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**OECD WORKING PARTY ON MANUFACTURED NANOMATERIALS
OECD Expert Meeting on Categorization of Manufactured
Nanomaterials**

Washington (USA) 17-19 September, 2014

Background Document

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OECD WORKING PARTY ON MANUFACTURED NANOMATERIALS

OECD Expert Meeting on Categorization of Manufactured Nanomaterials

Background

The goal of the meeting is to develop a categorization approach for manufactured nanomaterials in order to improve regulatory decision making. The expected outputs of the meeting are recommendations on how manufactured nanomaterials could be categorized, by endpoint, for purposes of testing, read across/Structure-Activity Relationships (SAR) for use in hazard assessment and exposure assessment, risk assessment, and how these can be used to better target risk management. The development of categories for nanomaterials will provide an important tool to increase transparency and help streamline the regulatory decision-making process for both regulators and those subject to regulations. In addition, given the limited data on nanomaterials, categories can be used to help fill data gaps, thus improving decision making. Lastly, working collaboratively under the OECD WPMN will ensure the development of a consistent approach to categorization of nanomaterials and minimize overlaps.

While the grouping of chemicals, particularly for purposes of hazard assessment, is used in many jurisdictions, nanomaterials introduce additional challenges, due to intrinsic and extrinsic differences in physical and chemical properties and differences among nano-forms of a chemical species, and between nano and non-nano forms. Further, they often do not exist as distinct species; rather the populations of the materials can consist of distinct species and agglomerates and aggregates and their properties are dependent upon the medium in which they are found. Thus, in looking at how to group nanomaterials, in addition to chemical composition and shape there are also considerations of properties, such as surface charge, which add complexity to the exercise. The context of this OECD Expert Meeting is regulatory, and regulators typically distinguish substances under their respective laws based on a molecular identity (material) focus as opposed to only a properties focus. Thus, while a consideration of properties should also be considered in developing the scheme, any categories being proposed at the workshop should also be based on molecular identity. Specifically, the framework of the categorization scheme should start with molecular identity as shown in the categorization scheme below.

The following categorization scheme is a starting point for the discussions that will occur in the breakout session for each of the following focus areas that the workshop addresses: physical-chemical characterization; fate, exposure; ecotoxicity; human health toxicity; and risk assessment. It is anticipated that a revised categorization scheme will be developed for each focus area. It is not expected that the revised categorization schemes from each of the focus areas will be the same. In addition, the revised categorization scheme may also incorporate one or more properties, e.g., surface reactivity.

- 1) Inorganic Carbon Based Materials
 - a. Fullerenes (other than CNTs)
 - i. Fullerenes
 - ii. Fullerenes modified with organic functional groups
 - b. Carbon nanotubes
 - i. Multi-walled Carbon nanotubes
 1. Number of walls
 2. Functionalized
 3. Unfunctionalized
 - ii. Single-walled Carbon nanotubes
 1. Functionalized
 2. Unfunctionalized
 - iii. Carbon nanofibers
 - iv. Complex arrays of carbon nanotubes
 - c. Graphene and graphitic sheets
 - d. Carbon black derivatives
- 2) Metalloids
 - a. Coating
 - i. Coated/Treated
 - ii. Uncoated/Untreated
- 3) Metalloid Oxides and other metalloid compounds
 - a. Coating
 - i. Coated/Treated
 - ii. Uncoated/Untreated
- 4) Metals
 - a. Coating
 - i. Coated/Treated
 - ii. Uncoated/Untreated
- 5) Metal oxides and other metal compounds
 - a. Coating
 - i. Coated/Treated
 - ii. Uncoated/Untreated

b. Solubility

6) Quantum dots

7) Organic Compounds¹

Heterogeneous substances with multiple components such as inorganic cores, shell structure, multiple functionalisations, etc.

The questions that the experts at this meeting will be asked to address include:

- Is the proposed categorization scheme presented a reasonable starting point for general categorization in a regulatory context? For further categorization?
- Are the proposed categories a reasonable starting point for further sub-categorization as is?
- What specific activities would be needed to provide sufficient evidence for the use of categorization in these questions for risk assessment and risk management?
- What information is required for assessing the validity of each (sub)category?
- What categories are applicable across multiple endpoints?
- To what extent can SAR be used?
- Are there categories that are relevant for an occupational setting?
- How can the data from the OECD WPMN Sponsorship Programme for the following materials be used to support categorization efforts?
 - Fullerenes (C60)
 - Single-walled carbon nanotubes (SWCNTs)
 - Multi-walled carbon nanotubes (MWCNTs)
 - Silver nanoparticles
 - Iron nanoparticles
 - Titanium dioxide
 - Aluminium oxide
 - Cerium oxide
 - Zinc oxide
 - Silicon dioxide
 - Dendrimers
 - Nanoclays
 - Gold nanoparticles

¹ Many organic substances are on the nanoscale but not all are engineered on this size to exploit a nano-specific property (e.g., organic dyes, polymers, organic pigments). There are some organic nanoscale substances which do take advantage of a nanoscale property (e.g., tensile strength of nano-cellulosic substances) and it is these which are included in the 'organic' category above.

Structure of the meeting

Focus areas for this expert workshop address the full range of risk assessment-relevant endpoints.

The meeting is structured to address categorization for the range of nanomaterials and the range of endpoints that are considered in assessing nanomaterials in a regulatory context. These include both hazard- and exposure-relevant endpoints and parameters.

The stage for the expert discussions at the meeting will be set by a series of keynote lectures that address the importance of categorization or grouping for use under regulatory regimes. While the regulatory regimes will vary and the extent to which they use categories and read-across in their regulatory regimes vary, the science upon which hazard, exposure and risk assessments and thus regulatory decisions are made should be consistent. The keynote lectures will provide background or address a key aspect of each of the areas that will be addressed by this expert meeting.

To help provide regulatory context for the expert discussion to follow, there will then be plenary discussions on the use of categories in risk assessment and whether risk management tools can be refined given the current state of knowledge and uncertainties.

The expert discussions will start with a session on physical-chemical characterization. The physical-chemical session will occur first and will not be parallel with other sessions because the proper characterization of physical-chemical properties is key to each of the other sessions –whether identifying the most important physical-chemical properties for each type of endpoint or proper characterization of the nanomaterial to be tested. The most important physical-chemical parameters have been identified for environmental fate, ecotoxicity and human health endpoints. This first breakout session will focus on these.

The session on physical-chemical characterization will be followed by breakout sessions on environmental fate, environmental toxicity, human health endpoints, and exposure assessment. There will then be a plenary session on risk assessment on the final day to draw on the knowledge and outcomes from the other breakout sessions.

Session 1: Context for the Need for the Use of Categories and Perspectives on their Application to Nanomaterials

Importance of Categorization for Risk Assessment and Risk Management

Maria Doa (Environmental Protection Agency, United States)

Categorization of traditional chemicals has been an important tool for purposes of testing, read across/Structure-Activity Relationships (SAR), and hazard assessment. Not only has this aided in conducting assessments with limited data and reducing the amount of testing required, it has helped promote the design, development, and application of safer chemicals and processes. It has also accelerated the market introduction of many new substances. These considerations then have an impact upon the potential risk management tools that would be used to address the chemical. These benefits are also those that are anticipated from a categorization scheme for manufactured nanomaterials. The categorization of manufactured nanomaterials is a goal for many but is still in its infancy. Categorization of manufactured nanomaterials will improve risk assessments because it will help reduce uncertainties in both hazard assessments and exposure assessments, decrease the amount of data needed for individual manufactured nanomaterials. This will support more targeted risk management approaches for different types of manufactured nanomaterials.

Testing OECD member countries' approaches to develop or use concepts of grouping, equivalence and read-across based on physical-chemical properties (GERA-PC) of nanomaterials for their hazard assessment in regulatory regimes

Takuya Igarashi (Research Institute of Science for Safety and Sustainability, National Institute of Advanced Industrial Science and Technology, Japan)

This lecture will give a snapshot of approaches within the OECD sphere to develop or use concepts of grouping, equivalence and read-across based on physical-chemical properties (GERA-PC) of nanomaterials for their human health and ecosystem hazard assessment in regulatory regimes, based on the results of a questionnaire-based survey conducted by the WPMN from October to December 2013. The respondents included Australia, Canada, Denmark, Germany, Japan, Switzerland, the United Kingdom, the United States, the European Union, and the Business and Industry Advisory Committee to the OECD.

Four member countries and the European Union responded that GERA-PC concepts were either in use or being prepared for use in hazard assessments in their regulatory regimes. Six member countries and the European Union reported various R&D activities aimed at supporting the development of GERA-PC concepts for regulatory purposes.

Respondents also addressed the needs and challenges in the development and regulatory implementation of GERA-PC concepts as well as views on the limitations of, and alternatives to, those concepts; including the need for scientific knowledge; mechanistic understanding; comprehensive and reliable data-sets with standardised testing methods; sample preparation and material characterisation; and dealing with surface modifications/properties.

Use of Category Approach and Groupings of Nanomaterials Regionally and Nationally: 1) The Canadian and United States experience with the Regulatory Cooperation Council; 2) European Union experience

Development of a Classification Scheme under the Canada-United States Regulatory Cooperation Council Nanotechnology Initiative

Brad Fisher (Environment Canada, Canada) and Jim Alwood (Environmental Protection Agency, United States)

The focus of this presentation will be on the the development of the classification scheme under the Canada-US Regulatory Cooperation Council (RCC) Nanotechnology workplan. This classification scheme was developed in collaboration with informed stakeholders in Canada and the US with the objective of increasing transparency in decision making, and to provide more targeted information needs for the assessment of industrial nanomaterials. The objective of this Work Element was to provide consistency and clarity in risk assessments through the development of a Classification Scheme. This Classification Scheme will be presented and explained to participants through discussion on the rationale behind its creation, how it was developed, its applicability and implementation in both countries and path forward. Further, participants will be invited to provide their feedback and consider the development of classes/categories from an international perspective

Categorisation under REACH

Jenny Holmqvist (European Chemicals Agency, European Union)

REACH is the European Union Regulation on chemicals and their safe use (EC 1907/2006). It entails the elements of Registration, Evaluation, Authorisation and Restriction of chemicals, and entered into force on 1 June 2007. Although REACH does not explicitly addresses nanomaterials, it implicitly governs these as they fall within the definition of substance under this regulation. Currently, based on concerns from the European Parliament and Council, REACH is undergoing a review in the context of nanomaterials. This is to ensure that nanomaterials are sufficiently addressed by the regulation and their safe use can be demonstrated. This work has identified a need to find pragmatic and scientific justified approaches for categorise and group nanomaterials to minimise e.g. unnecessary animal testing and cost for industry.

However, such approach must not compromise on safe use of these materials on the European market.

Under REACH the registrants have the possibility to use read-across, grouping and categorisation to bridge data gaps when conducting their hazard characterisation. Under the EU regulatory framework, existing approaches for grouping (categorisation) of conventional substances is foremost based on structural similarity and physicochemical properties as a first step. As a next step the read across hypothesis needs to be further strengthened with data of effects on human health and the environment that illustrates a similarity in behaviour. Considerations has been given by EU Member States on how a justification for a scientifically robust read-across for nanomaterials could look like when it is done to fill data gaps as part of hazard characterisation of the substance;

- Consider the quality of the (experimental or modelled) data used in the rationale in a transparent manner
- Terminology and purpose are important and therefore, clarify the purpose and boundaries of the read-across justification
- Solubility is a key parameter for toxicity but can be affected by coating and surface treatments
- Although exposure is part of REACH data requirements and to some extent can be used to waive certain tests the group agreed that “no exposure” argument has very limited use in a justification for read-across.

These initial discussions at EU level for how read-across, grouping and categorisation of nanomaterials and forms thereof can look like will be presented and consideration from the participants will be welcomed.

Physical-chemical Characterization Highlights of the OECD Meeting on Nanomaterials Physical-Chemical Parameters: Measurements and Methods

Monique Groenewold (The National Institute for Public Health and the Environment, The Netherlands)

In June 2014 the OECD Meeting on ‘Nanomaterials Physical-Chemical Parameters: Measurements and Methods’ was held in Washington. About 40 experts from member countries and Industry were present to assess the methods applied for testing the physicochemical endpoints in the OECD-WPMN testing programme. The aim of the meeting was to assess the applicability of the methods used for the specific nanomaterials as well as their general applicability, and provide recommendations for potential modifications of OECD Test Guidelines as well as the need to develop new OECD Test Guidelines.

In preparation of the meeting and to build further on the experience from the sponsorship programme and the expertise of the experimenters and other physical-chemical and metrology experts the submitted physicochemical data from the OECD-WPMN testing programme were assessed by these experts partly prior to the meeting.

During meeting the experts present were able to determine very concrete recommendations to the OECD WPMN for modifying existing and developing new test guidelines for Nanomaterials. In this lecture and overview of the high lights of the OECD Meeting on Nanomaterials Physical-Chemical Parameters: Measurements and Methods will be presented.

Grouping of Nanomaterials for Fate Assessments: Novel properties as an organizing concept for categorizing engineered nanomaterials for regulatory purposes

Mark Wiesner (Duke University Center for the Environmental Implications of NanoTechnology (CEINT), United States):

The observation that some materials exhibit properties at the nanoscale that are different from the properties observed for bulk materials is at the very heart of many applications of nanomaterials that take advantage of these novel properties. It will be proposed that one basis of a nanomaterial categorization scheme should be the novel properties exhibited by, and often specifically exploited in, manufactured nanomaterials. Such novel properties are responsible for both the purposeful interactions that drive performance of engineered nanomaterials as well concern and investigation with regard to collateral nanomaterial environment, health and safety concerns. These properties will drive interactions and transformations within environmental compartments and biota, and they will be the basis of decisions to develop and utilize, or abandon, individual manufactured nanomaterials. As such, this categorization would orient the discussion toward real-world decisions on manufactured nanomaterials.

Practically speaking, the performance of manufactured nanomaterials is what will drive their adoption and utilization in applications. The criteria utilized in determining which materials to develop and utilize are performance based, and the performance will often be related to the effective harnessing of these novel properties.

Categorizing materials according to novel property allows orientation of discussions in terms of the actual decisions being made regarding these materials. Which photocatalyst is the best one to utilize? What material responds to the most finely tuned frequency of drug delivery activation materials? What is the driver of the analyses and research? Decisions about which material to use will entail material comparisons based on performance, based on successful exploitation of the novel properties. Decisions about green chemistry approaches will also center around optimizing

performance while controlling the properties that create undesirable by-products or impart collateral damage upon release. Decisions about modelling material behavior and mode of action, and translating these into regulatory action, will be based on exposure and hazard potentials driven by the uniquely nano-scale properties interacting with the environmental and biological receptors.

Under this schema, some materials (e.g. TiO₂) would fit into multiple novel property categories (e.g. photocatalytic activity and UV absorption), which is appropriate because in those cases it is likely that multiple different attributes of the material must be considered separately from a risk perspective, and may correspond to different decision contexts with regard to material development, use and management.

Bridges: Implication and Application; Discussions and Datasets.

Mechanistic understanding of these novel properties will likely be critical to predicting their behavior not only in the engineered systems in which they are being designed to perform, but also within the natural systems that will be the ultimate sink/receptors/for released nanomaterials and their transformation products.

Categorizing materials according to novel property bridges the discussion of what the nanomaterials are being utilized to do in the first place with consideration of what collateral impacts may be expected, which is important both mechanistically and practically.

If the same data categorization schemata can be utilized to answer questions that inform decisions about performance (which material absorbs the widest spectrum of light?) as well as decisions about assessing and managing risks (which material generates the most net reactive oxygen species?), we can hope that the property-based categorization could serve as a common language to facilitate discussions across the application vs. implication divide. Longer term, datasets captured within this schema could be more interoperable across the application/implication divide, opening the possibility to find efficiencies by linking previously disparate datasets.

Grouping of Nanomaterials for Ecotoxicity Assessments. Novel techniques for toxic nanoparticle categorization

Suman Pokhrel (Foundation Institute of Materials Science (IWT), Department of Production Engineering, University of Bremen, Germany):

The unique properties of manufactured nanomaterials can have adverse bio-impact. The safe utilization of nanotechnology governing environmental health and safety is a multidisciplinary task that goes beyond the traditional risk assessment procedures. One approach for countering the impacts is to probe the number of newly emerging nanomaterials and their wide range of properties by using a high-throughput

screening platform that utilizes manufactured nanomaterials libraries exhibiting a range of compositions and combinatorial properties to study their relationship to a specific injury responses as well as exploiting computational methods to assist in the establishment of quantitative safer-by-design approaches. The development of conceptual paradigms in environmental and health assessment has been recognized that the physical-chemical properties of manufactured nanomaterials play a key role in their fate and transport, human and environmental exposure, and hazard generation. As an attempt, 24 metal oxide manufactured nanomaterials from different groups and periods within the periodic table were chosen and assessed based on their potential overlap of conduction band energy and cellular redox potential (-4.12 to -4.84 eV). The assessment of the cellular response was performed using mammalian cell line, sea organisms (zebra-fish) and bacteria²³. The reasonable correlation observed within these wide test models provided clear evidence that these 24 metal oxide nanoparticles could be categorized as toxic or non-toxic according to their specific physical-chemical properties. Results acquired from these models showed⁴ (1) conduction band energy overlapping with the redox potential in the cellular interior are toxic (2) metal oxide nanoparticles ionizing in the cells and chelating with the biological species are toxic (3) metal oxide nanoparticles having hydration energy > -70eV are toxic. The acquired knowledge in this area (through extensive categorization of the nanoparticles) will offer new opportunities to remediate the environment through multi-disciplinary research between several fields of science.

Report from “Nano Risk Analysis II: A Workshop to Explore How a Multiple Models Approach Can Advance Risk Analysis of Nanoscale Materials”

Jo Anne Shatkin (Society for Risk Analysis):

The Emerging Nanoscale Materials Specialty Group (ENMSG) of the Society for Risk Analysis (SRA) recently held a workshop to investigate the use of alternative testing strategies (ATS) for exposure and risk analyses of nanoscale materials (<http://www.srananoworkshop.org>). The workshop convened a diverse group of international experts to discuss how current and evolving *in vitro* assays might be applied in a “multiple models” approach to inform risk assessments of novel nanoscale materials, including assessing hazard, potency and exposure potential. Participants examined the availability and applicability of novel ATS methods for a multiple-models

² H. Zhang, Z. Ji, T. Xia, H. Meng, C. Low-Kam, R. Liu, S. Pokhrel, S. Lin, X. Wang, Y.-P. Liao, M. Wang, L. Li, R. Rallo, R. Damoiseaux, D. Telesca, L. Madler, Y. Cohen, J. I. Zink, A. E. Nel, Use of metal oxide nanoparticle semiconductor properties and band gap to develop a predictive paradigm for the oxidative stress injury and acute toxicological potential in cells and the murine lung, *ACS Nano*, 2012, 6(5), 4349–4368.

³ S. Lin, Y. Zhao, Z. Ji, J. Ear, C. H. Chang, H. Zhang, C. Low-Kam, K. Yamada, H. Meng, X. Wang, R. Liu, S. Pokhrel, L. Mädler, R. Damoiseaux, T. Xia, H. A. Godwin, S. Lin, A. E. Nel, Screening to reveal the mode-of-action of metal oxide nanoparticles toxicity in zebrafish embryos, *Small*, 2013, 9(9-10), 1776-1785.

⁴ C. Kaweteerawat, A. Ivask, R. Liu, H. Zhang, C. H. Chan, C. Lowkam, H. Fischer, Z. Ji, S. Pokhrel, Y. Cohen, J. Zink, L. Mädler, P. Holden, A. Nel, H. Godwin, Toxicity of metal oxide nanoparticles in bacteria correlates with conduction band energy and hydration energy, *Environ. Sci. Technol.* 2014, Submitted

approach to toxicity, environmental and exposure analyses of emerging nanoscale materials in the risk analysis paradigm. The presentation will describe the findings and recommendations of the expert deliberation and elaborate shared strengths and gaps in support of weight-of-evidence methods that rely on ATS to inform context-specific assessment and management decisions for novel nanoscale materials. Recommendations and guidance for using ATS in hazard characterization and risk assessment for those decisions will be shared.

Grouping of Nanomaterials for Health Assessment.

Highlights from the OECD WPMN Expert Meetings on Genotoxicity; Toxicokinetics, and Inhalation

Maria Donner (DuPont, Business and Industry Advisory Committee).

This presentation summarizes the outcome of the WPMN Expert Meeting on Genotoxicity of Manufactured Nanomaterials (November 18-19, 2013, Ottawa, Canada) and places it in the context of current requirements and guidelines for assessment of genotoxicity endpoints. A grouping of nanomaterials is a logical next step, and would be advantageous for identifying potential genotoxic properties and human health hazards that might be associated with nanomaterials. However, it is premature to attempt this based on the currently available genotoxicity data, based on their inconsistency. Positive results are almost the rule in the *in vitro* comet assay and in a limited array of other genotoxicity tests. On the other hand, data from *in vivo* experiments are often contradicting and do not necessarily support the *in vitro* findings. It has been generally agreed that the current OECD testing guidelines should be evaluated and adapted as appropriate for manufactured nanomaterials. Any revision must be carefully considered and data-based, as well as preceded by expert opinion on how the unique physicochemical properties of nanomaterials will be taken into account, and how to proceed with identification of critical similarities and differences. A successful categorization will facilitate these distinctions, and provide an aid for selection of exposure and testing conditions, and the construction of a training set to use for computational approaches. This categorization is considered to more likely to be driven by other aspects of nanomaterials, such as physicochemical properties, than genotoxicity.

Health effects of nanomaterials state of the art and their regulatory implications“

Tom Van Teunenbroek (Ministry of Infrastructure and Environment, The Netherlands)

The last 15 years the majority of nano-toxicity research is focusing on *in vitro* systems. Many questions have been raised regarding the quality of the *in vitro* nano-tox research due to disregarding transformation of the manufactured nanomaterials under experimental conditions and lack of thorough quality control. Thus, an understanding of

the relationship between the physical and chemical properties of the nanostructure and their *in vivo* behavior needs to provide a basis for assessing nano-toxicity and lead to predictive models for nano-toxicity assessment.

The WPMN has in response to a increasing number of publications on the possible implications of nanomaterials on human health, started in their exploratory phase of its existence the “Sponsor-projects-program”. Since the start of WPMN initiatives like the Nano-Safety-Cluster (NSC) of the European Union and similar programs in the United States (Environmental Health and Safety-National Nanotechnology Initiative) and other OECD-WPMN members (i.e. Japan, Australia, Canada, Korea) have been funding research manifold over the budget of the Sponsor-project. Industry has stepped up their participation the most recent being the Nano-release project.

Due to these on-going efforts many studies have become available of *in vivo* nanoparticle-exposure. Predominantly based on inhalation as exposure route, which is perceived as the most critical route of exposure.

This keynote on the health impacts of nanomaterials will present recent results of *in vivo* work and explore their implications for regulatory purposes.

Example of the Grouping of Nanomaterials for Health Assessment in a National or International Regulatory Context. How to extend/verify the Concept: “Taquann” whole body inhalation system for speeding up the toxicity studies for categorization of manufactured nanomaterials

Jun Kanno (Division of Cellular and Molecular Toxicology, Biological Safety Research Center, National Institute of Health Sciences, Japan)

Using the Mitsui MWNT-7 as a *de facto* standard multi-wall carbon nanotube for fiber-type nanomaterial toxicity studies, we first monitored its mesotheliomagenic potential by the intraperitoneal injection model of p53 heterozygous mice⁵. These studies suggested that non-granulomatous chronic inflammatory microlesions are closely related to the “frustrated phagocytosis” originally proposed in the past for the asbestos carcinogenesis.

In general, however, there is no pre-existing toxicity data on a new nanomaterial; its toxicity should be studied on a case-by-case basis. Meanwhile, since inhalation is the major route of exposure for humans, the whole body inhalation exposure study is the first choice for assessing its “hitherto unknown” toxicity.

⁵ Takagi, A., Hirose, A., Ishimura, T., Fukumori, N., Ogata, A., Ohashi, N., Kitajima, S., Kanno, J. Induction of mesothelioma in p53^{+/−} mouse by intraperitoneal application of multi-wall carbon nanotube. *J. Tox. Sci.* 2008, 33, 105-16, Takagi A, Hirose A, Futakuchi M, Tsuda H, Kanno J. Dose-dependent mesothelioma induction by intraperitoneal administration of multi-wall carbon nanotubes in p53 heterozygous mice. *Cancer Sci.* 2012; 103: 1440–4.

We adopted MWNT-7 as the first sample to develop a new whole body inhalation system. A major problem with generating MWNT-7 aerosol was the agglomerates and aggregates in its bulk sample. We developed a method to generate aerosol of highly-dispersed MWNT-7 single fibers without aggregate/agglomerate and a small scale inhalation system for it (Taquann Method and Taquann Direct Injection System)⁶. Aerosol in the inhalation chamber was composed of single fibers of the same size distribution to the fibers in the original sample. Fibers of the same length distribution were found in mouse lung, and histology showed no aggregates/agglomerates and no granulomatous changes. The fibers were also found in the microscopic lesions located on the surface of parietal pleura; the microlesions were similar to what we monitored in the intraperitoneal injection studies.

The Taquann system is relatively cheap, simple and easy to operate, and is a sealed system and therefore easy to keep the facility clean. There is no sample loss after liquid phase filtration so that this system is ideal for testing the new nanomaterials of low-product-volume. The system can be applied to various types of nanomaterials; we have tested TiO₂ nanoparticles to gain a highly dispersed aerosol.

In conclusion, Taquann system should speed up the whole body inhalation toxicity testing of the new nanomaterials and facilitate the process of categorization of manufactured nanomaterials using *in vivo* respiratory toxicity data. And, timely transfer of the toxicity data to the manufacturers and to the future users will contribute for the development and acceptance of the safer new products⁷.

Grouping of Nanomaterials by Release Type

Thomas Kuhlbusch (Institute of Energy and Environmental Technology e. V. (IUTA), Air Quality & Sustainable Nanotechnology Unit, Duisburg, Germany, and Center for NanoIntegration (CENIDE) University Duisburg-Essen, Germany)

Current assessments of possible exposures of workers, consumers, the population and the environment are mainly based on the direct measurements of nanomaterials in the corresponding exposure media. In cases where measurements are very tedious or near to impossible, models are applied to derive realistic exposure values. The current situation is that 1) measurements can be expensive, 2) measurements are not really possible and models have to be applied, 3) basic data on the release of nanomaterials are lacking for these models, and 4) predictions of the likelihood of exposure are difficult to make without knowledge on possible release rates.

Hence, basic developments and studies are currently on-going investigating release mechanisms and rates as well as the environmental transport. Additionally,

⁶ Taquahashi, Y., Ogawa, Y., Takagi, A., Tsuji, M., Morita, K., Kanno, J. An improved dispersion method of multi-wall carbon nanotube for inhalation toxicity studies of experimental animals. *J. Tox. Sci.* 2013, 38, 619-628

⁷ Supported by the Health and Labour Sciences Research Grant, MHLW, Japan

national and international harmonisation and standardisation activities have started in recent years to facilitate the understanding and prediction of possible release.

One problem, inherent to manufactured nanomaterials is their high versatility with regard to the basic material and their modifications (e.g. morphology, surface functionality). It is near to impossible to test all manufactured nanomaterials and their products for all possible scenarios. Hence, a grouping or categorization of manufactured nanomaterials and their product classes are needed. To allow a grouping some specific questions have to be tackled:

- 1) Which release mechanisms/processes can be differentiated and possibly used for grouping in view of release probabilities and rates?
- 2) How can the link from release (testing) to exposure be established and used for grouping of exposure scenarios? and
- 3) Which metric/particle parameter has to be used in view of sensitivity and possible impacts?

The presentation will deliver an overview of recent advancements and discuss suggested concepts.

Exposure Assessment

Charles L. Geraci (Nanotechnology Research Center; National Institute for Occupational Safety and Health, United States)

Meaningful exposure assessment is a critical element for accurate risk characterization and for the development of effective risk management strategies. Exposure to engineered nanomaterials can occur across a broad range of populations as the material progresses down its life cycle. Human exposure can occur to researchers, manufacturing personnel, product consumers, and in the general population. The specifics of the exposure scenario will be dictated by a combination of the characteristics of the exposed group and of the nanomaterial itself. Logical groupings can be created for human exposure groups along the material and product life cycle. It should also be possible to approach the broad array of nanomaterials introduced into commerce by grouping them into categories based on basic physical and chemical characteristics. Creating a matrix made up exposed populations matched up with groupings of nanomaterials, weighted by parameters such as production volume and nature of the product using the nanomaterial will identify priority intersections where exposure assessment will be most meaningful. Moving this thinking into environmental exposure assessment will require a matrix of the environmental medium matched against many of the same characteristics used to group nanomaterials for human exposure. Additional factors such as agglomeration/deagglomeration, transformation,

matrix degradation, and bioavailability will impact exposure assessment in both the human and environmental arenas, but will likely need to be addressed differently.

Session 2: Risk Assessment and Risk Management

Session 2a: Risk Assessment

Co-chairs: Yasir Sultan (Chair WPMN Steering Group on Risk Assessment and Regulatory Programmes, Environment Canada, Canada) and Maila Poulamaa (European Chemicals Agency, European Union)

Risk assessments involve addressing a combination of endpoints and exposure/release probabilities and frequencies to determine if a substance presents or will present an (unreasonable) risk to health or the environment⁸. These end-points include, but are not limited to identification, physical-chemical properties, fate in the environment, human health or environmental effects, exposure pathways to humans, and end-of-life disposal/recycling. The risk assessment may cover a whole life cycle of the substance or selected parts and conclusions are made through balanced considerations of hazards and exposure. This paradigm holds true for both nano and non-nano chemicals. The participants in this expert meeting are invited to review the public OECD documents ENV/JM/MONO(2012)8 and follow-up prioritization in ENV/JM/MONO(2013)18 for more context on ongoing issues associated with the risk assessments of nanomaterials.

The categorization of risks provides new means to better assess, manage and communicate them. Qualitative risk categories are useful in case of problems in measurability, while quantitative ones will provide higher degree of precision to the risk assessment and risk management measures. Independently from the risk categorisation methods, their applicability areas and limitations should be defined as appropriate to enable a meaningful evaluation, management and communication.

Manufactured nanomaterials, in contrast to traditional chemical substances (non-nano), is an emerging area for which there is a paucity of information and experience. Thus, there are uncertainties associated with almost every aspect of risk assessment. Risk assessment practitioners continue to 'learn on the fly' and use 'innovative approaches' in the presence of ambiguous data, uncertainties, and knowledge to make decisions.

⁸ Chandler, R.; *BioEssays*, 1987, 5, 176.

One of the primary challenges for nanomaterials is that they offer an almost limitless variety⁹ of combinations in terms of physical (size, shape, etc.) and chemical (surface, core, core-shell chemistries, etc.) properties. While scientists are quite good at making all the different types of nanomaterials which can exist, their properties in different environments remain largely in the realm of the unknown. In addition, there is practically little to no information on the releases of nanomaterials and exposure pathways are very difficult to predict. As an interim approach, risk assessment conducted on nanomaterials is done on a substance-specific basis, *i.e.*, data is generated on each combination of nanomaterial. The lack of an appropriate nomenclature system may limit our ability to develop applicability domains to each set of data. While substance-specific information is useful, it is not ideal since it limits our ability to leverage existing information to increase our weight-of-evidence in risk assessments by reducing uncertainties and does not give us the ability to develop trends and in turn predictions through models.

Over the past few years, there has been a steady increase in scientific literature suggesting ways to group nanomaterials into categories for the purposes of risk assessment. These include works building on qualitative or quantitative assessments of hazards and exposure by Kuempel et al.¹⁰ on the development of categories for occupational exposure limits based on similar physical-chemical properties, biological mode of action, and comparative potency analysis; Madl et al.¹¹ who have suggested grouping for human health safety based on physical behavior including releasability from the matrix, exposure pathway, bioavailability, biopersistence and severity of health effects. Stone et al.¹² as part of the NanoImpactNet initiative identifying a chemical-based categorization system as a 'reasonable starting point, with some modifications'; Nel et al.¹³ who have started to develop categories based on toxicological modes of action governed by physicochemical properties; Wang et al.¹⁴ on ranking and profiling bioactivity of large number of diverse nanomaterials; Foss-Hansen et al. (2008) on the development of categories based on the location of the nanomaterial on/in the product¹⁵

⁹ Kuempel, E.; Castranova, V.; Geraci, C.; Schulte, P. Development of risk-based nanomaterial groups for occupational exposure control. *J. Nanopart. Res.*, 2012, 14, 1029.

¹⁰ Kuempel, E.; Castranova, V.; Geraci, C.; Schulte, P. Development of risk-based nanomaterial groups for occupational exposure control. *J. Nanopart. Res.*, 2012, 14, 1029.

¹¹ Madl, A.K.; Unice, K.; Kreider, M.; Kovochich, M.; Bebenek, I.; Abramson, M. TechConnect World Conference, May 12-16, 2013. Washington DC. ISBN 978-1-4822-0586-2 Vol 3:485-488.

¹² Stone, V.; Nowack, B.; Baun, A.; Brink, N.; Kammer, F.; Dusinska, M.; Handy, R.; Hankin, S.; Hassolov M.; Joner, E.; Fernandes, T. *Sci. Tot. Env.*, 2010, 408, 1745.

¹³ Nel, A.; Xia, T.; Meng, H.; Wang, X.; Lin, S.; Ji, Z.; Zhang, H. Nanomaterial Toxicity Testing in the 21st Century: Use of a Predictive Toxicological Approach and High-Throughput Screening. *Acc. Chem. Res.*, 2013, 46, 607.

¹⁴ Wang, A.; Berg, E.L.; Polokoff, M.; Yang, J.; El-Badawy, A.; Reif, D. Kleinstreuer, N.; Marinakos, S.; Badireddy, A.R.; Gavett, S.; Rotroff, D.; Gangwal, S.; Rabinowitz, J.; Matson, C.; Tolavmat, T.; Mark Wiesner, M.; Houck, K. Poster, *Ranking and profiling nanomaterial (NM) bioactivity by ToxCast high-throughput screening (HTS)*, EPA (2014) http://www.epa.gov/ncct/download_files/sot/posters/2013/Wang%202013%20SOT%20Poster7.pdf

¹⁵ Hansen, S. F.; Michelson, E.; Kamper, A.; Borling, P.; Stuer-Lauridsen, F.; Baun, A. Categorization framework to aid exposure assessment of nanomaterials in consumer products. *Ecotoxicology*, 2008, 17(5), 438.

categories of nanomaterial risks (NanoRiskCat)¹⁶ for further ranking and communicating on nanomaterials; Madl et al. (2013)¹⁷ used a similar kind of approach for both industrial and consumer applications of nanomaterials and O'Brien et al. (2011)¹⁸ for metallic nanomaterials of environmental concern in view of aquatic exposure and behavior. The comprehensive review by Jahnel, Fleischer, and Seitz in 2013¹⁹ on the merits of different types of categorization schemes adapted from traditional approaches for evaluating risks of nanomaterials, including the Swiss Precautionary Matrix assessing risk and indicating risk management options²⁰.

The various risk categorizations facilitate risk prioritisation and management as well as enhance communication between authorities, stakeholders and general public. However, they also require varying amount of data, tools and guidance to ensure consistency in data capture, assessment, modelling and reporting. It is unclear if all these different categorizations can be combined in an intelligent fashion to give a better picture of how/when to use categories in regulatory risk assessments.

Participants are expected to review the primary literature cited in this document and others in literature prior to the workshop.

Session 2b: Risk Management

Panel: Maria Doa (Environmental Protection Agency, United States), Henrik Laursen (European Commission, European Union), and Brad Fisher (Environment Canada, Canada)

The development of robust health and safety data for manufactured nanomaterials, an emerging area, is still in its initial stages. Assessments are further complicated because in addition to assessing the distinct species of manufactured nanomaterials, there must be a consideration of the related bulk chemical (if there is a related bulk chemical) and a consideration of the distribution of materials (distinct species, agglomerates and aggregates). These factors and the gaps in health and safety data impact the precision of the assessments and thus the risk management tools that can be used. Further complicating risk management is that manufactured nanomaterials may be regulated under authorities more tailored to chemicals rather than manufactured materials. The lack of information (and thus a higher level of

¹⁶ Hansen S.F., Jensen K. A., Baun A. J. NanoRiskCat: a conceptual tool for categorization and communication of exposure potentials and hazards of nanomaterials in consumer products. *J. Nanopart Res*, 2014, 16(1) 1-25

¹⁷ Madl, A.K; Unice k.; Kreider M.; Kovoichich M.; Bebenek I.G.; Abramson A.A (2013): TechConnect World Conference, May 12-16, 2013. Washington DC. ISBN 978-1-4822-0586-2 Vol 3:485-488.

¹⁸ O'Brien, N.J., Cummins, E.J. A risk assessment framework for assessing metallic nanomaterials of environmental concern: aquatic exposure and behavior. *Risk Analysis*, 2011, 31(5), 706-726

¹⁹ Jahnel, J.; Fleischer, T.; Seitz, S. 2013, 429, 012063.

²⁰ Swiss Precautionary Matrix <http://www.bag.admin.ch/nanotechnologie/12171/12174/index.html?lang=en>

uncertainty), the complexity of manufactured nanomaterials, and the existing statutory frameworks may result in risk management decisions that may not be tailored appropriately for the material even though the hazard characterization, hazard assessment or risk assessment was conducted for individual manufactured nanomaterials. Thus, the risk management decision may be inconsistently insufficiently conservative or overly conservative. This session will provide background on the limitations regulators are dealing with in treating these complicated materials within a framework primarily established for chemicals.

Session 3: Physical-Chemical Properties

Co-chairs: Vicki L. Colvin (Rice University, United States) and Angela Hight Walker (National Institute of Standards and Technology, United States)

Physical-chemical characterization of nanomaterials is paramount in order to correlate biological/toxicological responses with these properties. The purpose of this session is to assess to what degree physical-chemical properties can or should be used to guide the categorization of manufactured nano-objects. Another goal is to ensure direct communication between the characterization and toxicology communities. The session will also identify guidance on necessary measurands and potential strategies to enable sufficiently thorough and proper physical chemical characterization of manufactured nano-objects.

Physical-chemical parameters collectively define how a nanomaterial will behave within a given system and therefore likely influence the environmental and human health impact of nanomaterials. Since the properties of nanomaterials can influence their functionality, behavior and potential effects, physical-chemical characterization is inherently linked to all of the other break-out sessions.

The following questions will be addressed:

- Thorough and proper physical-chemical characterization of nano-objects is a necessary step in order to properly interpret data from biological and environmental fate outcomes. Is there sufficient evidence to suggest that physical-chemical parameters can be used to predict or categorize manufactured nanomaterials based on the potential for biological or environmental impact? To what extent can physical chemical characterization be used to inform risk assessment or risk management?
- Physical-chemical characterization has the potential to encompass a vast number of tests that may or may not have relevance to a specific risk associated with a given manufactured nanomaterial. To what extent should physical-chemical parameters like composition be used to guide other “necessary”

physical-chemical parameters? To what extent should fate and exposure or biological endpoints be integrated to assist in guiding physical-chemical analysis?

- What physical-chemical endpoints are most applicable for accessing potential environmental impact? Potential human health impact? How can the test regiments remain relevant despite the increasing complexity of materials and enhanced capabilities in terms of material design and synthesis?
- What are their barriers to implementing detailed physical-chemical characterization into toxicology assessments, i.e. expense, availability, time, multidisciplinary nature?
- Composition-based categorization schemes, such as the proposed categorization scheme, provide a framework that lends itself to the development of specific physical-chemical test guidance based on a material's assigned category. This form of categorization attempts to simplify necessary testing based on bulk chemistry and integrates well with existing categorization paradigms. However, is the proposed categorization scheme a reasonable strategy for manufactured nanomaterials? Should physical-chemical parameters like composition lead categorization or should it support other schemes?
- It is not always possible to directly compare physical-chemical characterization data from one study to another. To what extent is there a need to standardized test protocols, media and reference materials? How does one introduce a suitable amount of flexibility to ensure that the recommended procedures are valid within a given test substance? How can compliance with the recommendations be ensured?
- How should physical-chemical testing be implemented? Is there a need for a tiered-approach integrating parallel biological/environmental endpoints?

Session 4: Environmental Fate

Co-chairs: Willie Peijnenburg (Laboratory for Ecological Risk Assessment National Institute for Public Health and the Environment, The Netherlands) and Elijah Petersen (National Institute of Standards and Technology, United States). Rapporteur: Steffi Friedrichs (Nanotechnology Industries Association, Business and Industry Advisory Committee)

The focus of this breakout group will be to discuss the possibilities of grouping of nanomaterials with regard to their environmental fate. The discussion will be on the characteristics of groups of nanomaterials that determine the main processes that jointly determine the fate of a nanomaterial in water/soil/sediment. It is expected that as most information on these processes is available for the water compartment, the discussions on grouping will focus on this compartment.

Relevant endpoints

The environmental fate of nanomaterials is affected by the composition of the environment in terms of the physical-chemical composition of the environmental compartments, and the chemical and physical-chemical composition of the nanomaterial. In modelling and assessing the fate of nanomaterials, it is common to use a bottom-up approach in which the basic processes/mechanisms are integrated in an overall fate model that is typically applicable to a specified class of chemicals. A schematic overview of the important fate processes for nanomaterials in the aquatic environment is given in Figure 1.

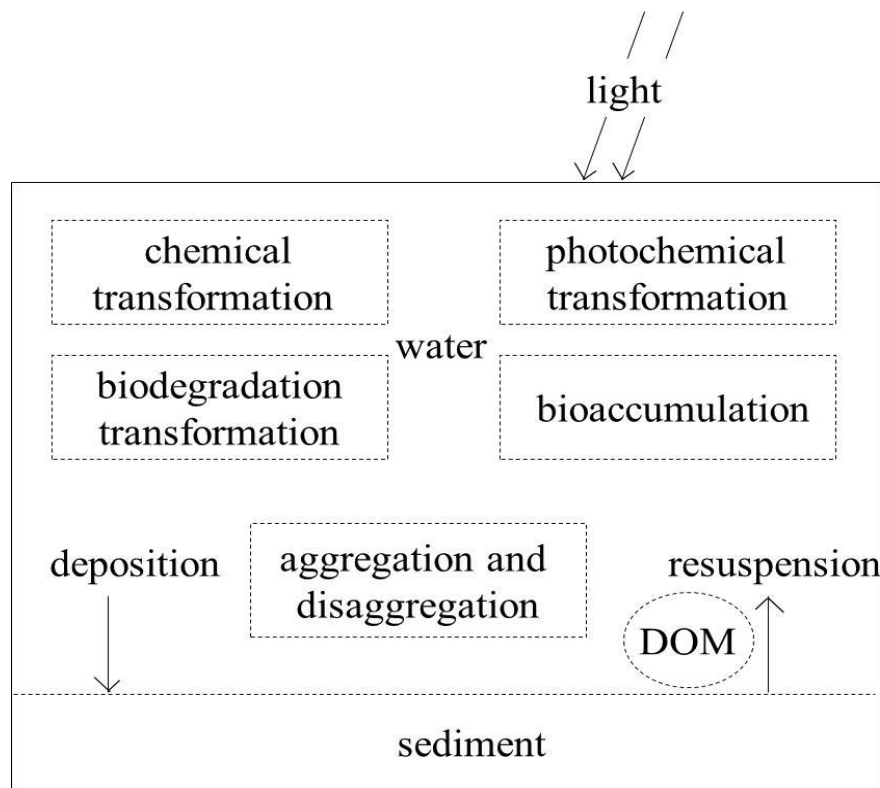


Figure 1. General overview of the main processes determining the fate of a nanoparticle in a stagnant aquatic environment.

As discussed by Baalousha et al. (2014)²¹ the main processes determining environmental fate, include:

- Aggregation/disaggregation
- Dissolution/precipitation
- (Bio)degradation
- Diffusion/sedimentation
- Nanoparticle coating, aging/weathering

The first issue of the breakout group will be on the completeness of this list of processes, taking account of differences among groups of nanomaterials.

Particle properties affecting environmental fate and factors of subcategories that impact multiple endpoint groups

The main part of the discussions within the breakout group will be on the nanoparticle properties that affect environmental behaviors for subcategories of manufactured nanomaterials and, *vice versa*, on the factors within subcategories of manufactured nanomaterials that impact the most important endpoints related to fate assessment. On the basis of the outcome of these discussions, appropriate types of testing for categories of manufactured nanomaterials and recommendations for grouping of nanomaterials with regard to environmental fate will be assessed.

The main goal of grouping manufactured nanomaterials is to identify groups of nanomaterials that allow filling in data gaps by using information from other ('related') nanomaterials, subsequently providing guidance on what information is needed for actual read-across and for data-interpolation. As mechanism-based insight in the relationships between physical-chemical parameters and fate-determining processes is currently still in its infancy, the members of the breakout group are expected to discuss the best strategy for grouping of nanomaterials for this purpose. As such a distinction may be made between what is achievable in the near future and the way forward in the long run. An important consideration in this respect will be on the properties of nanomaterials that affect the various processes, such as the following:

- Charge
- Size
- Morphology
- Coating/natural corona
- Chemical composition

²¹ Baalousha, M., Cornelis, G., Kuhlbusch, T., Lynch, I., Nickel, K., Peijnenburg, W.. Validation of model formulations for fate and behavior assessment of engineered nanomaterials. Environmental Science and Technology, in preparation, 2014.

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When combining the most important fate determining processes and general knowledge on particle composition, some potential categories of nanomaterials may be distinguished beforehand. For example:

- Dissolution is an important process with regard to the fate and the toxicity profiles of nanoparticles and could be substantial for some metallic nanoparticles like AgNPs, CuO NPs, but is less likely for AuNPs, TiO₂ NPs, and not applicable for carbon nanomaterials. In this respect, a strategy is needed for dealing with potential reformation of nanoparticles after dissolution, such as for AgNPs, taking account of new shapes that potentially may be formed.
- Biodegradation is only of potential relevance for carbon nanomaterials, although the coatings of other nanomaterials could also be degraded.
- There may not be sufficient information available to begin grouping for other processes such as aggregation, sedimentation, bioaccumulation, etc. based on the nanomaterial properties.

Recommendations

The final part of the discussions is expected to focus on the best way forward in grouping of nanomaterials with regard to assessing their environmental fate.

Session 6: Environmental Toxicity

Chair: Greg Goss (University of Alberta, Canada). Rapporteur: Eric Bleeker (The National Institute for Public Health and the Environment, The Netherlands)

This breakout group will focus on the grouping of nanomaterials with regard to their ecotoxicological effects. The discussion will be on the characteristics of groups of nanomaterials that jointly determine the environmental toxicity of a nanomaterial towards aquatic, terrestrial and sediment species. Similar to non-nanomaterials it can be expected that for risk assessment the main focus will be on aquatic ecotoxicology and thus this will form an important part of the discussions in this breakout group. In addition this breakout will benefit from the discussion on environmental fate and grouping of nanomaterials as the environmental toxicity of materials will in part be determined by their fate, both in the environment and in the test system.

Aquatic endpoints

During the OECD Expert Meeting on Ecotoxicology and Environmental Fate in Berlin (January 2013) it became apparent that knowledge on particle behavior in test media is still limited. Nevertheless it was concluded that dissolution and dispersability

are important processes, which resulted in the recommendation from this workshop to develop (guidance for) a decision tree that helps in decision towards the most relevant ecotoxicity testing (e.g. aquatic, sediment, or terrestrial testing).

Work on such a decision tree has started, including an Expert Workshop on the “Guidance Document on Aquatic (and Sediment) Toxicology Testing of Nanomaterials” that was held in Washington in July 2014. A presentation on this workshop will be given along with the key findings and discussion points from that workshop. The key goals of the workshop were to define as best as possible uniform and consistent bioassay data to inform future test guidelines and risk decisions, partly building on the OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures. Four phases were identified that need further guidance:

- The generation of a particle stock medium, needed for spiking test media (this should meet certain dispersion / stability criteria that need to be defined);
- The production of exposure media and dosing method (optimization is needed that takes into account particle stability and organism health);
- The conduction of the assay itself (which needs identification of acceptable methods, including monitoring frequency);
- The analysis and reporting of the data (which may need guidance on interpretation and dosimetry).

Each of these steps may inform potential possibilities for grouping nanomaterials as well.

The workshop in July decided on a preliminary decision tree for a priori decisions of how to conduct tests and which tests are needed to conduct. To some extent similar questions may lead to grouping of the nanomaterials.

The usefulness of such an approach for grouping of nanomaterials will be further discussed.

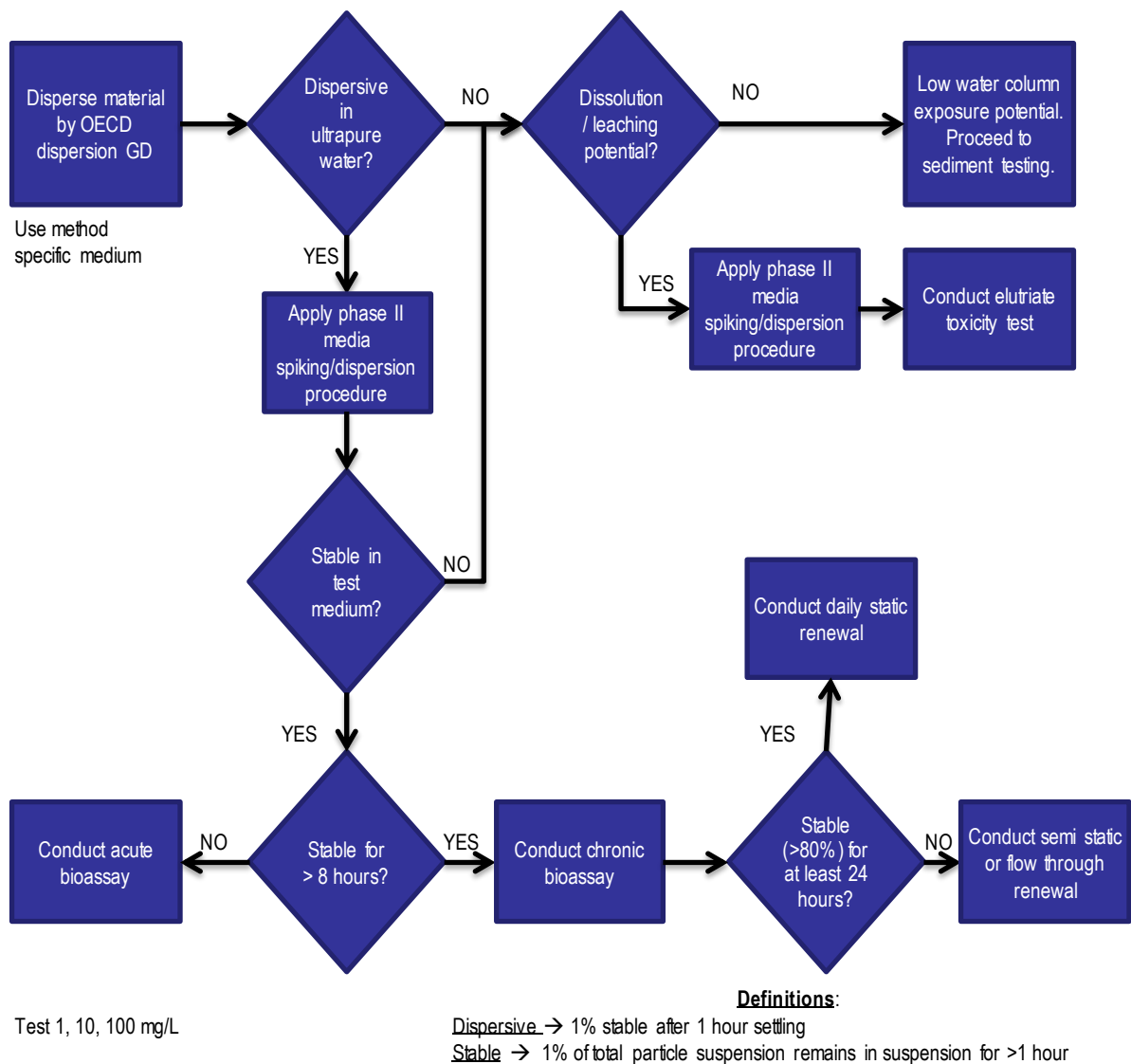


Figure 2. Preliminary decision tree for a priori decisions of how to conduct tests and which tests are needed to conduct as decided in July 2014

Terrestrial endpoints

The second part of the discussion will focus on environmental toxicity tests with terrestrial organisms. In the complex soil compartment toxicity is even more influenced by availability of the nanomaterials and thus the discussions on environmental fate will be important for these endpoints as well.

Nevertheless, specific modes of action (e.g. ion release) may be of influence in determining the most influential factors determining terrestrial toxicity, thus informing potential parameters for grouping of nanomaterials.

Recommendations

The final part of the discussions is expected to focus on the best way forward in grouping of nanomaterials with regard to assessing their toxicity in the different environmental compartments water, sediment and soil.

Sessions 5 & 7: Human Health

Co-chairs: Jenny Holmqvist (European Chemicals Agency, European Union) & Juergen Schnekenburger (Biomedical Technology Center of the Medical Faculty Münster, Germany) and David Warheit (DuPont, Business and Industry Advisory Committee) & Phil Sayre (Environmental Protection Agency, United States);

Rapporteurs: Myriam Hill (Health Canada, Canada) and Agnes Oomen (The National Institute for Public Health and the Environment, The Netherlands)

Nanogrouping or “Nanocategorization” is a concept (and a daunting task) that, going forward, will be critically necessary to implement as a framework in order to focus testing. To be specific, as new products containing manufactured nanomaterials come into commerce - it is unrealistic to assume that the safety profile of each of these individual components can be adequately tested or verified, for all of the relevant hazard endpoints in a timely manner. In theory, to implement this type of nanogrouping approach, closely related nanomaterials are assessed as a group or category, rather than as individual nanoparticulates so that not every manufactured nanomaterial-type would require individual testing for each of the relevant safety endpoints.

Implementing a framework for categorization that can be used in a regulatory context will be a complex process, due, in large part, 1) to the relative paucity of hazard information (for any relevant route of exposure) on many of the new manufactured-types; 2) insufficient information or criteria for assigning a particular manufactured nanomaterial-type to a particular group; 3) absence of relevant exposure information for the manufactured nanomaterial-type in question; 4) a variety of additional factors.

Where to begin? The development of functional categories (nanogrouping) for manufactured nanomaterials for the purposes of Read Across evaluations will require, at the outset, development of a trial and error approach and subsequent iterative process. The similar chemistry represents the most commonly used approach for categorization, although in very specific cases the fiber paradigm may be also considered. As more information is developed, this process will inevitably be refined.

The human health breakout sessions will focus on addressing the following issues/questions:

- How can the *in vivo* hazard data/outcomes derived from the WPMN I Safety Testing Programme be systematically utilized/incorporated to develop a strategic pathway or general framework for implementing a nanocategorization programme.
- Representative manufactured nanomaterials were tested in the OECD Testing Programme. Can the results from these studies contribute to the database from which a nanocategorization process can be initiated?
- Given the relative paucity of hazard or exposure information on individual forthcoming/new manufactured nanomaterials, how can we commence a process or framework to ultimately use “Read-across” methodology for assessing the safety of manufactured nanomaterials?
- How similar or identical is the hazard or risk profile of the manufactured nanomaterials vs. the bulk materials of identical chemical composition? Many of the bulk materials have previously been safety tested or extensively evaluated – do they provide important clues on the hazards of manufactured nanomaterials for purposes of grouping?
- Can certain categories of manufactured nanomaterials with similar composition (e.g. metal oxides) be grouped for the purposes of hazard assessment via non-testing approaches (despite evidence some published data suggesting that certain forms of TiO₂ may have different pulmonary toxicity profiles?) Does this vary depending upon type of manufactured nanomaterials, e.g., grouping may be more appropriate for subsets of CNTs?
- What are the minimum hazard information datasets necessary to adequately conduct hazard assessments via non-testing approaches? Are there any that can be done now?
- Given that, in general, there are different (*i.e.*, reduced) safety concerns associated with dermal and/or oral routes of exposure to manufactured nanomaterials relative to inhalation exposures of the same materials; can one separate the “Read-Across” processes to expedite the development of this framework for certain applications/anticipated exposure routes?
- Given the relevant paucity of *in vivo* hazard information on specific manufactured nanomaterials -types at relevant exposure concentrations (*i.e.*, to which humans will be exposed) and that some regulatory authorities continue to request *in vivo* testing given the limited alternatives -how can the those within the OECD encourage the initiation and development of studies that address the impacts of manufactured nanomaterials exposures on a variety of endpoints in complex systems? Moreover, how can studies that can link *in vitro* studies to relevant *in vivo* findings be developed? What are the best ways to facilitate data sharing broadly? How can the *in vivo* testing that will continue to be generated in some jurisdictions be best used?

- How can a set of screening tools that reflect important characteristics or toxicity pathways for each of the relevant routes of exposure be developed? How can these tools provide important mechanistic data that can be utilized for non-testing approaches to hazard assessment? Important criteria for the experimental design of these studies include: for *in vivo* studies: robust physicochemical characterization of the manufactured nanomaterials of concern; relevant exposure/dose and appropriate dose-metrics; dose-response characteristics (e.g., zinc-oxide particles); time course studies; benchmark controls for better interpretation of data;
- For transitioning of *in vivo* results to *in vitro* investigations, relevant cell types that reflect the appropriate route of exposure (oral, dermal, pulmonary – co-cultures vs. single-cell cultures; appropriate doses and dose metrics; dose-response characteristics; time-course studies; (critical) benchmark controls for necessary interpretation of hazard data.
- How should the proposed categorization be modified?

An important consideration in developing an approach will be on how the properties of nanomaterials affect various biological. The key physical-chemical parameters are:

- Particle size and size distribution (wet state) and surface area (dry state) in the relevant media being utilized – depending upon the route of exposure
- Crystal structure/crystallinity;
- Surface charge
- Agglomeration status in the relevant media;
- Surface functionalization/Composition/surface coatings;
- Surface reactivity;
- Dissolution/Solubility
- Method of nanomaterial synthesis and/or preparation including post-synthetic modifications (e.g., neutralization of ultrafine TiO₂ particle-types);
- Purity of sample

Do the results of the physical-chemical session result in changes to this group of physical-chemical parameters?

Breakout Session 1 will focus on an overview of the issue, purpose, use of sponsorship data, limitations and challenges of group. This session will discuss an overall approach. This session will also address the following issues.

- Define the purpose of the grouping/category
- Identify requirements for a regulatory decision
- Assessment and format of available data

- Minimum criteria for a scientific justification of the boundaries of the category/group

There are differences in what criteria should be considered and used to justify a decision on the possibilities to group and construct categories of nanomaterials and/or forms. However, despite these differences, it is recognised that a proper physical-chemical characterisation (structural similarities) is a fundamental start. Depending on the level of uncertainty, further information may be needed. In case of read-across to fill data gaps in a hazard characterisation of a substance, there is currently a need to strengthen the justification by also presenting toxicological data indicating similar behavior. A prelude to a read-across approach may be to examine the results of shorter-term test protocol results, in conjunction with results of standardized tests, in order to group materials and/or reduce further testing needs.

Breakout Session 1 will analyze the criteria, approaches and tools for manufactured nanomaterial categorization to allow for a combined evaluation of available categorization criteria. Breakout Session 1 has a focus on the data for different material properties and toxicological endpoints.

Following the discussion in Breakout Session 1, Breakout Session 2 will consider hazard endpoints in more detail, and attempt to build on the findings of Breakout Session 1. Presentations will focus on pulmonary toxicity and the construction of categories for relative toxicity evaluation. The working hypothesis is that physical-chemical properties alone will not be sufficient to build scientifically justified categorization of nanomaterials or forms of a certain nanomaterial. The questions to be addressed include:

- To what extent can categories be formed for pulmonary toxicity, based on (1) in vitro, and short-term in vivo, data; (2) physicochemical traits; and (3) other approaches such as mode-of-action groupings?
- How can these categories for pulmonary toxicity be used in reduced testing and/or read across contexts?
- How can similar approaches to reduce testing needs be used for other health endpoints?

Session 8: Exposure Assessment

Chair: Vladimir Murashov (National Institute for Occupational Safety and Health, United States); Rapporteur: Kim Rogers (Environmental Protection Agency, United States)

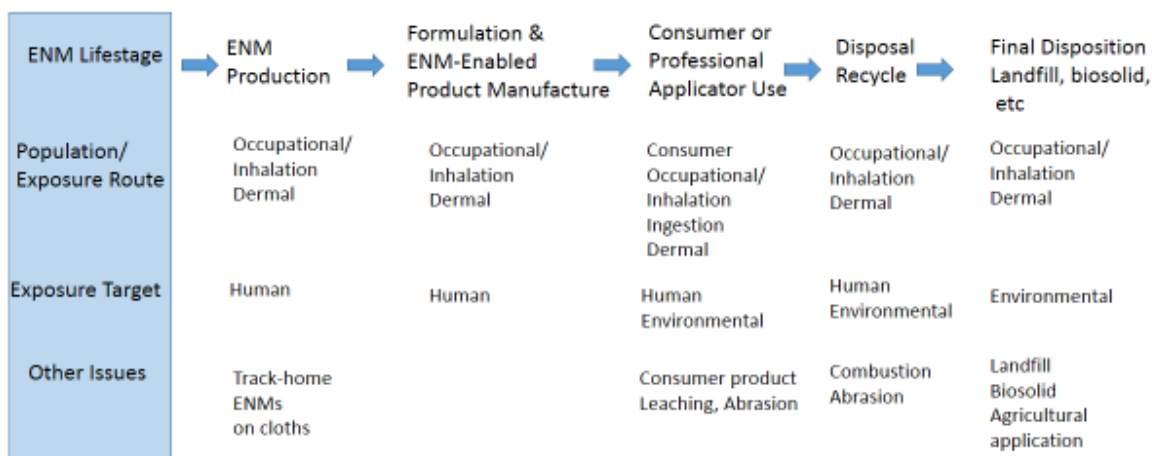
Where there is a corresponding bulk form, manufactured nanomaterials are considered separately from their bulk elemental or compound forms primarily because of their size which endows them with unique physical and chemical properties (*i.e.*,

<100 nm in one dimension [plates], in two dimensions [fibers and tubes] or in three dimensions [particles]). Specifically, manufactured nanomaterials have a very high surface area per unit mass, as well as unique optical, electrochemical and chemical reactivity that can vary significantly with the size, shape and surface coatings even for particles of essentially the same core elemental composition. These characteristics present a significant challenge for categorizing manufactured nanomaterials with respect to their potential for human and ecosystem exposure.

Although occupational exposure through the inhalation pathway is likely to produce the most significant exposures and potential hazard, a wider range of potential human and environmental exposures should be considered. A more comprehensive approach becomes particularly relevant as manufactured nanomaterials-enabled products and processes increase in scope, number of product types and total production volumes. One way to view this challenge is to consider the exposure potential for specific types of particles from manufacture to final disposition. A potential advantage to a lifecycle approach is that it may provide a more comprehensive treatment required by regulators who are routinely petitioned to provide risk analyses for specific particle types proposed for specific industrial or consumer-based applications. In addition to occupational exposures during manufacture and formulation, this approach may also direct attention to potential human exposures and environmental contamination that may occur during use, disposal/recycle and final disposition which have not always been considered by manufactures. Figure 3 represents a schematic framework that may begin a discussion concerning a lifecycle approach to exposure assessment of manufactured nanomaterials.

Figure 3

Framework for Exposure Assessment Based on ENM Lifecycle



One of the charges for the Exposure Assessment Breakout Group is to provide recommendations for categorization of nanomaterials based on exposure assessment considerations which is an integral part of the assessment and management of risk. Risk analysis for manufactured nanomaterials requires information concerning physical-chemical properties, hazard and exposure as well as a clear understanding of how features and events from these areas interact with each other²². Manufactured nanomaterials physical-chemical characteristics and their effect on exposure to target organisms together contribute to risk. The influence of both manufactured nanomaterials characteristics and exposure scenarios on risk (to both humans and ecosystems) exist along separate continua. As a simplistic example, a highly hazardous type of manufactured nanomaterials may rarely encounter a target organism, or by contrast, a target organism may be chronically exposed to a manufactured nanomaterial that shows only a moderate acute biological response. In any case, risk assessment must include contributions from physical-chemical properties, hazard and exposure.

A wide range of physical-chemical properties have been used to characterize ENMs for a diverse range of endpoint requirements. With respect to manufactured nanomaterial exposures, certain physical-chemical characteristics tend to be more

²² Stone, V., Pozzi-Mucelli, S., Tran, L., Aschberger, K., Sabella, S., Vogel, U., Poland, C., Balharry, D., Fernandes, T., Gottardo, S., Hankin, S., Hartl, M.G.J., Hartmann, N., Hristozov, D., Hund-Rinke, K., Johnston, H., Marcomini, A., Panzer, O., Roncato, D., Saber, A.T., Wallin, Hakan, Scott-Fordsmand, J.J. ITS-NANO-Prioritizing nanosafety research to develop a stakeholder driven intelligent testing strategy. *Particle Fibre Toxicol.* 2014, 11, 9.

influential than others²³. For example, dustiness, defined as the propensity of a material to generate airborne dust during its handling, is one of the determinants of exposure and re-suspension potential of a nanomaterial. Dustiness is not an inherent ENM characteristic and can vary considerably depending on environmental conditions such as humidity. It can, however, be assessed using standardized techniques²⁴. Similarly, in the liquid media, one of characteristics that influences environmental fate and transport and resulting environmental exposure potential is the ease of nanomaterial dispersal. This feature is also dependent on multiple inherent properties such as particle size distribution, surface charge and surface potential²⁵. For example, there are a number of indicators of exposure related to manufacturing processes and consumer product use that are largely independent from physical-chemical and toxicological characteristics (Figure 4)^{26,27}. Also, in the case of exposures related to consumer products or industrial processes, the physical-chemical properties of the matrix may also become an important consideration²⁸. This is particularly important for manufactured nanomaterials that are dispersed in food or personal care products; embedded into polymers used to construct various rubber, plastic or glass products; coated onto surfaces such as textiles; or used for industrial or professional products such as exterior coatings for buildings or added to concrete.

²³ Kuempel, E.D., Castranova, V., Geraci, C.L. Development of risk-based nanomaterial groups for occupational exposure control. *J. Nanopart. Res.* 2012, 14, 1029.

²⁴ Evans, D.E., Turkevich, L.A., Roettgers, C.T., Deye, G.J., Baron, P.A. Dustiness of Fine and Nanoscale Powders. *Ann. Occup. Hyg.* 2013, 57, 261-277.

²⁵ Hendren C.O., Lowry, M., Grieger, K.D., Money, E.S., Johnston, J.M., Wiesner, M.R., Beaulieu, S.M. Modeling Approaches for Characterizing and Evaluating Environmental Exposure to Engineered Nanomaterials in Support of Risk-Based Decision Making. *Environ. Sci. Technol.* 2013, 47, 1190-1205

²⁶ Hristozov, D.R., Gottardo, S., Cinnelli, M., Isigonis, P., Zabeo, A., Critto, A., van Tongren, M., Tran, L., Marcomini, A. Application of a quantitative weight of evidence approach for ranking and prioritizing occupational exposure scenarios for titanium dioxide and carbon nanomaterials. *Nanotechnol.* 2014, 8, 117

²⁷ Clark, K., van Tongeren, M., Christensen, F.M., Brouwer, D., Nowack, B., Gottschalk, F., Micheletti, C., Schmid, K., Gerritsen, R., Aitken, R., Vaquero, C., Gkanis, V., Housiadas, C., Lopez de Ipina, J.M., Riediker, M. Limitations and information needs for engineered nanomaterial-specific exposure estimation and scenarios: recommendations for improved reporting practices. *J. Nanopart. Res.* 2012, 14, 970.

²⁸ Ibid

Figure 4

Exposure-relevant ENM / Process Characteristics

(Adapted from Hristozov et al., 2014)

- **Physicochemical Characteristics**
 - Dustiness, Size (distribution), Shape, Surface area, Surface charge, Surface coating, Reactivity, Solubility-Dissolution
- **Manufacturing Process Features**
 - Physical Environment, Weight-fraction, Duration of handling,
 - Automatic processing, Ventilation, Respiratory protection
- **Consumer Product**
 - Use-dependent exposure route, Leaching of ENM or Ions from matrix,
 - Use by vulnerable populations
- **Ecosystem Issues**
 - Entry point into Ecosystem, Frequency and amount of release, Transport Potential
 - Transformation of ENM in Environment, Vulnerable indicator species

Session 9: Risk Assessment

Chair: Yasir Sultan (Environment Canada, Canada); Rapporteur: Kirsten Rasmussen (European Commission Joint Research Centre, European Union)

The purpose of this session is to discuss the merits of the different types of categorization schemes in the scientific domain and identify commonalities and differences between these schemes, agree on which are the most appropriate for specific application domains and closest in terms of maturity for use in risk assessment (in the short, medium, and long-term), and their applicability domain *i.e.*, where in the risk assessment process has been already used or can be used to increase weight-of-evidence and reduce uncertainties. The discussion will not be limited to using categorization schemes to inform hazards, but rather how a more balanced risk determination can be made by increasing knowledge on both hazards and exposure through categories. In addition to identifying gaps associated with using these categorization schemes for risk assessment, participants will be asked to identify how to address the gaps, *i.e.*, through the development of test guidelines and/or guidance, engagement with ongoing scientific collaborations/activities, or new research directions.

The session will have stimulus presentations from co-authors of primary literature. The session co-chairs will provide an overview of the schemes available in literature. Participants should come prepared to critically evaluate the existing schemes or provide insights into the development of new ones. Following the stimulus

presentations, there will be a discussion amongst participants followed by a panel discussion of key experts to generate consensus.

Outcomes from this session will include a prioritization of categorization schemes, *i.e.*, which ones are the most appropriate for risk assessment and are the closest to being mature. Follow-up work will be initiated within OECD WPMN SG-AP and interested experts to further integrate these schemes into risk assessment processes.

Lastly, an opinion will be prepared for publication in peer-reviewed literature summarizing the discussion of the break-out session and identified path forward.