Report of the scientific conference:

Science Based Support for Regulation of Manufactured Nanomaterials

Organised by Prosafe and hosted by the OECD

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INTRODUCTION: BACKGROUND AND SCOPE OF THE SCIENTIFIC CONFERENCE

1. The ProSafe project (02/2015-04/2017) is an EU H2020 coordination and support action (CSA) which amongst other things was created to give support to a review of regulatory relevant results from NANoREG, the OECD Working Party of Manufactured Nanomaterials (WPMN), and other relevant projects of the NanoSafety Cluster funded within the EU FP7.

2. The main objective of the 2 years ProSafe project with 11 partners, is to coordinate and support the aims of EU Member and Associated states in their EU and international efforts (OECD, COR, EU-USA) for risk assessment, management and governance by streamlining data acquisition, collection and management on regulatory orientated safety testing of nanomaterials, exposure monitoring, LCA, and disposal and treatment of waste nanomaterials.

3. ProSafe contains a number of specific actions, of which the key action is to develop a White Paper specifically for the EU Member states with recommendations for policymakers and regulators on the measures needed to regulate manufactured nanomaterials and products.

4. The White Paper will be backed up by a technical annex called the Joint Document which comprises a review of selected and relevant results, protocols, and guidance documents, from NANoREG, OECD WPMN activities, and other EU FP7 NanoSafety Cluster funded projects (Annex 1). This review was carried out by a Task Force (TF) of international experts, who analysed data according to relevant themes such as physical-chemical characterisation and identification, human and environmental exposures, fate modelling, health and ecological effects, computational methods as well as risk assessment strategies.

5. As support the objectives of the ProSafe project and of the Joint Document, a major joint conference called *Science based support for regulation of Manufactured Nanomaterials* was jointly organised by the ProSafe project and OECD. The meeting also formed the final conference of the FP7 NANoREG project. It took place on the 29th November - 1st December 2016 at the OECD Headquarters (Conference Centre) in Paris.

6. Main objective of the conference was the discussion of the regulatory relevance of new and State of the Art research and initiatives in the field of nanosafety and to identify the outstanding and future regulatory challenges. This included EU FP7 as well as Horizon 2020 research projects, but also non-EU research and activities related to OECD and ECHA. For this aim scientists, risk assessors, and legal advisors from EU member states and OECD member states and involved in FP7/H2020 projects or OECD WPMN activities met and discussed in groups the regulatory relevant areas of concern, including physicochemical identification and characterisation, exposure, fate and kinetics, ecological and health effects as well as testing and assessment strategies. The findings from the Joint Document on regulatory relevant and reliable data, protocols and guidance from research projects and initiatives regarding nanosafety were used as an input for the discussion. Key Questions which arose from the review of the Task Force were deduced to guide the discussion on regulatory relevance and needs.

7. This report summarises the discussions at the conference and highlights the findings of the experts. It also includes summaries of the presented lectures and a plenary panel discussion. It features a compilation of conclusions and recommendations for regulatory questions regarding assessing risk for human health and environment. This compilation will be considered in subsequent focused workshops for risk assessors and policy makers.

8. The program of the conference is presented in Annex 2. Annex 3 contains a reference list. Word-for-word notes of the group discussions are available on request.

SESSION 1: SUMMARIES OF ALL KEY NOTES AND INVITED TALKS

Chair: P. Kearns, OECD WPMN Secretariat

Welcome Remarks

(N. Van Hulst, Ambassador, Permanent Delegation of the Netherlands to the OECD & A. Wyckoff, OECD Director for Science, Technology & Innovation), Introduction to the Structure of the Conference & Housekeeping Information

9. *Mr Van Hulst* welcomes all participants on behalf of the Dutch Government and the Ministry of I&M to the scientific conference organised by OECD and ProSafe Projects. This is the final conference of NANoReg, in which more than 80 parties from more than 18 countries collaborate in developing methods to test and assess nanomaterials (NMs) from a regulatory point of view. The Dutch Ministry of Infrastructure and Environment coordinates both projects. The objective is to evaluate and discuss the scientific results of 5-10 years of nanosafety research to come to a common understanding and conclusions regarding the best way to assess environmental health and safety effects with a view to provide a science base for developing clear and predictable regulations. This will help regulatory bodies and industry to test and assess and diminish risks and reduce the uncertainty in this field and enhance innovation procedures. The results of the conference will form the basis for setting priorities for further research and harmonisation of test methods. This conference brings together the "crème de la crème" of the nanosafety science community, but it also marks a milestone in collaboration in the nanosafety community. Compared to other fields of science like astronomy or climatology, big data sharing within the nanosafety community is still in its infancy state. Most of the generated data is poorly accessible, but the good news is that things are changing and there is an increasing awareness that collaboration is key to addressing the challenges. NANoREG has proven that it is possible to develop a concerted research effort generating agreements on test methods and quality control to create reliable and comparable data. Open data has been a big priority of the Dutch government during the presidency of the EU last year. The project has demonstrated that there is a willingness to share information and as one of the first EC projects, the NANoREG will make all the data and deliverables available for interested parties. Hopefully this example will be followed by other nanosafety projects.

10. *Mr Wyckoff*, OECD Director for Science, Technology and Innovation, cares passionately about innovation and how it sculpts productivity and standards of living. Scientific technology is fundamental to that. The week before this conference there was a high levelled conference in Sweden, called the next production revolution, a 2-year running project which highlighted the importance of NMs and innovation. A presentation on 'Revolutions need materials!' is offered to the participants. Nanotechnology is a great enabler of new materials, presents essential tools and processes for the circular economy and tools for the quality control of advanced high-throughput manufacturing. NMs are the lacking tissue between the digital revolution on the factory floor and the high precision manufacturing between the material world and the digital twin. The objective of the conference is to solve, enable and harmonise test methods for nanosafety. It will have a direct connecting link to science, technology and innovation and beyond the field of nanotechnology to the next production revolution.

11. The structure and the agenda of the meeting is introduced by *Ms Doris Völker*.

Introduction to the OECD WPMN: Test Guidelines Activities

(P. Kearns, OECD WPMN Secretariat)

12. *Mr Kearns*, responsible for the nanosafety at OECD, chairs the meeting and introduces the nanosafety programme. The test guidelines activities are very much connected with the ProSafe work. Like all OECD bodies, the member countries of OECD are the main participants, but collaboration also takes place with non-members and countries that are involved in the work, as well as with industrial and intergovernmental organisations. Standardised testing guidelines and principles of good laboratory practice together lead to a mutual acceptance of data, which is of great importance when working on NMs. This avoids duplication of testing by industries and non-tariff trade barriers and reduces duplication of tests which is good for animal welfare. In 2013 a council recommendation of OECD specifically on NMs, was adopted by member countries to use OECD testing guidelines also for the testing of NMs, however some of these test guidelines might need to be adapted as appropriate to consider the specific properties of manufactured nanomaterials. It also recommended to make use of OECD principles of good laboratory practice. An annex to the council recommendation provides tools for the adaptation of the existing chemical regulatory frameworks or other management systems to the specific properties of manufactured NMs with regard to testing, exposure assessment and risk assessment. A report on the implementation of the council recommendation and updating the annex will be going to the council. The testing programme is completed and data is available in dossiers at the nanosafety webpage of OECD (http://www.oecd.org/chemicalsafety/nanosafety/testing-programme-manufactured-nanomaterials.htm).
WPMN has organised several expert workshops on specific issues with regard to nanosafety, like inhalation toxicity (October 2011) and ecotoxicity and environmental fate (January 2013). Currently test guidelines are identified that need to be adapted and/or amended or need to be newly developed. Recent projects concern a proposal for a guidance document on the test guideline for leaching in soil columns and adaptation of a test guideline on particle size and size distribution. Mr Kearns shows a list of recent publications available on the website. Subsequently, Mr Kearns introduces the team members.

Introduction to EU NanoSafety Programme

(G. Katalagarianakis, EU COM)

13. *Mr Katalagarianakis*, EU COM, introduces the EU NanoSafety programme. The Horizon 2020 is a single programme with three pillars: excellent science, industrial leadership and societal challenges. The priorities of commissioner Moedas are open innovation, open science and open to the world. The translation in jobs and growth is priority number one which is made possible by policies on the re-industrialisation of the EU, the digital single market, the EU Energy Union and Circular economy. Nanotechnology innovation can only succeed if all possible or perceived as possible risks are convincingly managed. The historical footsteps in nanosafety research since 2004 are highlighted. The nanosafety research policy is implemented in a way ensuring completeness, consistency and efficiency. Continuity within the EU is a challenge and global continuity is an even bigger challenge. Two recent projects, ACEnano and npScope are to be launched early next year. NANoREG and ProSafe is a joint action supported by public funding from the EU, member states, associated states and industry. ProSafe started on 1-2-2015 for 2 years and complements NANoREG and supports the EU-USA CoRs. The regulatory research roadmap on quantifying and assessment of risks is presented. The next step is to bring this progress to market level. The EC4SafeNano which started on the 1st November 2016 has this aim. Also, the EU-US cooperation on Nano safety is explored. Achievements of strategic importance in this domain are the research community building, the close cooperation with member state programmes, the integration of scientific research, the close cooperation with regulatory authorities and agencies and the EU and the strong cooperation in the international scene. The next step is risk governance. Governance is primarily consisting in defining a goal or a consistent (or at least not-self-contradicting) set of goals after which policies towards the goal can be implemented. The first steps are collecting of

information and conveying this to all other agents, and communicating the information in content and shape, necessary for comprehension at different levels. The next steps will be planning and feedback and then process monitoring which is the most difficult operational phase of governance, but most important for success.

Introduction to ProSafe, Aims and objectives

(T. van Teunenbroek, Min. I&M, NL)

14. *Mr Van Teunenbroek* hopes that this conference is the first step to governance in the programme, Horizon 2020. The aim of the ProSafe coordination action is adding value to individual nanosafety projects by integrating results of "nanosafety activities" in the EU and other countries. A "white paper" will be developed with recommendations for policy makers and regulators regarding the risk assessment of nanomaterials for the short, medium and long term. What will happen next? Many of the contributions of NANoREG must still be uploaded in the database, but hopefully by the end of December this process will be concluded. Nearly all NANoREG deliverables have been submitted to and accepted by the management committee. Apart from NANoREG there are many other big projects already finished or in their final stage. The discussions of this conference will be noted and included in the Joint Document. The finalisation stage of the final Joint Document is foreseen for the end of January 2017. Mr Van Teunenbroek will compose a draft white paper with short and long term policy recommendations within the ProSafe coordination action. The draft white paper is a policy document with short and long term policy recommendations as well as R&D gaps to support the regulatory development, where until now no novel techniques have been applied in the regulatory arena. The draft white paper will be discussed in two stages between risk assessors and risk policy makers. The final Joint Document amended with the comments of the workshops, provides a basis of how regulatory relevant data can be generated and can be used as a guidance and/or as requirements to generate new or existing data. The utilisation of quality data in a tiered framework approach or the so-called how question, will be incorporated in the expert framework proposal from NANoREG. This framework proposal will be discussed in the risk assessors and risk policy makers workshop at RIVM, NL. The feedback of the workshops/break out groups will be processed and included in the redrafting of the white paper. The second workshop is between policy makers of innovation and policy makers of risk assessment on risk analysis and technology assessment. Data is an integral part of business sense to develop progress in the circular economy. Regulatory readiness is a corner stone of market acceptance. The focus of the workshop is on regulatory requirements and their influence on innovation policy.

Questions and remarks:

15. *Ms Hellsten* is working on a Swedish project to create a platform for communication to get authorities working together with other scientists in the industry. In the past she used to work for the Commission. She wonders if with the white paper there is a continuation, now that it is a research project with a final consensus of recommendations. How are policy makers at EU-level being approached?

16. *Mr. Van Teunenbroek* refers to the advisory body with national coordinators. Most of them are scientists from the member states or policy makers on risks. This community is added to NANoREG to discuss with the policy makers who are going to be invited to participate at the workshops. For the risk assessors, these are the people who deal with the NM working group. He would love to have a joint meeting, because many policy makers don't understand enough about toxicology and that is an argument – with regard to the framework proposal whether it could work – that needs to be signed off by the risk assessors in the institutes. Many institutes were partners inside the project, so he will reutilise the network structure inside the NANoREG to continue the work, that needs to be followed up and therefore the commitment of member states is necessary. The white paper is meant to be discussed on a higher European level between member

states. The white paper is meant to create a European position which makes possible some real governance through collaboration.

Introduction to ProSafe Task Force for Review of data

(K. Steinhäuser, GER)

17. *Mr Steinhäuser* mentions that the contents of this presentation concern the status quo, nanomaterials vs. conventional chemicals, procedures, results and perspectives. Status quo: increasing number of publications on Nano safety, more than 100 research programmes in EU and US over the past decade, regulatory relevance of the results mostly not examined and contradictory and misinterpreted results. Why is Risk Assessment with Nanomaterials different? Nanomaterials are chemical substances! Risk paradigm holds for nanomaterials, methods and tools for risk assessment apply. The great variety makes read-across, grouping, tiered schemes necessary with higher relevance of *in vitro* tests, acellular assays, HTS, modelling and *in silico* approaches. Areas of concern are physicochemical characterisation, human exposures through the lifecycle, exposure modelling, environmental fate and bioaccumulation, ecological effects, health effects and biokinetics *in vivo* and *in vitro*, computational methods and risk assessment. The taskforce studied many programmes in the EU and US, approx. 1,000 reports and publications were examined and reviewed. The recommendations on the Joint Document that is discussed during this conference will be considered by regulatory authorities and the OECD. The question sets over the 9 areas of concern, are included as annex 1 with the Joint Document on Reliability of Methods and Data for Regulatory Assessment of Nanomaterial Risks. As examples the results of specific characteristics are presented, e.g. on physicochemical properties, functional assays (FAs), framework of releases, inhalation toxicity and risk assessment frameworks. Selected areas for future research are e.g. an assay to determine the likelihood of hetero-aggregation as a major fate process in environment, validated testing schemes to determine aging and data sets/measurements for the development and validation of exposure models and (Q)SARs with a specific focus on nanomaterials. Validation of regulatory relevant methods and their inclusion in regulations have just begun. Nanomaterials are reaching the marketplace in increasing volumes and high product diversity, the nanomaterials' structure will become increasingly complex in the future; functionality will dominate properties of NM; keeping pace with scientific and technical progress is a challenge for researchers and regulators and will make further Nanosafety research necessary.

Introduction to NANoREG

(H. Crutzen, EU JRC & T. van Teunenbroek, Min. I&M, NL)

18. *Mr Van Teunenbroek* mentions that NANoREG is a collaboration between 87 partners, predominantly institutes that are linked to governmental organisations and 15 member states of the EU and 2 associated states, Switzerland and Norway. On top of it there exists a collaboration agreement between South-Korea and Brazil. Small and big companies participate, like BASF. One has established a collaboration link with ECHA. For regulatory purposes, guiding questions are needed with regard to the delivered output. Some NANoREG facts and figures are shown. The basic philosophies of the programme are a strong focus on both regulatory needs and scientific needs: methods and data that can be used in a regulatory context and a top-down approach to assure the basic conditions for linking *in vitro* and *in vivo* data, categorisation, read across, etc. For the demand driven side of the project 16 regulatory questions were formulated by NANoREG partners, Member States and industry relating to e.g. measurement, characterisation, identification, transformation, (intrinsic and media depended properties), dose metrics, metrological aspects and kinetics (vivo/vitro). To fulfil demands on reliability, comparability and exchangeability, partners shall comply with a Guidance Document and agreements regarding test design and data management. The results of the programme can be identified in 60 scientific deliverables which will be

publicly available at the end of the project, as well as 70 SOP's and methods and factsheets for deliverables, the draft final report NANoREG and NanoEHS data.

19. *Mr Crutzen* states that the NANoREG framework for the safety assessment of NMs offers a frame of reference for the safety assessment of nanomaterials, focused on REACH, inclusion/use of concepts such as read-across, grouping, control banding, tiered approach, decision trees, review and identification of the Nano specific hurdles in REACH, implementation for nanomaterials, propose innovative and efficient testing strategies that support and facilitate REACH implementation, offering new perspectives with both regulators and industry as users. Key NANoREG results are: a NANoREG Harmonised Terminology: a review of about 40 key NM safety assessment terms, answers to selected questions of regulatory relevance (public at project end), a toolbox of reviewed, working tools from NANoREG itself and many other initiatives at national and European level and an acknowledged key role by all partners of the 'scientific work packages 2-6'.

20. *Mr Van Teunenbroek* proceeds by saying that the way forward entails building on results achieved and answering the question what is necessary and available and which concerted action to expand knowledge under the conditions of institutional aspects, data management and harmonisation is required. The mind-set is aiming at collaboration and data sharing and one-off approaches need to be prevented. All participants are requested to use the dataset and help to expand it. Hopefully all the work that has been done will be continued in the NANoREG-2 programme.

Questions and remarks:

21. *Mr Oberdörster* acknowledges that there are more than 70 SOP's that have been developed and that are at different stages of development and acceptance. Are there any plans to keep further validation of the SOP's, so they can be used by others as well?

22. *Mr Van Teunenbroek* proposes the following. With regard to what is going to be done with the SOP's a further discussion will be proposed to determine whether we can adhere to the SOP's and turn them into guidances. The other thing is that in many projects a great number of SOP's has been developed. There are too many SOP's around. Therefore, a meeting on the NanoSafety Cluster in Europe will be organised to bring all SOP's on the table and transfer them into a fuller body and in a more agreeable standard and then hopefully the researchers will decide to make them mandatory for the next Nano projects, otherwise one cannot build on these results.

Introduction on the current status of EU regulation

(Andrej Kobe, EU COM)

23. *Mr Kobe* mentions that the presentation relates to the work in progress of EU regulation. Three components are highlighted: NMs in EU regulation, definition of a nanomaterial and REACH. The second regulatory review offers a frame on how EU regulation functions, where it stands and what still needs to be done. The main topics were the fact that NMs are considered as any other chemicals but risk assessment paradigms require certain adaptations. When specific provisions needed, use of 2011/696/EU, REACH is the most appropriate framework to improve access to information. NM in EU regulation refers to chemicals and workplace legislation, consumer product safety legislation and environmental legislation. Whether NM provisions exist or not, regulation should be knowledge based and supported by guidance on implementation. Deliverables are supported by a number of opinions (EFSA, Scientific Committee on Consumer Safety), positive listing of NMs (with conditions linked to physical characterisation/form) in cosmetics, biocides, food contact material, ECHA evaluation decisions and no specific provisions for NMs included in environmental legislation (e.g. waste streams, air or water quality standards or monitoring). To date there are no 'restrictive' management measures. A Commission's assessment led to the conclusion that there is no

EU registry necessary and existing databases in particular REACH, are sufficient. The first regulatory definitions are included late in decision making processes. Some regulations applied to the definition, other ones haven't until now and wait for the end of review. Results of consultation will be used to finalise the revised recommendation to be adopted as early as possible in 2017.

Invited talks

Introduction to NANoREG2

(E. Frejafon, coordinator NanoReg2, INERIS, FR)

24. *Mr Frejafon* mentions that the objectives of **NANoREG2** are firstly to compile regulatory driven tools for a robust information on safety requirements of an innovation, all along the value chain, then to build on these regulatory driven tools Safe-by-Design principle addressing 3 pillars (safe / products, processes & uses) and demonstrate its applicability on several (5 or more) industrial case studies. For this we will integrate knowledge and expertise of innovators (academia and industry), risk assessors and regulators for an optimum efficiency to check if an innovation is safe & demonstrated gains achieved. Expected outputs are notably Hazard regulatory oriented tools, Phys-Chem descriptors & End-points, an Intelligent Testing Strategy (Grouping, in-silico, HTS, *in-vivo*, *in-vitro* ...), a Safe by Design (SbD) implementation strategy defined on the 3 main pillars which rely on the above mentioned regulatory oriented tools and which will be validated on several Industrial case studies such as Hazard reduction (dustiness, toxicity), enhance process safety (modify inputs) or primary product exposure reduction (master batch).

25. Outcomes will be disseminated to all stakeholders, including help on request strategy definition. Outcomes will be also used to promote pre-validation capabilities of regulatory driven tools. Up to now we have proposed grouping criteria and strategy, based on existing approaches such as MARINA and including regulatory requirements which were reviewed by a panel of international experts. Several industrial case studies were defined and available tools for hazard & risk assessments are under identification. Last but not least, an ambitious data management action is ongoing as a necessarily tool for grouping strategy and SbD strategy implementations, addressing technical challengers on data format and data curing beside serious legal difficulties (access and use available data). Interested parties have the capability to attend a meeting dedicated to Safe by Design industrial implementation, which is co-organised by NIA and numerous on-going project and which will take place on 24-25th April 2017 in Gaiker Technological Centre in Bilbao – Spain.

26. *Mr Frejafon* mentions also that following the necessity to build regulatory driven strategy for a safe innovation, the harmonisation of the corresponding expertise is a crucial point to take into account and that **EC4SafeNano** (European Centre for Risk Management and Safe Innovation in Nanomaterials and Nanotechnologies), had just stared, coordinated by INERIS, operated together by major European risk institutes and numerous associated partners, gathering all stakeholders involved in Nanomaterials and Nanotechnologies (regulators, industry, society, research, service providers...). EC4SafeNano will promote a harmonized vision of expertise in risk assessment and management for the public and private sectors to enable the safe development and commercialisation of nanotechnology. The main objective of EC4SafeNano is to design mechanisms and rules to sustainably build harmonised expertise in risk assessment and management. For that, the project will gather stakeholder needs, will map all resources available, will define harmonised expertise gathering resources and addressing needs. It will demonstrate the efficiency of the proposed solution on case studies within the project and in relation with a Joint Call initiative launched in parallel. Interested entities are welcome to join the project as Associated Partners and interested funding agencies are welcome to enter in the joint call.

GUIDEnano: A Tool for risk assessment of Nano-enabled products

(S.Vázquez- Campos, LEITAT Technological Centre, SP)

27. *Ms Vázquez* mentions that the Risk Assessment Tool in the project now is available in version 2.5, and the final version will be available at the end of the project (end of April 2017). This Tool will guide the users, i.e. nano-enabled product developers (industry), into the application of the most appropriate risk assessment and mitigation strategies for a specific nanomaterial/ nano-enabled product. The different modules/ frameworks of the Tool were presented, starting with the nanomaterials framework: First the user should describe the activities (scenarios) and materials to be assessed and, based on this information, the Tool can predict associated NM release (quantities and material forms) to different compartments. A wizard helps the user to select the most adequate activity from a library of 300 activities/processes. The usage of the library is presented and highlighted in a particular case study. The roles for predictions and those roles that connect the different modules are coming from organising data collected from literature in tables, decision trees to be fed into the tool. The system has been built to allow description of complex materials and contains graphical representations to guide the user. The goal of the NM Hazard Module is to predict hazard reference values for human & environmental health. In comparison with other tools it is comprehensive and includes all phases of the risk assessment, focuses on environmental and human Risk Assessment and can be done for an activity or for the whole life cycle, allows assessments with different levels of data availability, Risk Assessment estimate is quantitative, is has a flexible structure to allow extensions and updates following scientific/ regulatory progress.

Questions and remarks:

28. *Mr Sayre* asks if the worker exposure component actually includes current data on protectiveness and e.g. engineering control data from NANoREG in order to get some fairly realistic exposures to workers.

29. *Ms. Vázquez* confirms that the data available is included in the Tool. At the moment, it contains the data that is available on engineering control efficiency for NM and non-NMs because the team has been working on the effectiveness of PPEs (personal protective equipment) for example, modifying the values for NMs. NANoREG data is already included in the Tool.

Species Differences in Pulmonary Responses to Subchronic and Chronic Exposures to Low Solubility Particulates: Comparisons of Biokinetic and Pathophysiological Responses in Rodents, Non-Human Primates, and Coal Miners

(David Warheit, Chemours, US and Gunter Oberdörster, University of Rochester, US)

30. *Mr Warheit* is putting the following on the table. Whilst we very much welcome the use of modelling and translational toxicology from rat data to assist prediction to human hazard and risk assessment, we would argue that more consideration should be given to using both a weight of evidence and fit for purpose approach which take into account the totality of the data; in particular species differences and human findings from working populations exposed to PSPs under relevant occupational conditions that have been extensively investigated. Outline of evidence: Interspecies differences in lung responses to inhaled particulates. Accordingly, the objective of this brief presentation is to provide important insights on the fundamental differences in pulmonary responses between experimentally-exposed rats, other experimental species and occupationally-exposed humans. Briefly, five central factors are described by the following issues.

1) Interspecies differences in lung responses of rats vs. other rodents, triggering different adverse outcome pathways (AOPs). Rats are a particularly sensitive species in pulmonary responses to

particle overload inhalation exposures, and a unique species in developing lung tumors following chronic inhalation (particle overload studies). Mice and hamsters do not develop similar responses.

2) Interspecies differences in inhaled particle kinetics in rats vs nonhuman primates and humans triggering differential particle-related pulmonary responses. In 2-year studies with rats and monkeys exposed to the same aerosol concentrations of aerosolized dusts, rats also develop hyperplastic and hyperinflammatory lung responses while nonhuman primates are known to develop "normal physiological", macrophage-mediated responses to chronic dust exposures, with little detectable pulmonary inflammation. Perhaps more importantly, the potential "smoking gun" difference between particle-exposed rats and monkeys/humans are the particle kinetic differences. In rats, the inhaled particles remain within macrophages and epithelial cells on alveolar duct surfaces; this produces sustained alveolar inflammation, hyperplasia and cell proliferation – leading to the pathological sequelae which ultimately cause lung tumors. In contrast, inhaled poorly soluble particulates (PSPs) which deposit on respiratory bronchiole/alveolar regions in monkeys and humans generally transmigrate from epithelial sites of deposition to interstitial compartments of the respiratory tract, which severely diminishes any inflammatory or cell proliferative effect in the lungs following chronic exposures.

3) Advanced and updated human respiratory tract deposition and retention models allowing more realistic particle translocation/retention estimates. The updated ICRP model by Gregoratto and co-workers confirms the morphometric findings by Nikula et al. that inhaled radioactive dusts in workers become interstitialized following particle deposition. This leads to a prolonged lung clearance rate vs. those measured in rats but "discourages" inflammatory and cell proliferative responses.

4) Differences in morphologies and characterisations of rat vs. human pulmonary tumour types and locations within the respiratory tract. Human production workers do not develop lung cancers following chronic exposures to poorly soluble particulates – as rats do following chronic inhalation exposures. Asbestos fibres and cigarette smoke-related lung cancers in humans occur at bronchiolar sites in the respiratory tract. Alternatively, particle overload-exposed related lung tumors in rats occur in alveolar regions of the lung and frequently develop keratinizing squamous features - which does not occur in humans.

5) Comprehensive in-depth analysis of available epidemiological data from PSP production workers that demonstrate no correlations exist between particle exposures and lung cancers or other non-malignant respiratory diseases.

31. Conclusions: The most plausible conclusion that can be reached is that results from chronic particle-overload inhalation studies with PSPs in rats have no relevance for determining lung cancer risks in production workers exposed for a working lifetime to these poorly soluble particulate-types.

Questions and remarks:

32. *Mr Cassee,* RIVM asks whether Mr Warheit argues that the rat is not the appropriate species, and that the technical guidelines allow also other species. Is he promoting using hamsters for toxicity studies?

33. *Mr Warheit* has thought about that a lot and he thinks the issue is not exposing the rats at overload concentrations, particularly in chronic studies which we may never see anyway. The rat is still a pretty good model, having said that the overload of concentrations is not relevant for humans. The rat studies at reasonable concentrations will provide good information. In particular, inflammation may be a useful indicator for rat studies, because, although the pulmonary kinetic mechanisms between rats and humans are

different, an exposure level in rats that does promote sustained pulmonary inflammation will not result in cell proliferation, septal fibrosis and the pathological sequelae that leads to lung tumors in rats.

34. *Mr Oberdörster's* presentation concerns lung particle overload of micro and Nano of hazard and risk by chronic particle inhalation in rats which resulted in induction of lung tumours. The exposure concentration, retained dose and the inflammation, particle kinetics and morphology results are presented. The volumetric particle overload concept is generally well-accepted by the scientific community, however, its application for establishing exposure limits generated heated, sometimes emotional discussions. Follow-up studies to modify the overload hypothesis have been published, in particular using particle surface area or specific surface area reactivity as dose metric. Although the focus is on microscale poorly soluble low toxicity particles represented by pigment grade TiO_2 and on concepts of inter- and intra-species extrapolation, consequences for nanoscale TiO_2 are also pointed out. Using dosimetry extrapolation modelling, Occupational Exposure Limits (OELs) for microscale TiO_2 have been derived ranging widely from 0.3 mg/m^3 to 2.4 mg/m^3. This presentation provides rational, objective discussions of the lung particle overload concept, addressing topics of exposure-dose-response relationships for inhalation toxicology, exposure modes, predictive particle deposition models, dose metrics, dosimetry extrapolation modelling, risk assessment.

SESSION 2: MN[1] IDENTIFICATION AND CHARACTERISATION AS BASIC INFORMATION REQUIREMENTS FOR AN APPROPRIATE REGULATION

Chair: T. van Teunenbroek, Min. I&M, NL

Stimulus talk by G. Lowry

[Lead Expert of the TF on Physicochemical methods (Greg Lowry, Carnegie, US)]*

35. *Mr Lowry* outlines the different validated methods for the identification and characterisation of physicochemical properties of engineered nanoparticles. He explains that, for two reasons, reliable and accurate methods are needed to measure the effects of these properties: to determine if a material fits into the legal definition of NM and to enable read-across for NMs. The NM properties that matter, can be sorted in terms of what they are (chemical or physical identity), where they go (fundamental fate behaviour) and what they do (reactivity). However, the system properties are as important as the NM properties in controlling their behaviours. The highly system dependent properties are termed extrinsic properties, whereas the NM properties are independent of the system (or less dependent) are termed intrinsic properties. Both the material properties and the environmental system they are in, have to be considered to understand the effects.

36. Important for the identity of NM, are the particle size distribution (PSD), VSSA (Volume Specific Surface Area), shape and chemical identity (structure). The proposed definition of NM says that a material is NM if at least 50% of the number concentration of primary particles has a dimension of less than 100nm, or if the VSSA area is $60 m^2/cm^3$. There is currently only one way to reliably measure particle size distribution: EM (electron microscopy). Other methods all measure different things. One question is if the different methods can be used to get an accurate measure of primary PSD. Is it possible to measure size distribution with a certain method and relate it back reliably to the real primary PSD to satisfy the definition of NM?

37. There are several encouraging techniques to accurately measure intrinsic properties, like automated EM image processing (for PSD). Other methods also show promise, e.g. measuring absorption of gas (VSSA), but need more validation. Combinations of ICP-MS, TGA, EM and XPS can be used together to determine the chemical identification for chemical composition, but they all have some limitations and disadvantages. There is no simple, rapid one-size-fits-all solution for the chemical identity characterisation problem.

38. Many of the extrinsic (system dependent) properties of nanomaterials are measured with FAs (functional assays). There are FAs available to measure, e.g. dissolution rate, zeta potential, or (homo)agglomeration potential. FAs are often designed to measure rates of processes, e.g. dissolution, under the system conditions that the NMs are used in. They provide values for the parameters used in fate or toxicity models. Most of the processes that are measured for nanomaterials are not at equilibrium, so rate matters a lot.

39. Robust EM methods are emerging for characterising PSD for simple NMs. Many NM properties of interest have reliable measurement methods. But there remains a gap with protocols for sample preparation and measurement and with data / metadata reporting guidelines. Data and metadata both have to be collected and be properly reported in order to do comparisons between studies. The absence of data reporting standards

[1] MN: Manufactured Nanomaterials

is hindering the ability to do read-across. Characterisation methods have to be reliable (and therefore reproducible), accurate and relevant for the decision-making process.

40. The relevance of most of the NM properties for decision making about risks and regulation remains to be validated. It remains unclear what properties and processes are most significant, and how important the NM characteristics are. The list of properties has to be narrowed; then it is possible to start measuring the things that matter most.

41. In reaction to the presentation of Mr. Lowry, *one of the auditors* remarked that it is incorrect to presume that a suitable method should be available for low costs for risk assessment of NMs. Any method giving important answers, must be used regardless of the costs. It is important to find methods that can give, now or in the future, the answers that are needed. EM has a lot of development potential and can be made cheaper, faster and more user independent. Instruments have to be developed, whatever they may cost. *Mr Lowry* referred to the war between regulation (looking at costs, doing business, and trying to be as simple as possible and getting a-good-enough answer) and science (wanting to understand how things really work). Low cost criteria came from the regulatory side.

BOG Identification summary

Break Out Group on MN Identification (based on the EU recommendations for a definition), Co-Chairs: K. Jensen (NRCWE, DK) and A. Patri (FDA, USA), Commentator: H. Rauscher (JRC, EU)

42. This group has been focusing on a list of seven questions on the identification of NMs.

Key Question 1: How can the preparation methods for (Electron Microscopy) EM analyses best be standardised and automated for a wide range of NM types?

43. For identification purpose, this question is narrowed down to metals and metal oxides in pristine state. Later the discussion can move forward to more complex matrixes and additional challenges. According to NANoREG, seven SOP's (standard operating procedures) have to be followed for EM (electron microscopy), including sample preparation to bring the NMs into dispersion. The critical part is to get a suitable sample that can be analysed.

44. For dispersing NMs generally applied industrial methods, like material-specific mediums or agents for the preparation of dispersions, are applicable. For metal and metal oxides, water and some critical sonication energy will give enough dispersion to break the forces. Powders can be dispersed in a certain polymer. Bringing an NM into dispersion and putting it on a grid is not always critical. It can be much easier to go to dispersion from a plastic matrix. An option is to do zeta potential measurements, adding sonication energy, and using centrifugation methods.

45. At CEN, some projects are going on about the preparation of samples. One of them is focussed on samples for EM. There is already an ISO guideline for sample preparation for EM (ISO TR16196:2016), but further guidance and SOP development are needed; certainly, a round robin testing will be necessary for validation. To standardise a methodology, quality monitoring with certification of procedures will also be needed. In that respect, further work must be done on odd shapes, such as fibres.

46. The first question can also be approached from the perspective of a modeller or the industry. Designing a new particle starts with creating a virtual library of NMs that are different by structure, properties and activity. In virtual design, the properties can be predicted and the most efficient properties are selected from the library. Knowing the characteristics of the pristine NM, the modellers can estimate how it will behave in different media and environments. They should link intrinsic and extrinsic properties to each other when designing an NM in a certain system and they should develop a model to determine which medium to

use for dispersion. This approach can be useful for primary particle size distribution, but can't be done for agglomerated materials. To be able to adhere to the EU recommendation for a definition, a paradigm or a procedure has to be developed for standardisation of criteria to enable sizing by EM.

47. Regulation requires a minimum size distribution analysis. An analytic method that can be used in a standardised way has to be developed and solutions for automated dispersions have to be found. The key is to get the material as dispersed as possible to do sizing on agglomerates and aggregates. The best dispersed state is suitable, but when preparing samples, the suitable concentration also needs to be considered. To get to the smallest dispersible size of an NM, the overall guidance is to take it to a plateau dispersion and send energy that is enough to break the particles apart, but not enough to push them back together. With the plateau for many fully dispersible materials, a good correlation with EM can be obtained. When materials are not fully dispersible, hydrodynamic methods don't give a good correlation with EM.

48. The agglomerate size depends on the sequence of media that surround the NM. When the primary size and the properties of the media are known, it is possible to predict the size of agglomerates in different media. Not everybody agrees on this vision: An aggregate is a material that can't be broken apart. Theories say that, going to minimum dispersible sizes, only aggregates (and no agglomerates) are present.

49. Depending on the properties which are needed, the industry always chooses the most suitable methods to determine the sizes. Light scattering is for instance used for scattering properties and the hydrodynamic diameter for segmentation properties. If the necessary values can't be obtained with one technique alone, a combination of techniques is used. A benchmark technique has to be defined to be able to compare size distributions from different methods. Then a validated technique can be used to estimate the value under EM.

50. When it comes to automation, there are examples from the industry already having commercial solutions on the market to disperse particles. They might be adaptable for NMs. A key issue will be to define the benchmark technique and quality criteria for preparing dispersion through automated processes.

51. As for some NMs (for example beam sensitive materials), using EM techniques may not be optimal, other methods are also needed.

Key Question 2: Is it possible to relate characterisation of size distribution measured by ensemble methods to those measured by EM method?

52. Ensemble methods measure the whole sample at the same time. The answers to this question were mixed. They focussed on identification and sizing of the NM and not on identification of NM`s sizes in exposure / environmental mediums. Related questions are whether ensemble methods are needed, and under what circumstances the various size measurements should be taken and being related to each other.

53. Not many organisations can afford EM. Easier and cheaper alternatives could be related to DLS (Dynamic Light Scattering) or nanotracking. But they measure different things: EM measures particle core and DLS hydrodynamic size. Maybe the hydrodynamic diameter of density can be related back to the primary particle size distribution as measured by EM. From those that have been measured until yet, the EM particle size couldn't be predicted.

54. Some ensemble methods are applicable for some ranges. EM gets into other ranges. Whether ensemble methods can be used, depends on what has to be measured. Analyses of a number of materials and their measurements showed that, under certain circumstances, they can be quite reliably applied. For specific cases and under specific conditions, ensemble measurements can be used to find out if a material is NM according to the definition. Usually they run into trouble when some broadness occurs in the size distribution or in case of irregular morphologies.

55. Whether ensemble methods are necessary, depends on the purpose of the measurements, e.g. if an NM falls under the EC definition. If an ensemble method cannot distinguish between constituent particles and agglomerates, and if it gives a result of 50% below one hundred nm, it is pretty sure (even without measuring the exact size distribution) that a material is NM. In that case, it is not necessarily to exactly relate. A general result is sufficient.

56. The US FDA (Food and Drug Administration) cannot require the use of specific equipment, but has to accept and to analyse relevant data from validated measurement methods. A solution phase measurement like DLS, can indicate that a material is NM (in the EU definition) if it comes up well below one hundred nm. If the result is around one hundred nm, it is not sure if it is NM because the primary particle size may be less than one hundred and the agglomerate size may be more than one hundred.

57. Round robin tests on colloidal silica samples as well as colloidal gold and colloidal silver with TEM and SEM as well as normal SMTS (scanning mobility particle sizer) or regular DMA show good correlations. When the material is well behaving and well dispersed, analogue methods can be used if there is a correlation function. Broader unusual shapes lead to more uncertainty in the measurements. Ensemble methods can be used if one knows the material and if it is validated against a reference technique.

58. As long as the distribution is narrow, it is in principle easy to correlate different techniques. The problems start with a broader distribution and get worse for by-model distribution. When there are big particles, small particles won't be seen in light scattering. Then binary distribution could be used. In multi-angle DLS, it is possible (if the size is not too big) to separate different distributions of different populations.

59. Symmetric Flow FFF might be a helpful method, too. It can check size distribution from one nm up to almost one micrometre. Multiple detectors can be used to get all results in a single shot. The analyses can be done quite quickly. However, the dynamic range of FFF is less than expected. Instruments cannot reach one micron. They go up to twenty-five nm, or maybe to forty nm in a specific set-up.

60. For proper identification, a complete size range has to be determined. Even in EM, it can be a challenge to size very accurately at the lower limit of around one nm. Other methods might be required to cover the lower end of the method. The project NanoDefine analysed methods to characterise NMs and developed a tiered approach using ensemble methods.

61. Procedures should start with determining for what purpose measurements are performed and what properties need to be measured as they will determine the methods to be used. There is not one approach to measure everything: methods have to be combined and sometimes two methods have to be used for measuring one property.

62. The characterisation of NM should be focussed on the real world. The definition has to serve as a border in risk assessment In this context, it is also relevant to realise that some materials can, under standard conditions, be characterised as NM (having an average size of fifty nm in the distribution) while they are no longer NM in interaction with a cell (having for example a size of two hundred nm).

63. NMs are not just chemicals. They feature a much more dynamic set of properties, defined by the system. Media characterisation and harmonisation of media should be elevated to the same level of necessity as the harmonisation of the NMs themselves. A set of meta data and descriptive data (e.g. in a form of a wiki) might help to create a uniform number of characteristics. Construction of the data set could be cloud sourced. NANoREG and NANoREG2 already worked on these thoughts.

Key Question 3: What level of uncertainty is acceptable for classifying a material as NM or not NM?

64. Based on an interlaboratory study under NANoREG, it is currently possible to size materials with an overall uncertainty of less than five to ten percent. The question is if this uncertainty will be acceptable. The answer depends on the perspective of the stakeholder, e.g. manufacturers might accept some variability between batches instead of admitting each batch to TEM.

65. In many cases the NM is not determining whether a material is an intoxicator or not. Identification as NM is not enough to evaluate the risks. That might imply doing extra work and having an extra set of data requirements. If a material is an NM or potentially an NM, certain properties have to be investigated: Additional tests might be required to find out whether any hazard or risk is associated to the material.

66. The EU definition doesn't warrant safety. In the US, the definition is about materials being approximately one to one hundred nm, being engineered, and having novel properties. Materials above one hundred nm show a significant change in the pharmacokinetics. At a certain size, the toxicological and physical chemical properties are different. Instead of being more toxic, NMs can also be less toxic.

67. Although discussions are usually focussed on the size, the surface is also relevant. Small changes in size or surface can completely change behaviour, e.g. immunological behaviour. Batches can look identically in TEM, DLS and zeta potential while they have different biological properties. For characterisation with respect to regulatory needs, the definition of a size range is important. When a size has been defined, the acceptable level of uncertainty can be determined. In this context, a data set is relevant. Part of the question is also the uncertainty/reproducibility of data between labs. They have to remain within certain boundaries, although they can be certified at different compliance levels.

68. The question has to be addressed whether regulation can accept a declaration of a registrant with a certain error or not. The issue about uncertainty is mainly a legal one. When can people be legally bound to say that a material is nano? Manufacturers have to understand all off the constituents of their products and to take their responsibility. They should always go for safety and come up with extra data when their products are nearly NMs.

Key Question 4: Can tiered schemes for NM identification be used in place of quantitative size-distribution data?

69. This question is referring to the tiered approach for NM identification as developed under NanoDefine. Tiered schemes for NM identification can be used in place of quantitative size distribution. Another option is to use them to avoid expensive EM in certain situations; if a material passes certain thresholds, it moves to higher tiers with other characterisations efforts. One goes from the most simple, easy and cheap end stepwise to more complicated and more thorough methods, ending with EM. In certain cases, ensemble methods can determine whether a material is an NM or not. The final question could be: what tiered scheme can be used to avoid EM on all materials? It is also important to realise that a lot of materials don't lend themselves for EM. There have to be found ways to characterise the sizes of those materials that don't show up well by EM methods.

70. Assuming that a tiered approach will work, the differentiating parameters of those tiers have to be determined. For environmental regulation, it is for instance relevant to take the release rate into account; whether a material is released immediately or gradually over a long period, has consequences for the measures to be taken. Such measures also depend on the bio-availability: how much of the released material can be taken up by environmental organisms and does it pose an environmental harm? There are further parameters to differentiate between tiers in a nanospecific approach, e.g. crystallinity, catalytic activity, surface, shape. Specific FAs (functional assays) to determine these parameters have to be developed.

71. Nano safety research has learned that certain fibres, respirable fibres, are less toxic in a thin form (diameters below fifteen nm) than the tickers ones. An NM can be more toxic than the bulk form. Application of size criteria instead of hazard criteria may drive innovation into a wrong direction. Producers might choose to make thicker, more hazardous materials to avoid the NM label and according regulation. Thus, for certain groups of NMs with respect to specific protection targets (e.g. respirable fibres in human health assessment) a tiered approach (e.g. starting with respirable fibres) might be better than looking for accuracy in the NM definition in terms of sizes. Some uncertainty is acceptable; no toxicological test has an uncertainty of less than ten percent.

72. In the future, energetic parameters might be relevant too. Different polymorphic phases, for example of TiO_2, are totally different in terms of toxicity and reactivity. Surface energies are very strongly correlated to phase transformation. In terms of hazard, the crystallinity of different phases as a parameter should also be taken into consideration.

73. For the sake of human health, smaller and medium enterprises have to learn about new materials and their health risks. A dustiness test, to find out if there is a risk of respirable particles releasing, should become mandatory. In the supporting guidelines of REACH, dustiness testing and morphological characterisation are recommended, but they should be part of the regulation.

74. Probably the tiered approach can be used for screening procedures, but not for high concern materials. This means that one has to define what “high concern materials” are. Tiered schemes can be approached as risk-based or as sized-based. From a regulatory point of view, particle size is only meant to identify the NM and not the hazard category of the NM.

Key Question 5: Can volume specific surface area be suitable for identifying NMs?

75. Both NANoREG and NanoDefine worked on an operational procedure to enable to distinguish between porosity and surface area. VSSA (volume specific surface area) is rather suitable for identifying nanostructured materials than NMs. It can be used under certain well-defined conditions. In a tiered approach, VSSA might be one way to determine if a material is an NM. Some say that VSSA can only be suitable in combination with quantitative size-distribution or as an additional supporting identifier for NM in dry powder form.

76. An issue are porous materials which have different surface measurements. What are the implications for highly porous materials if VSSA is adapted as an identifier for NM? Based on VSSA highly porous NMs might be larger than according to the definition, while they are in fact NMs. NANoREG and NanoDefine looked for an optimized approach to VSSA. They tried to identify what fraction of the surface is related to porosity (which is possible with analysis of the adsorption isotherms). However, there are the quickly phenomena that change the measurement of the surface area, like functionalisation. In some circumstances, VSSA can be used as a screening method if one knows the material and how it has been modified. In other cases, with coatings and smaller sizes, the method cannot be used.

77. NanoDefine went into the question if VSSA measurements can be related to size distribution. Surface area is important and might even be more important and more risk related than size, but has a poor correlation with size distribution. VSSA measurements can only be related to size distribution under certain well-defined conditions, for instance that the material is not too polydispersed. In most cases VSSA is not sufficient to decide whether something is a NM or not. Therefore, it is not recommended by the Task Force as single parameter to identify an NM. A publication about this work item of NanoDefine is in preparation.

Key Question 6: What research is needed to be able to streamline the determination of NM structure (e.g. core-shell and shape) and organic coatings to quickly and inexpensively characterise NM identity?

78. The question is about NMs having core-shell and coatings. In NANoREG a draft guidance has been developed to enable identification and quantification of impurities and surface chemical modifications as coatings and functionalisation. The procedure was developed for unknown NMs.

79. Surface coatings are very critical, but the biomedical area doesn't have many methods for characterisation of such NMs. Industry is better equipped to analyse and characterise its own materials. But often materials have to be characterised without knowing their original characteristics. When the industry doesn't give all information about its own products and doesn't provide methods for characterisation, a kind of expensive forensic route is necessary to figure out the composition of the materials. From a regulatory perspective, it is important to know the surface chemistry, i.e. clarifying comparability of nanoforms as an essential parameter for e.g. read-across.

80. It is not only important to know what components an NM consists of, but also where they are located in the structure of the NM. The position plays a big role in the behaviour of the NM. Suitable methods are needed to investigate the distribution and location of the components in the NM.

81. Usually, the distribution of components in an NM originating from an industrial process is known. If NMs are not fully coated, it is necessary to step back to separation techniques. Separation is critical for understanding the surface coating of NMs, but is not often coupled with the characterisation of NMs. There are many separation techniques, like the film flotation methods, that work well for certain classes of NMs and particle materials.

82. The stakeholders want better understanding of the surface condition and surface structure, for example in liquid dispersions. Processes should be speeded up because it is important to have the relevant information. It is also relevant to realise that even cerium oxide or ironed materials can have different redox states and XPS.

Key Question 7: What criteria can be used to define monoconstituent substances, multiconstituent substances, and unspecified substances and mixtures?

83. The seventh question was skipped because there wasn't enough time left. Written comments made clear that the different opinions exist on this issue. The variation of composition / structure of separable or distinguishable parts, the ECHA criteria, and the physicochemical criterion were suggested as criteria.

Comment on the work of the BOG

84. *Mr. Rauscher* concludes that the criteria for identification of NM can be found in the text of the definition. The properties that characterise the material are equally important, but they are not directly relevant for the decision whether something is an NM or not.

85. The European approach, with its definition of NM, helps to distinguish between the identification criteria and the characterisation criteria. The definition is a consequence of the precautionary principle which is fundamental in European legislation: identifying a specific class of materials and then looking at the associated problems. It doesn't mean that identifying something as NM, automatically means that it is hazardous. Nor does it mean that it is not hazardous if it isn't identified as NM. But it helps regulators and manufacturers to look closer at a specific class or a material. Worldwide, there are other approaches which are completely valid. However, this discussion was about the European approach.

86. Regarding sample preparation for EM, several ways seem to work. The case by case approach is seen as the best one. Groups that deal with EM analyses, should share their knowledge. The ISO TR16196 meant an intervention in sample preparation.

87. The acceptable uncertainty of size distribution hasn't been determined yet. For legal classification, it is import to say if a material is NM because that triggers certain requirements, tests, and information. Manufacturers have the responsibility to do everything to be sure that their products are safe, whether they are NMs or not. The definition for NM classification is a help for regulators and manufacturers and doesn't automatically release them from their responsibility. They should not hide behind a legal text and a specific uncertainty, but they should do everything to be sure that the material or the product is safe. Again: Classifying a material as NM, doesn't automatically mean that it is hazardous. If it isn't classified as NM, it isn't automatically safe.

88. Concerning the measurement methods, there was a discussion about the relation between the non-counting methods and the counting methods. In the last couple of years, tremendous progress has been made in measuring sizes of NMs and their size distributions. The possibilities and the limitations of the methods are understood, but this knowledge is still scattered. However, this knowledge is needed for risk assessment and regulation to take the right decision. Projects as NANoREG and ProSafe are on the right way. It has to be made sure that, when these projects are over, the results are sustained.

Contributions from the participants

- When the projects are over, hopefully the guidance and tests developed will keep the results living.
- There was not enough time left to discuss the chemical composition and the way to analyse the structure of NMs. Such information should come from the industry as producers know their NMs. It is a tremendous work if that has to be analysed again.

BOG Characterisation summary

Co-Chairs: I. Lynch (University of Birmingham, UK) and E. Valsami-Jones (University of Birmingham, UK)
Commentator: J. Riego Sintes (JRC, EU)

89. This group has been focussing on a list of four questions on NM characterisation as basic information requirements for an appropriate regulation. The questions are about extrinsic, system dependant, properties of materials. The purpose of measuring them, is to set up parametrized models that can be used for decision making.

Key Question 1: How important are the selected NM properties for regulatory purposes? Are there any important properties missing?

90. The discussion went into intrinsic versus extrinsic properties. It is not always clear whether a property is intrinsic or extrinsic. Sometimes it can be both.

91. In the next five years, a lot of new information is expected about surface chemistry and simple methods to characterise surface chemistry. Measuring the affinity of a particle with a macromolecule in a certain environment, should be done with an FA. The probability that an NM, when it hits a surface, will stick to that surface is related to attachment efficiency, but it deserves its own separate test. There are already beginners of that sort of FAs.

92. A distinction can be made between the affinity of the NM to something, and the affinity of something to an NM. Both have an affinity constant, which means that the affinity can be intrinsic (from the

nanoparticle) or extrinsic (from the other material). Until yet, all the work around corona and protein binding has been related to the NM surface characteristics.

93. Another vision is that attachment efficiency can no longer be seen as an intrinsic property, as it always depends on the counterpart, for example another particle. In different systems, the interaction partners have different affinities. However, the FA doesn't have to cover all systems, but only the systems that matter for the particular regulatory concern.

94. Attachment of a particle to something or vice versa, are part of the same process. What exactly happens will always depend on the particle size. In that respect, an assay measuring electric constancy might be useful. Energies can allow the prediction of the association constant, which also depends on particle concentration. This is a more fundamental approach which will be available only on the mid to long term. A near term approach might be to directly make measurements in the system. The results are not completely transferable to other systems, but hopefully they will be transferable within the system. FAs don't provide answers for all environments, but some data are needed in the short term because regulatory decisions have to be taken.

95. A lot of information is needed: attachment efficiency, the corona effect, agglomeration rate, sedimentation. Some of them are related to each other, like attachment efficiency and agglomeration rate. Thus, they might be clustered.

Key Question 2: What other validated methods are available for measuring and reporting of these properties?

96. It is important to go as far as possible with FAs and other tests in order to understand the key characteristics. A point that has to be taken into consideration, from a regulatory point of view, is that FAs should be able to be carried out in expert labs. Also from an economic point of view, many labs around the world must be able to deal with the recommended methods. FAs have to be reproducible. Simple assays can be used and reproduced by anyone in the field. Medium level assays could be run with good reproducibility by confident labs. Automation might resolve many reproducibility problems and analytical problems.

97. Validation and certification of methods is needed. Pilot production facilities in publicly funded projects should be encouraged to use validated methods, but most methods are only evaluated and not yet validated.

98. The focus is on a faster and more efficient approach for creating good data and enabling regulators to come to conclusions and decisions. A lot of resources, money and time are needed to come to validation and standardisation. It would be good to have an international committee focussing on a limited number of well-defined assays and their promotion to a level that is really useful for regulation. First it has to be determined which properties need to be measured. The list of properties identified by the Task Force, must be narrowed to some key properties.

99. Exposure and hazard are important criteria in the decision which FAs are worth to further evaluate and standardise. Some FAs (like dissolution rate and agglomeration behaviour) are already in the process of standardisation by OECD.

Key Question 3: What functional assays need to be developed to ensure they cover the endpoints required for regulatory use?

100. The link between requirements of regulators and the proposed FAs is not always clear. If for instance the industry is asked to provide for all products the attachment efficiency, the industry would like to know how the regulator is going to use this information. In this respect, the NANoREG roadmap can be

used for clarification. All FAs have got specific goals. It is clear where they fit into the overall model of exposure, fate, hazard and risk.

101. FAs may get a role in tiered testing schemes, determining whether further testing will be necessary. If an NM is only used in specific conditions, some FAs might be irrelevant. Which FAs have to be done, could be NM specific and use specific. The results of FAs can be used for developing models that are able to predict e.g. behaviour and exposure.

102. The suggestion of a tiered structure including FAs is promoted. Only if needed, the higher tier is started. High-level screening whether a material is going to be a risk or not, will be very useful in order to avoid having to measure all endpoints and all possible scenarios. Assessing NMs along their lifecycle is a favourable approach to see where FAs might be relevant, would link closer to regulatory issues and lead to a quicker realisation of what parameters and scenarios might be most relevant.

103. There is a need for globalised regulatory needs. The EU has no harmonised regulation on NMs, because everybody is waiting for final agreement on the definition of NMs and an addition to the REACH legislation. Guidance and regulation will allow flexibility for new scientific developments. Therefore, people want the EU to speed up. However, it is highlighted that guidance development already started. There is also an ECHA guidance on NM. Priority should be given to effectively gathering information. The Task Force has picked this up and will provide a list with FAs that become suitable for use for regulation.

104. One of the FAs that has to be developed is on attachment efficiency. An FA for both attachment efficiency and agglomeration behaviour may be necessary. The draft OECD test guideline on agglomeration behaviour that is under development, may help to cover this need. An FA to measure in one go the attachment efficiency of a particle to different surfaces (e.g. oxide, metal, sugar coated) would be helpful to get a picture on the potential interaction of NMs. A publication related to this subject will come out in the near future.

105. In due course, one might be in the position to make a corona FA. Adsorption onto macro molecules is an importing thing to understand. There is already some progress in understanding the protein corona, linking that to cellular uptake, and developing (Q)SARs. There is some evidence in literature that incites to start making FAs. However, the protein corona is just a screening assay and not a predictive assay. More fundamental data are needed to predict corona formation for the multitude of proteins in plasma. From a fundamental point of view, the issue is interesting. But from a regulatory standpoint it isn't. A lot of clarification is necessary to understand how the corona formation is influencing bio kinetics and toxicity.

106. Another subject for a FA is related to transformation rates. A research group is already working on it. Ideas for a general guidance on how to do a bio uptake rate are very welcome. Nano Fate tries to address the modelling of uptake into the organism and tries to predict which parameters are most important. There is no single assay available. The modified Rose-Bengal can be used as an assay for hydrophilicity / hydrophobicity, linked to surface affinity. The parameter in interaction that needs to be understand, is the hydration energy. Hydrophilicity and hydrophobicity could be relevant. There are several ways to measure it, although they have limitations (for example Rose-Bengal, hydrophobic interactive chromatography).

107. Interesting is also to link the redox cycling of an NM to the ROS generation or the oxidation potential. If the band gap has a certain potential and is in the same potential as the physiological range, redox cycling could potentially happen. Whether it actually does happen, would have to be measured in an FA. The band gap is an intrinsic property, but the ability for redox cycling would be an extrinsic property. Surface is also relevant for redox cycling. The surface properties are strongly related to the energetic parameters. FAs

108. There is a need for the FAs to reduce complexity and to narrow the current list to a smaller set of assays. They have to provide input to the model of exposure, fate, hazard and risk. The assays should also have a predictive capability.

Key Question 4: What is the best way to make the functional assays flexible for use over a range of exposure conditions?

109. FAs are a way to help regulation moving forward. The mechanistical approach starts from the other end and hopefully they meet in the middle. A balance has to be found between the needs of today and the understanding of things in the future.

110. The question is how to design a FA to cover a wide range of behaviours, for example measuring in the lungs, in a waste water treatment plant, in sedimentary soil. Ideally, a FA goes as far upstream as possible to avoid having to do too many measurements. More standardised environments (that still have to be defined) might be needed to be sure that valid and robust data are generated. They have to meet back to a regulatory point of interest or a modelling point of interest to be useful.

111. Some NMs, for instance silver NMs are difficult to track and to follow. The FA has to be designed as measurable and as realistic as possible. There are all kinds of interferences coming in for some of the kind of measurements.

Comment on the work of the BOG

112. *Mr Riego Sintes* (JRC; EU) focussed his comment on the applicability for regulations. In the discussion about methods for identification and characterisation, a distinction should be made between nice to know, need to know, and possible to know (in a reasonable time and at reasonable efforts).

113. There is a demand for FAs, which are mainly screening methodologies for extrinsic properties. They should be integrated in testing strategies and can also be used as tools to build hypotheses, e.g. for grouping and read across or mechanistical studies.

114. Often, it is not clear for which regulatory information requirement an assay is proposed. That is why the distinction has to be made between nice to know and need to know. Nor is always clear which stage in the lifecycle of an NM is addressed by an assay. This is important to put the right priorities in place. Action on this has already been taken e.g. for agglomeration behaviour and dissolution rate by developing standardised OECD test guidelines for these endpoints.

115. It is a long way to develop certain methods to the level that they provide information which is directly relevant and applicable in risk assessment. In the case of NMs, the interaction with the surroundings is very important. An example is the study of corona formation. Such a study is very interesting and important. But standardisation or information about how to apply it for risk assessment of NM, is still far away.

116. It is necessary to get clarity about the requirements that are addressed by FAs. There is also a need to have well-defined strategies that will address and lead to answers for a particular endpoint in the information requirements. The NANoREG project will help to provide this clarity. A recurrent theme of the discussion was that many factors and many scenarios will influence the behaviour of NMs. From a scientific point of view, everything is interesting. But from a regulatory point of view, one should focus on a limited number of critical scenarios and try to develop some methods that are easy (and if possible automatable) to apply for testing and assessing NMs with a reasonable effort. One by one testing of NMs or nanoforms should be avoided. Focussing on a number of scenarios and methods puts emphasis on the need to carefully

control and define sample preparation and the dose and metrics that are used in order to be able to use the results in a risk assessment.

117. Talking about methods in general, there is always the recurrent problem of validation and the need to test representative reference materials. Methods should be carried out and incorporate requisites for well-controlled conditions and well-defined protocols. Hopefully some of the current projects will deliver standard operation procedures that will support protocol standardisation. Acceptance to use a set of common protocols could be the initial step to standardisation of procedures.

118. Keeping a set of data and meta data (to be able to interpret the results of functional tests (FAs) and other) must be considered. How do they have to be reported and how can their quality be assessed? What will be done to ensure that these data will survive the research efforts and standardisation efforts to be further used for current and future methods, including *in silico* developments?

Contributions from the participants

119. After this final conclusion, there were some remarks from participants:

- For the safeguarding of databases, a more constructive and strategic approach is prepared. Discussions with the Commission about a foundation to preserve and maintain the databases are going on. One idea is to integrate them into the plans of the nano-observatory. Issue is the money.

- A common approach with regard to ontology aspects is also necessary. In this respect, there is collaboration with the NanoNet approach and Duke University. The data will become available for everybody. It would be nice if the Commission would require that, at the end of a project, all data have to be uploaded. This should be done according to the ontology and ISOTAP nano system. Ontology is foundational work and has been picked up by eNanoMapper.

- Currently, there is discussion about how to reproduce FAs across different labs and how many labs would be involved. This issue is relevant, however, needs to be tackled later. First it should be determined what an assay should look like to capture the information. Once the assay is in place, validation and reproducibility have to be considered.

- There has been some discussion about environmental fate modelling and FAs. The scientist wants to parameterize models, while the regulators are mainly interested in what matters for risk prediction. So, it will probably become a balance between these things.

SESSION 3 MN EXPOSURE AND FATE: RESULTS OF REGULATORY RELEVANCE

Chair: K. Steinhäuser, GER

Stimulus talk by T. Kuhlbusch

[Lead Expert of the TF on Exposure through the life cycle (Thomas Kuhlbusch, BAuA, GER with assistance from Susan Wijnhoven, RIVM, NL and Andrea Haase, BfR, GER)]

120. *Mr Kuhlbusch* outlines the exposure through the life cycle of NM. This life cycle starts with the production of the NM and ends with waste management. In case of exposure, there has to be some form of release from the composed material. During the life cycle, different processes can lead to release, for instance combustion. The material can end up in soil, water and/or air, and lead to exposure and potential hazards of flora, fauna, humans and habitat.

121. The Task Force has identified following areas of achievements as well as some needs for needed improvements for regulatory use:

- *Exposure related measurements*: new priceworthy and portable (personal exposure) measurement devices have been developed for nanoparticle measurements at the workplace. They can measure different metrics, reproducible and comparable (~30% for e.g. number, size, surface area). The first SOPs are being written, so they can be used in everyday life, at work places and for regulatory purposes. Testing on robustness still has to be done for the different areas and usable standards have to be developed. Currently, there are still some problems with the calibration.

- *Particle metrics* (particle number, surface area, volume, mass and reactivity): particle mass concentrations are currently mainly applied (e.g. Announcement 527, NIOSH) since the value is 'conservative' and fits to the current regulation. Improved correlations to effects are not really proven for other metrics. But measurement devices for other metrics have been and are being improved to facilitate future evaluation.

- *Tiered approach*: as measurements of exposures at the workplace are expensive, there have been about ten or twelve suggestions for tiered approaches. A diagram has been made with three tiers: information gathering; basic exposure assessment; and expert exposure assessment. The steps only have to be made as needed. The first harmonised guidances and SOP's are available. For the first tier a "framework of release" is necessary to access possible exposure. There is also a need for harmonised accepted assessment values.

- *Framework of releases*: this has multiple purposes, e.g. workplace, consumer and environment protection, safer by design and exposure assessment. Evaluation of the current test methods is needed: what methods are available? How do they fit together? Which workplace or release scenario do they actually represent? Then a ranking can be made of the amount of exposure. Also, linkage to exposure is needed.

- *Exposure reduction measures*: the general concept of reduction measures (technical, operational and at last personal protection) does apply. Technical measures for gases often also work for NM exposure (e.g. dust reduction, ventilation, closed systems) and appropriate filter for airborne and liquid nanoparticle dispersions are available. Better guidelines needed if and when specific equipment has to be used. The effectivity of PPE still has to be tested for ENM.

122. The overall aim of all developments is to ensure a safe use of ENM. Facilitating the assessment of possible release and exposure throughout the life cycle is an important issue to achieve this aim.

BOG on Consumer and Occupational exposure summary

Co-Chairs: M. van Tongeren (IOM, UK) and T. Thomas (CPSC, US) Commentator: R. Packroff (BAuA, DE)

123. This group has been focussing on a list of five questions concerning the pathway from source to exposure: NM - release – transport/transformation – exposure – dose – effect. Safer by design is often believed to concern only the design of the NM, but it also includes products and processes, as well as reduction in release and exposure potential. Therefore, tools and methods have to be developed that allow making predictions of future exposures in order to intervene before products come to the market.

Key Question 1: What is the dominant consumer and worker exposure route of concern for nanomaterials (inhalation, oral, dermal) and where are the primary data gaps/needs? What product types are likely to contribute to exposure, and what are the potential routes of exposure?

124. The dominant exposure routes for consumers and workers concern inhalation, dermal and oral. The question about dominant exposure routes may be misleading, because there are no data to determine which route does concern most. Existing toxicity data is mostly acute data or sub chronic data. The relevance of the different routes on the adverse effect (short or long term) are hardly studied at all. There are some studies on materials in native status. But when these materials are released, they tend to accumulate e.g. in dust to which consumers (e.g. children) and workers are exposed.

125. More information about products is necessary. What products contain NMs? What kind of matrices contain them? For materials that are important for consumer exposure and of which high quantities are produced, like titanium dioxide and carbon black, inventories should be made to a much higher level of precision. Stakeholders agree about the importance of inventories, but such inventories should be harmonised across Europe, to minimize the workload.

126. Ideally, such European wide inventories contain information on production figures, but also data on NM content (type, amount, etc.) that consumer products contain. In Europe, consumer exposure has already been tightly regulated for different sectors like cosmetics and food. Not everybody agrees on that. There is not much data available on NMs in food, which makes it difficult to figure out to what level people are exposed to NMs.

127. The importance of an exposure route depends on the population exposed: the whole population, or specific groups like children or workers. Usually, the type of product also determines the population (industrial or consumer goods for general or specific target groups).

128. There seems to be a blind spot in the inventory process, although it is possible to make estimations *in silico*, with some reasonable assumptions about the relative sizes and classes of production. However, the supply chain and the use are more important. Interesting is to know the exposure route from a user's perspective and to understand why NMs transform, which is rather thinking from the end of life cycle than from the start.

129. When doing a risk assessment on consumer exposure, a knowledge gap occurs in estimating the release of NMs. It makes quite some difference if all NMs in the product are released or just a fraction. There is very little data available to guide such assumptions. Therefore, empirical data about release and systematic outtakes through certain barriers are highly important. Inventories can't fix this gap, although information on the composition of the product and general use conditions would allow determining the likelihood of any release.

130. There is not much information about exposure uptake, because there are no analytics available for these complex materials such as NMs. A lot of development work is necessary to assess the properties of the NM introduced to the product and its alterations after a certain time and after digestion. Recently a project has been started with the Federal Institute of Food Safety in Switzerland to develop analytical methods for identification of NM in consumer products. Information about the starting materials is very important, but hard to get. Inventories would improve this information gap.

131. General inventories may not address the specific questions about exposure routes and exposure assessments for workers and consumers. From a regulatory point of view, there are three points to consider for prioritisation: the endpoint, acute or chronic; the level of exposure; and the uptake. To these priorities, maybe a fourth point has to be added: susceptible populations.

132. In terms of occupational exposure, inhalation is probably the most important route of concern as inhalation is leading most likely to an uptake. Dermal uptake is not the biggest concern, because the skin is a good barrier. For consumer exposures, there is more uncertainty. The primary exposure route of concern depends on the type of product/matrix, product use, population and susceptibility.

Key Question 2: Are environmental media significant sources of nanomaterial exposure for humans? What are these media and what are primary routes of exposure? Do we have important data gaps?

133. Although environmental exposure to NM may be negligible, there is a degree of combined exposure to NM that is processed or embedded in a matrix like rubbers or tyres or used in fuel for vehicles.

134. In addition, NMs may become part of the food chain. Data don't indicate a high uptake, but it has to be taken into consideration because there might be some persistent material that if taken up in the food chain may be relevant in the long term. Application of NMs in agriculture can lead to exposure through the food chain and through bystander exposure.

135. An issue relevant to consider with respect to consumer and worker exposure is the end of life. With the absence of labelling, nobody knows whether or not products are nano enabled. If inventories are established, they should include information on NM enabled products. There are projects that focus on what happens at the end of life, when NM enabled products are incinerated. They might release in the air, which requires risk management measures. There are still quite some unknowns concerning releases and fate of NM enabled products. Releases of NMs will depend on the technology of incinerators (temperature) and the treatment and disposal of ashes etc. NM enabled products are applied in buildings and in case of uncontrolled fires, engineered as well as process-generated NMs could be released.

136. Accidental releases of nano-sized particles can occur at any stage of the life cycle. Incidental releases (e.g. from combustion, traffic) are widespread; these are general process generated nano-sized particles although emission from engineered NMs (e.g. fuel additives) is also possible. Inhalation can only be expected for a limited time and a limited distance. In case of accidents, there might be high levels of releases. However, these are very localised.

Key Question 3: Are harmonised tests to assess the release of nanomaterials from products during the life cycle needed for regulatory assessment? What are the main data gaps for consumer product/product matrices or nanomaterials in terms of release? What harmonised test methods already exist and what tests need to be developed? (e.g. ageing: UV, humidity, heat, abrasion, mouthing, etc.)?

137. Release / exposure testing is important as it can inform / prioritise hazard testing and risk assessment. It also informs on the routes of exposure. Release tests for some processes (e.g. ageing, abrasion, dustiness) along the product life cycle do exist, but they are not harmonised. Some of them are from ISO,

others are textile standards or OECD test guidelines. Reliable tests have to be developed for many of the release processes, products and NMs (and combinations of these). There is currently no conceptual approach for linking the different types of tests. Hence, they can't be compared and used for purposes as exposure assessment (as essential part of risk assessment) or safer by design. Part of the complexity is the fact that specific methods for detection of NM portions are missing. It hasn't been decided whether methods that are used for conventional materials would also work for NMs.

138. For utilisation in a regulatory context, reliable tests for parameters as release and ageing are needed which can be used for the multitude of products that contain NMs. In fact, methods do exist, but the validation step is missing. Analytics and data presentation has to be harmonised. Although a huge variety of products has already been tested, a lot of polymers and textiles still need to be tested. Powerful techniques are necessary to characterise and quantify the complex materials that are released under different conditions. Labs must have the right equipment and tests must be reproducible. The NANORELEASE project showed that this is challenging.

139. Regulatory standardisation organisations should attempt to develop harmonised approaches and a paper describing how to make the tests comparable and robust.

140. As there are so many different products, it will be impossible to test them all for their release of contained NMs. Therefore, it is necessary to develop valid models to predict releases from the variety of products containing NMs.

141. For some pristine NMs, the toxicity data are known. However, pristine, well dispersed NMs might not represent the worst case situation as changes in surface functionalisation or photo activation will influence the toxic outcome. Using these materials in matrices and processes, might change the toxicology profile. The bottom line is that the worst scenarios should be studied. One has to understand exposure, releases and toxicology to identify the worst case. NMs agglomerate and can be inhaled. It makes a difference if somebody inhales one big particle or several small ones.

142. A distinction has to be made between occupational and consumer scenarios. Of course, the matrix of NMs and eventually the hazard always has to be considered. Regarding risk assessment for consumers, exposure is evaluated and then toxicological assessment follows. Important is to find out what humans are exposed to in terms of particle size distribution as well as the differences between humans and animals in responses. The understanding of release from the matrix and exposure in real life is going to be critical for toxicology.

143. There is a considerable overlap between occupational and consumer scenarios. Occupational exposure also involves professional use of products. Agglomerates of NMs have a higher surface reactivity and activity than particles that are micro sized. This is especially important to realise for worker's exposure. For bigger agglomerates, oral exposure could be more important than inhalation exposure. The difference of surface reactivity has to covered, with special attention for aggregates and agglomerates.

144. Another approach is to start with the NM enabled products. From there, the life cycle specific scenarios that have to be tested can be defined. The challenge is to develop exposure platforms that enable the generation of realistic exposure. Methods that simulate real world release scenarios and exposure must be developed. An integrated approach should start with families of NM enabled products, e.g. those in building materials. Product families should be studied one after another to determine their kind of exposures and the toxicology profiles. Grouping approaches for release testing are necessary because it will be difficult to test all individual products. There are so many products that a registry as well as data sharing is important.

145. Certain studies have to be done on a product by product base, for example for children's products. Here mouthing and other routes have to be taken into consideration. Looking at the type of use and the susceptible population to determine the exposure route and magnitude of actual release, is part of a tiered matrix. A roadmap for a harmonisation across methods, has to be set out. In this process, the differences in abrasion, ageing and weathering between simulations and the real world have to be considered.

146. For release testing, a tiered approach could be envisaged: after a first screening whether there is any potential for release, a higher tier might be necessary to quantify the release. At some point, it is necessary to know how the variability in the use affects the variability in exposure. There has to be understanding of all levels, and for each level tests and harmonised approaches should be available.

Key question 4: Regarding workplace exposure assessment: should a tiered approach be implemented? Which information is needed? What are the next steps needed?

147. Everybody agrees that a tiered approach has to be implemented. The next question would be: what is the purpose of the tiered approach and how can it support reliable decision taking?

148. Regarding the proposed tiered scheme, there are some questions about the use of the tier 2 instruments and the values they give. Concerning tier 3 (addressing variability and distinguishing from background), people wonder if the instrumentation is really as good as necessary.

149. The detailed chemical and physical analyses of airborne NM exposure in tier 3 requires the use of personal sampling equipment as well as highly sophisticated particle monitoring devices.

150. For tier 1 assessments, handheld devices are available, which can be used in the workplace area to check if there are any hot spots. Not all of them have good coverage of the full range of enabled particle sizes up to ten microns. Some portable optical detection devices can be used for personal sampling or measuring the variability and contrast in exposure between workers. As they are limited to certain size ranges, it might (in certain situations) be necessary to use more than one device. Background levels of nano-sized particles can be a major problem as many of the devices are non-specific and specific measurements strategies are required that measure airborne concentrations in the background as well as near to the source of the NM release.

151. The tiered approach requires informatics and data sharing to improve the data quality. A suggestion is to create integrated data sets for multiple purposes so that data could be reused. For tier 1 decisions (whether there is NM), data can be used from other tier 2 or tier 3 assessments. If no nanoparticles are released, it stops with tier 1. Risks can come from nanomaterial, nanoparticles, and nanomaterials in a non-nano sized form. NM may contain some nanoparticles or nano objects. They may as well be embedded in a matrix and a larger size of agglomerates. The discussion is about NM and it doesn't make sense to talk about nanoparticles and nano objects. NM has to be linked with existing risk assessment and exposure assessment schemes.

Key question 5: Exposure models for nanomaterials are currently semi-quantitative and probably rather crude. Are they currently useful e.g. for risk management and/or risk assessment? What higher tier and/or specific exposure models are required/need to be developed?

152. Crude models are available for screening: either nano-specific or existing models for conventional chemicals. Models exist for occupational and for consumer exposure. Currently, models for environment are being developed. However, validation of models is generally missing.

153. Whether those models are useful or not, depends on the purpose: useful for management of exposure or for regulatory risk assessment? For risk assessment, regulators need models that give a relatively

robust and possibly quantitative estimate of exposure. When it comes to estimating exposure to aggregated and agglomerated particles, the existing (non-nano specific) exposure models should be able to determine the exposure.

154. When NM characteristics and concentration data are available, tests can be done to determine where it will go, how it will accumulate and how transformation might take place. Bottleneck is the input information that is required to drive those models. Another issue is consistency in parameters of surrounding media. If these items are tackled, existing models can be improved. An important functional assay to develop is on how to predict what will be released with a certain type of matrix or type of manufacturing process. Understanding of exposure pathways can be used to develop higher tier models.

155. Models can predict if a group of workers will be exposed to certain parameters. However, correlation studies between models and actual measurements of airborne NM concentration suggest that models are not always good in predicting the level of exposure.

156. A key point is how to collect the data for further development of the models. Some important parameters are generally not well recorded when carrying out measurements. Robust data of the highest quality, gathered with reliable and reproducible methods are required. Harmonised databases are required to store data and encourage data sharing.

157. For worker's models, the lack of information about the effectiveness of control measures and personal protective equipment is a problem. Field studies are necessary to get more reliable data and corresponding protection measures.

Comment on the work of the BOG

158. *Mr. Packroff* remarks that it is important to distinguish between regulatory science and regulation. Regulatory Science implies high scientific quality, complex requirements, qualified and good scientists. It allows the improvement of (new) techniques (like measurement) and must keep pace with innovation. In regulation, a high legal certainty is needed. Simple requirements are needed, also to improve regulation compliance. Regulation must be addressed to "practitioners" and not to scientists and should be limited to standardised techniques. Regulation mostly lags behind of (materials) innovation.

159. Exposure assessment in regulatory science should consider the current scientific knowledge from risk research (e.g. toxicological grouping approaches); use and correlate different metrics (to improve scientific hypotheses on risks for man and environment); compare new and established measurement techniques (to find out the best for regulatory purposes); correlate exposure and release measurements; help to make exposure models better (information about the background and protection measures is needed, mostly these things are not well-documented); and gather reliable data for safety-by-design of products and processes.

160. Exposure assessment in regulation should start from the potentially harmful entity as a whole (e.g. respirable particle fraction, WHO fibres); must lead to clear and unambiguous decisions (because of liability, compliance et cetera); needs (legal) benchmarks or exposure limits; should be based on simple and well-established models or measurement techniques (tiered approach) because it is very complicated and a long way to go to implement new techniques to regulation; should make use of easy available information (e.g. from (M)SDS, release testing results); and should fit to control banding approaches. It has to be kept in mind that SME (and consumers) regularly do not carry out exposure measurements!

161. From the session it can be concluded that inhalation of respirable particles is the most important concern for workers. Standardised dustiness/release tests may offer a reliable starting point for risk

assessment. There are still significant scientific gaps to be filled in for consumers; especially for the end of the life cycle.

162. In a regulatory science, that keeps pace with innovation, there is need to generate reliable exposure data for NM. These data are necessary to improve models et cetera. This is a permanently ongoing task. But one needs to consider that specific regulation for NMs might be misinterpreted. People might think that NMs are a common group of concern, which is not the case. From current scientific knowledge, one can wonder if nano specific regulation is justified?

Contributions from the participants

163. After this final conclusion, there is one question from participants:

- Are there programs going on in Europe to monitor household NM in dust or other non-worker exposures?
  There seem to be measurements on dustiness testing planned in households too, but there are no common measurement programs regarding dust in households. For dust, it is very difficult to discriminate between sources. Usually, the potential exposure is estimated from release potentials of consumer products rather than by direct measurements. The US Consumer Product Safety Commission studies the deposition in dust if there are releases of materials in the indoor environment. Potential concentrations and accumulations in home environments are studied as well.

Stimulus talk by A. Baun

[Lead Expert of the TF on Environmental Fate, Persistence and Bioaccumulation (Anders Baun, DTU, DK)]

164. *Mr Baun* gives an outline on environmental fate, persistence and bioaccumulation. He provokes the participants by stating that persistence and bioaccumulation are only of minor regulatory relevance. Distribution or fate as such are not, unless something can pick it up. All data that can be generated for environmental fate processes are of course of very high scientific interest and are needed, but if there are no models or any other ways to handle those in the regulatory context, the regulatory relevance is not so high.

165. How relevant inclusion of the different compartments in chemical fate modelling is, depends on the environmental transformation process. NMs act in another way than dissolved chemicals, for which the present models were developed. These models were based on assumptions that are not true for NMs. The scientific basis for the models that are going to be developed, is not known.

166. The situation for the environmental distribution and transformation processes of NMs is dynamic. There aren't any equations to support it yet. The mix of environmental conditions is very complex. It will be difficult to suggest a simple test design that can solve that once and for all. For regular chemicals, this has been done in FAs (functional assays). They are a sort of a black box; nobody is quite sure about the processes, but experts trust the numbers that come out. OECD draft test guidelines for environmental fate endpoints are on the way and will contribute to increase the relevance and reliability of tests.

167. Two processes of environmental behaviour of NMs are identified as key: dissolution and agglomeration. Both are currently addressed by drafting respective OECD test guidelines specific for NMs, however, hetero-agglomeration is not yet addressed for such test guidelines. For modelling, sedimentation considerations have to be included into the equations for the estimation of fate, if agglomeration is ongoing.

168. Transformation processes are a big topic. They are actually not very well-supported by literature. On biodegradability, the OECD "inherent-functional-assay-defined-property" is only relevant for carbon-

containing NMs. Biomodification will take place under environmental conditions, but very little is known about the processes themselves or how biomodification will influence fate and behaviour (and effects) of NMs in the environment.

169. There is a table available that shows the relative importance of transformation processes for modelling of environmental fate of AgNMs. Such a table might help moving forward in determining which processes are to be included in a model.

170. Bioaccumulation is a very important process in ecotoxicology and in risk assessment of NMs in the environment. Direct toxicity is important too, but bioaccumulation has been studied far less. Although it is such an important process for regulation, it hasn't been studied that much. So, the draft OECD guidance document that is on the way on dietary exposure is highly needed, as well as more study on intake versus uptake and in other organisms than fish.

Stimulus talk by B. Nowack

[Lead Expert of the TF on Modelling of Environmental Fate and Exposure (Bernd Nowack, EMPA, CH)]

171. *Mr Nowack* gives an outline on the modelling of environmental fate and exposure. The goal is to get, in the end, environmental exposure to predict environmental concentrations. At the moment, the environmental exposure of engineered NM (ENM) can't be measured. So, modelling is the only option.

172. There are two kinds of models. Material flow models (MFA) predict releases from production, fate in technical systems and final release to the environment. Environmental fate models (EFM) describe the further fate in the environment and distribution within environmental compartments. The EFM rely on the input from the MFA. In the end, they lead to PEC-values (predicted environmental concentrations).

173. The Task Force considered eight MFA models, from simple algorithms to advanced web-based models. They were published between 2007 and 2016 and applied to a variety of ENM. Also, eight EFM models, published between 2012 and 2016, were considered. These models were on different scales (well mixed compartments, spatially explicit, and watershed); steady state and fully dynamic. In view of main fate processes, most of them considered hetero-agglomeration, sedimentation, dissolution and sediment transport.

174. The concepts behind modelling are described well. The algorithms or codes are the basis: for some models a scientific paper describing the codes is available. Some codes can be found online and others are not openly available. The input parameters are often given as extended supporting information. How the input parameters were obtained, is not always transparent in MFA studies.

175. MFA models are robust, although input parameters might be uncertain. In some models uncertainty is not covered, and in others probability distributions are used. Production volume and product distribution have the highest uncertainty. As data from release experiments is quite incomplete, it is not very useful for MFA. Only few materials and product categories have been experimentally studied.

176. Environmental fate models can be separated into three tiers. Tier 1 are equilibrium models. Their relevance is highly debated in the literature. Tier 2 concerns steady-state models. A hypothetical scenario is modelled, that allows comparisons between different substances. This approach is accepted by regulators, e.g. SimpleBox. SimpleBox4Nano is based on SimpleBox. Tier 3 provides dynamic and spatially resolved models: MendNano, NanoDUFLOW, Rhine/Rhone models.

177. MFA is a standard method to quantify flows of materials. It is rather a way to organise information than a model. All fate models use an MFA approach to get the input, although this is normally not called MFA. There are no nano-specific parameters in MFA; one just follows a material. MFA results should

therefore, without any reservation, be acceptable for regulatory purposes. EFM should also be acceptable. SimpleBox, as steady-state compartment model, is accepted by ECHA. Therefore, SimpleBox4Nano (that is based on SimpleBox) will likely be accepted, too.

178. There are no analytical methods available to specifically detect ENM in environmental matrices. Current methods to detect NM can't see whether it is engineered or natural, which means that PEC values – and thus the MFA and EFM models – can't be validated. However, a validation of processes can be performed based on lab or mesocosm studies. But, modelling and analytical studies are able to provide an orthogonal view.

179. One of the knowledge gaps concerns experimental data to parameterise the models. Such data are often missing. For hetero-agglomeration, only some data are available that are useful for modelling. And how to include chemical transformation? Another knowledge gap is the input on size distribution. EFM models need this input, but nobody knows what the size distribution of actually released NM is. It is assumed that it is equal to the pristine NM, although many nanoparticles that enter the environment may be in the form of "chunks" with embedded nanoparticles. The third knowledge gap is that, so far, only "generic" nanoparticles, like titanium dioxide, are considered in the models. Specific forms (mineral forms, coatings) may behave in a completely different way.

180. Production/product distribution is the main unknown input parameter for MFA. As the definition of NM is central, only sources that follow the EU definition, should be used. In an ideal situation, not only NM would be modelled but just particles; not only the nano-size, but the complete size distribution of a material. Quantitative data for product distribution, going beyond consumer products, need to be obtained. MFA models should include data on material characteristics, such as form, size distribution and transformations.

181. When it comes to EFM, intensification of collaboration between MFA and EFM model developers is necessary. The output from certain models serves as input for other models. The input to the environment needs to be separated into dissolved ENM, ENM contained in matrix fragments, free ENM, and transformed ENM. Size distribution of all forms should be available, and separation of a generic ENM into different forms with different chemical identify or coatings is needed.

182. Basic parameters such as dissolution rate and hetero-agglomeration need to be provided by experimentalists, in a way that they are also useful for modelling. Process validation of models can be done by mesocosms or small-sale tracer studies.

Contributions from the participants

- The outcome of a previous session was that, for NM, mandatory registration or inventory on an EU level (beyond what is already part of regulation) won't be conducted because it hasn't been proven an effective step forward. This will impact the ability to use these MFA models as input to the transport models. Inventories of productions are the basis for the MFA models. There are some national registries that can be used and complemented with market studies.

BOG on Environmental Exposure, Behaviour and Fate (including modelling) summary

Co-chairs: P. Westerhoff and F. Von der Kammer, Commentator: J. Ahtiainen.

Key Question 1: Which processes do you see important for environmental fate and which data are necessary to take them into account in EFM models? Please consider specifically: how flexible should

environmental fate models (EFM) for NM be with respect to include processes that are different compared to models for conventional chemicals? Are fate estimations based on pristine NM relevant? (Aging and interactions may rule the fate)

183. Written answers on this question say that important properties of pristine NM should be considered, as well as how they are released into the environment, in terms of aggregation or agglomeration, and (bio)degradation of coating. Dissolution, bioaccumulation and biotransformation of the NM are important, as well as surface chemistry and attachment deficiency. Ion release is relevant in terms of how much particulates will be sustained in the environment. Maybe waste management, end of life, recycling and accidental release should also be incorporated into the environmental fate modelling. Hazard should be estimated for transformed materials as well as pristine.

184. The Breakout Group had no big reservations about the models themselves. EFM should be tiered, progressing in conjunction with toxicity. A tier 1 screening might tell where a NM ends up, while tier 2 is a larger and more complicated model. In tier 2 specific rate constants are needed that might vary once there is more information on certain intrinsic properties. Tier 1 is a screening level and tier 2 is detailed, mechanistic and site specific.

185. It looks like NM models will always be dynamic, whereas for conventional chemicals in most cases equilibrium partition based models can be used. Concerning the EU definition for NMs, there is a grey area. NMs smaller than about twenty nm may feature the highest bioavailability. They feature more effects and reactivity than other ones. Around one or two nm, NMs have really high diffusivities compared to the larger ones. They start to distribute themselves in a matter that may well approximate by K_{ow}.

186. NM are different forms of chemicals and therefore have different behaviour, although some conventional chemicals like radionuclides aren't fit for equilibrium models either. Depending on the tested organism, the behaviour and effects, and the system certain kinetic time frames for such chemicals, as EPI Suite (a series of EPO models that break half-lives into minutes, hours, days, weeks or years), might also be suitable for NM. The questions about lifetime (of the nanoform), residence time (of a particle in a product or environment compartment) and how they relate to each other are relevant.

187. In this context, equilibrium shouldn't be confused with steady-state. It is important to determine when the steady-state should be considered. A model for steady-state doing mass flow with a lot of uncertainty about production amounts, could be valid to figure out environmental fate for new NMs. Another kind of model, considering different timeframes, will be necessary for currently produced NM where some of the reaction constants will be more important.

188. Fate models should be flexible. Research projects on exactly designing how transformation happens, are very expensive. In most cases, it will be sufficient to know that within certain parameters, a transformation will always happen. Such leaps must be justified by functional assays (FAs) or chemical understanding. For slower reactions, the hazard value for the soil should be set on the transformed form of the particle: fate models should explain that a certain nanoparticle can only get into the soil through certain reactions. Models should have some expert judgement to identify critical mechanisms that need system level complexity.

189. Models provide information about the important processes and parameters. Pristine NM is easy to work on, but the relevance of utilising data of pristine NMs is questionable. In the process, it is relevant to consider the variability of what happens to the NM and the variability of the environment. A model has to be able to take both into account, on a temporal level. Modelling pristine nanoparticles is a good first step, but many products may not contain pristine NMs as they might transform. Such transformations are important and should be classified (hydrolysis, et cetera).

190. To define the aging process, which is part of the fate process too, is challenging. There is an endless number of permutations for different environmental conditions that effect the aging process. A first step might be to look at standardised toxicity testing, which features harmonised conditions. Some additional parameters like pH and salinity (as in the standard tests for chemicals) might be included in an NM ageing test. Some of the fundamental parameters which will control ageing will include agglomeration, behaviour, dissolution or coating potentials. In a toxicity test mimicking natural environment, NMs will be altered by media ingredients, which means different kinds of fate processes will take place. It is not clear which ones are the most relevant. One should also realise that, before entering the environment, the form might already be completely transformed. The pristine material (the particle size distribution obtained from the manufacturer), is probable not what is needed to model the processes in the environment.

191. The only way to deal with the importance of all processes, is to do sensitivity analysis of the model. That will allow to determine on which processes the most accurate estimates are needed and which ones could be less relevant under certain conditions. Validation of such models is, even for conventional chemicals, impossible. However, there must be an insight on how to validate models and how much accuracy is needed for the input.

192. Ideally, environmental fate models and tiered testing strategy on environmental fate should resemble each other as much as possible. A tiered approach will be necessary to get relevant information. The guidelines for environmental fate of normal chemicals are going into the right direction.

193. For conventional chemicals, many different EFMs exist (spills, large spatial distribution, metals, organics) and they are somewhat application specific. One model may not be the sole objective for NM. Scenario relevant fate models should be developed. For each particular scenario, the parameters, the type of materials, the concentrations and the levels have to be considered.

194. An issue is the understanding of the interfaces between different environmental compartments and the handling of those interfaces in existing models. In some cases, expert judgement will be enough to determine where the mass of a material goes. However, for multi-media models (air/water/soil/biota), the connection and interaction with NMs might need studies on a higher level.

195. With a certain level of knowledge about specific models for each of the processes, a global fate model can be stitched together. Those stitches might rely on some expert judgement. Release pathways for certain scenarios with certain products or NM forms can be predicted. It won't be necessary to cover every detailed model for all products. Linking all models together, is very difficult. It is not realistic to expect NM modellers to do so. Nevertheless, one should think about linking models.

Key Question 2: What validation of models do regulators require in a situation where PEC values cannot be validated analytically?

196. Actually, concentrations of NMs can't easily be measured in environmental matrixes. There were only a few written answers on the validation question. Harmonised test methods are needed to estimate or measure input parameters.

197. Rather than regulating NMs, nano-phases should be included in models for conventional contaminants. The overall fate models for conventional chemicals are not validated because it is virtually impossible to do so. Not all necessary measurements can be done and not all information about sources and individual processes is available. For the moment, validation of overall fate models for NM won't be possible either. The best solution is to validate individual processes for specific scenarios or specific NMs, considering specific types of environments. The multitude of validated processes can give an overall estimate

of how well a certain model might perform. Validation should be broken down to a process level, and probably combined with some sensitivity analyses.

198. Breaking down is meant to learn to understand basic principles and processes. The work should go on with as few unknowns as possible. Understanding of certain processes, can probably be transferred to other types of NMs without doing all experiments again. The information about parameters and their relationship that is obtained from breaking down processes, can be put into SimpleBox4nano.

199. Confidence in models is gained from understanding the fate or being able to predict the fate in simple experiments or mesocosms. For fate, some predictive models that work in mesocosms have already been developed.

200. Validation of models by data from microcosms control tests are more important than validation of the models in the field. The focus should rather be on validating EFMs for micro/mesocosms than on natural systems (which are mostly very dynamic). Such validation would identify processes and models to prioritise mechanisms that need better data. However, some monitoring in the field is appropriate because it would trump any modelling data. It is not possible to analytically differentiate engineered, pristine, natural NMs. However, field monitoring should not be overlooked, but not used to validate EFMs.

201. Relevant is also to realise what the purpose of a model would be. The European concept of the generic PEC generation gives a generic value for a whole region. PECs derived from EFMs can support hazard testing in terms of choosing relevant test concentrations. Size specific risk assessments can't always be related to generic models. In regulation, measured environmental concentration always outranks predicted concentration.

202. Regulators need harmonised methods for the input parameters (to get well-validated data) and perhaps simplified process experiments. For certain cases, there might be methods to derive PECs available in the near future. Regulators have to be convinced that models are transparent enough to explain what is happening, but it is not possible to go much further in terms of validation.

Key Question 3: Are we able to account for dynamic and complex changes in a realistic way in current tests? Are current test guidelines applicable for a fate prediction, looking at the simplicity of tests and the complexity of EFMs?

203. This question is about the input, which could vary from intrinsic properties to more behavioural properties or functional assays. Test guidelines can parameterise EFMs or validate EFMs. The current test guidelines for chemicals are not adequate for NM. Test guidelines and guidance documents how to use existing test guidelines for NM that are now under development by OECD are important and will improve the situation.

204. Many NMs are metal-based; they may have similar fate and further behaviour as the metals. The tools to deal with the metals, may also be used for NM. The definition of soluble with respect to soluble NMs is relevant. Metals are not really soluble, but small metal particles are equally bioavailable as metal ions. That's why, for risk management, the kinetic dissolution rate in various media has to be included in models.

205. The hypothesis that NMs will behave differently, should not be put away although those that have been looked into more detailed, seem to behave similar to normal metals. Produced metal nanoparticles look, in clinical tests, completely different. They have specific surfaces which result in certain behaviour. Some metal particles behave like organics because of their surface coating. Maybe it is true for the aquatic compartment that metal NMs behave the same as metals, but this might not be the case for soil or terrestrial ecotoxicity data in terms of bioavailability on the long term.

206. Experimental data from a UK-US project with an ionic form of silver and a particle form of silver, is available. Toxicity tests in sewage sludge in the field after six months showed that, for the ion species, the effects were as expected from the total silver levels. But the effects for the NM fraction were much higher. The difference couldn't be explained. At considerable time scales, NMs doesn't seem to behave the same as metals. A conclusion might be that current test guidelines don't include the right time span or the starting material that is put in the test has to be re-considered. One has to know the fate timeline of the NM and test it in the soil when it will transform to its most toxic component.

207. Nano phases have to be incorporated in existing models for metals. The reason why nanoscale metals provoke different responses with respect to toxic effects than the dissolved forms, might be that it can go to places and deliver little packets of the toxic ion.

208. The length of test durations is an issue. For metals, the test duration seems to be not long enough to allow stable chemistry to develop in test systems. Therefore, metal risk assessment has to include a specific factor for uncertainty. Not only the action per se is relevant, but also the delivery, transport mechanisms, bioaccumulation. Those things need to be understand, which means that lots of data and meta-analysis are necessary. Obviously, the same is true for metal NMs.

209. The focus on environmental behaviour and fate has been on relatively simple (metal) oxide NMs. One needs to move forwards to more complex materials as e.g. used in batteries as properties like dissolution behaviour of these materials can be totally different. When it comes to mixed metal, NM behaviour and effects cannot be predicted from behaviour and effects of ions in an aquatic environment. The assumption that all metals behave in exactly the same way as the simple ones that have been studied until yet, is not correct.

210. There is a need for tests that are independent of the time frame and of the concentration of NMs. Test can give information about the complexity of a system and can be done in a simple idealised system with pristine nanoparticles or in a complex system. Surface affinity of NMs needs to be addressed in such tests. The surface affinity can be scaled up to any time frame and to any concentration of particles. Relevant are the surface of the particle and its interaction with other surfaces and encounters.

211. Interaction with surfaces is a fundamental NM related process with different ways to measure it. However, interpreting data on surface interaction tests is challenging with respect to environmental significance and outcome.

212. The interpretation of test guidelines results has to be changed. When assessing metal NMs and their dissolved ions, it is important to know how and where NM delivers its metals. Using existing tests for NMs, whether they are fate or toxicity tests, might not necessarily give the worst-case scenario. Normal assumptions that would be used to put the PEC and the PNEC together might don't work as expected. Such precautions have to be taken into consideration. The tests and the spectra of environmental variables that are used as input for the tests may resemble those that are used to test metals. However, it has to be figured out if and what NM specific considerations have to be included in the test performance.

213. Current tests on environmental behaviour do not accurately represent dynamic or complex systems. Once the full complexity of processes and the different factors determining the processes of NMs in the environment is understood, the important parts need to be included in a nano specific test guideline.

Key Question 4: Is bioaccumulation testing necessary or is prediction by functional assays possible?

214. There were several written answers on this question. One says that bioaccumulation of NMs can't be done by prediction, which means that testing will be necessary. The general opinion was that first the processes have to be understood more in detail before a simplified approach (FAs) can be developed. More

and more is learned about the processes and the parameters that control bioaccumulation. On the way investigating bioaccumulation, the controlling parameter similar to K_{ow} can be identified that control the uptake.

215. Testing and interpreting data on bioaccumulation for NMs is complicated. Organisms can change the speciation and the dissolution rates as well as the properties of the NMs. This makes the test systems more complex and dynamic. Until yet, this hasn't been studied in detail. Tests have to be done for metal NMs and organic NMs. Testing might end up in some FAs that are able to predict bioaccumulation.

216. Bioaccumulation can be linked to the effect, to how much toxicity it causes to the organism in the corresponding test Data on bioaccumulation will investigate in detail how NMs will distribute in the organisms body. In case of metal NMS, they might deliver their metal ions in different ways and might accumulate in different parts of the organism. There is no clear understanding where NMs will accumulate in organisms, how bioavailable they will be and will distribute in the body, and how stable they remain. Organic chemicals can't be used that simply as an analogy. A process has to be set out for developing methods to enable the characterisation of NM after entering an organism (target site of accumulation, alteration of physical chemical properties of accumulated NMs…). For carbon-based organics, labelled tracers are relatively easy to use.

217. Species differences exist with regard to bioaccumulation of NMs, e.g.in the amounts of copper that they accumulate, depending on whether they have hemocyanic blood or not. Certain traits in species will decide how NMs accumulate. Understanding of such processes is necessary to be able to predict trait. Trait-based ecotoxicology is just starting. Trait-based models to predict the real effects in organisms still have to be developed. That will take a long time, but traits for species will provide information about bioavailability and uptake. In this context, other definitions (bio-accessibility and bioreactivity) might be relevant, as well as alternative and/or additional properties of the NMs that enter the organism, their transformation, and what happens inside the organism.

218. Bioaccumulation is influenced by the ratio of two rates (uptake and depuration). It happens within organisms and across the food chain. A guidance document, with tiers, is being developed on how to apply dietary approach to test NMs bioaccumulation using fish. This guidance is needed as the standard bioconcentration or bioaccumulation test (OECD TG No. 305) in fish, is probably not that relevant for NM.

219. There is a call for more scientific insight and basic understanding of the whole process of bioaccumulation of NMs. Data adequate for regulatory use need to be reliable (reproducible) and relevant. FAs are supposed to answer relevant questions. It seems tempting to define the investigation of surface affinity as a FA because surface affinity is an underlying basic for toxicity and might take the role of the K_{ow}.

Comment on the work of the BOG

220. *Mr Ahtiainen* summarises the session. He got the impression that the test guideline for agglomeration is in order. There might be some structural changes, but there has already been a public consultation in OECD member countries. Hopefully the same applies to the draft dissolution rate test guideline, which should be available soon for public commenting. Another guidance document – on how to use the data and the methods for agglomeration behaviour and dissolution rate in aquatic fate - is also well-advanced and had been available for public commenting. The guidance document for bioaccumulation is currently on hold, but the discussions at the meeting show there is a lot of need for it.

221. A harmonised method on surface affinity seems to be necessary. In developing harmonised methods, people should ask themselves if these FAs only serve for environmental fate modelling or can also help in (eco-)toxicology testing. In some cases, they might perhaps serve both.

222. Environmental fate models should have tiered approaches and options. To run the model and to see where the material goes, it won't always be necessary to get all the input data. Testing strategies, including the test methods for fate and the input data for the models, should be tiered as well. They should resemble each other, so that answers for the data gaps can be found.

223. Bioaccumulation testing in fish is based on OECD Test Guideline No. 305 (normal bioconcentration testing for chemicals in aquatic conditions), which has now been updated including bioaccumulation by dietary exposure. This guideline is useable. A guidance document on how to apply it to NM, is not available yet.

224. The best that can be done in terms of validation, is to harmonise methods and validate the input parameters as well as possible. Then simplified process experiments can be set up. If possible, real world measurements should also be done. The only way forward, at least to get input parameters for the models, is to have good harmonised and validated models, hopefully at OECD level.

Contributions from the participants

- Harmonised tests are needed, but what works for one NM doesn't have to work for another NMs. Validated tests must have enough flexibility to work over a range of NM's. They also must balance between simplicity and complexity (measuring different processes and complicated matrices). The existing test guidelines can be used for effect testing. But for guidance in OECD guidance documents, a matrix type of approach has to be considered. It won't be easy to get all the input in guidances, but the world has to be informed about the best practicalities that are known. This information should be published in guidance documents. If necessary, these guidance documents can easily be updated in contrary to the test guidelines. Certain guidelines won't be able to tackle all NMs. Whether a different experimental approach would be necessary for other NMs, has to be decided case by case. Harmonising shouldn't go that far that tests don't provide good answers anymore.

SESSION 4: MN HAZARD: RESULTS OF REGULATORY RELEVANCE

Chair: K. Steinhäuser, GER

Stimulus talk by G. Oberdörster

[Lead Experts of the TF on Human Health and biokinetics in vivo (Günter Oberdörster, University of Rochester, US)]

225. *Mr Oberdörster* states that respiratory tract dosimetry requires knowledge about exposure -> dose -> response relationships. Importantly, one needs to know the dose, to be able to come up with a reasonable risk assessment. *Mr Oberdörster* shows a classical risk assessment and risk management paradigm modified for engineered nanoparticles, consisting of hazard identification and hazard characterisation, exposure assessment (extremely important) and risk characterisation; all of these are required for risk management decisions. Risk, being a function of hazard and exposure, is the basis of this concept, and what needs to considered is to have appropriate data on dose-related toxicity.

226. Regarding physico-chemical and functional NM properties of relevance for toxicology, *Mr Oberdörster* focuses on solubility or dissolution rate and surface reactivity. He points out that specific surface reactivity, i.e., response per cm^2, is a most appropriate metric.

227. *In vitro* and *in vivo* assays with nanoparticles show a good correlation (*in vitro* cell-free ROS and *in vivo* inflammation) which is quite promising. It is suggested to further pursue validation of this concept of specific surface reactivity.

228. A graphic of ROS/cm^2 response of anatase TiO_2 in cell free assays as a function of particle size, shows that response per surface area can be size-dependent. There is a big jump in ROS activity between 10 and 40 nm, which might be due to surface defects.

229. It is important to define positive and negative benchmark material. It would be best to have a quantitative endpoint to measure the effects; in addition it should be mandatory to get information about the retained lung burden. Only then, one can really establish an exposure-dose-response relationship and eventually extrapolate to chronic exposure levels in rats, and then to derive the human equivalent concentration and eventually compare *in vivo* and *in vitro* tests.

230. Regarding biokinetics, it can make a very big difference if NM is inhaled or administered as bolus.

231. When comparing *in vitro* and *in vivo* dose metric extrapolation in nanotoxicology for respiratory tract exposures (see included graphic), a key issue for both *in vivo* and *in vitro* is the deposited dose, rather than the exposure concentration in the medium (*in vitro*) or in the air (*in vivo*).

Questions

232. When dealing with chronic diseases and chronic exposure, the dose-rate is the key issue. It makes a huge difference in the response if for example a worker is exposed to a certain accumulated dose in forty years, or if that same dose is given in a fraction of a second as a bolus in *in vivo* or *in vitro* toxicity tests. It is not scientifically acceptable to use the argument that the deposited or retained dose in the lung inhaled over a long period of time, should be considered in an *in vitro* study or in an *in vivo* instillation study as equivalent.

BOG on Human Health *in vivo* and toxicokinetics summary

Co-Chairs: L. Tran (IOM, UK) and F. Cassee (RIVM, NL), Commentator: W. Kreyling (Helmholtz Centre Munich, GER)

233. In this Break Out Group five key questions on human health have been discussed.

Key Question 1: It seems to be desirable to establish benchmark materials for facilitating predictive toxicity testing. If you agree, what properties should benchmark materials have?

234. There is a need for benchmark materials. Benchmark materials should be robust, easy to handle and there should be different benchmarks for different endpoints. Furthermore, the material has to be very well characterised as either positive or negative controls. Positive and negative controls can be different for different biological/toxicological endpoints; it can even be a soluble chemical. When it comes to 'well characterised' in terms of their effect on biological parameters, one dose may not be sufficient.

235. A big constraint in testing is that animal testing is that it often is not allowed to test a chemical that has already been tested extensively. In addition, *in vivo* studies can become very expensive and it is not done at present with regular chemicals either. It was suggested to incorporate at least one dose level of a benchmark material in order to make a better comparison with other studies. For *in vitro* studies there is a lot more flexibility.

236. If one or several benchmark particles are selected, also some round robin tests need to be done. Afterwards, this can be used as guidance to another dispersion protocol, to another dose or to other particles. The round robins should result in standard operating procedures (SOP).

237. Since the availability of benchmark materials may be limited, one needs to specify for which purpose the material can or should be used.

Key Question 2: Should acute toxicity *in vitro* studies, as proposed by Wiemann et al (2016) and validated by the results of STIS (short term inhalation study) be standardised for identifying toxic vs benign NMs?

238. The short answer is yes. Today, standardisation can only be defined for poorly soluble, granular low toxic materials. It is still too early to define it for other and more complex materials. Moreover, although rapidly emerging, the dosimetry issue has not been resolved yet. Often it is not known what the biological effective dose is in *in vitro* test systems.

239. Short-term inhalation studies (STIS) should be seen as qualitative studies, only suitable for hazard identification and ranking. There is no consensus yet on the optimal design, preventing also standardisation by OECD. Besides that, a difference should be made between assays just for hazard ranking purposes and to identify potentially toxic material and assays for risk assessment. Regulators would prefer predictive assays and scientists would prefer assays that are as close as possible to the biological reality and address mechanism of action.

240. The question remains what kind of *in vitro* studies need better standardisation to identify toxicity and to what extent materials can be ranked in toxicity. There should be a whole battery of tests; not just one that would allow to get a good understanding of the mechanism of action and link to adverse outcome pathways.

241. The complexity of the 'whole' body still prevents us to rely on *in vitro* tests only.

Key Question 3: Dose metric vs static and abiotic dissolution assays vs bio dissolution: what is the significance of determining it, which approach should be favoured?

242. For an *in vivo* system, where equilibrium will never be reached, *in vivo* dissolution tests are needed, in order to understand what is happening in the body. The toxicity is not an effect of the particle per se but can also be the result of the solubilized fraction. It is suggested to use a single dose and then study the clearance very qualitatively to get an answer on the dissolution as well as clearance kinetics.

243. Given dynamic exposure as well as flows in our body, and compartments e.g. lysosomes in cells it is recommended to use bio dissolution i.e. dissolution in the organism as input for kinetic modelling, no static or artificial dissolution systems.

Key Question 4: Dose metric extrapolation modelling of NM risks: what are the requirements, and how to proceed?

244. Assay the delivered dose, for which also different metric can be used depending on the port of entry. For lung the dose per unit lung surface area might be helpful.

245. The retained dose should be included in *in vivo* studies, whether it is oral or inhalation, because it helps to extrapolate from animal to human study. The retained dose after 90 days would be a good measure to come up with. This can also be related to a secondary organ dose and to the clearance kinetics; then extrapolation modelling makes sense. One issue with NMs to consider is that at higher doses, the degree of agglomeration increases, and the absorption or uptake can relatively decrease. So, there is no dose proportional uptake. Still the retained dose is important, especially in cases of a partially soluble agent that is deposited in the lung for a certain time. Then there is a chance that the dissolved irons might be taken up through the lung, and afterwards distribute in the organs. At this moment there are no tests available yet for new NMs.

246. A No-Observed-Adverse-Effect. This can be helpful to assess the Human Equivalent Concentration and uncertainty.

247. Next, there is a wish-list to assess the dose for clearance kinetics at multiple dose levels, multiple time points during and after an exposure, multiple organs, delivered dose, to confirm the relation between exposure and dose in the lung. There are different clearance kinetics. Particularly NM can be highly deposited in the conducting airways, but it will also be eliminated very rapidly from there. So when analysing the doses, a distinction should be made between the cleared material and the substantial uptake. Part of the dose will also be exhaled from the respiratory tract. Usually the exhalation part is not measured in studies. Last but not least, there is also the difference in species.

248. In drug development, a lot of oral toxicity testing is done. It might be interesting to share this knowledge.

Key Question 5: What`s specific about nanotoxicology?

249. Nanotoxicology is not specific in the way that there are not infinite possibilities for cells or tissues to respond to toxic stimulants. NMs are only size specific which affects the delivered dose as well as the biodistribution and clearance kinetics. Once they are in a certain tissue, the toxic effects are most likely conventional, either particle (physical) or solubilized components (chemical) driven. Nobody has ever observed any nano specific toxicities so far and we might not be able to find any. What we do know is that these particles may show different kinetics, and may end up in different organs. However, in guideline testing, toxicokinetics come rather late in the game; this is already an issue with NMs. A good guideline/guidance for biodistribution studies should be developed.

250. A big difference between micro- and nanometer size is that the microparticles are efficiently captured by and retained in macrophages present on the epithelium, while a fraction of NMs can interact with epithelial cells, can translocate into the interstitium and even be cross this barrier and reach endothelial cells and the blood. So there is a different distribution – translocation behaviour. Therefore, the adaptation of the current guidelines should be speeded up.

251. Maybe very tiny, highly localised doses of dissolving NMs could be very, very dangerous (many manganese miners develop a kind of dopamine impairment). So researchers who take concern for particle toxicology should upfront ask for biokinetic data. This changes the toxicity profile, because particles get to other target organs. OECD TG 417 for toxicokinetics already exists for oral intake. Maybe an addendum should be created for inhalation. From a scientific point of view, a new TG 417 would be the way to go, but this is very difficult to realise. From a regulatory point of view, one has to make informed decisions, and if there is no big probability that there will be a translocation to other organs and no systemic toxicity, there would be no need for this kind of TG 417 testing. At present there are only a few studies to guide such a decision.

252. Another nano specific risk is the exposure to mixtures. Particles will allow some other chemicals (by adsorption) to go to organs or places where they are not normally going. NMs can become mixtures and carriers of other pollutants.

253. Moreover, in pregnant animals, effects in the offspring were found. Therefore, dosimetry, biodistribution (kinetics) and size considerations remain important issues. Studies should preferably be long-term, at least 90 days to investigate long term and delayed type of effects.

254. The Break Out Group mentions the following aspects to be taken into the white paper:

- Make a distinction between scientific interest and what is really needed for regulatory purposes.
- Find additional evidence to build read-across or weight of evidence.
- Surface treatments: if you modify something on the surface, is it still the same nanoparticle including unchanged physicochemical properties?
- Start with a biodistribution/biokinetics study.
- Consider to give some solid advice on what to avoid, promoting a safety by design product. A sort of avoidance list.
- Since there is REACH and CLP, and ECHA connecting all the data and relevant information, producers themselves should also collect available information.
- All specialists should work together on this.

Contributions from the audience in the plenary session:

- Ranking in the potency of NMs depends on the extrapolation of the findings for *in vitro* towards *in vivo* animal and from *in vivo* towards humans. When it comes to hazard, just recently a correlation between the *in vitro* and animal *in vivo* is seen. There is a lot of data and the ranking can be confirmed, but extrapolating from animal data to humans is still very far away, because there are still many uncertainties. However, a lot of studies reports describe that *in vitro* and animal *in vivo* do not correlate very well likely due to wrong dose assumptions, so caution is needed here.

- *In vitro* data should still to some extent predict what happens *in vivo*, whether it is in human or in animal. Therefore, there is a challenge for *in vitro* work to come up with tests that allow better predictions. When it comes to *in vitro* and *in vivo*, there is also a difference in terms of hazard versus risk characterisations. Hazard seems to correlate quite well with lots of *in vivo* data, whereas a risk characterisation with *in vitro* results is not possible yet. With *in vivo*, this can be done.

- Benchmark material is necessary, because a lot of issues are not specific for NMs. If benchmark material shows more or less the same patterns, fewer studies may be necessary.

- Once benchmark material has been established and validated, testing on animals is less necessary, because historical controls can be used as a comparison.

- There are a number of bio-distribution studies showing that particles are going to the brain and that they also interact very much with the immune system. Therefore it is useful to have a good bio-distribution study before starting any toxicity testing, because then one knows which target to look for. If there is a real concern that the material might have the potential to cross the blood brain barrier, then there might be the necessity to do a neurotoxic test.

- Another aspect is the issue of cardiovascular problems. LDL particles are an NM, NMs may mimic effects of endogenous particles.

Stimulus talk by B. Rothen Rutishauser

[Lead Expert of the TF on Human Health and biokinetics in vitro (Barbara Rothen-Rutishauser, University of Fribourg, CH)]

255. *Mrs Rothen – Rutishauser* gives an outline on "Human health and biokinetics *in vitro*". In cooperation with Barbara Drasler, she reviewed no less than 120 papers on this topic.

256. In cell culture experiments, several key factors have been identified: nanomaterial (NM) properties of pristine materials, dispersion of NMs, behaviour in complex environments and dose metrics, choice of relevant cell types, characterisation of growth and differentiation and relevant endpoints, including controls.

257. For the characterisation of NM it is important to know the source of the materials and their physicochemical properties analysed by a combination of different techniques. The most relevant parameters are size and size distribution, shape, agglomerate/aggregate state, (effective) density, surface area/charge/reactivity, solubility and crystalline phase.

258. Storage, "aging" of the nanomaterials (NM), is also very important. If they are produced, how do they behave over time? Which changes do they undergo if they stay in labs and fridges for years? Dispersion protocols for NMs have to be standardised.

259. When NMs are localised in complex environments such as cell culture media, other components (proteins, amino-acids, iron et cetera) can induce changes in the primary NM properties, like aggregation, dissolution and the formation of corona.

260. In view of determination of the delivered dose onto the cell surface, *Mrs Rothen* mentions the dosimetry approach, "*In vitro* Sedimentation, Diffusion and Dosimetry model" – ISDD. The dosimetry approach should be much more encouraged, because there is the need to know how many NM really reach the cell surface and how many NM are taken up by the cells. Air-liquid approaches which are currently more often used to assess effects in lung cells allow to directly measure NM mass delivered onto the cell surface.

261. It is necessary to apply realistic NM concentrations and avoid overload concentrations. More data from industry – that has done a lot of *in vivo* experiments - is required; it can be very difficult to find reliable data from *in vivo* or human assessment data, to enable count backs on how much NM have to be applied in the cell culture experiments.

262. It is recommended that the physicochemical characterisation of NM has to be done in the pristine form, and during and after the experiment. Investigation is required of the behaviour and interaction of protein or biological corona, to assess the secondary properties of NM, the stability or biopersistence of NM in cell culture media, or physiological fluids.

263. It is recommended to use at least two dose metric parameters to really describe the cell culture experiments and conditions.

264. The use of occupationally relevant concentrations in cell culture experiments is needed.

265. Regarding cell cultures, it is important to report in publications on cell source passage number and the precise cell culture method. One should characterise and report cell growth (proliferation rate) and differentiation; different cell types require different differentiation protocols.

266. Cell types should represent the primary organs of entry. Disease-related endpoints should be included; HeLa cells should not be used anymore.

267. Standardisation and interlaboratory comparison is needed, for which the use of cell lines is recommended. The final goal here is the use of primary cells and more complex models; this part really is not satisfactory in all the publications and there is a lot of work to be done.

268. On biokinetics (uptake and intracellular fate, different time points and long-term analysis) *Mrs Rothen* mentions that evidence from intracellular localisation indicating a specific cell response, was not found. Therefore, this might not be relevant for regulatory purposes, however different time points have to be considered.

269. Where do the particles end up (biopersistance)? They may be trapped in the mucus, or intracellular in the lysosomes. Omic tools are recommended for the identification of mode(s) of action (MOA).

270. Omic data present the first step in identifying adverse outcome pathways (AOP's). Again, more *in vivo* data is needed, to validate what has been found or described so far *in vitro*.

271. Cytotoxicity is described a lot in the reviewed reports. There are many colorimetric and fluorescent based assays; interference problems are often seen, so false-negative or false-positive results are seen a lot. In one paper, colony forming efficiency (CFE) assay was considered to be one of the most promising tests for general NM toxicity evaluation; could this also be applicable for confluent cells types? This could be discussed in the breakout group.

272. Other found endpoints are impedance-based measurements, oxidative stress and (pro-) inflammation.

273. Many papers have also looked at genotoxicity. Bacterial based tests, i.e. the Ames test, are not appropriate, because non-soluble NM cannot penetrate the bacterial wall. Therefore, comet assay and micronucleus assay done with mammalian cells are recommended.

274. A combination of these tests is needed: two to three representative cell lines, five concentrations of NM and included negative and positive controls. After certain time points, comparison of different

methods is needed. In general, for cell culture assays, it is recommended to use several cell types, several concentrations, endpoints and different tests, and of course negative and positive assay controls, benchmark controls and particle controls. For 'omics approaches, a non-biased selection of endpoints is recommended, in order to not miss relevant reactions.

275. From a regulatory perspective, different genotoxicity and mutagenic endpoints are needed, in order to not miss one important event.

276. On regulatory relevance *Mrs Rothen* mentions testing strategies and she refers to the functionality-driven decision-making framework for the grouping and testing of NM (DF4nanoGrouping) and the stepwise approach to categorise NMs (from the NANoREG project). So far, none of the reviewed frameworks have been approved. However, there are some very interesting ideas and concepts which should be looked into.

277. Final conclusions: so far, *in vitro* test results are not sufficient for regulatory decisions. Many different parameters have to be considered for cell culture experiments. However, they can be helpful for the ranking of NM and to report key events, which might then lead to adverse outcome pathways (AOPs). It is very important to focus more on characterising the particles before, during and after the test performance. There is need to use well-characterised cell models and to choose relevant endpoints, as well as more than one assay for one endpoint.

BOG on Human Health *in vitro* summary

Co-Chairs: A. Haase (BfR, GER) and B. Fadeel (Karolinska, SE) Commentator: M. Sharma (PETA, UK)

278. In this breakout session, the participants focus on four key questions. The questions will be addressed from the point of view of relevance for regulation.

Key Question 1: Which physicochemical characteristics of NMs before, during and after *in vitro* test performance are most relevant? We have noted that the main ones for the pristine NM include: size, size distribution, shape, agglomerate/aggregate state, density, surface area, surface charge, surface reactivity, solubility, crystalline phase. Additional parameters to characterise the NM in the *in vitro* system may include: hydrodynamic size, protein coating and hydrophobicity.

279. The main characteristics for the pristine nanomaterial include: size, size distribution, shape, agglomerate/aggregate state, density, surface area, surface charge, surface reactivity, solubility and crystalline phase.

280. Additional parameters to characterise the nanomaterial in the *in vitro* system may need to be included, like hydrodynamic size, protein coatings, and hydrophobicity.

281. All parameters listed here represent the most important parameters that need to be assessed. Not all of them are necessarily changing during the exposure, so the question is which parameters really need to be measured during and after the exposure. One more parameter may be added, namely impurities.

Key Question 2: Which exposure time point, i.e. hours, days, weeks, sampling frequency, i.e. biokinetics and NM exposure concentrations, i.e. dosimetry aspects have to be considered for *in vitro* test assays? How can we move forward with respect to improved *in vitro*/*in vivo* correlation? Should *in vitro* dosimetry calculation like it is done with ISDD or similar models be an integral part of all *in vitro* testing approaches?

282. The discussion can be summarised as follows:

1) Dosimetry models are available; their use should be encouraged to supplement every *in vitro* study in order to derive the exposure concentration on top of the standard models.

2) Also for *in vitro* studies a fulltime course is needed (which time and how many time points may be endpoint specific)

3) Are chronic *in vitro* models available? (cell lines vs primary cells).The use of primary cells may have to be considered.

Key Question 3: What are the most important considerations, for each aspect of *in vitro* testing noted below? Which cell types should be used: cell lines or primary cells? What about advanced model systems including co-cultures, 3D cell spheroids or organ-on-a-chip models?

283. There are two different opinions on how to start testing:

- Screening/ testing established endpoints: Start with cell lines and then validate them with primary cells.

- By using primary cells: Start in the lab by putting together complicated models that try to mimic what really happens *in vivo*, to have relevant endpoints and results.

Tissue types, i.e. which routes of exposure are most important for the tested NM: inhalation, oral or dermal?

284. Before starting testing *in vitro*, it has to be considered how humans may be exposed to the tested NMs and then to choose the corresponding cell type for testing.

285. This raises the following questions and remarks:

- Can exposure route and system be mixed or should they be kept separate. Multi-cellular *in vitro* exposure should be taken into consideration, because particles can circulate and the exposure may be through secondary messengers, or a combination of secondary messengers and translocated NMs.

- Do NM trigger specific pathways? Use existing AOP's as a starting point.

- Other target organs should be kept in mind. For instance, if NMs can translocate into the brain, what is the effect on neurons?

Which functional assays are most important: cytotoxicity, genotoxicity, 'omics assays? Which endpoints are most important?

- Immunotoxicity, inflammation and fibrosis are relevant endpoints which need to be addressed in corresponding assays.

- Cytotoxicity should always be done in order to get the adequate dose range.

- Genotoxicity is also important.

- When it comes to inflammation, the investigation over a time course is very important. Inflammation is a normal defence response that goes up and then resolves. Chronic inflammation

is going to be pathological, so without a time course, no distinction can be made between "good" inflammation and "bad" inflammation.

- It is important to distinguish assays from endpoints. What is actually meant by a 'functional assay'? What is an endpoint? What is needed to assess and interpret the data, and what do we actually measure?

- Tests should address the most sensible endpoint.

Is there need for negative and positive NMs controls and benchmark materials? (to rule out interference of NMs with assay reagents)

- There is no generalised benchmark material or generalised positive control material yet. But there is definitely a need for it.

- The purpose will define what kind of negative/positive control material or benchmark materials are suitable. This depends very much on the endpoints.

- Interference testing is needed, but there is no guidance on that yet.

Do you agree that, where justified, at least two *in vitro* tests should be applied to assess the same endpoint?

286. The participants confirm this question.

Key Question 4: Do you agree that the following testing strategy elements are relevant for regulation?

287. Several points are added to this question:

Physicochemical characterisation of NM, protein (bio-) corona, analysis of NMs biopersistance/biotransformation in relevant physiological fluids

288. The protein (bio-) corona a parameter was proposed as relevant parameter in a test strategy as information on the corona formation will deliver information on the characteristics of the NM and support parameters used for predictive models. Regulators need to know adverse outcomes *in vivo* and whether there are any actual links between adverse outcomes and protein corona assessments has to be clarified.

289. The question is whether at the moment, such a testing strategy with protein corona assessments is needed. For *in vitro*, corona assessment can be relevant. However, for *in vivo*, relevance of corona assessments is still unknown. It may not be robust and predictive enough for assessing *in vivo* hazards. Thus, characterisation of corona might currently not applicable for regulation at this moment.

290. Biopersistance depends on the cells that are used for investigation, rather than the physiological medium. Biopersistance is a very important characterisation feature. The question is raised whether there are some kinds of simple cell models that can determine biopersistency of a NM. Can biopersistence be measured *in vitro*? For carbon based NMs this is actually already done within the NANOSOLUTIONS project, both using cell media and also simulant fluids.

291. The difference between biopersistence, biodurability, dissolution and biotransformation needs to be clarified. All these phenomena are related and part of the same discussion, but they are not the same thing. If the difference is not clear, the discussions are mixed up.

292. It can be concluded that physicochemical characterisation of NMs is urgently needed for consideration in a testing strategy for NMs including *in vitro* data.

Choice of relevant cell models including thorough characterisation of cell growth and differentiation, and relevant functional assays

293. This item was not discussed.

Assessment of cellular nanomaterial uptake and biokinetics, i.e. cellular fate

294. Here too, the protein corona plays a role, because the uptake into a cell is very much defined on the surface of the material. If the corona is modifying this strongly, then this has a tremendous effect on the speed of the uptake, the mechanism of uptake etc.. Upon entering the cell, then again the surface including the protein or the opsonization, which is a better term, determines the interaction inside the cell which should be of regulatory interest.

Use of (high-throughput) screening methods using conventional or validated end-points and system biology approaches using ´omics" methodologies to identify (novel) end-points, in support of AOPs

295. How can we speed up the validation process to really implement the *in vitro* testing approaches into regulatory testing frameworks?

296. The solution may be the use of IATAs, Integrated Approaches for Testing and Assessment. Decisions should be taken, whereas there is no time for new methods to be developed or validated. However, the regulatory agencies are not ready to accept data which comes from an assay that is not validated. For chemicals there are different rules: running a full validation is not needed; a catch-up validation will do. It is suggested to apply catch-up validation also to NMs.

297. Another idea for speeding up validation is by selecting between accepted assays. However, it is not very simple to find model NMs for such validation.

298. There was not enough time to discuss the relevance of ´omics" approaches. However, the group highlighted that system biology approaches and ´omics methodologies should be considered as relevant methods in the ProSafe review (Joint Document).

Comment on the work of the BOG

299. In the plenary session, *Ms Sharma*, remarks that three things were brought up multiple times throughout the conference:

1) What do we actually need to know and what would be nice to know?

2) We are not really applying all the techniques.

3) Existing tools can mostly be applied to NMs considering the NM properties are taken into account.

300. We have to keep in mind that the number of NMs that are being or will be used in commercial products is increasing and it is impossible to test each NM. Therefore, we have to use existing tools or we have to develop new ones. We have to consider relevant dose metrics and dose. There are a number of validated *in vitro* methods that are used for chemicals that can be applied to NMs. It is agreed that a single *in vitro* assay cannot predict what happens in humans after exposure to NMs. We have to think of *in vitro* assays in terms of a battery of assays to assess a complex biological outcome rather than relying on a single

assay. To predict better outcomes, existing adverse outcome pathways (AOPs) should be used where possible. AOPs are not specific to a chemical or NM. AOPs can be combined with other existing information to develop integrated approaches for testing and assessment or IATAs. But for all of this to work, we need to standardise methods and possibly speed up validation, which is not an easy task. In absence of validated methods, we can make use of a cause and effect analysis that was recently published by Rösslein et al. The cause and effect analysis looks at the different steps in *in vitro* methods and identifies where variability can arise and then reports it. In the end, *Ms Sharma* likes to add that researchers are looking to understand how NMs might affect human health. Therefore, they need to figure out how to use the available tools. Integrated methods are needed that apply all the tools together to predict what happens to humans after exposure to NMs.

Contributions from the participants

- *A participant* is curious about the *in vitro* method validation in the European context, what is the controlling body that is the most acceptable to regulators concerning the validation process, besides OECD and ECVAM?

- *Ms Haase* answers that there is no single entity that has the power to do single validation, but in Europe ECVAM (European Centre for the Validation of Alternative Methods) is involved. OECD does not organise validation; validation is handed over to ECVAM or ECPH and OECD continues or stops.

- *A participant* has a question about the validation of *in vitro* methods. If we ever get to a standardised *in vitro* method that could predict lung information, what would be the way to validate that? Would *in vivo* studies be used as a golden standard to compare *in vitro* results to?

- *Ms Haase* answers that there are two issues. For any kind, a selection of benchmark materials, positive and negative controls, is needed. This is the first problem with NMs. Negative controls are not as complicated as positive ones. Besides that, the process should be speeded up with catch-up validation, but positive controls only refer to methods that are validated for chemicals. If the political pressure is there and high enough, processes can go very fast, so a validation process can be speeded up.

Stimulus talk by A. Baun

[Lead Expert of the TF on Ecological Effects and biokinetics (Teresa Fernandes, Heriot Watt University, UK)] > replaced by Anders Baun.

301. *Mr Baun* gives an outline on aquatic ecotoxicity and biokinetics (focus on aquatic compartment due to lack of time for this presentation to also focus on soil and sediment).

302. A lot of papers are said to be of poor quality, which may be due to lack of regulatory reliability and relevance. Perhaps there is also a lack of insight whether the data fits to the regulatory needs. *Mr Baun* and his colleagues tried to take all the data published on ecotoxicityof TiO_2 NM in the open literature and tried to rank it according to regulatory adequacy. There are some criteria for that and there are few studies that fit, but that does not mean that the other ones are of low quality. Regarding quality, *Mr Baun* urges people to consider whether it is a bad study or whether it does not fit the desired purpose. This is very important, because the whole idea of ProSafe is to talk about regulatory adequacy of data.

303. After the ProSafe review eight recommendations were given:

1) NM dispersion stability is key for reliable ecotoxicity testing.

   – A lot of tests have been done where the dispersion was not stable.

2) From “control” to “describe” (test dispersion stability before, during, after testing).

3) Quantification of NM solubility and dissolution kinetics is crucial for disclosing ecotoxic effects.

4) Physical versus chemical effects of NM in aquatic toxicity tests.

5) Pros & cons of addition of dissolved organic matter for stabilising test dispersion.

6) Chronic toxicity (and organism uptake) is feeding dependent.

7) Data selection for dose-response assessments should be structured, reproducible and transparent.

   – Data that can be used for hazard identification purposes, so for classification and labelling, require high test concentrations. That is a need for regulation. In order to meet that requirement tests are modified and therefore they are unrealistic.

8) Environmental realism versus generic data with high reproducibility for regulatory purposes.

   – There is a call for environmental realism, but what are the costs? It is possible to lose control and that researchers violate the concept of high reproducibility.

304. Some quotes from *Mr Baun* in his slide presentation on aquatic ecotoxicity and biokinetics:

- The fundamental principle in ecotoxicology is, like in toxicology: everything is a poison; it just depends on the dose.

- We want to study effect as a function only of the dose or concentration.

- ISO is much more rigid than the OECD test guidelines. In the preamble for the method is written: “This method is applicable to: chemical substances which are soluble under the conditions of the test, or can be maintained as a stable suspension or dispersion under the conditions of the test”. So it is clear: if a substance cannot stay stable, it is not suitable for the test.

- If we cannot control the experiments, we will have to describe what is going on.

- A unifying concept for reliable determination of NMs effects: four “pieces of the puzzle” need to be in order and fit: dissolution, internalisation in the organism, physical effect and known NMs Mode of Actions.

305. The expense of environmental realism is that one loses control. The developed standardised tests were founded on control. So, when doing hazard identification, labelling and classification of chemicals, tests are used that are deliberately unrealistic for the purpose of ranking and identification.

BOG on Ecological Effects summary

Co-Chairs: R. Klaper (University of Wisconsin, US) and N. Hartmann (DTU, DK), Commentator: A. Kennedy (US Army, Corps US)

Key Question 1: An important aspect of nanomaterial ecotoxicity testing is dispersion stability.

To which extent should one attempt to maintain concentrations >80%? What are the constraints/challenges?

Which mixing method (stir, sonication method) is appropriate in what circumstances?

How should nanomaterials be added to aquatic and terrestrial test systems, respectively (wet, dry, diet) and depending on what purposes?

Should dispersions of certain nanomaterials be prepared differently, e.g. addition of NOM for CNTs and recognition that certain methods of dispersion may lead to changes in nanomaterials (e.g. probe sonication and CNTs)? Furthermore, what are acceptable trade-offs for dispersing nanomaterials for ecotoxicological testing (considering stability vs environmental realism)?

How should the stability and concentrations of nanomaterials be characterised in suspensions to define actual exposure? How might this change with the test conditions and organisms used (pelagic, sediment dwelling etc.)?

306. First some slides are shown with the results of the survey using the key questions to open up the discussion. Some topics from this presentation:

- In the Joint Document it is stated that artificially forcing conditions (i.e. dispersion stability) may lead to artefacts that may well influence the effects observed. Important to discuss is: How far should one go to maintain a stable dispersion? What are acceptable methods and how is the 20% rule applicable to nanomaterial testing.

- Mechanical methods to disperse NMs in test systems seem to be fairly acceptable. Not too many artefacts should be introduced.

- pH can be varied within certain limits to enhance stability.

- On the utilisation of natural organic matter no clear agreement was reached.

- Media modifications or other exposures could be applied, like semi-static, flow through or circuit circulation systems.

- Sonication may generate some pit falls, some risk of artefacts.

- It should be accepted that agglomeration and sedimentation occur; that is a property of NMs, so that should be allowed in the test.

- A general question is how much the system should be controlled, as there is the issue whether realism is more important than stability.

307. The Break Out Group comments as follows.

308. Agglomeration and sedimentation of a NM in a test system depends on the dose and therefore the dose is relevant for an organism that is floating in the water column. Measuring and figuring out the real dose is difficult, especially for NMs that are not metals. The real dose can just be determined after all 'reactions', e.g. agglomeration and settling behaviours of a NM in the test media. What is left behind and staying stable after agglomeration, might not represent the total population of the particles. If there is some fraction that remains stable, one might should consider to do the exposure on the stable fraction, to characterise what that is.

309. Many NMs tend to adsorb to test organisms which introduces an unclear exposure situation to the test system. In the different guidelines, there are suggestions for various ways of dealing with this, but this varies from guideline to guideline.

310. Another challenge is doing a time weighted approach in a multiple factorial dynamic situation. How should this be measured throughout the course and integrated into a dose response? Regulatory testing should be straightforward, easy, with high throughput; it should not end up into a complex research situation. For regulatory testing one should simulate and measure simplified situations. It is suggested to distinguish between different states of the NMs in the system to get closer to the real exposure situation. In general, a procedure should be found to describe systems over time.

311. The utilisation of flow through systems when testing NMs was discussed as alternative to static renewal of test suspensions. It was stated that semi-static might be a solution to find the right balance between renewal periods and stable concentrations.

312. The choice of the test media will influence the physical-chemical properties of the tested NMs and thus the bioavailability and in turn the results on ecotoxicity. As a compromise, test media could be used in two steps: First use a highly standardised test medium for every test organism. This allows comparing all the results of the different NMs and interlaboratory differences. Secondly, a more refined environmental relevant medium could be used to enhance environmental realism in the single test.

313. The utilisation of standard ecotoxicity tests are indispensable with regard to regulatory risk assessment ("need to know"). On the other hand, scientific research focuses on a broader understanding of mode of actions explaining the observed effects ("nice to know") which results in data not always addressing the needs for regulatory risk assessment. Closer cooperation is needed.

314. There is a need for standardised dispersion methods regarding stability and possibly more environmental relevant media standards (more realistic exposure conditions).

Key Question 2: For nanomaterials that tend to dissolve how should we deal with the dissolved fraction of nanomaterials in ecotoxicologal assays (aquatic and terrestrial)? What is the consequence for the interpretation of dose-response data for dissolving and partly dissolving nanomaterials?

Furthermore: how do we account for the breakdown of surface chemistry and release ligands or other materials from the nanomaterial's surface?

315. Dissolution rate is a key parameter for interpreting the ecotoxicology effects of dissolving NMs. Data in dissolution are valuable for interpretation and should be taken into account for grouping of NMs. Ideally, one should measure dissolution kinetics in the presence of test organisms in the corresponding test media as the organism itself might influence the dissolution of NM.

316. Some nanoparticles dissolve immediately and other ones are poorly soluble. Each of these cases may involve different measurement approaches. It is suggested to distinguish NMs based on kinds of dissolution rates and decide on appropriate needs for analytics and data interpretation.

317. The survey answers covered a wide range of different opinions:

- Dissolution is relevant, time dependent and can be used to predict (and explain) toxicity by differentiating between fractions.
- Dissolution is relevant, depending on the NM, dissolution processes and rate.
- NMs should be considered as a whole and one should not differentiate between fractions.

318. The attendants agree that knowing the dissolution is important to interpret data, but the question is whether to refer the effects to the concentration of the released ions or to the concentrations of all of the whole NM.

319. It is also important to consider a potential altered effects of aged NMs in the test suspension and finally, impurities are very relevant to consider and measure when ecotoxicity tests of NMs are carried out and the results are evaluated.

Key Question 3: What are the best (most sensitive) aquatic/sediment/soil test organisms?

320. The immediate recipient of NMs will be in most cases the water compartment, but the settling behaviour shows that exposure of sediment compartment will be relevant as well.

321. Some answers from the survey were:

- There are not any best organisms, but the more sensitive ones are the pelagic organisms.
- Comparable to traditional chemicals the sensitivity can vary across organisms. Daphnids, algae and bacteria are the most sensitive organisms.
- More information on alternative endpoints is needed, because the current endpoints are not suited for identifying all nanoparticle effects.
- Chronic effects are more sensitive than acute endpoints, and have a higher weight in hazard assessments which should be considered when choosing the appropriate test organism.

322. The Break Out Group discussed this question as follows.

323. The life stage of certain organisms is important to consider as it will affect sensitivity towards and/or bioavailability/uptake of the NM. Often standard test organisms are less sensitive as sensitive species are per definition more difficult to keep under a laboratory conditions.

324. Fish embryos are used to avoid animal testing, but they seem to be rather insensitive, especially with regard to NMs in case they cannot pass the chorion. Thus, they cannot serve as overall surrogate for fish testing. Another way to reduce fish testing is using organisms like algae and daphnia, like it is done for traditional chemicals. However, if only lower species are used for testing, certain endpoints can be missed and relevant trophic levels are not adressed. More information on appropriate alternative endpoints should be collected, since the current endpoints may not be suited for identifying all possible NM effects.

325. The best test organism is probably the one that gives the answers that the regulators needs; so it is best to use a range of organisms with different sensitivities, phylogenies. Tests with sediment organisms and soil organisms are needed. Like it is done for traditional chemicals, extrapolation from aquatic pelagic testing

to sediment should be tested for appropriateness for NMs, too. Testing on conventional test organisms should remain for making comparisons from a regulatory perspective. Chronic endpoints feature more sensitive data than acute endpoints and as such have also a higher weight in the hazard assessment. Chronic testing will become more important in hazard assessment, also with respect to modelling and grouping of NMs. Unfortunately, there is currently no good correlation between acute testing and chronic testing of NMs, thus no extrapolation can be done.

326. From a data-integration point of view, capturing and communication artefacts occurring in (standardised) testing would be really helpful in the context of harmonising protocols and methods. It would support researchers considering copying pre-standardised methods and trying to avoid challenges and mistakes that have been made before.

327. Positive and negative benchmarks can be useful for NM ecotoxicological testing. They might also capture differences in dispersion preparation. However, conventional chemicals as reference substances might be sufficient.

Key Question 4: Are there *in vitro* tests that may be appropriate for nanoecotoxicology and what considerations need to be made regarding the types of exposure conditions?

328. The Joint Document states that progress in this area is promising, but there is still further need for comparisons between *in vitro* and *in vivo* systems to evaluate the validity of *in vitro* environmental assays in regulatory testing with respect to the investigation of human health effects. However, *in vitro* testing is not likely to replace *in vivo* models for risk assessment purposes.

329. The survey results indicated that *in vitro* tests are not appropriate assays for ecotoxicology.

330. The Break Out Group mentioned the following:

331. The correlation and interpretation of results from *in vitro* or *in vivo* testing is not a nano-specific problem. It is a generalised problem that the toxicology community is facing. In ecotoxicology, the focus on using *in vitro* methods should be on describing mechanisms; ecotoxicology tests are cheaper than tests used in human toxicology and whole organ testing in ecotoxicological test settings poses less ethical issues compared to tests with mammals: This is why there is not the same 'push' as for human toxicology.

332. However, benefits for some *in vitro* tests are a higher throughput – but this is also achievable with some whole organisms.

333. For *in vitro*, cell cultures might be relevant to identify some points of entry. Thus, to develop cell lines for different points of entry for different organisms as they exist for fish. Additional types of cellular based assays for other test organisms that represent interaction points between biological surfaces and NMs would improve understanding of these interactions on a cell basis.

Question 5: Distinguishing between effects vs artefacts.

a) What is the best way to design and conduct ecotoxicity studies that allow the distinction between different types of effects and artefacts in ecotoxicity tests with nanomaterials (such as physical effects, effects of shading)?

b) What is the best way to deal with feeding in long-term studies (as this may interfere with nanomaterial behaviours, ingestion and excretion)?

334. Joint Document states that “In general, guidance on separating different types of effects caused by either physical interaction or by dissolved ions is necessary to elucidate the toxic mechanisms, but also in addressing concentration dependent effects which do not correspond to the dose-response paradigm. Physical effects must be accounted for, by including shading controls in algal tests as addressed in the OECD guidance for aquatic testing of NMs (OECD, 2016a)”.

335. Answers from the survey using this key question include: conducting more round-robin tests may help to identify artefacts; the occurrence of artefact probably depends on NM characteristics; and artefacts like physical effects or effects of shading are up for debate as one might accept them as effects due to the particular property of the NM;

Contributions from the Break Out Group:

336. Physical effects can depend on the life stage and they are quite important to measure and consider. However physical effects can also be seen as artefacts, which leads to the discussion about the difference between physical effects and artefacts. An artefact could be defined as something that messes with the test system that would not be happening in the environment. However, even if it is a physiological effect, it is an effect and not an artefact. One should report artefacts in the reporting section of the test guidelines documents. Another issue is to differentiate between what is the actual mode of toxicity, whether it is a physical effect or a chemical effect.

337. For algae testing it is a little bit more complicated to say whether it is an artefact or not. The used dose is often at such a level that effects of shading rather that intrinsic toxic effects of the tested NM are measured which probably doesn`t need consideration for risk assessment. However, there is a difference between shading of the algae due to adsorption of the material on the algae or the turbidity of the test dispersion which will lead to shading. It was recommended to distinguish between method artefacts and the instrumental artefacts.

338. As artefacts are dose-related, overloading the test system will intensify the introduction of artefacts. This could be avoided by preparing the test dispersion which is allowed to settle to an extent that represents a semi stable or maximum concentration.

339. It is recommended not to use limit test for determining upper effect concentration ranges for NM as high concentrations of test substances are used for limit tests. A bioavailability of NMs at higher concentrations might be lower than for lower concentrations due to greater extent of agglomeration and limitations this introduces wrong results.

Comment on the work of the BOG

340. Mr Kennedy comments on this issue as follows.

341. Currently a OECD Guidance Document on Aquatic (and Sediment) Toxicity Testing of NMs is under developed which focuses on guidance and recommendations for testing NMs using the existing OECD test guidelines on aquatic and sediment toxicity testing of chemicals. The following recommendations were mentioned in the BOG which also address the draft guidance document:

- Measures need to be taken to make the tests more consistent, reliable and replicable.

- Dissolution kinetics ideally should be measured in presence of the test organisms. Pre-tests (no organisms) allow planning to avoid pitfalls; the tests should be monitored extensively. It is more accurate to consider the dissolution rate, rather to measure dissolution at one time point.

- The quality criterion of 20% recovery of the test substance is a challenge for testing NMs. There a different options to deal with this criterion. The BOG concluded that both agglomeration and sedimentation is system dependent and in the same time properties of the NM. Thus, both need to be considered during testing and reporting.

- Organic matter should perhaps not be used as an additive because it may mask toxicity. If DOC is used to stabilise NMs, an additional test without DOC should be conducted as control.

- The influence of food needs to be addressed.

- Phototoxicity is beyond the scope of the current version of the guidance document. It might be added in a next revision.

- Alternative endpoints also need to be addressed in a revision.

342. In order to get environmental realism, one has to acknowledge mesocosms. Mesocosms can simultaneously consider the exposure, fate and hazard of the test substance. However, the value of microcosms that don't represent the environment is that they often provide a worst case hazard value and a comparative hazard value relative to other substances that are evaluated.

343. Some overarching questions were mentioned by the commentator based on the experience working on the draft guidance document:

- It is simpler to focus on the worst case hazard value instead of focusing on the stable concentration. It is suggested to first promote stability and if that doesn't work, allow the NMs to settle. Stability can be promoted either through medium manipulations or adding energy to the system and allow the particles to settle. It is important to kinetically measure and describe the exposure.

- In terms of describing the exposure, when the particles simply cannot be controlled, the suggestion is to look at nominal arithmetic means, geo means and time weighted average, depending on the nature of the exposure.

- Alternatively, considering the stable fraction for hazard testing only will focus on the hazard only rather than on hazard combined with a complex exposure situation which will complicate hazard assessment.

- Guidance on pretests should be given to inform on stability. During the bio assay, once a decision is made, promoting and providing guidance for kinetic sampling of the exposure is necessary to describe it.

SESSION 5: MN TIERED TESTING AND TOOLS FOR RISK ASSESSMENT: RESULTS OF REGULATORY RELEVANCE

Chairs: K. Steinhäuser, GER and Phil Sayre, US

Stimulus talk by A. Oomen

[Lead Expert of the TF on Risk Assessment and Management (Agnes Oomen, RIVM, NL)]

344. *Ms Oomen*, RIVM presents a short overview of the risk assessment and management frameworks, which subject will be extensively discussed during the BOG of Thursday, December 1. The details are mentioned in the joint document.

345. Nanomaterials (NMs) can vary in their physicochemical properties. As a consequence, a large number of different nanoforms with different physicochemical properties exist now and even more may exist in the future. These different physicochemical properties can result in a different fate, toxicokinetic behaviour, hazard and consequently different risk. However, it will not be possible to test each nanoform extensively. Hence, there is an urgent need for efficient information gathering methods for risk assessment of NMs. This can be achieved by efficient Risk Assessment (RA) frameworks and/or grouping and read-across approaches.

346. The present RA frameworks for NMs are diverse in their aim, applicability domain, basic assumptions and alignment to regulations. For example, some frameworks are quantitative and others are qualitative, some are directed towards information for regulatory evaluation and others are directed toward the innovation chain, etc. A best RA framework can therefore not be indicated.

347. A review of the current regulatory framework in view of NMs is provided in NANoREG D1.10/1.11. The ECHA/JRC/RIVM approach on read-across between nanoforms outlines overarching principles on read-across for NMs.

348. Given the diversity in scope, a few RA frameworks can be indicated as better screening-level and specific RA frameworks.

349. The better screening-level RA frameworks are the NANoREG nanospecific approach as described by Dekkers et al. (2016), focussing on materials, and the NanoRiskCat as described by Foss Hansen et al. (2011), focussing on products. These frameworks are considered most transparent and elaborated: choices are underpinned using scientific information (as far as possible) and build upon existing approaches for normal substances. It should be noted however, that they remain qualitative.

350. The better specific RA framework is the DF4nanoGrouping framework to assess inhalation risks as described by Arts et al. (2015ab). This is the only fully elaborated specific risk assessment framework, i.e. including clear decision criteria, triggers/cut-off values and tools. However, an independent evaluation of these triggers and methods has not been conducted, and the framework does currently not integrate easily into existing processes specified by regulations such as REACH. Hence, the regulatory acceptability is unclear.

351. RA frameworks in between screening-level and specific that also seem useful are the risk banding framework by Oosterwijk et al. (2016), and general screening for environmental risks by Hund-Rinke et al. (2015).

352. There are several issues to consider when trying to find ways to come to structures that allow for efficient information gathering in risk assessment of NMs.

353. First, physicochemical identification and characterisation of each nanoform is the starting point for information gathering for risk assessment and finding options for grouping and read-across. This information is thus required.

354. Second, science is not sufficiently advanced to allow for the derivation of scientifically-based cut-off values and decision criteria. There are concerns particularly on chronic effects due to NMs, a.o. related to persistency and accumulation, which will only increase slowly in time. As a consequence, the likelihood of false negatives in RA frameworks cannot be assessed.

355. Third, as there is an urgent need for increased efficiency in risk assessment of NMs and science is not sufficiently advanced to make informed choices that can increase the efficiency, we should ask ourselves the question if pragmatic, partially scientifically underpinned choices are feasible.

356. Finally, it is known that physicochemical properties of NMs relevant for their potential risk may change during their life-cycle. It is anticipated that hazard studies with well-dispersed, pristine materials often represent a worst-case. It should be considered if hazard studies should in general be performed with the pristine material, or if other options and arguments should be made possible or required.

357. For the path forward, the following considerations and options are proposed:

358. The number of NMs with a safety issue could be reduced by further encouraging considerations on human health or environmental risks in the innovation chain, as is for example outlined in NANoREG D6.4.

359. For NMs that reach regulatory evaluation, two options can be considered.

360. Option 1: continue in line with current regulation. Here, some increase in efficiency is possible via grouping and read-across. The outline according to the ECHA/JRC/RIVM scientific document can be used as a basis.

361. Option 2: focus on NMs with greatest potential for hazard/risk. To that end, pragmatic, partially scientifically underpinned choices for decision criteria are needed, e.g. cut-off values, trigger values, tools and methods. This would require cooperation between different stakeholders and international agreement. As a starting point, the aspects most likely influenced by nanospecific properties can be used, as for example in the NANoREG nanospecific approach described by Dekkers et al. (2016). Alternatively, an independent investigation of the decision criteria, their order and the tools and tests in the DF4Nano Framework can be used as a starting point.

362. Option 2 may be applied to NMs already on the market to gain experience with this approach. The thus obtained insights can be used in the first place to regulate the most hazardous nanoforms according to the current understanding. Subsequently, a more generic understanding on the behaviour of NMs can grow continuously. This will add to the feasibility of pragmatic, partially scientifically underpinned choices for decision criteria, tests and tools.

BOG on Risk Assessment Frameworks and Grouping Strategies summary

Co-Chairs: A. Sips (RIVM, NL) and F. Le Curieux (ECHA), Commentator: C. Studer (BAG, CH)

363. This group has been focussing on a list of seven questions regarding risk assessment and management frameworks for NMs.

Key Question 1: There are several frameworks as well as grouping and read across approaches that can aid in efficient information gathering for risk assessment of nanomaterials.

a. Is this enough?

364. A framework is needed that has been validated by others. The present frameworks contain the cut off values and specifics needed, but validation is missing. A robust framework based on robust data is needed. For regulators and industry, it is also preferable to use a less flexible framework which allows for some concrete predictions. The exact triggers and methods still need work. In addition, coverage of environment effects is still limited for the present frameworks.

b. Are pragmatic, partial scientifically based decision criteria (e.g. cut-off values, trigger values, application of tools and methods) needed and feasible?

365. The feasibility of the decision criteria comes down to robustness of the methods. One approach is to view robustness as the currently best available science. Stakeholders should be involved in the discussion to agree on what decision criteria are acceptable. Uncertainties in the risk assessment should be transparently communicated. Key points here are more dialogues and combined scientific effort to tackle current impedances.

c. Are such pragmatic approaches acceptable for regulatory purposes?

366. The acceptability is mainly determined by provisions of regulation. Concrete and robust justification are needed, e.g. for read across to other forms of a substance, i.e. nanoforms, which is currently not well provided in the REACH database. Current legislation allows industries to be less transparent about this information. Ideally, the data provided in REACH should clearly convey relevant information with regards to hazard and risk assessment.

d. What do we need to achieve this (both technically and process related)?

367. In terms of the process, scientific discussions may be beneficial to combine knowledge to reach the common goals. Issues related to approaches used to fulfil information requirements in REACH may be easier resolved by having dialogues with member states and the industries. This is already part of the dossier evaluation of REACH by ECHA.

Key Question 2: Considering the uncertainties on the nanomaterial fraction that reaches the target site, is information on the effective dose at the target site needed for risk assessment of a nanomaterial? Is it feasible and proportional to request this information in regulatory settings?

368. Information on the effective dose is good to know, but is not proportional. With regard to human health *in vivo*, it is suggested to do an experimentation and thorough study on the retained dose and calibrate experiments against that. Technical issues still make the collection of this information infeasible. Modelling may provide an outcome for acquiring this information, though the levels of uncertainty will increase. As a side note, it may be beneficial to focus scientific effort on understanding toxicity mechanisms, which allows grouping and reduces *in vivo* testing.

Key Question 3: How would you consider changes in physical chemical properties of a nanomaterial during the life-cycle in regulatory settings?

a. Can pristine well-dispersed nanomaterials be used for hazard testing as a worst case?

b. Are hazard studies with released materials (called fragmented products) useful in regulatory settings?

369. When using pristine NMs for hazard testing as a worst case, the underlying assumption is that the pristine NM is the most hazardous form of that substance, which may be incorrect. The hazard testing should focus on the entire product. Research should be focussed on the location and form of the material.

370. Hazard studies with released NMs are already being performed. It may be useful in regulatory settings, but some open issues remain. The effect of the specific NM is difficult to assess: in real-life exposure scenarios, many other NMs are present. More dialogues between scientists, industry and regulators are needed to establish the exact effect of the relevant form of the NM and to communicate this to the public.

Discussion points in the plenary

371. Are frameworks, including grouping and read-across approaches or test strategies necessary and are they enough for risk assessment of NM?

372. Frameworks should enable regulatory decisions on further testing and should be accepted by regulators; the role of frameworks under existing legislations should be clarified. Additionally, a dialogue between scientists and regulation is necessary with regards to linkages between frameworks and the existing endpoint specific testing strategies. Further case studies are needed for all frameworks for proof of principle and performance. *In vitro* assays used in frameworks should be clearly defined and validated. Future work includes exploration of frameworks based on key events of specific Adverse Outcome Pathway (AOP).

373. Is information on the effective dose at the target site feasible and proportional in regulatory settings?

374. Getting measurement data on internal distribution of NM *in vivo* is currently infeasible. A temporary solution could be the usage of models to estimate distributions.

375. How to consider changes in physicochemical properties of NM during life-cycle in regulatory settings

376. Pristine NM do not always cause the worst case effect, hence hazard assessment with released NM should be done on a case-by-case basis.

377. Less attention was spent on environmental effects during the discussions. Since the human health effects and environmental effects have some differences and approaches on risk assessment of NMs with regard to human health are more advanced, it would be preferable to have more discussion on the environmental effects.

Stimulus talk by E. Burello

[Lead Expert of the TF on in silico Strategies and (Q)SAR Modelling (Enrico Burello, IT)]

378. *Mr Burello* presents a short overview of the *in silico* strategies and (Q)SAR Modelling which will be extensively discussed during the BOG of Thursday December 1.

379. First, a list of available (Q)SAR models for NMs is outlined, these models are briefly discussed with respect to their relevancy and reliability with respect to regulatory needs. The major conclusions are that:

- The majority of models comply with the five OECD validation principles: from a statistical viewpoint models are robust and reliable. However, the statistical significance of descriptors in the models is not reported;

- Often calculated descriptor values do not correlate with experimental measurements; in addition, they are calculated on ideal structures, rather than from structural information derived from actual physchem characterisation: in this sense the structural variability and extrinsic properties of NMs are lost/not considered;

- For several models the considered endpoint is not relevant for use in a regulatory context;

- The most plausible mechanisms of toxicity for metal oxides are solubility (i.e., ion toxicity/reactivity) and particle's reactivity (i.e., interference with cellular redox equilibrium/radical formation);

- Model development does not include a complete panel of descriptors that can account for different structural features of NMs (of note, calculation of hydrophobicity or wettability is missing); in this way, other mechanisms or processes leading to toxicity might not be captured by the model.

380. In general, two main knowledge gaps are evidenced:

- Lack of descriptors which can account for nanomaterial's structural variability and extrinsic properties (effect of environmental conditions);

- Lack of data for modelling;

381. While for the lack of data, several initiatives are already devoted at the collection and curation of data to develop databases for modelling, the development of descriptors that can account for nanomaterial structural properties requires both experimental and theoretical insights.

382. Finally, the importance of (Q)SAR modelling for NMs is briefly discussed. The (Q)SAR technology can be useful for:

- Read-across;

- Development of functional assays (e.g. with or without cells) possibly for high-throughput screening methods;

- Development of Adverse Outcome Pathways and Mechanisms of Action;

- Design of safe NMs during the R&D phase by combining information on functionality and safety/toxicity.

BOG on Computational Methods and (Q)SARs summary

Co-Chairs: P. Demokritou (HSPH, US) and T. Puzyn (University of Gdanzk, POL) Commentator: C. Hendren (Duke University, US)

383. In this BOG the computational methods and approaches will be discussed. This is a neglected tool in the field of nanotoxicology and nanosafety in particular, but it needs to be developed urgently in order to reduce the uncertainty around nanosafety.

384. Some slides were shown to introduce to (Q)SARs. It was distinguished between two types of data: activity data (from experimental toxicology) and structural descriptors. Scientists are looking for a mathematical function that will connect these two kinds of data. It is important to know which particular characteristic of NMs is responsible or is important for which particular toxicological effect. That these particular characteristics are responsible for toxicity can be proven by using (Q)SAR according to the OECD guidelines. Besides that, (Q)SAR can be used for making predictions when designing new NMs, even before synthesis. Creating a large library of thousands of possible NMs *in silico*, would enable to select the most promising candidates for being synthesised and then tested.

385. There are two general types of descriptors: The first are physicochemical properties, measured somehow during the experimental characterisation. The other type are calculated descriptors based on computational chemistry methods. This means that some properties of NMs can be derived not from real NMs, but from a virtual model of an NM. These properties can be correlated with toxicity. Based on these properties predictions of toxicity can be made, which is much more useful from the view of (virtual) designing of new NMs.

386. A (Q)SAR method is a method for the interpretation of data. (Q)SARs help to understand which structural features are responsible for the activity and after establishing the relationship, one can start to predict activity or properties for untested chemical structures.

Key Question 1: Which relevant or promising descriptors/physical chemical properties and mechanisms of action from existing (Q)SAR studies do you see most helpful for application in Nano(eco)toxicology?

387. The consensus is that the relevance of descriptors and physical chemical properties depends strongly on the endpoints tested. In addition, descriptors for NMs may need to be combined to properly describe the observed effect. The specific evolution of the system should also be taken into account, which may modify the descriptors.

388. Properties of the system containing the NMs can have significant influence on the descriptors of the (Q)SAR and thus the toxicity estimation. This may pose an issue for the applicability and generalizability of the (Q)SAR models. This led to the discussion of the role of (Q)SAR models in toxicity estimation and prediction. (Q)SAR models may be seen as a tool in helping to explain the mechanisms that play a role in the data obtained experimentally. Additionally, they may help to pinpoint the most significant descriptors for a specific problem. They could be adopted into a decision tree for specific purposes alongside other models.

389. Several important properties for the (Q)SAR models were mentioned: dissolution rate, surface functionality, aspect ratio, particle reactivity, ion reactivity, surface chemistry, bio-persistence, band gap, protein corona, media influence. In addition, key mechanisms of action that were mentioned are particle reactivity and ion reactivity.

Key Question 2: Which aspects do you consider key to strengthen the collaboration between experimentalists and modellers? How should experiments/assays be designed to be helpful for developing (Q)SARs?

390. For currently available datasets, estimating the representativeness of the studied NMs is difficult and in addition, the quality of the dataset needs to be assessed. To make the (Q)SAR model useful, high quality *in vitro* and *in vivo* data is needed. How to salvage current datasets for (Q)SAR models is still a question.

391. To lower the barrier between experimentation and modelling, a data reporting template may need to be developed by cooperation between modellers and experimentalists. In addition, test protocols may help to standardise the test conditions between experiments. These conditions tend to differ largely between publications in the past. This will improve data quality used as input for (Q)SARs models.

392. The ISA-TAB-Nano and NANoREG formats for reporting are currently being used. The criticism for the ISA-TAB-Nano format is that it is too extensive for practical purposes. The NANoREG is more applicable. The ISA-TAB-Nano format is being updated, for which input is welcome.

393. Selection of which NM to test can be approached in different ways. One may select most structurally diversified NMs or most distant NMs in terms of similarity. Currently, the selection of the studied NM seems to be somewhat arbitrary. This may be remedied by involving modellers in the design of the experiments. Stronger collaboration between experimentalists and modellers is needed in this respect.

Key Question 3: How can (Q)SAR models be used for developing functional assays and tiered testing strategies?

394. A dialogue is needed between experimentalists that produce datasets and modellers, to avoid wrongful usage of the dataset. The (Q)SAR models need to be more closely connected to the experiment. But this connection is technically difficult due to the complexity that arises from functional assays. Intermediate steps may be needed, using empirical, statistical tools to derive properties which can be related back to the (Q)SAR model. This may be the first step in (Q)SAR model development, to derive structural properties from NMs using empirical models, and try to predict these properties using (Q)SAR models. In turn, connections to higher level biological/ecological activities can be predicted from the models.

395. *In silico* computational approaches for establishing NM properties are not feasible yet. The dependence on system properties poses the main problem here. Functional assays may be used to support validation of the models. (Q)SAR models may be used as a means to group NMs, which can improve read-across. Additionally, they help driver safer by design principles for nanomaterial synthesis.

Key Question 4: Given the difficulties of obtaining descriptors for nanomaterials, which scientific developments would you foster with respect to regulatory application of (Q)SARs?

396. Ideally, larger groups with similar properties can be found. Inclusion of information on the (test) environment is important in the development of the models and grouping of NMs. Differences between cell lines make grouping more difficult.

397. A key development is the EMMC (European materials modelling council) attempt to community approved standardisation of calculating descriptors for general use. Another idea is to have a repository of tested NMs, maintained by the (Q)SAR community. This repository can then be used to characterise NMs with future methods. Also, general datasets for testing the models are needed to allow for comparison between models. These datasets should contain data adapted to the specific needs of the nano research field. The question is still whether the data obtained in the past should also be included in these standardised repositories for datasets as they were not generated taken nanospecific considerations for testing into account.

Comments from the plenary

398. Tools need to be developed for both sides (experimental and modelling) of the heuristics-mechanistics continuum. Prioritisation is needed where to put effort on developing tools within this continuum. This may be performed iteratively, where the effort may shift between both ends. The collected data should meet the needs for both sides.

399. Key efforts to be made for developing (Q)SAR models are:

- bringing together quantification efforts in experiments as well as model estimates,
- adding to the structural harmonisation in order to interpret the relationship between the structure and the activity,
- determining which activity is of interest: specific modes of action or specific biological or ecological activities ,
- assessing which relationships matter. Interrogating the relationships between new structures and the important activities depends on a growing critical mass of consistent, harmonised data, captured with “premeditated interoperability”

400. For combining (Q)SARs with the ´omics there seem to be two possibilities:

- take the molecular initiating event, for which chemistry can be used to describe it, and take a specific endpoint and model/predict what happens and the endpoint
- apply system biology to formulate a hypothesis on a mechanism, then use this to select descriptors for the (Q)SAR’s.

SESSION 6: CONCLUSIONS

Chair: T. van Teunenbroek, Min I&M, NL

Panel discussion

Chair: B. Diderich (OECD), Panellists: G. Katalagarianakis (EC DG RES), J. Holmqvist (ECHA), P. Kearns (OECD WPMN), P. Sayre (ProSafe Task Force, US), J. Alwood (EPA, US), G. Moore, (KEMI, SE), T. van Teunenbroek (Min I&M, NL), J. Arts (AkzoNobel, NL) and L. Friedersdorf (NNCO, US), Mr Sung Ik Yang (KHU, KOR)

401. *Mr Diderich* concludes that there is a need for adequate risk assessment methodologies and adaptations of regulations applicable to all kinds of NM which are coming on the market. He would like to know what the panel sees as the take home message to improve current risk assessment methodologies and to adapt the regulations.

402. *Mr Sayre* refers to all existing and emerging protocols, methodologies and approaches. The review performed by the ProSafe TF was extremely helpful in pulling this out. For instance, the Guidance Document on aquatic toxicity under development by OECD will address a lot of key issues in testing NMs. One particular area which is controversial, has a high value and urgency, and needs data for regulation to be picked out: the inhalation toxicity of NM. There was one suggestion to use valid sub chronic inhalation tests. More alternative (including *in vitro*) methods could be brought in to speed up the review and to consider animal welfare issues.

403. *Ms Holmqvist* highlights the challenge to implement current or existing legal frameworks for NM. There is still a lack of agreement how REACH should be implemented. The main question is what would be relevant in a regulatory context. Under REACH, ways have been found to bridge over existing uncertainties. Experts are getting closer on important issues in terms of identification and characterisation and on the FAs that are needed. Research initiatives like NANoREG will provide SOPs that, from a regulatory perspective, will be very useful and important.

404. *Mr Katalagarianakis* remarks that a voluminous work has been completed with a strong effort and very strong outlook. The community building is impressive: a community of laboratories around the globeis working on succeeding in getting reproducible test results and elaborate reasons for failed reproducibility of results on nanosafety. Now this community has to increase in size and substance by involving laboratories in the EU and in other areas of the world that haven't been participating in NANoREG.

405. *Ms Friedersdorf* was impressed by the Draft Joint Document. The focus is no longer on data gaps, but on bringing information and knowledge together and on creating a community. Knowledge is analysed and state of the art science documents are developed. A couple of years ago, thinking about data sharing was scary. Now it becomes the way of doing science. With ISA-TAB-Nano, the community is even moving towards a common format.

406. *Mr Van Teunenbroek* remarks that still meaningless activities are going around in the field of nanosafety. An overarching topic is the disconnection between the academic toxicologist and the regulatory community. Actually, there is scientific reasoning behind all regulations on chemical safety and their methodologies to assess the implied risks. Bridges have to be built between these communities, and the commission might be of help to reach this goal.

407. *Mr Yang* concludes from the Joint Document, the presentations and the discussions, that great advance has been made in nanosafety research with relevancy for regulation. The proposed test methods can identify NM and are helpful for their regulation. Detailed assessment is difficult because of their physicochemical complexity. To characterise NM for regulatory use, valid FAs are required. Further research is needed for environmental modelling, computational methods and (Q)SAR. Harmonisation and evaluation are a good strategy to examine the nano EHS.

408. *Mr Moore* was impressed by the Draft Joint Document. There is a common goal and good cooperation. Progress is made. Although chemical assessment and regulation in the EU and the US are different, they are both represented and understand each other relatively well as they are facing the similar challenges regarding the regulation and assessment of NM. The next step should be to get more cohesion and cross talk in developing test guidelines, methods and guidance. It will be necessary to think out of the box. Regulation, the test guidelines and pure science and innovation must go hand in hand and keep contact because nanotechnology will be a fast-moving field.

409. *Ms Arts* wants to stress the difference between nice to know and need to know. Scientists want to know a lot that is, from a risk assessment point of view, just nice to know. The industry may end up with lots of laborious studies, using lots of animals. With regard to hazard assessment, a general correlation between *in vitro* and *in vivo* testing hasn't been found yet; nor for NMs, nor for chemicals in general. A good starting point for the assessment of inhalation exposure could be the decision framework for grouping of NMs as starting point for grouping and read across of NM instead of doing studies for each single chemical and form.

410. *Mr Alwood* stresses that the level of uncertainty on how to assess the risks of a NM is much lower than 10 years ago. He takes home the agreement on some key physicochemical properties. As new materials are developed, a few more properties will be added to the list. One of the biggest needs at EPA going forward, is how to do environmental assessments. The approach of cooperation and sharing of information to answer immediate questions of regulators, is key.

411. *Mr Kearns* remarks that, from the OECD perspective, the development of adapted test guidelines have a very high priority. Guidances for aquatic toxicity studies, alternative methods and physical chemical characterisation still remain a need. Another issue is the communication between the regulatory and scientific community on the issue "need to know" versus "nice to know". The community has to pay more attention to regulatory needs and academic possibilities.

Recommendations from the panel for the regulation of NM

412. To start with, everybody has to speak the same language. The US is more liberal on its definition than the EU. It is necessary to clearly define what is a NM and what are nanoforms and to perform an adequate characterisation of that materials in order to be able to perform a valid risk assessment and decide on an adequate regulation in a confident way.

413. In order to do hazard assessment and hazard characterisation, existing test methods must be reviewed for their applicability for investigation nanomaterials. In general, most of them will be adequate for NM with some adaptations. If the test methods don't give confidence for testing fate and effects of NMs, there is a huge issue that will hamper the implementation of regulations.

414. NMs can (based on certain physical chemical parameters and surface functionalisation) exist in different forms. As it is impossible to test them all, acceptable approaches for read across have to be found. Concepts on *in vitro* screening methods or short time inhalation studies may be a way to find bridges between different nanoforms with respect to the inhalation exposure of humans. One of the challenges is to find an

agreement on approaches on how to perform read across for NM. There is the need for some fine-tuning in terms of lacks of methods.

415. Besides *in vivo* and *in vitro* for inhalation toxicity, there are some major issues like understanding the main exposures in the environment. With such understanding (based on adequate information on production, use, release and disposal), better predictive models can be built and input data would be more effective to estimate actual exposure. But also reliable methods to measure what is really in the environment need to be developed and considered in risk assessment.

416. As nanotechnology is progressing fast, new materials are expected to enter the market, there is the need to know more about the modes of action. Thus, one promising way forward are *in silico* approaches. Even though effective models are available, there is a lack of real data sets to try test methods or models to see if they really work out. In NANoREG and in the Joint Document, tools are identified in order to generate reliable data. Adhering to those tools, protocols or guidelines in order to create such a set of data, and pulling and sharing that data, might give a glimpse into the future to correct them not *in silico.*

417. Databases on nanosafety data are recommended, if there is a way to sustain. Often, they run into funding problems and as a consequence not maintain. For better exploitation, lasting databases with continued IT provision are needed.

Remarks from the plenary

418. There were some questions and remarks from the participants.

- Concerning data bases and data base stability, there are some underlying problems. Copyrights hinder data mining and data sharing. If it is allowed to share data, there won't be a database problem. Existing databases disappear because there are copyrights and nobody wants to maintain them. Open data is much cheaper.

  Because it is hard to collect data, there is the sense of ownership. However, data usually have a short life. They become outdated soon. People who have been open with data sharing, gain recognition. Methods and criteria are named after them and they earn money from consulting. Data sharing is very productive. Being open on data, is the best way to keep any database and thus, information alive. Having open databases and continuing to feed them with new data, is the best guarantee for sustainability and quality.

  For sharing data, there seemed to be a general agreement that ISATAB Nano is the good format, but there is still a lot to be done there. Common reporting procedures with templates or frameworks have to be developed, agreement has to be found on which kind of data and metadata have to be included in eventual databases etc. This is not only a regulators' and scientists' activity; the industry has to invest and demonstrate its openness as well.

- Data-openness is a pre-requirement to control and validate the quality of data and methods. Often, the data behind publications is not available for quality control. NKI (NNI nanotechnology knowledge infrastructure group) already developed a data maturity ranking system with data readiness levels (DRL) that are similar to the technology readiness levels (TRLs) that have been used by NASA and DOD. The DRL document isn't perfect yet, but it is a start.

  A next step will be to develop a data ranking system so that users can see which data to avoid. Scientists would like to see raw data and not just the nice pictures. It has to be considered that next to very good data, also a portion of the existing nano literature is not good. False data have been

published to drive competitors off the road. A data curation step on the quality of data might be needed before these data feed processes.

A way to find data and have them quality assessed, might be to make industry submit publicly available data when they register substances (as e.g. under REACH). ECHA has a huge open database which is searchable and where information on all registered materials and substances can be found.

One of the main barriers for creating input, is that it isn`t mandatory to create input in a consistent form that will capture everything in a way that is interoperable and flexible enough to be updated as methods are evolving. Researchers will only be compelled to spend extra time on adding data to a specific tool, when it helps them to answer their questions. Funding agencies should integrate a requirement for utilisation and consideration of valid tools and/or proven data bases for awarding academic grands. In the NANoREG project this was already a mandatory requirement. As a prerequisite for funding, researchers were obliged to upload their data developed in the research project. Data sharing should become part of the culture of science.

- The manufacture, import and use of chemicals in Europe are regulated in the European Chemicals Regulation REACH. It is broadly accepted that its approaches, tools and methods provide a suitable framework also for NM. However, adaptations are needed to take the complex features of NM into account. Different forms of dialogue platforms are currently installed to exchange the different opinions on the needs for adaptations. Getting into a dialogue with regulators is important to decide on the information needed for a valid risk assessment. With regard to industry, ECHA does invite companies to comment and discuss draft decisions of substance evaluations before they become final. As such a dialogue is beneficial for all parties. With regard to science, channels of communications are needed to discuss what is needed to efficiently implement the existing regulations to NM. Scientists and regulators (ECHA but also representatives from member state authorities) currently meet in scientific projects, e.g. projects like NANoREG and other FP7/Horizon 2020 projects to address these issues. In addition, OECD as an important organisation to facilitate these kind of discussion in particular in the context of revising test guidelines.

  There is the need to understand the state of science and the boundary conditions. In order to keep regulators up to date on the scientific state of the art regarding nanosafety the dialogue on science is very important. The same is true for companies who have to protect their workers and need to install safety procedures based on best scientific knowledge.

- On the brink of Horizon2020 and the need for governance, platforms on nanosafety do emerge in many European countries. They are like an interface linking research, regulatory aspects but also innovation policy. These kind of platforms can be a sort of translation or interface for the regulators, the real pure scientists and other actors such as industry, to start communicating, more casual and more focussed, sort of like the ‘need to know’ type of question.

  While waiting for an adequate regulation for NM to be put in place, dialogue platforms can also serve as a guidance for innovators. They can give signals to inventors of what could be sustainable ways to move forward. Platforms might fill the gap that is caused by the uncertainty about which NM is safe(r) or not. Those platforms can also support legislation and the filling of gaps.

  It has to be expected that in the future, thousands of SME companies will be developing all sorts of applications using a multitude of NMs. However, their knowledge regarding nanosafety is often single minded. Countries develop platforms for helping such innovators to become more aware of

regulatory and risk aspects. In addition a pre-regulatory dialogue is needed to improve the utilisation of concepts on safer-by-design for NMs.

- The development of certain regulations is always a process. Usually the desire for regulation is strong after events with adverse effects. With NMs, there haven't been causal links to such events. With regard to predicting of what might be the risk and hazards of NMs, exercises like the EU-US Communities of Research are very useful. With regard to NANoREG and other research projects of regulatory relevance, it is necessary to pass from research to the implementation of knowledge into regulation. The way to go on, is to speculate about the future with different scenarios, virtually supposing certain adverse effects and then act in preparedness.

  There is complementary between the aims of ProSafe and the work of the OECD WPMN of the last 10 years in defining gaps, setting priorities, and providing information that is needed. Now it is started to answer the gaps that were identified in the evaluation of NM. At OECD, work has started to address issues e.g. on NM behaviour in the environment or inhalation toxicity.

- Regarding the conflict of "nice to know" of research and "need to know" of regulation, this distinction between regulatory research and scientific research is an artificial one. It is less a distinction, but a continuum. Regulators have to solve immediate questions, while it takes time to get complete results. The focus is on need to know. However, scientific research will provide knowledge for regulators that might not support them to take a quick decision now but might offer support to decisions in the future on unforeseen regulatory needs.

  Nice to know information, coming from research benefits and developments of better methods, might become need to know or even mandatory for other arguments or other needs in the future. Such information might become quite handy to avoid tests and the development of (alternative) methods. In addition, academic research helps to find a way to develop simple, faster and cheaper methods to assess procedures and frameworks.

- Nanotechnology will be developing very fast with a lot of small and medium and also big enterprises bringing a lot of materials onto the market. Innovators in industry should consider whether some investments in providing additional information (which is not mandatory for regulation) could be a good investment for themselves in order to get better, cheaper and faster methods that may need less animals or resources.

- Alternative testing is needed (for NMs and chemicals in general). Acceptance of alternative testing by regulators should be speeded up. Both in the EU and the US, initiatives have been developed to speed up validation and acceptance of methods. This has to be done rigorously.

  All other options to get the necessary information without testing every form and every substance have to be exhausted as well. It just should be made sure that these tools are effective. Some tools are already in place.

- Regulation relies upon academia to resolve certain questions. But the business model of academia is not made to develop new tools for regulation or standardised methods for risk assessment. With academic freedom, questions are only partially answered.

  A sort of body should be set up in which needs are defined and results are obtained in a demand driven fashion. That means procurement: buying the research that is wanted. NANoREG showed that such an approach works, even among eighteen countries and more than sixty institutes. This

can be done with dedicated funding towards certain objectives. There has to be funding to buy tools in the market and procure them in a research fashion.

The question is not whether a NM is hazardous or not. The question is whether there are methods to assess the hazard and behaviour of these types of materials. The funds should be focused on this. Money for dedicated development of tools is urgently needed.

Conclusions - Final Plenary

Summary of final conclusions and recommendations to regulators and policy makers based on the conference discussions

(K. Steinhäuser, GER/P. Sayre, US)

419. The final conclusions and recommendations of this three-day conference reflect the conclusions of the Joint Document as well as the outcomes of the discussions during the conference.

420. One of the goals of this conference was to review the Joint Document that summarises the available information which is applicable for regulatory needs. The responses on the Joint Document were very positive and hopeful. This will help further discussion.

421. Another goal was to identify methods and protocols that are reliable and relevant for regulatory use; to identify methods that are (at this moment in time) the best candidates for regulatory use; and to identify gaps and paths forward, particularly in the context of REACH.

Physicochemical Properties for NM Identification

422. It is clear that EM is the method for primary particle size distribution. One of the issues is how to prepare the samples for EM. An ISO standard ("NT compilation and description of sample preparation and dosing methods for engineered and manufactured NM" ISO/TR16196:2016) is applicable, but further validation of the guideline is needed.

423. Ensemble methods measure different diameters. They have difficulties with polydispersity, but may serve as a first-tier assessment. In a tiered scheme, which is proposed by NanoDefine, these ensemble methods can be applied for definition purposes if they identify a material as NM.

424. For definition purposes, VSSA is not appropriate as an alternative for particle size distribution; it is only an additional supporting identifier for NMs in dry powder form.

425. Methods for the determination of chemical composition (some also for spatial information) are available but they are expensive if complex NMs are analysed. There are limitations with functionalised NMs. Further research will be needed. However, information about the chemical structure of NMs should be provided by producers.

426. The commentator of the Breakout Group on NM identification advised to be aware that the information is available but scattered and should be pooled and made available for regulation.

Physicochemical Properties for NM Characterisation

427. Zeta potential is relatively easy to measure, but is dependent on pH, ionic strength, et cetera. In order to provide a regulatory metric that can be understood most specifically, however, the electrophoretic mobility is likely the best measure to indicate zeta potential (Lowry et al. 2016). Since electrophoretic

mobility is an extrinsic property, by virtue of its measurement being medium-dependent, it should be derived using standardised protocols that specify the test conditions and parameters to be measured and recorded. In this way, for example, two or more NMs relative electrophoretic mobility may inform the relative fate of the two NMs if similar protocols and test conditions are employed. While electrophoretic mobility is an extrinsic property, it could be used also to identify an as-produced nanomaterial as being subject to regulatory oversight or not, as long as similar protocols and test conditions are used.

428. To determine the extrinsic properties of NM, which depend on medium and time, functional assays (FAs) will be necessary. They have to be predictive. A clear definition of FAs will be provided in the final document. For some properties, it is not clear whether they are intrinsic or extrinsic (e.g. hydrophobicity). For hydrophobicity, the Rose Bengal method was suggested. Some tests, e.g. agglomeration behaviour and dissolution rate in aquatic media, are already in the standardisation process of OECD. An additional FA on corona formation has been proposed. Clarification will be necessary on: types and amounts of proteins, and predictively for biodistribution. More clarification is also necessary on attachment efficiency/surface affinity (e.g. for hetero-aggregation). There is no validated test protocol yet. Further development and standardisation of tests for regulatory purposes has to be done.

429. The commentator of the Breakout Group on NM characterisation stressed that well defined testing strategies are necessary and advised to concentrate on 'need to know' parameters.

Consumer and Occupational Exposure of NMs

430. A three-tiered approach (OECD Guidance) is a useful construct for the assessment of worker exposure, principally through the inhalation route (scoping; handheld device measures, more detailed exposure assessment).

431. Regarding consumer exposure there isn't much data about this topic. It is not yet clear how often there would be a high concern for consumer exposure to NM.

432. Some modelling methodologies are working now and can be useful in a screening context for worker and consumer exposure (ConsNano and Stoffenmanager, etc.). They fit within the three-tiered approach. Control Banding for worker exposure may be too general for formal regulatory use.

433. Portable devices for aerosol measurements work well, but might not give the exact precision that is needed for detailed risk assessment. They must be comparable and reproducible. SOPs have to be available, standards have to be developed, and calibration is needed. Portable devices have gaps (diffusion devices up to 400 nm; optical devices for larger particles up to 10µm) and there are still background concerns. Fixed, filter based devices are part of the three-tiered OECD protocol because they will be more precise. As they are not portable, they don't give a full image of Worker Exposure over time.

434. Generally mass-based particle metrics are preferred, but they have a disconnect with particle count devices. There are exceptions (fibre counts). Other metrics (particle number, lung deposited surface area) may be more appropriate, reflecting toxicological concerns.

435. A lot of work has been done on personal protective equipment (PPE) and more modern estimates on releases from consumer spray applications for NM. Information on engineering controls and nanomanufacturing application equipment releases is still largely lacking. Worker PPE generally works; but needs more verification for NMs.

436. Inhalation is the dominant exposure route for workers. In terms of consumer exposure, inhalation and ingestion might be important, but there are still a lot of knowledge gaps in this field. This leads to a discussion about the need for inventories of NMs in nano-enabled products. We need also for consumer

exposure tiered approaches for prioritisation. The focus should be on chronic exposures and the indoor environment.

437. In terms of environmental media and exposure to consumers, a few potential sources are highlighted as combustion or agricultural products. However, there aren't many hard data about them yet.

438. A lot of discussion was about standard tests for releases of materials from nano-enabled products and how doable such studies are. General protocols do exist for dustiness, abrasion, incineration and leaching, but they may not be adequate. Will a grouping approach help for release testing? A problem is how to link releases to exposure analyses.

Environmental Exposure, Behaviour and Fate of NMs (including Modelling Environmental Exposure and Fate)

439. Parameters like dissolution rate, agglomeration behaviour and transformation are key to determine the fate of NM in the environment. Current standardisation activities on dissolution rate, agglomeration behaviour and activated sludge retention will provide valuable information for the future.

440. Test guidelines should also reflect some other points, such as realistic worst case situations. This may be a future point for the OECD Working Party on NMs (WPMN). In defining worst case scenarios for NM, time must be taken into consideration as an important factor. Test guidelines need to reflect dynamic and complex systems. For FAs, standardised guidelines are necessary to determine important parameters, independent of concentration and time. Interaction with surfaces is an additional key process of NM which should also be reflected in a test guideline (heteroagglomeration).

441. Guidance on how to address transformation processes for regulatory purposes is still lacking. Transformation processes should be ranked and classified.

442. Concerning bioaccumulation, there is already a draft for a guidance document studying accumulation in fish (TG 305, dietary in fish but also for sediment and soil organisms), but the document is still currently on hold.

443. Material flow analysis (MFA) and Environmental Fate Models (EFM) that are suitable for NM should be robust. Input parameters might be uncertain, data incomplete and thus the application of the models not fully predictive for concentrations of NMs in environmental media. Therefore, quantitative data and data on production is needed, as well as data on characteristics (form, size, transformation) and key fate parameters as dissolution rate or hetero-agglomeration. MFA and EFM models are accepted for regulatory review of conventional chemicals. EFM models for NMs exist (e.g. SimpleBox4Nano).

444. Models should contain a screening and a detailed tier. Both have to be complemented by expert judgement. For the validation of models, the methods for generating input parameters urgently need to be harmonisation (agglomeration behaviour, dissolution rate) and experiments to validate processes need to be conducted (e.g. lab and mesocosm studies).

445. The commentator of the Breakout Group on Environmental Exposure, Behaviour and Fate advised to decide how much harmonisation is needed versus the flexibility and versus the environmental reality. Tiered testing strategies and modelling are needed.

Human Health in vivo of NMs

446. Except for the requirement to collect lung burden data, the current drafts of the OECD Inhalation TGs for subacute and subchronic toxicity of NMs are complete. Lung burden data are needed, where feasible,

to understand the retained dose. The value of lung burden data also allows a better understanding of *in vivo* biokinetics and enables read-across. It also allows a common dose metric link to bridge to data from alternative test methods. There is potential to use the results from the new OECD draft subacute inhalation test guideline as a substitute for data from a subchronic study. However, there is not sufficient data currently available to evaluate correlations between 28-day subacute tests and subchronic nanomaterial tests conducted in accordance with OECD test guidelines for nanomaterials. .

447. The potential to link *in vivo* and alternative method test results using common dose metrics is possible, and could be considered as alternative means of ranking relative potency in the near term. Longer term possibilities could go beyond ranking and grouping. Such a system would benefit from benchmark NM standards and lung burden data that would allow the ranking of relative potency from inhalation study results, and the anchoring of the results of *in vitro* studies and shorter term inhalation studies.

448. There are protocols available for biodissolution whose results could limit the need for biokinetic studies, in a tiered approach. In a situation where a material does not biodissolve, it would contribute to the decisions on further testing. However, the exact protocols for biodissolution must be carefully prescribed to enable such approaches.

449. The use of benchmark materials and gathering lung burden data may increase animal usage in the near term, but decrease usage in the longer term due to increased acceptability of alternative test method results by regulators.

Human Health in vitro of NMs

450. Detailed standardisation principles were identified for conducting *in vitro* health tests for NMs. These principles were largely agreed to by participants. Principles identified addressed a number of parameters, including the following: dispersion methods, physicochemical characterisation of NM, dose metrics and dosimetry, details of cell culture, relevant endpoints, and cell types; “effective density”, impurities, hydrophobicity, and Characterisation “during” test performance should include dissolution, aggregation and protein corona evaluation.

451. Some participants prefer to choose the cell type for an *in vitro* test based on disease mechanisms (perhaps primary cells first if the mechanism isn’t known). The majority prefer the standardisation of cell lines. Chronic exposure assessments with *in vitro* systems are currently challenged by cell proliferation when cell lines are used, but the meaning of results on primary cells is questioned for chronic endpoints.

452. A minimum of three doses should be tested (for receiving a dose-response curve). The use of dosimetry models was pushed for the liquid culture tests. It is recognised that the protein corona may be relevant because it can affect toxicity *in vitro*. Biodurability should also be considered.

453. Performance controls and benchmarks of NM are necessary to allow comparability between studies, both intra- and inter-laboratory. They can vary with the test system. Benchmark materials do not necessarily need to be NMs.

454. At least two independent methods per individual tested *in vitro* endpoint need to be performed, with multiple relevant cell types or co-culture models. A battery of standardised cytotoxicological assays may be used.

455. Three most promising near-term liquid suspension assays were identified for cytotoxicity (on cell membrane leakage or cell reproduction/death) and five for genotoxicity.

456. There is a shortage of *in vitro* methods for oral route toxicity evaluation, some immunotoxic effects and fibrogenicity. There is an urgent need to validate *in vitro* methods for regulatory use via ECVAM and OECD. Eventually more realistic *in vitro* assays should be employed including ALI (air-liquid interface) and multiple cell culture systems. ´Omics assays should also be further developed.

457. *In vitro* assays, using the identified standardisation principles, could be integrated in frameworks such as those proposed by NANoREG or ECETOC.

Ecological effects of NMs

458. The draft of the aquatic toxicity guidance document was welcomed. A standardisation of dispersion methods is needed. A compromise has to be found between environmental realism and stability. The addition of organic matter was seen of use only for a limited set of NMs. For stable dispersion, there may be a small window of opportunity regarding the dependency of the zeta potential on the pH.

459. Dissolution kinetics is also a key parameter. The interpretation of the data and effects is an issue. Results of NM with significant dissolution should be depicted as effect of a NM as a whole, not differentiating between dissolved and not dissolved parts. However, for interpretation of the results the examination of both fractions is important.

460. Recommended aquatic test organisms for NM are algae, daphnia, bacteria for most of the NMs the fish embryo toxicity test might be too insensitive. The so-called Kd conversion sediment/freshwater is not useful for NM because no equilibrium will be reached.

461. Chronic toxicity data is needed for risk assessment, but may be challenged by the dependence on feeding of the test organisms.

462. Differentiation between artefacts and adverse effects is possible. There are some quite good solutions, although it remains difficult to differentiate physical and physiological adverse effects (e.g. NM attached to algal cells, gills).

463. There was a recommendation against limit tests because agglomeration is concentration dependent and bioavailability may decrease with higher concentrations.

464. In terms of reference/benchmark materials, the conventional chemicals as described in the test guidelines might be sufficient.

465. *In vitro* tests are not an alternative for *in vivo* testing, but may provide additional (e.g. mechanistic) information in future. Their correlation with *in vivo* is not yet studied.

466. The commentator of the Breakout Group on Ecological Effects provided updated information on the development of the draft guidance document on aquatic (and sediment) toxicity testing for NMs.

467. NM depositories should be more established and used for developing datasets.

Risk Assessment Frameworks and Grouping Strategies

468. There are two different options on this topic:

- *Option one* is to increase efficiency of risk assessment of NMs by applying qualitative grouping and testing schemes, such as the ECHA/JRC/RIVM guidance for read across or the NANoREG framework. This approach is also useful in the phase of development of a NM, prior to regulatory

submission. It is a rather flexible case-by-case approach with individual materials and groups of materials, but it has less predictively for regulators and industry because there are limited clear quantitative criteria that are applied to these qualitative approaches

- *Option two* is an approach for NMs that reaches regulatory evaluation with a focus on NM with the greatest potential for hazard/risk by applying a more quantitative approach that applies. specific triggers and protocols. The DF4Nano of ECETOC is actually the only example in this category. It is also accompanied by nanomaterial case studies which test how the DF4Nano framework functions in a regulatory decision-making context. However, currently it is limited to inhalation toxicity. If such a quantitative approach is pursued, then three or more separate frameworks are required. Beyond inhalation other frameworks are needed for consumer exposure (e.g. oral exposure) and (aquatic) ecotoxicity. Alternative methods, such as that proposed for inhalation toxicity testing, can be incorporated. Implementing this second option in a regulatory sense will be more complicated. There are also questions about the methods and the triggers in the DF4Nano approach which need independent evaluation. Option 2 framework gives a compromise between science-based assessment and efficiency. Decision criteria and methods are only partially scientifically underpinned. These frameworks have a clearer structure to predict outcomes. This fits better the needs of regulators and industry by providing more certainty on the concerns for a given material and the likely outcome of a decision process. Although this option is more difficult to match with the current REACH framework, it might also be useful for grouping, read across, and tiered risk assessment of NMs. Generally, when tiers, triggers and protocols are properly validated, it seems to be a good option for providing transparency and understanding on which way to go.

469. To move forward, stakeholders should provide case studies and independent review of these frameworks. There is concern about the robustness and uncertainty of the frameworks, which should be addressed and requires more specific risk assessment frameworks. An open dialogue should be created, and more pre-notice meetings between companies and regulators may be organised in an early stage of the design process. An intermediate body (such as the TLV (Threshold Limit Values) Committee of the American Conference of Governmental Industrial Hygienists, ACGIH) could be created between regulators and industry.

470. A critical need concerns the funding for regulatory test method research to allow the development of alternative *in vitro* and *in vivo* methods in order to progress through validation. Difficulties to predict chronic effects by alternative methods are of concern. Other issues are the need to know the dose at target site. More attention should be given to the assessment of false negatives, and the question whether pristine materials really represent the worst case.

471. The concept of retained dose is useful, but it is not clear if it would be required for all NMs and if it would be feasible to do. There is a need for validation of biokinetics (applying mainly to particles with limited solubility). Life cycle issues have to be considered with the testing of pristine NMs versus NM-product matrix combinations. Problems exist in obtaining enough fragmented material.

Computational Methods and (Q)SARs of NMs

472. It is important to comply with the five OECD principles on computational methods. All-purpose solutions don't exist. Groups with similar characteristics can be built and then (Q)SARs can be developed and applied.

473. (Q)SARs basing on energy band gap and dissolution rate are most promising. Other descriptors might be available and worth to be tested. There are sixteen properties for characterisation of NM discussed

in the Joint Document. (Q)SARs may help to decide which properties are really important for toxicity and fate. This may be a multidimensional problem, but the modelling specialists expect that it can be resolved.

474. ´Omics methods provide a possibility to get datasets which include different modes of action and should be examined for linking with (Q)SAR approaches.

475. Further OECD guidance on data curation would be welcome. Cooperation of experimentalists and modellers is urgently needed to develop datasets which help to develop reliable (Q)SARs.

476. Virtual production and characterisation of NM, followed by synthesis and testing, might be a way to get datasets which are suitable for modellers. Standardised tests with standardised conditions and standardised reporting formats would facilitate the development of (Q)SARs. This may prevent problems with medium and time dependency (Q)SMARs). (Q)SARs can also be used to develop FAs and in turn FA results may help to develop (Q)SARs for biological and ecological effects on human and environmental health.

Summary of conclusions of the panel discussion

477. One of the main conclusions is that academic researchers and regulators have to be brought together for moving regulatory science forward and for better using science findings in regulation.

478. The Task Force has done good work. There is much more knowledge and much less uncertainty than ten years ago, but there is still some complementary work to be done, e.g. by the OECD WPMN with respect to test guideline and guidance development. Relevant is to decide what is needed to know and what is nice to know. Also important is the maintenance of databases for a range of aims like effects, physical chemical properties, nano-enabled products and fate aiming to allow extrapolation, model building and read across,.

479. As most important issues are regarded grouping, read across, characterisation, understanding and assessing exposures, environmental risk assessment and modelling. There is a need for tiered testing schemes and grouping approaches. Tiered schemes for health effects can help to allow for use of alternative test method results. FAs will be useful and important.

480. Surface modification is huge challenge under REACH. More complex NMs will be a challenge for future regulation. Safer by design may help to minimize risks in a pre-regulatory phase. Test guidelines and guidance documents have a high priority for regulators.

481. The industry sees difficulties in enhanced costs, e.g. difficulties to conduct inhalation studies with lung burden and clearance rates. Dialogue platforms, including regulators, science and industry, are recommended.

482. Although many questions still have to be answered, the scientific instruments for assessment of NMs with respect to regulation are yet available or near to be available, which means that there is good evidence that better regulation and enforcement have become possible.

ANNEX 1: BACKGROUND DOCUMENT

1. The Joint Document (as part of the ProSafe White Paper) was used as a background document of the conference. The document comprises a review of selected and relevant results, protocols, and guidance documents, from NANoREG, OECD WPMN activities, and other EU FP7 NanoSafety Cluster funded projects. This review was carried out by a Task Force (TF) of international experts, who analysed data according to relevant themes such as physical-chemical characterisation and identification, human and environmental exposures, fate modelling, health and ecological effects, computational methods as well as risk assessment strategies.

2. The final versions of this conference report, the Joint Document as well as the White Paper are available at the ProSafe Results Repository:
http://rivm.nl/en/About_RIVM/Mission_and_strategy/International_Affairs/International_Projects/Completed/ProSafe/ProSafe_Deliverables1/WP_5_and_WP_1_The_OECD_ProSafe_Joint_Scientific_Conference_the_Joint_Document_and_the_White_Paper

ANNEX 2: AGENDA

DAY 1:

08:00-09:00	**Registration at OECD headquarters**
09:00-09:30	Welcome Remarks (N. Van Hulst, Ambassador, Permanent Delegation of the Netherlands to the OECD & A. Wyckoff, OECD Director for Science, Technology & Innovation), Introduction to the Structure of the Conference & Housekeeping Information (CC9)
Session 1 (part 1): Introductory lectures (Chair: P. Kearns, OECD WPMN Secretariat) (CC9)	
09:30-09:50	Introduction to the OECD WPMN: Test Guidelines Activities (P. Kearns, OECD WPMN Secretariat)
09:50-10:20	Introduction to EU NanoSafety Programme (G. Katalagarianakis, EU COM)
10:20-10:40	Introduction to ProSafe (T. van Teunenbroek, Min I&M, NL)
10:40-11:00	**Coffee break**
11:00-11:20	Introduction to ProSafe Task Force for Review of data (K. Steinhäuser, GER)
11:20-11:40	Introduction to NANoREG (H. Crutzen, EU JRC & T. van Teunenbroek, Min I&M, NL)
11:40-12:00	Introduction on the current status of EU regulation (A. Kobe, EU COM
12:00-13:00	**Lunch**
Session 1 (part 2): Invited talks (CC9)	
13:00-13:20	Introduction to NanoReg2 (E. Frejafon, coordinator NanoReg2, INERIS, FR)
13:20-13:40	**GUIDEnano: A Tool for risk assessment of nano-enabled products** (S.Vázquez-Campos, LEITAT, SP)
13:40-14:00	Species Differences in Pulmonary Responses to Subchronic and Chronic Exposures to Low Solubility Particulates: Comparisons of Biokinetic and Pathophysiological Responses in Rodents, Non-Human Primates, and Coal Miners (D. Warheit, Chemours, US and G. Oberdörster, University of Rochester, US)
Session 2 (part 1): MN Identification and characterisation as basic information requirements for an appropriate regulation (Chair: T. van Teunenbroek, Min I&M, NL) (CC9)	
14:00-14:30	Stimulus presentation in the plenary [Lead Expert of the TF* on Physicochemical methods (G. Lowry, Carnegie, US)]
Session 4 (part 1): MN Hazard: Results of regulatory relevance (CC9)	

14:30-15:00	Stimulus presentations in the plenary [Lead Experts of the TF on Human Health and biokinetics *in vitro* (B. Rothen-Rutishauser, University of Fribourg, CH) and *in vivo* (G. Oberdörster, University of Rochester, US)]	
15:00-15:30	**Coffee break**	
15:30-17:30	**Session 2 (part 2): MN Identification and characterisation as basic information requirements for an appropriate regulation**	**Session 4 (part 2): MN Hazard: Results of regulatory relevance**
	Break Out Group on MN Identification (based on the EU recommendations for a definition) (CC9) Co-Chairs: K. Jensen (NRCWE, DK) and A. Patri (FDA, USA) Commentator: H. Rauscher (JRC, EU)	**Break Out Group on Human Health (*in vivo* and toxicokinetics)** (Auditorium) Co-Chairs: L. Tran (IOM, UK) and F. Cassee (RIVM, NL) Commentator: W. Kreyling (Helmholtz Centre Munich, GER)
17:30	**End of day 1**	
	Buffet (Salon du Parc)	

DAY 2:

Session 3 (part 1): MN Exposure and Fate: Results of regulatory relevance (Chair: K. Steinhäuser, GER) (CC9)		
08:30-08:45	Stimulus presentations in the plenary [Lead Experts of the TF on Exposure through the lifecycle (T. Kuhlbusch, BAuA, GER)	
08:45-9:00	Stimulus presentations in the plenary [Lead Expert of the TF on Environmental Fate, Persistence and Bioaccumulation (A. Baun, DTU, DK)]	
9:00-9:15	Stimulus presentations in the plenary [Lead Expert of the TF on Modelling of Environmental Fate and Exposure (B. Nowack, EMPA, CH)]	
09:15-11:15	**Session 2 (part 2): MN Identification and characterisation as basic information requirements for an appropriate regulation**	**Session 3 (part 2): MN Exposure and Fate: Results of regulatory relevance**

	Break Out Group on MN characterisation (CC9) Co-Chairs: I. Lynch (University of Birmingham, UK) and E. Valsami-Jones (University of Birmingham, UK) Commentator: J. Riego Sintes (JRC, EU)	**Break Out Group on Consumer and Occupational Exposure** (CC7) Co-Chairs: M. van Tongeren (IOM, UK) and T. Thomas (CPSC, US) Commentator: R. Packroff (BAuA, DE)	**Break Out Group on Environmental Exposure, Behaviour and Fate (including modelling)** (Auditorium) Co-Chairs: F. von der Kammer (University of Vienna, AT) and P. Westerhoff (Arizona State University, US) Commentator: J. Ahtiainen (FIN)
11:15-12:15	Plenary: Reports from the BOGs** of Session 2, discussion, final comment (CC9)		
12:15-13:15	**Lunch**		
13:15-14:15	Plenary: Reports from the BOGs of Session 3, discussion, final comment (CC9)		
Session 4 (part 3): MN Hazard: Results of regulatory relevance (Chair: K. Steinhäuser, GER)			
14:15-14:30	Stimulus presentations in the plenary [Lead Expert of the TF on Ecological Effects and biokinetics (T. Fernandes, Heriot Watt University, UK)] (CC9)		
14:30-16:30	**Break Out Group on Human Health (*in vitro*)** (CC9) Co-Chairs: A. Haase (BfR, GER) and B. Fadeel (Karolinska, SE) Commentator: M. Sherma (PETA, UK)	**Break Out Group on Ecological Effects** (Auditorium) Co-Chairs: R. Klaper (University of Wisconsin, US) and N. Hartmann (DTU, DK) Commentator: A. Kennedy (US Army, Corps US)	
16:30-17:00	**Coffee break**		
Session 5 (part 1): MN Tiered testing and Tools for Risk Assessment: Results of regulatory relevance (Chair: K. Steinhäuser, GER) (CC9)			
17:00-17:30	Stimulus presentation(s) in the plenary [Lead Experts of the TF on Risk Assessment and Management (A. Oomen, RIVM, NL) and *in silico* Strategies and (Q)SAR Modelling (E. Burello, IT)]		
17:30-18:30	Plenary: Reports from the BOGs of Session 4, discussion, final comment		
18:30	**End of day 2**		
20:00	ProSafe Task Force Dinner (Task Force members only)		

DAY 3:

Session 5 (part 2): MN Tiered testing and Tools for Risk Assessment: Results of regulatory relevance (Chair: P. Sayre, US)		
08:30-10:30	**Break Out Group on Risk Assessment Frameworks and Grouping Strategies** (CC9) Co-Chairs: A. Sips (RIVM, NL) and J. Holmqvist (ECHA) Commentator: C. Studer (BAG, CH)	**Break Out Group on Computational Methods and (Q)SARs** (Auditorium) Co-Chairs: P. Demokritou (HSPH, US) and T. Puzyn (University of Gdanzk, POL) Commentator: C. Hendren (Duke University, US)

10:30-11:00	**Coffee break**
11:00-12:00	Plenary of Session 5: Reports from the BOGs, discussion, final comment (CC9)
12:00-13:00	**Lunch**
Session 6: Conclusions - Final Plenary (Chair: T. van Teunenbroek, Min I&M, NL) (CC9)	
13:00-15:00	Panel discussion on general conclusion and central messages chaired by B. Diderich (OECD) Panellist: G. Katalagarianakis (EC DG RES), J. Holmqvist (ECHA), P. Kearns (OECD WPMN), P. Sayre (ProSafe Task Force, US), J. Alwood (EPA, US), G. Moore, (KEMI, SE), T. van Teunenbroek (Min I&M, NL), J. Aarts (AkzoNobel, NL) and L. Friedersdorf (NNCO, US)
15:00-15:30	Summary of final conclusions and recommendations of the science to regulators and policy makers based on the conference discussions (K. Steinhäuser/P. Sayre)
15:30	**Closure**

*TF = Task Force
**BOG = Break Out Group

ANNEX 3: REFERENCES

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