the effect of different ENM, namely Carbon Nano Tubes (CNT) and TiO_2, to fish exposed through water. ENM tend to be deposited on gills causing occlusion and respiratory distress. The question was if there is a real nano-effect, or if the observed effects are linked only or mainly to respiratory distress. The second hypothesis should be verified, since the lack of evidence of Ti uptake (and thus no ENM absorption) by fish from water, and the absence of observed effects via dietary exposure suggest that there is an

effect linked to gill occlusion. This kind of effects should be compared to similar effects of aluminium and humic acid. At the same time, potential change in size and charge should be studied to assess any toxicity effects. In the case of metal ENM, it is relevant to consider the possibility of ion uptake through gills facilitated by the nano form association with the gills. Another aspect to be evaluated is the effect of ENM on the microbial community in the gut, and related systemic effects. ENM can also modify the bioavailability of co-contaminants, both in water and through diet. It seems from results for C60 and β ethynylestradiol, that bioavailability is reduced in zebrafish and shrimps.

Finally, a really important topic not sufficiently addressed in the literature is the potential biomagnification of ENM through food chains. According to the data presented, ENM can bioaccumulate in ecologically relevant receptors, and biomagnify under certain conditions. Data show that gold ENM can accumulate easily if taken up via food than via direct exposure. There are some indicators that can be used to identify the distribution of metal nanoparticles in the organism's body, such as for example metallothionein expression. It was used in nematodes to identify the distribution of ZnCl2 and nano ZnO, with the latter located only in some specific places. Collectively, these data indicate that to ascertain the potential risk, studies in more environmentally relevant conditions should be performed.

6.2. Abstracts

Challenges in managing ecological risks posed by nanomaterials: the balance between uncertainties and certitudes.

Kapustka L. (Larry) A.[1]

[1]*SLR Consulting (Canada) Ltd., 134-12143 40th Street SE, Calgary, Alberta T2Z 4E6, Canada*

Though considerable knowledge about the behaviour and effects of nanomaterials in environmental media has been gained in the past several years there remain more questions than answers. Yet, as the technology moves closer to widespread commercialization of products, regulation will have to proceed with significant knowledge gaps. The framework for ecological risk assessment provides a useful approach to sort through relevant information as we address the concerns of stakeholders. We can anticipate that without clear efforts to characterize the safeness and the risks of various nanomaterials the likelihood for a societal backlash to the whole industry is significant (Figure 1). In this presentation, I will summarize lessons learned in the assessment and management of risks of other novel substances as well as case studies of a select number of nanomaterials. Emphasis will be placed on what we know (certitudes) that can help us work through the many uncertainties about nanomaterials in various environments.

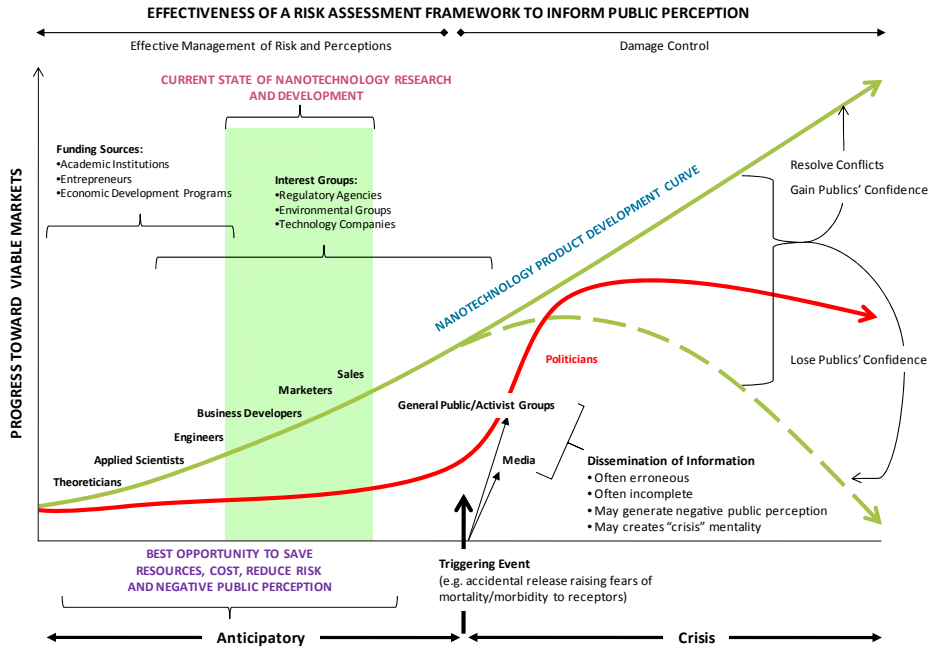

Figure 1. Stages in the continuum from concept to commercialization (from Kapustka et al. 2009).

Kapustka L, Chan-Remillard S, Goudey S. 2009. Developing an Ecological Risk Framework to Assess Environmental Safety of Nanoscale Products. pp 149-159 In Linkov I, Steevens J, (Eds.) *Nanotechnology: Risks and Benefits*. Springer, The Netherlands. 483 pp.

Integrating test strategy for ecotoxicity assessment

Hund-Rinke K.[1], Klein C.[2], Scott-Fordsman J.[3], Fernandes T.[4]

[1]Fraunhofer Institute for Molecular Biology and Applied Ecology, Schmallenberg, Germany; [2]Nanotechnology; Institute for Health and Consumer Protection; Joint Research Centre, Ispra, Italy; [3]National Environmental Research Institute of Denmark, Silkeborg, Denmark, [4]Heriot-Watt University, Edinburgh, Scotland, UK

For the adequate assessment of the environmental impact of nanomaterials (NM) a specific test strategy (ITS) is required. In the context of REACh an ITS for NM should consider the procedure established for conventional chemicals expanded to address the specific peculiarities of NM. Furthermore, a procedure comparable to the assessment of human hazards is desirable. Based on these specifications, the approach highlighted in Figure 1 is proposed.

Initially a comprehensive characterisation of the NM is performed. Then similarly to the standardized procedure established for conventional chemicals, toxicity and accumulation will be determined based on standardized laboratory test systems (Step 1a). A verification of the results considering realistic environmental exposure scenarios may be necessary (Step 2). The information acquired through the use of standardized test systems (e.g. according to OECD test guidelines), is mostly appropriate, although it is limited in what concerns mode of action. This information (Step 1c) is, however, required for grouping of NM as prerequisite for a reduced, but effective, testing effort which would lead to a practical assessment of the hazard of those NM. Given that NM can cover a wide range, such as size, surface modification, crystalline structure and others, it is important that groups can be defined. A group shall comprise NM with comparable behaviour and effects in the environment, as defined by the test strategy. It is proposed that a specific number of materials belonging to a derived specific group, will be studied in detail to provide comprehensive information on properties and effects of the group. For further materials, confirmed to be affiliated to the same group, only key parameters, such as the most sensitive endpoint have to be addressed (Step 1b).

A variety of methods and approaches concerning the assessment of unspecified toxicity and accumulation of NMs is available with others also now being developed. However, suitable test systems beyond standardized testing (Step 1b, Step 1c) still need to be defined. The same applies for the criteria (threshold values e.g. PEC/PNEC approach) to move from Step 1 testing (tier 1) to Step 2 testing (tier 2).

This presentation will discuss this proposed approach and will provide examples on how such methodology could be implemented.

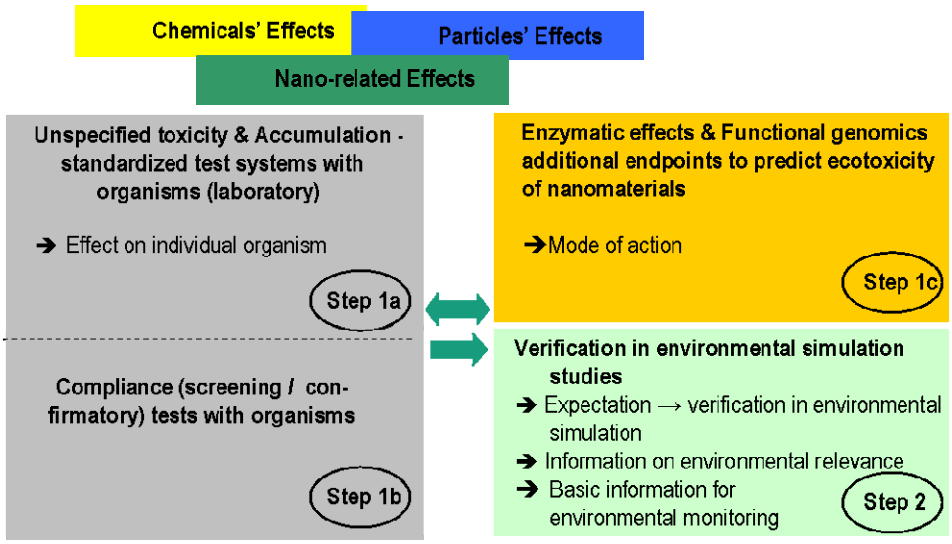

Figure 1: Proposal for a scheme concerning the assessment of nanomaterials

Ageing and Toxicity of real NM in aquatic environment

Rose J.[1,2], Auffan M.[1,2], Labille J.[1,2], Botta C.[1], Chaurand P.[1,2], Borschneck D.[1,2], Masion A.[1,2], Garric J.[3], Fouqueray M.[3], Vollat B.[3], Noury P.[3], Abbaci K.[3], Pielichowskiaze K.[4], Leszczynska A.[4], Michalowski S.[4], Njuguna J.[5], Sachse S.[5], Bottero J-Y.[1,2]

[1]CEREGE, UMR 6635 CNRS/Aix-Marseille Université, F-13545 Aix en Provence FRANCE; [2]iCEINT, international Consortium for the Environmental Implications of Nanotechnology, F-13545 Aix en Pce-FRANCE; [3]CEMAGREF Lyon UR MALY, Ecotoxicologie, F-69336 Lyon France; [4]Cracow University of Technology, Krakow, Poland; [5]Cranfield University , Bedfordshire MK43 0AL, United Kingdom

More than 1000 nanotechnology-based materials and products are on the market (www.nanotechproject.org) proving that these products have moved well beyond the laboratory. Indeed due to their unique size dependant physical-chemical properties (Auffan et al. 2009), nanoparticles (NP) are incorporated in a large number of nanomaterials (NM) and nanoproducts. The dedicated applications go from clothing, personal care products, cosmetics, pharmaceutics, and to automotive among others. Many types of NP can be added to materials (for instance TiO_2, SiO_2, Ag, Fe_20_3, Carbon nanotubes etc …). However the potential impact on the environment and human health of "nanomaterials" and "nanoproducts" is not yet fully well understood. Indeed even if there are evidences that bare NP can be toxic *in vitro* and *in vivo* most of bare NP are surface modified before being incorporated into products. So what about their toxic effects when surface modified? More over in most cases NP will not enter the environment in their pristine form but rather incorporated into matrix as by-products released during their life cycle.

In the present talk we will focus on two types of nanomaterials i) nano-TiO_2 incorporated as UV filter in sunscreens ii) nano-SiO_2 based composite (polymeric matrixes including polyamides, polypropylenes and polyurethanes as bulk materials). The objective of the work is to assess their mechanisms of degradation through their life cycle (aging experiment, crash tests, …) and to characterize the nature, the structure and the physical-chemical properties of the formed Residues (Auffan et al., 2010; Labille et al., 2010; Botta et al., 2011). Then from this description, the second objective is to determine the behaviour of these residues of degradation with a set of biological targets (micro-organisms, algae, invertebrates, fish, …) via different exposures and transfer by the water and the food. More importantly the aim is then to compare biological effects with pristine NP.

Note: Experiments were supported by the French national programs NANOALTER (INSU/EC2CO/CYTRIX), AGING NANO & TROPH (ANR-08-CESA-001) and the FP7 NEPHH project (CP-FP 228536-2).

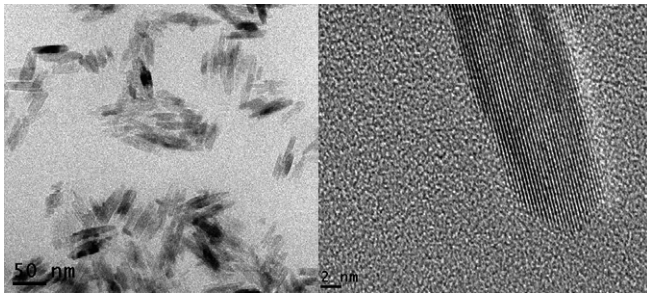

Figure 1. TEM images of nano-TiO_2 incorporated in commercial sunscreen (from Botta et al, 2011)

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Labille, J., J. H. Feng, et al. (2010). "Aging of TiO_2 nanocomposites used in sunscreen. Dispersion and fate of the degradation products in aqueous environment." Environmental Pollution **158**(12): 3482-3489.

Comparing the reproductive toxicity of ZnO nanoparticles, bulk ZnO, and $ZnCl_2$ to the earthworm *Eisenia Andrei*

Svendsen C.[1], **Spurgeon D.**[1]

[1]*Centre for Ecology and Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire OX10 8BB, UK*

The field of nanotechnology is evolving rapidly and the development is largely driven by the great possibilities for industries to improve the properties of their consumer products using nanoparticles (NPs). Fate modelling studies conducted for TiO_2, that have considered NPs release from sunscreens, has highlighted that the majority of NP releases to wastewater are likely to end up closely associated with the activated sludge during sewage treatment. Since much of this sludge is directly deposited to land in many countries, it is likely that a large volume of post production NPs will end up in soil.

With NP increasingly likely to be reaching the soil compartment, there is a potential for toxic effects on the soil and soil surface fauna and primary producers. To date studies of metal oxide nanoparticles in soil systems have focussed almost exclusively of first generation materials produced for use in consumer production. From the studies conducted with metal and metal oxide NPs, it has so far largely been seen that observed effects are likely to be associated with metal ion release during dissolution. Indeed our own studies conducted using a range of ZnO NP have confirmed that the prevalence of free ion, rather than NP associated, toxicity as the principal determinant of toxic effect. While initial evidence points to the importance of ionic effects, studies with soil organisms have to date been conducted only using standard artificial media rather than natural soils. This means there is uncertainty about how effect observed in standard tests relate to toxicity expected in natural soils in which key soil properties (pH, cation status, organic carbon content) can vary greatly. Studies to investigate such effect are a focus for current research.

While toxicity may be driven by free metal toxicity, studies conducted with soils species have shown the direct uptake of gold, copper and ZnO NPs. To understand the potential toxicity associated with metal effects, using both classic model species such as the soil dwelling nematode *Caenorhabditis elegans* and other ecological relevant taxa, analysis of expression change for pathways with a potential role in toxicosis (e.g. metal binding proteins, ROS defence) as well as identification of new pathways associated with toxicity are already proving informative.

The bioavailability and trophic transfer of manufactured nanomaterials in aquatic and terrestrial food webs: Research challenges and potential risks

Judy J.[1], Unrine J.[1], Tsyusko O.[1], Shoults-Wilson A.[1], **Bertsch P.M.**[1]

[1]*Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY USA 40502.*

Despite the benefits that are currently being manifested and those transformative breakthroughs that will undoubtedly result from advances in nanotechnology, concerns surrounding the potential negative impacts to the environment and human health and welfare continue to emerge. Information on the transport and fate of manufactured nanomaterials (MNMs) in the environment and on their potential effects to human and ecological receptors is emerging at an increasing rate. Notwithstanding these developments, the research enterprise focused on the environmental implications of nanotechnology is in its infancy and few unifying principles have yet to emerge. This lack of unanimity is related to many factors including, the vast diversity in chemical composition, size, shape, and surface chemical properties of MNMs, as well as the range of receptor species and cell lines investigated. Additionally, the large variation in exposure methodologies employed by various investigators as well as the discrepancies in the amount and quality of characterization data collected to support specific conclusions, provide major challenges for developing unifying concepts and principles. As the utilization of MNMs for a large variety of applications is currently in an exponential growth phase, there is great urgency to develop information that can be used to identify priority areas for assessing risks to humans and the environment, as well as in developing potential mitigation strategies.

There is a limited, albeit an increasing, number of studies that have examined the transfer of MNMs from one trophic level to the next, a critical measure of potential long-term ecological impact as well as a possible pathway for human exposure. These studies have ranged from relatively simple aquatic food chains under very controlled exposure conditions to more complex terrestrial food chains, to very complex mesocosm-based estuarine food webs. We have compiled/calculated bioaccumulation factors (BAFs), defined as the concentration in the tissues of a consumer divided by the concentration in the consumed media/prey from most of the available studies. In terrestrial food chains, values range from 0.006 for SWNT (soil→ earthworm) to 11.6 for Au NPs (plant → caterpillar), while in aquatic food chains they range from 0.009 for TiO_2 (Daphnia → zebra fish) to 5.37 for CdSe (bacteria →protozoa). Thus, It is clear that the propensity for MNMs to be transferred from one trophic level to the next is MNM and food chain specific. However, the evidence for biomagnification in diverse aquatic and terrestrial food chains for quite different MNMs, suggests that more work needs to be focused on the properties/factors (MNM and organism/food chain specific) controlling this phenomenon, due to potential risks to eco-receptors and human exposure associated with the transfer and biomagnification of MNMs in food chains.

Aquatic Toxicity in Fish: Chronic Studies, Sub-lethal Effects

Henry T.B.[1,2]

[1]School of Biomedical and Biological Sciences, University of Plymouth, Plymouth, United Kingdom; [2]The University of Tennessee Department of Forestry, Wildlife and Fisheries and Center for Environmental Biotechnology, Knoxville, Tennessee, USA

The development of nanotechnology and industrial production of nanoparticles (NPs) offers considerable benefits in many sectors of society; however, negative effects of NPs in the environment after release are important to consider and have become a priority in environmental science. The toxicity of the first manufactured NP, the C_{60} fullerene, has been investigated from various persepctives in fish and serves as an example of the challenges of evaluating toxicity of nanoparticles. Early reports of C_{60}-induced toxicity in fish have been revised and highlight the issue of vehicle solvents and decomposition products that can lead to erroneous reports of NP toxicity. As methodologies for testing toxicity of NPs are improved, a better picture of their toxicity has emerged; however, early reports of NP toxicity based on improper methods continue to contribute to a mis-understanding of NP toxicity in some cases. A prominent question within fish NP toxicity is whether absorption of NPs occurs across epithelial membranes. Evidence indicates that for some NPs absorption is either very low or not occurring, while some types of NPs may have greater potential to be absorbed. If NPs are not absorbed then issues of tissue distribution, metabolism, and excretion are not relevant; and concerns of NP bioaccumulation and biomagnification become less relevant. However, even if NPs are not absorbed there can be effects mediated at epitheilial surfaces and evidence from tests on various NPs in the aqueous phase indicate that occlusion of fish gill surfaces by NPs (or aggregates) can occur and lead to toxic effects. Respiratory effects of NPs in fish could be important ecotoxicologically, and it is possible that these effects are enhanced by the nano characteristics (i.e., a “nano effect”) of NPs. The accumulation of NPs on gills may also facilitate exposure to toxic ions from metal NPs (e.g., Ag-NPs) and the release of metal NPs may enhance the exposure of fish to toxic metals. The unique nature of NPs has led to the need for innovative assays to evaluate their toxicity and formulation of standardized ecotoxicity tests for NPs are still under development. Unique approaches for assessing toxicity of NPs will also be discussed and how they can contribute to understanding the toxicity of NPs in fish.

7. HUMAN TOXICITY

7.1. Summary record

The human toxicity session included presentations on biokinetics, genotoxicity, acute toxicity and other toxicity endpoints relevant for the human health risk assessment.

The toxicology session was introduced by presenting the results of a risk assessment appraisal performed with four kinds of manufactured nanomaterials under the ENRHES (i.e. Engineered Nanoparticles: Review of Health and Environmental Safety) project. The methodology to derive limit values following the REACH guidance (i.e. DNELs = derived no-effect levels) was compared to other methodologies for deriving limit values recently published. It was shown that depending on the methodology there can be big differences. Further, great variation exists in occupational exposure values reported in the literature. Consequently, depending on the methodology and exposure values considered, different conclusions on the presence or absence of a risk are reached. During the discussion it was highlighted that for the hazard and risk assessment, consideration of genotoxic effects of nanomaterials and whether these are threshold or non-threshold is crucial. Based on the available data there are however no generalisation possible across nanomaterials and each evaluation has to be made on a case by case basis.

During the consequent discussion and a later presentation on biokinetics it was highlighted that the exposure duration is an important factor for assessing the risk, as it is difficult to draw conclusions from either the absence of or relatively low toxicity observed under subchronic - chronic exposure conditions. For example, carbon nanotubes can be inhaled and deposited in the lungs as bundles, which may not be a stable form of the CNT, and some individual CNT can be released over time. Therefore adverse effects would only elucidate over chronic exposure, as it may be also expected for rather inert nanomaterials with no/low short term toxicity but possible adverse effects under chronic conditions due to accumulation. In addition, the possibility of translocation should always be considered. The dedicated presentation later during that session highlighted the importance of considerations of the ENM accumulation in secondary target organs following chronic exposure. Following pulmonary exposure, nanomaterials (20 nm TiO_2, gold, iridium and carbon) will predominantly be retained in the lung, but approximately between 1 and 10% can slowly be translocated across the air-blood-barrier. There are significant differences in translocation between different sizes and materials in relation to distribution to secondary organs, such as liver, spleen, kidneys, heart and brain, but also skeleton and soft tissues. The distribution pattern differs between intravenous injection and pulmonary exposure, however one study indicated that the biodistribution of inhaled and intratracheally instilled nanoparticles (20 nm gold) was similar, suggesting that intratracheal instillation studies could for many purposes replace the costly inhalation studies. This may however need further validation. Stable radiolabelling plays an important role in the detection of low nanomaterial concentrations in different organs, and it allows ENM detection over 5 orders of magnitude (important for chronic exposure). Better understanding of how size and composition affects absorption is needed, and based on that it might be possible to build models for estimating the translocation. Further work is also needed to understand the absorption of soluble nanomaterials such as nano-silver.

The next speaker presented the results of genotoxicity studies (Micronuclei and Alkaline Comet assay) on silica ENM, which had to be adapted to avoid interference of nanoparticles with the test result (e.g. interference with serum). The speaker concluded that the alkaline comet assay and the micronucleus (MN) assay with adaptations are suitable tests to study *in vitro* genotoxicity of insoluble ENM. The MN assay was also proposed as pre-validation study to be used for hazard assessment. The need of adaptation, however, implies that one has to be careful with the interpretation of published

genotoxicity data, since the experimental conditions might have been not optimal for ENM uptake by cells. Moreover, the selection of the dose metric is fundamental, since no effect was observed by using mass, but a statistical difference with the control was clear if measured by using particle number or surface area, and a dose-response curve was obtained. It was observed that ENM entered the cells as single particles, and that the mode of action of insoluble silica nanoparticles was due to interference with microtubules in the absence of ROS induction and without crossing the nuclear membrane. The fact that absence of serum increased the cytotoxic and genotoxic effects could be an indication that under physiological conditions in a body the protein corona would have a protective effect.

Within the ENPRA project, *in vitro* and *in vivo* models are developed to investigate dose-response relationships for pulmonary, hepatic, renal, cardio-vascular (including blood) and developmental endpoints. The result should provide further information on the relationship between physicochemical characteristics and toxicity, on the predictive value of *in vitro* tests, on the biological mechanisms of NM toxicity and on the sensitivity of target organs. Further they should allow establishing dose-response relationships for a number of endpoints and target organs and to derive benchmark doses to be used (as alternative to NOAEL) in the risk assessment process. The first results of studies carried out with a panel of selected ENPRA nanomaterials were presented. The studies were focusing on both, acute toxic effects *in vivo* following instillation of NM into the mouse lung, and *in vitro* testing in a human hepatic cell line. The investigated *in vivo* endpoints included: lung inflammation and cell damage and systemic toxicity (inflammation, effects on bodyweight), while *in vitro* endpoints included cytotoxicity, pro-inflammatory mediators (IL8, TNF-α, il6 CRP), ROS and GSH depletion and cell function. ZnO was identified as the most toxic particles for both the *in vivo* and *in vitro* studies. In *in vivo* studies, TiO_2 NP and nano-Ag were both not acutely toxic at the tested dose ranges (0-128 µg/mouse), while MWCNTs showed some lung inflammation at the highest dose (128 µg/mouse). The nano-TiO_2 testing was more problematic, because the nano-TiO_2 chosen as a positive reference at the beginning of the experiments was not toxic at all, and thus it was not a good choice. In general, to fully interpret the toxicity data there is the need to measure the physical-chemical properties in the exposure media.

The *in vitro* investigations in liver cells showed that nano-Ag and nano-ZnO were constantly more toxic than MWCNT and nano-TiO_2 with respect to cytotoxicity and cytokine production, as the latter ones only induced cytokine production at very high exposure concentrations which was associated with oxidative stress. The total levels of the antioxidant glutathione GSH decreased dose-dependently in all tested nanoparticles with greater effects for nano-Ag and nano-ZnO, confirming the production of oxidative stress. The need to investigate effects at sub-lethal concentrations was raised as it has been shown that cytokine levels were decreasing when cells were dying and this may lead to an underestimation of effects. The pre-treatment of cells with an antioxidant resulted in general in decreased particle induced cytotoxicity and decreased IL8 secretion. Nano-TiO_2 was able to induce DNA-damage at sub-lethal concentrations as shown by comet assays. Transmission electron microscopy pictures identified all particles in compartments within hepatocytes in vitro, however only one type (MWCNT) was found within the nucleus. The ENPRA project is further analysing the data with an aim to try understanding why nano-Ag appears very toxic *in vitro* but low toxic in the applied *in vivo* system.

Overall, from all presentations in that session it was concluded that it is of greatest importance to correlate all results from the different studies to all other data, that is currently generated, e.g. under ENPRA but also under the OECD test program, to gain most benefit out of the studies and to improve the overall knowledge about nanomaterials toxicity and the best ways to test it.

7.2. Abstracts

Challenges in nanomaterials human health risk assessment

Aschberger K.[1], Burello E.[1], Christensen F.M.[1]

[1]*European Commission, Joint Research Centre, Institute for Health and Consumer Protection, Ispra, Italy*

Nanomaterials risk assessment is still hampered by lack of data for both, hazard and exposure assessment. Moreover, current technical guidance documents for preparing risk assessments have only limited focus on substances in particulate and/or in nanoform.

Within the ENRHES project and follow up investigations, we have prepared basic human health risk assessment appraisals for 4 types of nanomaterials: carbon nanotubes, fullerenes, nano-silver and nano-titanium dioxide. These risk assessment appraisals followed the structure of a regulatory risk assessment and, where possible, we attempted to derive indicative human no-effect levels from key studies by applying assessment factors as suggested in the REACH guidance on "Information Requirements and Chemical Safety Assessment". Consequently we compared these values to available exposure values. Our investigations showed that based on current information, occupational inhalation risks cannot be excluded, but no generalisations on risks of nanomaterials are possible. It is thus recommended to perform the risk assessment on a case-by-case basis.

The results from our approach to derive indicative human no-effect levels were compared to other approaches that have recently been published. The main divergence was observed when evaluating the differences between animal and human exposure situations (including duration), when accounting for interspecies (rat to human) differences and in applying assessment factors for intraspecies differences for local effects. The REACH guidance gives preference to chemical specific assessment factors over default assessment factors if data allow and if scientifically justified. Deviations from default assessment factors thus require extensive chemical specific data and a good understanding of the fundamentals of the biological response to the material.

A step forward, moving to grouping of nanomaterials and read across among different nanomaterials and to bulk-materials requires likewise a profound understanding of the properties of nanomaterials and their mechanism of action in biological systems. As a starting point we will show a hypothesis model as an example for a basic approach for using QSARs for predicting nanomaterials mode of action and adverse effects based on their different properties.

Variation in acute toxicity for 9 engineered nanoparticles in murine lungs

Gosens I.[1], Jacobson N.R.[2], Stoeger T.[3], de Jong W.H.[4], Cassee F.R.[1]

[1]Center Environmental Health, RIVM, Bilthoven, The Netherlands; [2]National Research Centre for the Working Environment, Copenhagen, Denmark; [3]Comprehensive Pneumology Center, Institute of Lung Biology and Disease, Helmholtz Zentrum München, Germany; [4]Laboratory for Health Protection Research, RIVM, Bilthoven, The Netherlands

Within the EU project ENPRA (Engineered NanoParticles Risk Assessment), hazard identification for a panel of 9 different engineered nanoparticles is being performed after a single exposure via the airways. Dose-response curves were generated for a number of endpoints and target organs. From these relationships, a benchmark dose (BMD) for each endpoint was derived to assess acute toxicity. The biological endpoints include oxidative stress, inflammation, cytotoxicity, genotoxicity and developmental toxicity. Overlap between these endpoints and cell culture assays representative for target organs should allow in vivo validation of in vitro results generated elsewhere in this project. All nanoparticles were well characterized for physico-chemical parameters. In addition a dispersion protocol has been developed and was essentially the same for in vitro and in vivo studies.

Female C57BL/6 mice were exposed via intratracheal instillation for 24 hours to 0, 4, 8, 16, 32, 64 and 128 µg/mouse with 3 animals per group. Four different types of titanium dioxide (TiO_2), a coated and uncoated Zinc Oxide (ZnO), nanosilver (Ag) and two types of multiwall carbon nanotubes (MWCNT) were used and adverse effects were determined in lung, liver, spleen, kidney and cardiovascular system.

The most toxic particles (per µg) from this panel are both types of ZnO, based on reduced body weight and pulmonary and systemic inflammation. The TiO_2 nanoparticles were not acutely toxic at most endpoints, except for some pulmonary cytotoxicity for a 10 nm rutile type of TiO_2. Nanosilver was not acutely toxic at the dose-range tested here, while MWCNTs showed a trend towards some lung inflammation only at the highest doses. The BMDs will be used for extrapolation of in vitro to in vivo results and serves as an alternative for the NOAEL in the risk assessment process.

Assessing the toxicological impact of a panel of engineered nanomaterials for risk assessment purposes

Kermanizadeh A.[1], Gaiser B.K.[1], Hutchison G.R.[2], **Stone V.**[1]

[1]Heriot Watt University, [2]Edinburgh Napier University, UK

Following exposure via a number of routes (inhalation, instillation or ingestion) some nanomaterials have been shown to translocate to secondary tissues where they could be potentially toxic. The impact of this secondary organ exposure is unknown. One organ identified as a site for accumulation of blood borne particles is the liver. For this reason, this study has focused on liver cells, namely hepatocytes in the first instance. Future studies will investigate the phagocytic cells of the liver, Kupffer cells. As a model for human hepatocytes this study has focused initially on the C3A cell line (human liver cell line derived from a hepatoblastoma). The impact of a panel of engineered nanomaterials consisting of five titanium dioxide (TiO_2), two zinc oxides (ZnO), two multi walled carbon nano-tubes (MWCNT) and one silver (Ag) particles were observed on hepatocyte cytotoxicity, oxidative stress, and cellular function.

The silver particles elicited the greatest levels of toxicity (LC_{50} between 1.25 and 2.5 μg/cm^2) followed by the ZnO uncoated (LC_{50} between 5 and 10 μg/cm^2) and ZnO coated particles (LC_{50} is between 10 and 20 μg/cm^2). The LC_{50} was not reached in the presence of any of the other nanomaterials after exposure for 24 hours at concentrations up to 80 μg/cm^2. The C3A cells produced significantly increased levels of the pro-inflammatory cytokine IL8 following exposure to the Ag and both ZnO nanomaterials, with the protein production peaking around the LC_{50}. Meanwhile there was a dose dependant increase in IL8 levels after exposure to the low toxicity nanomaterials. We did not observe any significant increase or decrease in the levels of TNF-α, IL6 or CRP secreted from the hepatocytes after exposure to any of the nanomaterials investigated. Experiments were conducted to ascertain the potential mechanism driving pro-inflammatory protein production in C3A cells post nanomaterial exposure. Intracellular ROS levels were assessed (DCFH-DA assay) and shown to significantly increase following exposure of the C3A cells to MWCNT and TiO_2 particles but not after exposure to Ag and ZnO particles.

In order to evaluate the ability of ENPRA nanomaterials to induce oxidative stress, measured as a decrease in the antioxidant glutathione (GSH), C3A cells were exposed to the panel of nanomaterials at increasing concentrations. The cellular GSH content was determined using the fluorescent probe *o*-phthaldialdehyde. We found that there was a dose dependant decrease in levels of total GSH across the ten particles. This effect was greatest in the presence of the Ag and the two ZnO NPs, where it was most likely caused by cell death rather than oxidative stress. For the other seven particles the GSH depletion was not associated with cell death and therefore suggests oxidative stress.

Albumin and urea production were measured as markers of cell function. There was no significant increase or decrease in the levels of urea or albumin produced by the C3A cells in the presence of the investigated nanomaterials compared to the control, with the exception of a significant reduction in the levels of albumin produced at LC_{50} concentrations of both ZnO NPs. Furthermore, we aimed to investigate the effects of pre-treatment with an anti-oxidant on cytotoxicity and cytokine production. The cells were pre-treated with Trolox for one hour before being exposed to the four highest concentrations of the nanomaterials used in our previous study. We noted that pre-treatment with Trolox resulted in decreased cytotoxicity across nine of nanomaterials investigated (with exception 7 nm anatase TiO_2). We also observed that Trolox decreased IL8 secretion for all nanomaterials with the exception of Ag.

In conclusion, the *in vitro* hepatocyte model demonstrated that Ag and ZnO NPs were constantly more potent than MWCNT and TiO_2 nanomaterials with respect to cytotoxicity and cytokine production. For the MWCNT and TiO_2 nanomaterials, these particles only induced cytokine production at very high exposure concentrations which was associated with oxidative stress.

Biokinetics of Manufactured Nanomaterials in vivo

Kreyling W.G.[1]

[1]*Helmholtz Center Munich - Research Center for Environmental Health; Comprehensive Pneumology Center - Institute of Lung Biology and Disease and Focus Network NanoHealth, 85764 Neuherberg / Munich, Germany*

The inhalation and deposition of Nanoparticles (NP) in the respiratory tract provides a direct route of intake into the organism by crossing the air-blood-barrier.

To quantitatively determine accumulated NP fractions in such organs the ultimate aim is to balance the NP fractions in all interesting organs and tissues including the remaining body and total excretion. Since these gross determinations of NP contents in organs and tissues do not provide microscopic information on the anatomical and cellular location of nanoparticles such studies are to be complemented by electron microscopy analysis as demonstrated for inhaled titanium dioxide nanoparticles.

After inhalation of various 20 nm NP aerosols (iridium, carbon, titanium dioxide, gold) and based on quantitative biokinetics in rats we found small NP fractions in all secondary organs studied including brain, heart. Fractions per secondary organ were usually below 0.1 % of the administered dose but depended strongly on particle NP parameters. Interestingly NP which had crossed the ABB showed different patterns of organ distribution compared to intravenously injected NP supporting the hypothesis that the formation and the dynamics of protein conjugation mediate the fate of NP in the organism.

Current knowledge on systemic translocation of NP and their accumulation in secondary target organs and tissues of man and animal models does not suggest to cause acute effects of translocated NP but chronic exposure may lead to elevated NP accumulations resulting eventually in adverse health effects; these are likely to be triggered by mediators released in the lungs being the organ of intake.

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I. ANNEX I: LIST OF PARTICIPANTS

Nr	Last Name	First Name	Organisation	Country
1	AITKEN	Robert	Institute of Occupational Medicine	United Kingdom
2	ALESSANDRELLI	Maria	Istituto Superiore di Sanità	Italy
3	ALWOOD	James	Environmental Protection Agency	United States of America
4	ANDERSSON	Yvonne	Swedish Chemicals Agency	Sweden
5	APOSTOLOVA	Margarita	Institute of Molecular Biology, BAS	Bulgaria
6	ASCHBERGER	Karin	European Commission - JRC - IHCP	Italy
7	BERTSCH	Paul	University of Kentucky	United States of America
8	BORN	Roland	Syngenta Crop Protection AG	Switzerland
9	BROUWER	Derk	TNO Quality of Life	Netherlands
10	CARBO	Manuel	Ministry of Environment and Rural and Marine Affairs	Spain
11	CARLANDER	David	European Food Safety Authority (EFSA)	Italy
12	CHARLES	Sandrine	ANSES	France
13	CHEN	Chunying	National Center for Nanoscience and Technology	China
14	CHRISTENSEN	FRANS	European Commission - JRC - IHCP	Italy
15	COSKUN	Erdem	Gazi University of Ankara, Faculty of Pharmacy	Turkey
16	CRITTO	Andrea	University Ca' Foscari of Venice	Italy
17	DOOME	Roger	IMA-Europe / REACH Alliance	Belgium
18	ENGIN	Ayse Basak	Gazi University of Ankara, Faculty of Pharmacy	Turkey
19	ESPOSITO	Dania	Istituto Superiore per la Protezione e Ricerca Ambientale (ISPRA)	Italy
20	FERNANDES	Teresa	Heriot-Watt University of Edinburgh	United Kingdom
21	FOIS	Pierfrancesco	Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers (ETAD)	Switzerland
22	FRANCHINI	Fabio	European Commission - JRC - IHCP	Italy
23	FRIEDRICHS	Steffi	Nanotechnology Industries	Belgium

			Association (NIA)	
24	FURUNO	Shoko	Association of Natural and Organic Cosmetic (NATRUE)	Belgium
25	GARCIA	Patricia	Ministry of Health	Spain
26	GIBSON	Neil	European Commission - JRC - IHCP	Italy
27	GOETZ	Mario	Federal Institute for Risk Assessment	Germany
28	GOSENS	Ilse	RIVM	Netherlands
29	GROZDANOV	Anita	Faculty of Technology and Metallurgy	Former Yugoslav Republic of Macedonia
30	HANKIN	Steven	Institute of Occupational Medicine (IOM)	United Kingdom
31	HASSELLOV	Martin	University of Gothenburg	Sweden
32	HELLMER	Lena	Swedish Chemical Agency	Sweden
33	HENRY	Ted	University of Plymouth	United Kingdom
34	HOLMQVIST	Jenny	CEFIC	Belgium
35	HRISTOZOV	Danail	University Ca' Foscari of Venice	Italy
36	HUND-RINKE	Kerstin	Fraunhofer Institute	Germany
37	IVANKOVIC	Marica	University of Zagreb, Faculty of Chem. Eng.Technol.	Croatia
38	JENSEN	Keld	National Research Centre for the Working Environment (NRCWE)	Denmark
39	KAPUSTKA	Larry	SLR Consulting	Canada
40	KIRSCH-VOLDERS	Micheline	Vrije Universiteit Brussel	Belgium
41	KOBE	Andrej	European Commission - DG Environment	Belgium
42	KREYLING	Wolfgang	Helmholtz Center Munich	Germany
43	LARSEN	Poul Bo	Danish EPA	Denmark
44	LECOQ	Pierre	ANSES	France
45	LEUSCHNER	Cornelia	Ministry of Environment	Germany
46	LIEWALD	Heike	Eurocolour	Germany
47	LYONS	Daniel	Rudjer Boskovic Institute	Croatia
48	MICHELETTI	Christian	European Commission - JRC - IHCP	Italy
49	MLADENOVSKA	Kristina	University Ss Cyril and Methodius of Skopje, Faculty of Pharmacy	Former Yugoslav Republic of Macedonia
50	MOLDOV	Raili	Health Board	Estonia

51	MROZIEWICZ	Kinga	Bureau For Chemical Substances and Preparations	Poland
52	NORTHFLEET	Christina	Omya International AG	Switzerland
53	PALOSAARI	Taina	European Commission - JRC - IHCP	Italy
54	POLAND	Craig	Institute of Occupational Medicine (IOM)	United Kingdom
55	PONCE DEL CASTILLO	Aida	European Trade Union Institute (ETUI)	Belgium
56	POPESCU	Laura Madalina	National Research and Development Institute for Nonferrous and Rare Metals	Romania
57	PROYKOVA	Ana	University of Sofia	Bulgaria
58	QUINN	Bernadette	European Chemicals Agency (ECHA)	Finland
59	RASMUSSEN	Kirsten	European Commission - JRC - IHCP	Italy
60	RAUSCHER	Hubert	European Commission - JRC - IHCP	Italy
61	RIEDL	Alexander	European Commission - JRC - IHCP	Italy
62	RIEGO SINTES	Juan	European Commission - JRC - IHCP	Italy
63	ROSE	Jerome	CNRS CEREGE	France
64	ROSSI	François	European Commission - JRC - IHCP	Italy
65	SCHROEDER	Frauke	BAuA	Germany
66	SIMION	Monica	National Institute for R-D in Microtechnologies	Romania
67	SKAUG	Vidar	National Institute of Occupational Health	Norway
68	SOKULL-KLUETTGEN	Birgit	European Commission - JRC - IHCP	Italy
69	SPURGEON	David	Centre for Ecology and Hydrology	United Kingdom
70	STAMM	Hermann	European Commission - JRC - IHCP	Italy
71	STINTZ	Michael	TU Dresden, Inst. Verfahrenstechnik	Germany
72	STONE	Vicky	Heriot-Watt University	United Kingdom
73	TOUFEKTSIAN	Marie-Claire	Observatory for Micro & NanoTechnologies, OMNT (CEA/CNRS)	France
74	TRAN	Ignace Lang	Institute of Occupational Medicine (IOM)	United Kingdom
75	VAN TONGEREN	Martinus	Institute of Occupational Medicine (IOM)	United Kingdom

76	VOM BROCKE	Jochen	German Federal Institute for Risk Assessment	Germany
77	VAZQUEZ-CAMPOS	Socorro	LEITAT Technological Centre	Spain
78	VEGRO	Stefania	European Commission - JRC - IHCP	Italy
79	WIKSTAD	Emma	Swedish Chemicals Agency	Sweden

II. ANNEX II: WORKSHOP AGENDA

1ST DAY 08:30 – 18:00
OPENING **CHAIR: E. ANKLAM, EUROPEAN COMMISSION (JRC)**
Welcome and Introduction [E. Anklam, JRC]
Objectives and organisation of the workshop [J. Riego-Sintes, JRC]
The ENPRA Project: progress [L. Tran, IOM]
1ST SESSION: REGULATORY DEVELOPMENTS **CHAIR: B. SOKULL-KLÜTTGEN, EUROPEAN COMMISSION (JRC)**
01. Introduction: content and goal of the session
02. EU perspective [A. Kobe, DG ENV]
03. Nanomaterials in REACH registrations and C&L notifications [B. Quinn, ECHA]
04. Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain [D. Carlander, EFSA]
05. Regulation and assessment of nanoscale materials under the Toxic Substances Control Act [J. Alwood, US-EPA]
06. Current progress of activities and efforts on nanomaterials environmental health and safety in China [C. Chen, National Center for Nanoscience and Technology, China]
PODIUM DISCUSSION*
2ND SESSION: REGULATORY IMPLEMENTATION **CHAIR: F. CHRISTENSEN, EUROPEAN COMMISSION (JRC)**
01. Introduction: content and goal of the session
02. OECD Working Party on Manufactured Nanomaterials [K. Rasmussen, JRC]
03. REACH Implementation Project on Nanomaterials - RIP-oN2: information requirements [S. Hankin, IOM]
04. REACH Implementation Project on Nanomaterials - RIP-oN3: specific advice on exposure assessment and hazard/risk characterisation for nanomaterials under REACH [R. Aitken, IOM]

05. NANEX: development of Exposure Scenarios for manufactured nanomaterials [M. Van Tongeren, IOM]
06. Industry experience with conducting nanomaterial safety assessments [S. Friedrichs, NIA]
07. Life Cycle Assessment of nanomaterials in polymer nanocomposites (Nanopolytox approach) [S. Vazquez, LEITAT]
08. Health, Safety, and Environment: assessment methods [D. Hristozov, Università Ca' Foscari di Venezia]
09. The European Repository of nanomaterials and the NANOhub information platform [H. Rauscher, JRC]
PODIUM DISCUSSION*

2ND DAY **09:00 – 18:00**
3RD SESSION: CHARACTERISATION AND DETECTION OF NANOMATERIALS **CHAIR: H. RAUSCHER, EUROPEAN COMMISSION (JRC)**
01. Introduction: content and goal of the session
02. An example of the advantages and disadvantages related to producing a protocol database for nanoscience [I. Gosens, RIVM]
03. Primary and secondary characterization of ENPRA ENP [K.A. Jensen, NRCWE]
04. Characterization and detection in environmental media [M. Hassellov, Göteborgs Universitet]
PODIUM DISCUSSION*
4TH SESSION: EXPOSURE ASSESSMENT **CHAIR: M. VAN TONGEREN (IOM)**
01. Introduction: content and goal of the session
02. Sources and Release: exposure to nanofilm sprays [K.A. Jensen, NRCWE]
03. Application and adaptation of exposure models [D. Brouwer, TNO]
PODIUM DISCUSSION*
5TH SESSION : ECOTOXICITY & ENVIRONMENTAL FATE **CHAIR: T. FERNANDES (HERIOT-WATT UNIVERSITY)**
01. Introduction: content and goal of the session
02. Challenges in managing ecological risks posed by nanomaterials: the balance between uncertainties and certitudes [L. Kapustka, SLR Consulting Ltd, Canada]

03. Integrating testing strategies for ecotoxicity assessment [K. Hund-Rinke, Fraunhofer-IME]
04. Ageing and toxicity of real NM in aquatic environment [J. Rose, CEREGE]
05. Comparing the reproductive toxicity of ZnO nanoparticles, bulk ZnO, and $ZnCl_2$ to the earthworm *Eisenia Andrei* [D. Spurgeon, CEH]
06. The bioavailability and trophic transfer of manufactured nanomaterials in aquatic and terrestrial food webs: Research challenges and potential risks [P. M. Bertsch, University of Kentucky, USA]
07. Aquatic Toxicity in Fish: Chronic Studies, Sub-lethal Effects [T. Henry, University of Plymouth]
PODIUM DISCUSSION*

3RD DAY **09:00 – 14:00**
6TH SESSION: HUMAN TOXICITY **CHAIR: L. TRAN (IOM)**
01. Introduction: content and goal of the session
02. Challenges in nanomaterials human health risk assessment [K. Aschberger, JRC]
03. Exploring the genotoxic potential of engineered nanomaterials [L. Gonzalez, Vrije Universiteit Brussel]
04. Variation in acute toxicity for 9 engineered nanoparticles in murine lungs [I. Gosens, RIVM]
05. Assessing the toxicological impact of a panel of engineered nanomaterials for risk assessment purposes [V. Stone, Heriot-Watt University]
06. Biokinetics of manufactured nanomaterials in vivo [W. Kreyling, Helmholtz Zentrum]
PODIUM DISCUSSION*
CONCLUSIONS
Take-home messages and closing [L. Tran, IOM]

* Podium discussion: guided by chair and speakers, with the active participation of the audience.

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Technical Notes

Abstract
This report informs on the Joint JRC Nano event and 2nd ENPRA Workshop on Challenges of Regulation and Risk Assessment of Nanomaterials held on 10-12 May 2011 in Somma Lombardo, Varese (Italy). The workshop intended to provide an overview and a forum for discussion to regulators, scientists and other stakeholders on recent regulatory and scientific developments in the field of Environmental Health and Safety Assessment of Nanomaterials, in particular from the ENPRA FP7 project. It included sessions on regulatory development and implementation, nanomaterials characterization, exposure, and effects. Summary records of the sessions as well as abstracts of the oral presentations are presented.

The mission of the JRC is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.