Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development

04-Nov-2016

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

GROUPING AND READ-ACROSS FOR THE HAZARD ASSESSMENT OF MANUFACTURED NANOMATERIALS

REPORT FROM THE EXPERT MEETING

Series on the Safety of Manufactured Nanomaterials
No. 76

JT03404543

ENV/JM/MONO(2016)59 Unclassified
English - Or. English

OECD Environment, Health and Safety Publications

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GROUPING AND READ-ACROSS FOR THE HAZARD ASSESSMENT OF MANUFACTURED NANOMATERIALS

REPORT FROM THE EXPERT MEETING

IOMC
INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS
A cooperative agreement among FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris, 2016

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No. 35, *Inhalation Toxicity Testing: Expert Meeting on Potential Revisions to OECD Test Guidelines and Guidance Document (2012)*

No. 36, *Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials (2012)*

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No.59, *Developments on the safety of manufactured nanomaterials: 2013 (2015)*

No.60, *Current developments in delegations on the safety of manufactured nanomaterials - tour de table (2015)*

No.61, *Developments in delegations on the safety of manufactured nanomaterials - tour de table (2015)*

No.62, *Considerations for using dissolution as a function of surface chemistry to Evaluate environmental behaviour of nanomaterials in risk assessments (2015)*

No.63, *Physical-chemical parameters: measurements and methods relevant for the regulation of nanomaterials (2016)*

No.64, *Approaches 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: analysis of a survey (2016)*

No.65, *Physical-chemical properties of nanomaterials: Evaluation of methods applied in the OECD-WPMN testing programme (2016)*

No.66, *Categorisation of manufactured nanomaterials (2016)*

No.67, *Developments in delegations on the safety of manufactured nanomaterials - tour de table (2016)*

No.68, *Multiwalled carbon nanotubes (MWCNT): summary of the dossier (2016)*

No.69, *Fullerenes (C60): summary of the dossier (2016)*

No.70, *Single walled carbon nanotubes (SWCNTs): summary of the dossier (2016)*

No.71, *Silicon dioxide: summary of the dossier (2016)*

No.72, *Toxicokinetics of Manufactured Nanomaterials: Report from the OECD Expert Meeting (2016)*

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No.74, *Exposure Assessment of Nano-Silver (AgNP):Case Study*

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FOREWORD

The OECD Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology (the Joint Meeting) held a Special Session on the Potential Implications of Manufactured Nanomaterials for Human Health and Environmental Safety (June 2005). This was the first opportunity for OECD member countries, together with observers and invited experts, to begin to identify human health and environmental safety related aspects of manufactured nanomaterials. The scope of this session was intended to address the chemicals sector.

As a follow-up, the Joint Meeting decided to hold a Workshop on the Safety of Manufactured Nanomaterials in December 2005, in Washington, D.C. The main objective was to determine the "state of the art" for the safety assessment of manufactured nanomaterials with a particular focus on identifying future needs for risk assessment within a regulatory context.

Based on the conclusions and recommendations of the Workshop [ENV/JM/MONO(2006)19] it was recognised as essential to ensure the efficient assessment of manufactured nanomaterials so as to avoid adverse effects from the use of these materials in the short, medium and longer term. With this in mind, the OECD Council established the OECD Working Party on Manufactured Nanomaterials (WPMN) as a subsidiary body of the OECD Chemicals Committee in September 2006. This programme concentrates on human health and environmental safety implications of manufactured nanomaterials (limited mainly to the chemicals sector), and aims to ensure that the approach to hazard, exposure and risk assessment is of a high, science-based, and internationally harmonised standard. This programme promotes international co-operation on the human health and environmental safety of manufactured nanomaterials, and involves the safety testing and risk assessment of manufactured nanomaterials.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, pesticides and Biotechnology of the OECD.

TABLE OF CONTENTS

OECD EXPERT MEETING ON GROUPING AND READ-ACROSS FOR THE HAZARD ASSESSMENT OF MANUFACTURED NANOMATERIALS

Background and Objectives

1. The OECD Working Party on Manufactured Nanomaterials (WPMN) Expert Meeting on Grouping and Read-Across for the Hazard Assessment of Manufactured Nanomaterials was hosted by the European Union in Brussels, Belgium on 13-14 April 2016. This was one of the expert meetings agreed to be held as part of the OECD Programme on the Safety of Manufactured Nanomaterials. It was attended by a total of 69 experts from 22 OECD delegations.

2. The meeting was opened by Mr Kestutis Sadauskas, Director Green Economy DG ENV-A (European Commission) and by Mar Gonzalez, from the OECD Secretariat. Then the Chair of the meeting, Mr Juan Riego Sintes[1], reminded participants that one main purpose of the meeting was to further the common understanding of which specific aspects are to be considered in a regulatory context when applying grouping and read-across for hazard assessment of manufactured nanomaterials (MNs). In particular, the meeting would seek to discuss the feasibility of updating Section 6.9 "Initial considerations applicable to manufactured nanomaterials" of the OECD Guidance on "Grouping of Chemicals" (OECD, 2014b). This would be achieved through sharing experiences in applying different grouping/read-across approaches for nanomaterials under different regulatory regimes, with a focus on facilitating a common understanding of when data on one (already defined) nanomaterial/nanoform may be relevant to other materials/forms (hypothesis development and justification). The meeting should also try to identify common denominators between different approaches and frameworks for grouping nanomaterials for the purpose of hazard assessment in future. The target outcome of the meeting is practical, concrete recommendations of common principles to be utilised in regulatory context by translating current scientific knowledge, and providing elements for a roadmap and a timetable for the Steering Group on Testing and Assessment (SG-TA) and the WPMN, to come forward with OECD guidance.

3. The participant list is available as an addendum.

Introduction: Background definitions

4. Grouping may include formation of a "chemical category" or identification of (a) "chemical analogue(s)" (OECD 2014a). The terms "category approach" and "analogue approach" are therefore used to describe techniques for grouping of chemicals whilst the term "read-across" is reserved for a technique of filling data gaps in either approach.

5. The absence of relevant, reliable and sufficient experimental data for chemicals in a category may result in one or more data gaps that need to be filled in order to finalise the hazard and/or risk assessment. The following non-testing methods can be used for filling these data gaps:

- Read-across;
- Trend analysis and use of computational methods based on internal models; and
- Use of computational methods based on external models.

6. Read-across (according to the official definitions) is only one of the three methods of data filling.

[1] From the Joint Research Centre (JRC) of the European Commission.

7. When "read-across" is applied, there are four following schemes:

- One-to-one (one analogue used to make an estimation for a single chemical);
- Many-to-one (two or more analogues used to make an estimation for a single chemical);
- One-to-many (one analogue used to make estimations for two or more chemicals); or
- Many-to-many (two or more analogues used to make estimations for two or more chemicals).

Presentation: Outcomes of the OECD Meeting on Categorisation[2]

Presenter: Kenneth Moss (EPA/US), Contributors: Maria Doa[3], Tala Henry[4]

8. A summary of the OECD Expert Meeting on Categorisation of Manufactured Nanomaterials, hosted by the U.S. Environment Protection Agency (EPA) was presented. It took place on 17-19 September 2014, Washington D.C., USA (OECD, 2016).

9. The goal of the meeting was to develop and define categorisation of nanomaterials (MNs) in order to improve decision-making, decrease the amount of data needed for individual MNs, and better target risk management. Categorisation of MNs is not the same as categorisation for chemicals in general due to unique physical-chemical properties of MNs, differences among nanoforms of a chemical species, and differences between nano and non-nano forms. Because regulators typically distinguish substances based on a chemical/molecular identity approach as opposed to properties, any MN categories should also consider molecular identity (e.g. carbon nanotubes, metal oxides, quantum dots).

10. The Meeting was organized into 9 sessions:

- Session 1: Context for the Need for the Use of Categories, and Perspectives on their Application to Nanomaterials
- Session 2: Risk Assessment and Risk Management
- Session 3: Physical-Chemical Characterization
- Session 4: Environmental Fate
- Session 5: Human Health (Group 1)
- Session 6: Environmental Toxicity
- Session 7: Human Health (Group 2)
- Session 8: Exposure Assessment
- Session 9: Risk Assessment.

[2] See: USEPA. Chemical Categories Used to Review New Chemicals under TSCA. http://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/chemical-categories-used-review-new. January 2016.

[3] Chemical Control Division, U.S. Environmental Protection Agency, Washington, DC.

[4] Risk Assessment Division, Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, DC.

11. Refined categorisation schemes were then proposed for each focus area through discussions on physical-chemical characterization, fate, exposure, ecotoxicity, human health toxicity, and risk assessment, and many schemes incorporate multiple properties. A key question is, at what point of the lifecycle of a MN do we categorise (e.g. "from the vial," aggregate/agglomerate state, or as potentially released from products)? In principle, the results from *in-vitro* assays can be used as a basis for categorisation, but the assays need to be standardized and validated, and be able to link to biological behaviour and effects. Thus *in vitro* methods seem not yet to be fully ready and applicable for read-across use.

12. Recommendations from the meeting were: 1) to identify and develop methods for characterization of physical-chemical properties relevant for toxicokinetics, fate, hazard and exposure assessments; 2) to use methods that enable comparability and are reliable, and 3) to use the OECD Guidance on Sample Preparation and Dosimetry[5]; and 4) to agree on or develop experimental models (e.g. *in-vitro* and *in-vivo* assays) which are predictive of human health and environment effects and support categorisation.

13. General conclusions from the meeting were that 1) tools and methodologies for categorisation might be different for the different parts of the assessment of manufactured nanomaterials (MNs); 2) definitions and terminologies need to be clarified and consistently applied; 3) there is a need to adapt existing approaches for conventional substances to fit specificities of categorisation frameworks for MNs; and 4) there is support for developing case studies that inform categorisation schemes as they are developed and refined.

Presentation: Grouping for Read-Across of Chemicals Properties

Presenter: Andrew Worth (EU-JRC), contributor: Lara Lamon (EU-JRC)

14. This presentation reviewed some of the key concepts on grouping and read-across of chemical properties from the 2014 *OECD Guidance on Grouping of Chemicals*[6]. These concepts were put in the context of recent developments of computational tools for grouping and read-across prediction as well as reporting formats based on the identification and characterisation of uncertainties in the prediction process. Emphasis was placed on the different levels of chemical and biological similarity that can form the basis of chemical categories and read-across arguments. These levels of similarity are consistent with the representation of mechanistic knowledge by means of adverse outcome pathways (AOPs). Finally, it was argued that the experience gained in the grouping and read-across to fill in data gaps in the properties of chemicals in the non-particulate form can be usefully extended and applied to the prediction of properties of nanomaterials.

15. Based on an EU-Joint Research centre (JRC) review of the (scientific and regulatory) literature, it was found that categorisation and grouping approaches cover a range of different approaches, depending on the purpose and basis of the approach, and whether or not categories/groups are predefined or established on an *ad hoc* basis. For the purpose of this presentation, and in line with the European Chemicals Agency (ECHA) and OECD guidance (OECD, 2014b), the concept of grouping was restricted to the grouping of chemicals/MNs for the purpose of filling data gaps by read-across.

16. In principle, read-across can be performed in a qualitative or quantitative manner, depending on whether the data of the source chemical(s) are categorical (hazard potential) or numerical (hazard potency).

[5] OECD, 2012. Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials [ENV/JM/MONO(2012)40].

[6] OECD 2014, Guidance on Grouping of Chemicals, OECD Series on Testing and Assessment No. 194 [ENV/JM/MONO(2014)4]

In the case of qualitative read-across, both positive and negative predictions can be made, but a more substantial proof is generally required for negative read-across.

17. Chemical similarity can be defined at multiple levels: a) structure (same molecular backbone and/or functional groups); b) reactivity (same mechanism of chemical reactivity); c) chemical-biological interaction (same molecular initiating event such as receptor binding); d) downstream biological effect (key events in an Adverse Outcome Pathway). In the case of chemicals that are in the particulate form (including MNs), the notion of structural similarity needs to be expanded to cover intrinsic chemical and physical identity ("what they are"). Extrinsic physicochemical properties ("where they go" and "what they do") can also be used in a grouping approach. In this context, reactivity properties ("what they do") are related to level of chemical-biological interaction, whereas fate properties ("where they go") are related to upstream (toxicokinetic) behaviour.

18. In practice, read-across for MNs is difficult to apply, given the need to verify similarity at multiple levels in the face of multiple confounders, such as size and property distributions, and potentially changing characteristics throughout the life cycle.

19. Irrespective of the grouping and read-across approach adopted, to ensure consistency in regulatory assessments, it is important to establish a consistent and transparent approach for documenting read-across arguments, including the identification and characterisation of known uncertainties. Existing frameworks in OECD and ECHA guidance, including the Read Across Assessment Framework (RAAF) would provide a suitable basis for the development of such a reporting standard.

REGULATORY CONTEXT IN DIFFERENT JURISDICTIONS - CONCEPTUAL BASIS

Session Chair: Maria Doa (USA), Rapporteur: Kirsten Rasmussen (EU-JRC)

Presentation: Read-Across for hazard assessment in the EU with emphasis on nanomaterials

Jenny Holmqvist (EU-ECHA)

20. Grouping of substances and read-across are valuable approaches in regulatory frameworks in order to minimize costs and the use of test animals and fill potential data gaps in the hazard characterisation, where experimental data may be insufficient. Also for nanomaterials, these approaches may be an important means of information generation and addressing data gaps. Based on existing knowledge and approaches, ECHA has developed a strategy to substantiate read-across for nanoforms of the same substance with a focus on compliance with the EU REACH legislation. This strategy comprises six different steps, including (1) identification and characterisation of the nanoforms, (2) when possible, formation of initial groups of nanoforms based on similarity in physical-chemical parameters (e.g. aspect ratio, or water solubility and dissolution rate), (3) identification of available information and data gaps for each nanoform, (4) hypothesis-driven identification of source materials to read-across from, (5) where necessary, additional testing to substantiate the read-across, and (6) assessing the new data and remaining uncertainties to conclude on the read-across arguments. Where read-across cannot be substantiated, the strategy foresees re-iterating (some of) the steps, or performing appropriate testing to fulfil the information requirement(s) in REACH.

21. The presented strategy for read-across between nanoforms of the same substance points towards the need for data on physical-chemical parameters of each nanoform as the crucial starting point for obtaining a better understanding of the environmental behaviour, fate, toxicokinetics and (eco)toxicity of those nanoforms. Such proper understanding is the cornerstone of developing a scientific, robust justification for grouping or the use of data for read-across for any endpoint. To improve understanding of behaviour and fate of nanomaterials e.g. in environmental compartments, further (international) co-ordination and collaboration of research is advisable. Furthermore, the quality of the data is critical, and the monitoring of physical-chemical parameters during testing is therefore a key element. This also requires harmonisation and standardisation of *in-vitro* test methods for toxicokinetics in order to enable an understanding of similarities in behaviour in hazard endpoints.

22. ECHA took initiative, in collaboration with RIVM and JRC, to explore the scientific aspect of justifying when and how to use test data from (eco)toxicity studies on one nanoform to cover data gaps for another nanoform of the same substance. This resulted in a scientific reference paper which the ECHA Nanomaterials Working Group has been consulted twice upon and provided input to (ECHA, EU, RIVM, 2016). The paper will form a cornerstone of further discussions with the European Commission, Member States, Industry and NGOs and will feed into ECHA's internal processes for developing/updating ECHA guidance on the REACH Regulation.

Presentation: Nanomaterials under the Toxic Substances Control Act[7]

Presenter: Maria J. Doa, Contributors: Kenneth Moss, Tala Henry

23. In the United States, there is no legislation specific to nanomaterials. Nanomaterials are managed under existing authorities for chemicals, pesticides, food and drugs, and consumer products. Nanomaterials that are nanoscale forms of chemicals are regulated under the Toxic Substances Control Act by the United States Environmental Protection Agency (U.S. EPA).

24. EPA reviews about 1000 new chemical submissions annually, of which fewer than 20 are nanomaterials. These reviews include characterization of the physical-chemical properties and hazard of the chemical; assessment of production, processing (formulation), and uses; assessment of releases to air and water and of disposal; estimated worker risk, environmental risk, general population risk and consumer risk.

25. While companies are required to provide information on production/import volume, uses, human exposure information, disposal methods and estimates of releases to the environment, less than 15 % of the submissions include toxicity data because there is no requirement that testing be conducted to support an initial submission of a chemical. Given the paucity of data on the chemicals in these submissions, reviews are typically based on analogues and categories. EPA has developed 56 chemical categories to aid in the reviews of new chemical substances.

26. The reviews of nanomaterials are conducted similarly to other chemical substances. In reviewing nanomaterials, EPA uses one of the 56 chemical categories, i.e. "respirable, poorly soluble particulates", to review most nanomaterials. In addition to this category, EPA uses the NIOSH REL (Recommended Exposure Limit) for carbon nanotubes and carbon nanofibres, and considers toxicity of the components (e.g. cadmium) and surface functionalization of the nanomaterial.

27. EPA typically regulates nanomaterials by limiting the types of uses and requiring engineering controls, personal protective equipment for workers, limited or no release of the nanomaterial to the environment, notification to EPA before any new future use is commercialized, and the submission of an enhanced 90-day inhalation study once a certain production volume is reached.

Discussion

28. Within the OECD, several geographical regions are represented, all having their own regulatory context(s). The regulatory schemes in the USA and the EU were presented in this session, including overviews of the main pieces of legislation in these two regions and how read-across for nanomaterials would be performed in each, including an analysis of issues that still need to be addressed. Participants were reminded that ECHA has published the scientific background paper "*Usage of (eco)toxicological data for bridging data gaps between and grouping of nanoforms of the same substance. Elements to consider*" (ECHA, EU, RIVM, 2016), which will form part of the scientific basis for preparing guidance on grouping and read-across for nanomaterials in the EU under the REACH legislation. In answering questions after the

[7] See: USEPA, Office of Pollution Prevention and Toxics. Reviewing New Chemicals under the Toxic Substances Control Act (TSCA).http://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca

USEPA. Chemical Categories Used to Review New Chemicals under TSCA. http://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/chemical-categories-used-review-new. January 2016

USEPA, Office of Pollution Prevention and Toxics. EPA Actions to Reduce Risk for New Chemicals under TSCA. http://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/epa-actions-reduce-risk-new#SNUR

presentation, the U.S. EPA highlighted that the categories developed in the U.S. system pre-date the discussion on nanomaterials, and for example fibres (asbestos) are not included. The "partly-soluble" description is used in connection with inhalation toxicity and persistency in the lung.

29. Experts were also made aware on the development of the OECD document "*Assessment of Biodurability of Nanomaterials and their Surface Ligands*"[8], which aims to compile the relevant information on the biodurability of pristine and functionalised nanomaterials in biological and environmental media *in vitro* and *in vivo*, as well as to describe methods for measuring the stability and half-times of nanomaterials.

[8] To be published in 2016.

RESEARCH INITIATIVES – IMPLEMENTING TOOLS

Session Chair: Andrew Worth (EU), Rapporteur: Lara Lamon (EU)

Presentation: A simple approach to categorize nanomaterials

Thomas Gebel (Germany)

30. Intensive efforts have been undertaken in investigating the toxicology and safety aspects of nanotechnology in recent years. It is time now to challenge and review paradigms on still existing major knowledge gaps. A paradigm repeatedly emphasized is that nanoparticles may exhibit a specific toxicity due to their size. However, at present there is no evidence of 'nano-specific' mechanisms of action; no step-change in hazard has been observed so far for particles when their size decreases to below 100 nm in at least one dimension. Moreover, there is no evidence so far that fundamentally different biokinetics of nanoparticles would trigger a novel type of toxicity.

31. To facilitate hazard assessment, nanomaterials may be categorized into three basic categories according to route of exposure and mode of action.

32. One category focuses on rigid biopersistent respirable fibrous nanomaterials with a specific geometry and high aspect ratio (so-called "WHO fibres"). For these fibres, hazard assessment can be based on the experiences with asbestos.

33. Another category focuses on respirable granular biodurable particles (GBP) which, after inhalation, may cause inflammation and secondary mutagenicity that may finally lead to lung cancer. After intravenous, oral or dermal exposure, nanoscaled GBPs investigated apparently have not shown 'nano-specific' effects so far. Hazard assessment of GBPs may be based on the knowledge available on granular particles.

34. The third category comprises nanomaterials for which toxicity is mediated by the specific chemical properties of its components, such as released ions, catalytic activity or functional groups on the surface. Nanomaterials belonging in this category have to be evaluated on a case-by-case basis, depending on their chemical identity.

35. The proposed categorization system may facilitate future hazard assessments as a first step in the course of risk assessment.

36. It was discussed that biodurability and dissolution rate are relevant properties in hazard assessment of manufactured nanomaterials (MNs). Dustiness and shape (granular/fibrous) are also relevant parameters, whereas reported evidence shows that surface coating, corona formation and different MNs kinetics result in less relevance in hazard assessment.

Presentation: Fixed entities and flexible strategies for testing nanomaterials

Tobias Walser (Switzerland)

37. The presentation illustrated, firstly, a concept currently being developed in Switzerland to facilitate the distinction between similar nanomaterials, and secondly, a concept to establish tailored, tiered testing strategies with a focus on Adverse Outcome Pathways (AOP).[9]

38. Conventional chemicals have identities, defined by an unambiguous molecular structure and elemental composition. In contrast to conventional chemicals, nanomaterials cannot be said to have these two properties alone. Size, surface and morphology are other typical characterizers which must be known before the nanomaterial can be further evaluated. Even regarding "simple" nanomaterials we need to know many physical-chemical properties and environmentally relevant variables (e.g. dissolution rate) for a sufficient characterization for further hazard assessment. For registration and potential naming of nanomaterials, it is proposed to assign only the four properties: Composition of the core material, coating, particle size and shape. The measured values of these parameters, measured with standardized measurement protocols, will determine a MN's identity. Additional parameters need to be reported for further hazard assessment, e.g. solubility and dissolution rate, resulting in a full characterization of the nanomaterial. The number of characterizers required by the authorities should be kept to the minimum, yet sufficient number to confer the ability to induce hazardous particle–bio interactions.

39. There are an unlimited number of different nanomaterials and hence an unlimited number of identities. The continuous scale of their physical-chemical properties does not allow easy division of nanomaterials into groups of similar ones. Such division is necessary, however, in order to evaluate whether a nanomaterial requires a separate registration, i.e., whether it is distinct from another nanomaterial from a regulatory point of view. One solution is to transform the continuous scale of each physical-chemical property into a discrete scale. It is proposed to divide the four previously mentioned property classes into unambiguous categories, starting by establishing the chemical composition with a separate analysis of the coating, if one exists. The core and the coating may consist of single substances, compounds or of a mixture thereof. The fractions of chemical elements or compounds are automatically associated with predefined bands, similar to the existing classification scheme for mixtures, as described in the EU Classification, Labelling and Packaging Regulation[10]. Size distributions are analysed for their width (narrow, wide) and a distinction is made between size classes < 20 nm and 20–500 nm. These two bands take into account physical-chemical disruptions of continuous phenomena (e.g. quantum effects or band-gap phenomena and uptake mechanisms, which may go beyond 100 nm if justified). Shape classes include spherical(-like) particles, tubes and rods, or plates, with further distinctions if justified, e.g. asbestos-like high-aspect-ratio nanomaterials (HARN).

40. The combination of the categorized properties results in many, but limited, entities. Registrants will be asked to provide a characterization of the nanomaterial identity with an unambiguous assignment to an entity. However it may be difficult to define the borders between two similar entities. It has been shown that changing physical-chemical properties usually do not have abrupt influences on biological systems. But there is no way around a pragmatic, yet scientific approach to reduce the number of identities into a manageable number of entities, if we want to cope with the myriad of different nanomaterials. Consequently, each of these entities may include many similar nanomaterial identities which are considered the same from a regulatory perspective. Entities will ideally have codes or names based on a

[9] A detailed outline of the approaches can be found in "Sameness: The regulatory crux with nanomaterial identity and grouping schemes for hazard assessment published 2015 in Regulatory Toxicology and Pharmacology (doi:10.1016/j.yrtph.2015.05.031).

[10] CLP Regulation (EC) No 1272/2008, L 353, 1.1.3.6.

durable, internationally harmonized convention that allows to unambiguously assign data to the entities and to make sharing data between the entities easier.

41. It was discussed how the identities and entities are related to further evaluation and testing. All identities within an entity will have the same testing strategy, and read-across within an entity is always possible and desirable in order to minimise testing efforts. Even different entities may share a testing strategy, in case the nanomaterials follow similar patterns of toxicological action. In contrast to the fixed system of identities and entities, testing strategies should show flexibility in the sense that they can be easily adapted as new knowledge and test guidelines become available.

42. The aim of the testing strategies is to reduce the testing efforts as much as possible while maintaining a robust and high explanatory power. The "space" of testing strategies is called "clouds". Entities may behave very similarly and therefore require the same testing strategy and consequently fall into the same cloud, independent of their physical-chemical properties. Therefore, individual clouds can address (in EU terms) different forms of the same substance, but they can also combine nanoforms of different substances. In the proposed concept, clouds can include nanomaterials with very different physical-chemical properties as long as the testing strategy remains the same.

43. When comparing to ECHA's publication on grouping of nanoforms of a substance[11], it becomes apparent that there are few differences and many similarities. The similarities may be not directly apparent due to different terminology. In the proposed concept, each nanomaterial receives a comprehensive identity based on the physical-chemical characterization, while the proposal of ECHA is that each nanoform of a substance receives an identity (while still leaving the possibility to register a nanomaterial as a substance on its own). In our concept, nanomaterials will be registered as such, and not as nanoforms of existing substances. However, this is not necessarily different from being evaluated as "form of a substance", as long as the testing requirements are the same.

44. The goal is to embed AOPs into tiered testing strategies in order to make better use of *in-vitro* tests (e.g. by testing key events), and to further reduce *in-vivo* testing. It will support grouping nanomaterials of similar toxic action. In a first step, AOPs are tested in the context of the online-tool «Precautionary Matrix».[12]

Presentation: NanoReg2: Preliminary grouping criteria for regulatory purposes[13]

Blanca Suarez (Spain) & Rambabu Atluri (Denmark)

45. The NanoReg^2 initiative is unique in the sense that it takes into account the whole innovation chain within product development, proposing robust risk assessment strategies at all decision points within the chain. Thus safety is implemented into innovation strategies, as a way of promoting safe innovation. Grouping is presented as a tool to assist both industry and regulatory bodies, supporting the implementation of a structured framework for MN safety assessment. Moreover, grouping will contribute to safe innovation objectives as support to the identification of Safe-by-Design materials, products and uses.

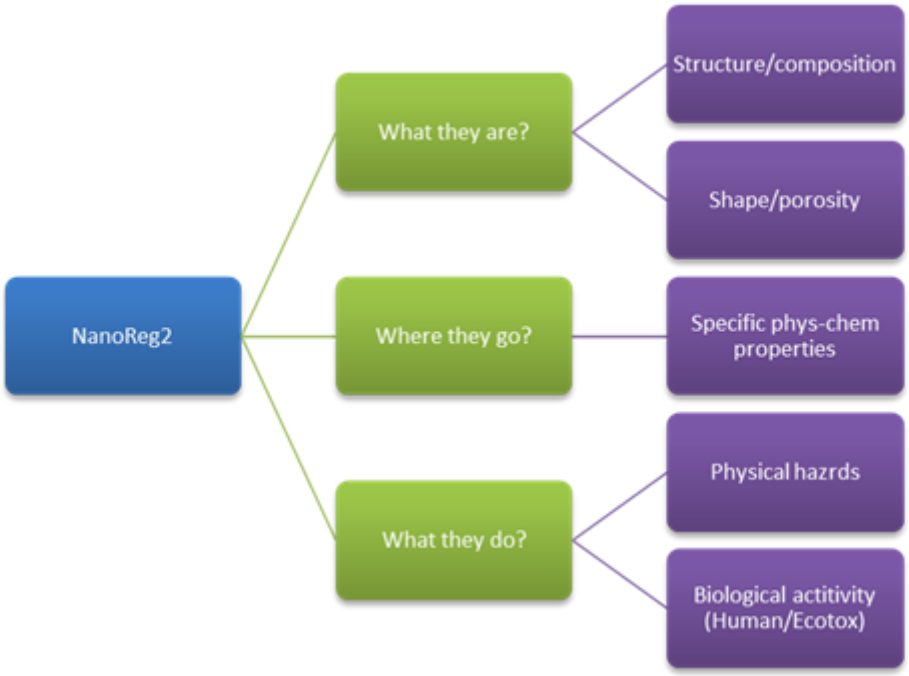
[11] ECHA, EU, RIVM (2016), Usage of (eco)toxicological data for bridging data gaps between and grouping of nanoforms of the same substance: elements to consider. This paper was jointly prepared by the European Chemicals Agency, Joint Research Centre, Dutch National Institute for Public Health and the Environment, 2016.

[12] More information can be found at www.infonano.ch

[13] This presentation was not given during the workshop.

46. Within this context, NanoReg^2 selected a set of criteria for grouping, taking into account both intrinsic and system-dependent changes of MNs along their life cycle (Arts et al, 2015; Oomen et al, 2015). The abovementioned set of criteria should be supported by a regulatory based approach (Sellers et al, 2015). The approach established under the MARINA FP7 initiative was elected as a starting point for the NanoReg^2 initiative.

47. The flowchart presented in MARINA was further modulated to incorporate information produced under the NANoREG scheme to meet the NanoReg^2 demands. By including both the MARINA and NANoREG grouping approaches, a preliminary set of criteria for NanoReg^2 grouping is proposed addressing the following questions; 1) What they are; 2) Where they go; and 3) What they do. See figure:



48. This initial strategy will be further elaborated once the NanoReg^2 project has collected more data from published reviews and internal case studies.

Presentation: The ECETOC DF4nano Grouping approach

Robert Landsiedel (ECETOC)

49. The safety of nanomaterials can be ensured even if animal testing is undertaken only as a very last resort. This is the premise and promise of the Decision-making framework for the grouping and testing of nanomaterials - DF4nano. The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) Nano Task Force (TF) has developed the DF4nanoGrouping based upon an earlier review of existing approaches for the grouping and testing of nanomaterials.[14]

50. The grouping concept developed by the ECETOC Nano TF aims at making the hazard assessment of nanotechnology-enabled products more efficient by using a stepwise procedure, resulting in a grouping concept that brings together substances with similar toxicological profiles. In an extensive review[15], the ECETOC Nano TF assessed all available concepts for the grouping of nanomaterials for human health risk assessment. Based upon this review, the ECETOC Nano TF proposes a functionality-driven Decision-making framework for grouping and testing nanomaterials that aims to group nanomaterials by their specific physical-chemical properties, biophysical interactions, biokinetics and their mode of action.

51. Ten properties, interactions and effects were selected and organised in three tiers. For each parameter, thresholds, methods and benchmark materials are given: In Tier 1, Intrinsic material properties (Water solubility, Particle morphology, Composition); in Tier 2 System-dependent properties (Surface reactivity, Dissolution rate, Dispersability in relevant media); Biokinetics (Uptake, Biodistribution, Biopersistence) and Cellular effects (Effects on Macrophages). DF4nano identifies nanomaterials as member of one of four Main Groups (MG): i) MG1: Soluble MNs; ii) MG2: High aspect ratio MNs; iii) MG3: Passive MNs; and iv) MG4: Active MNs. Some of these groupings may directly be of use for the subsequent risk assessment, whereas others may require further data and sub-grouping. The use of DF4nano was explored and advanced in the MARINA EU project.

52. The DF4nano for hazard assessment has been tested by using it in case studies involving 24 materials. The case studies confirmed the usefulness of DF4nano, as all materials with potential to be hazardous *in vivo* were identified by DF4nano. In a step-wise approach, DF4nano provides solid rationales

[14] Arts, Josje HE, et al. "A critical appraisal of existing concepts for the grouping of nanomaterials." Regulatory Toxicology and Pharmacology 70.2 (2014): 492-506

[15] See: Arts, Josje HE, et al. "A critical appraisal of existing concepts for the grouping of nanomaterials." Regulatory Toxicology and Pharmacology 70.2 (2014): 492-506.

Arts, Josje HE, et al. "A decision-making framework for the grouping and testing of nanomaterials (DF4nanoGrouping)." Regulatory Toxicology and Pharmacology (2015).

Bos, Peter M. et al. (2015). The MARINA Risk Assessment Strategy: A Flexible Strategy for Efficient Information Collection and Risk Assessment of Nanomaterials. International journal of environmental research and public health, 12(12), 15007-15021.

Arts, Josje HE, et al. "Case studies putting the decision-making framework for the grouping." Regulatory Toxicology and Pharmacology (in press)

Oomen, Agnes G., et al. "Grouping and read-across approaches for risk assessment of nanomaterials." International journal of environmental research and public health 12.10 (2015): 13415-13434.

Godwin, Hilary, et al. "Nanomaterial Categorization for Assessing Risk Potential to Facilitate Regulatory Decision-Making." ACS nano 9.4 (2015): 3409-3417.

for sub-grouping, including the needs for further data generation. The framework also proved to be efficient in sorting nanomaterials that could undergo hazard assessment without further testing.

53. This is the first comprehensive and pragmatic approach to the grouping and safety assessment of nanomaterials that has been presented to the scientific community. As with all innovative, new approaches, the framework is expected to attract both praise and criticism, which will facilitate the further refinement of this concept. The ECETOC organisation that sponsored the development of this framework is willing to provide guidance to companies dealing with nanomaterials on how to use this approach, should this concept in its present or modified form be or become acceptable to regulatory authorities.

CASE STUDIES, BEST PRACTICES AND RECOMMENDATIONS FOR READ-ACROSS FOR NANOMATERIALS IN A REGULATORY CONTEXT

Session Chair: Jenny Holmqvist (EU-ECHA), Rapporteur: Eric Bleeker (Netherlands)

BIAC: Industry Experience for Grouping and Read-Across Approaches for Nanomaterials

David Carlander & Blanca Serrano (BIAC)

54. An effective grouping and read-across strategy needs to meet the following four conditions.

- It should **leverage available data and experience with the chemical substance**. E.g. information from existing hazard classification frameworks and proven scientifically valid grouping for larger substances are in many cases useful for nanomaterials. The comparison of the behaviour of those particles dominated by surface interactions and those that are not can be used to identify the relevant parameters. E.g. the Peclet number (Pe) can be used to identify the surface interaction dominated regime (Pe<0.1).

- An effective strategy should also **remain flexible** to allow for the incorporation of relevant new information and methodological refinements to ensure the grouping structure remains current. It is imperative to ensure that focus is on fit-for-purpose experimentation and similarities.

- Science- and risk-based prioritization concepts to define required levels of similarity should be integrated. The amount of information required for the substances should be guided first by the intrinsic hazard of the substance and then by the available knowledge. The level of similarity required to group substances should also be related to intrinsic substance hazard and apparent risk.

- **Perception barriers to innovation should be avoided.** It is vital to realize that proposed boundaries will impact material design and consequently innovation. Rigid, pre-defined physical chemical grouping paradigms are likely lead to the avoidance of properties presumed to enhance risk, however limiting the design and innovation space. Therefore, it is imperative that any pre-defined physical chemical grouping paradigm be grounded on sound science. It is recommended that physical chemical parameters be used in combination with hazard and exposure outcomes (i.e. continual fit-for-purpose validation) in order to continue to build knowledge in this space and to encourage innovations in material design that leads to safer materials.

55. In conclusion, a number of strategies and perspectives to carry out read-across have been proposed by different actors in different regions, each with their own merits. In order to move towards a harmonize strategy to read-across we should ensure that grouping strategies rely on established methods and data. It is important to take in account that:

- Developing and use of alternative methods are very important but should be secondary to established guideline methods until fully validated.

- Existing data for the chemical substance should be used, were possible.

- Non-guideline / non-GLP research studies need to be considered with caution. A well-defined, structured data quality and utility framework should be implemented to ensure that the collective information used to categorize materials is of sufficient quality and is also fit-for-purpose.

56. The knowledge of chemical substance hazard potential and corresponding MoA for those materials should be used to inform the significance and relevance of physical-chemical parameters (e.g. weighing experience (evidence- based) over generalized assumptions, where valid)

Presentation: Grouping and read-across approaches for risk assessment of nanomaterials from the perspective of the European FP7 project MARINA

Agnes Oomen (the Netherlands)[16]

57. Physical-chemical properties of chemicals affect exposure, toxicokinetics/fate and hazard, and for nanomaterials, the variation of these properties results in a wide variety of materials with potentially different risks. To limit the amount of testing for risk assessment, the information-gathering process for nanomaterials needs to be efficient. At the same time, for each nanomaterial sufficient information to assess its safety for human health and the environment must be available. Grouping and read-across approaches can be utilised to meet these goals. In the presentation, different possible applications of grouping and read-across were outlined and discussed for nanomaterials within the broader perspective of the MARINA Risk Assessment Strategy (RAS), as developed in the EU FP7 project MARINA.

58. Firstly, nanomaterials can be grouped based on limited variation in physical-chemical properties to subsequently design an efficient testing strategy that covers the entire group.

59. Secondly, knowledge about exposure, toxicokinetics/fate or hazard, for example via properties such as dissolution rate, aspect ratio and chemical (non-)activity, can be used to organise similar materials in generic groups to frame issues that need further attention, or potentially to read-across.

60. Thirdly, when data related to specific endpoints is required, read-across can be considered, using data from a source material for the target nanomaterial.

61. Read-across could be based on a scientifically sound justification that exposure, distribution to the target (fate/toxicokinetics) and hazard of the target material are similar to, or less than, those of the source material. These grouping and read-across approaches pave the way for better use of available information on nanomaterials and are flexible enough to allow future adaptations related to scientific developments.

[16] On behalf of the partners involved in WP12 and 13 of the MARINA project

Presentation: USA MWCNT case study[17]

Presenter: Tala Henry (USA), Contributors: Iris Camacho, Doritza Pagan-Rodriquez, Rebecca Daiss, Jeff Gallagher, Karen Eisenreich, David Tobias, Susan Laessig, Rhema Bjorkland, Kelly Mayo, Maria Doa, and Ken Moss.

62. Under the Toxic Substances Control Act (TSCA), U.S. EPA regulates both new and existing industrial chemicals. For new chemicals, U.S. EPA must make a regulatory decision on each chemical, often with limited information on the specific chemical or a chemical class. U.S. EPA has relied on computational tools and chemical analogue and category approaches since the 1980s to make rapid decisions regarding risks associated with the manufacturing, import, distribution, use and disposal of new chemicals.

63. U.S. EPA's New Chemical Categories are formulated for chemicals for which sufficient assessment experience has been accumulated so that hazard concerns and testing recommendations vary little from chemical to chemical within the category. New chemical submitters and EPA reviewers benefit from the accumulated data and past decisions represented by a category. EPA considers all new chemical substances which fall within such categories on a case-by-case basis and uses the most appropriate structural analogue(s) to read-across concerns for health or environmental effects.

64. At this time, data are insufficient to identify relevant properties key to establishing a Carbon Nanotube (CNT) category. Barriers to grouping CNTs include: unclear test methods and relevance of results; linkage of chemical-structural and material characterization properties with biological properties; CNT samples are usually not composed of a single type of CNT, but rather are a distribution of structures (e.g. varying length) and also contain agglomerates or aggregates. Furthermore, a range of CNT characteristics may potentially affect toxicology, including: functionalization, length, end-capping, presence of catalyst metals and level of purity.

65. To date, U.S. EPA has primarily considered CNTs as part of the U.S. EPA "Poorly Soluble Respirable Particles" Category in recognition that the physical form of CNTs, at a minimum, present potential concerns about effects on human health or the environment. A number of other conservative assumptions have been used in conducting quantitative risk assessment in the absence of data for CNTs generally and Multi-walled Carbon Nanotubes (MWCNTs) specifically. As data have accrued for MWCNTs, U.S. EPA has adjusted some of these assumptions. For example, MWCNTs were previously assumed to have high bioconcentration/bioaccumulation potential; however, following a review of published literature, U.S. EPA now considers that MWCNTs will have low potential for bioaccumulation. U.S. EPA may also modify, on a case by case basis, assumptions about removal from wastewater treatment plants and destruction by incineration.

[17] See: Alloy MM, Roberts AP. 2011. Effects of suspended multi-walled carbon nanotubes on daphnid growth and reproduction. Ecotox Environ Safety. 74: 1839-1843.
Edgington AJ, Roberts AP, Taylor LM, Alloy MM, Reppert J, Apparao M, Mao J, Klaine SJ. 2010. The influence of natural organic matter on the toxicity of multi-walled carbon nanotubes. Environ Toxicol Chem 29: 2511-2518.
Murray, AR, Kisin E, Leonard SS, Young SH, Kommineni C, Kaga WE, Castranova V, Shedova AA. 2009. Oxidative stress and inflammatory response in dermal toxicity of single-walled carbon nanotubes. Toxicology 257: 161-171.
OECD, 2015. Preliminary Guidance Notes on Nanomaterials: Interspecies Variability Factors in Human Health Risk Assessment [ENV/JM/MONO(2015)31].
OECD, TG 413: OECD Guideline for the Testing of Chemicals: 90-Day (Subchronic) Inhalation Toxicity Study.

66. Regarding aquatic ecotoxicity assessment, U.S. EPA generally predicts "no effects at saturation" in recognition of the general insolubility of MWCNTs, but also identifies a concentration of concern of 1 µg/L. Empirical data from Edgington et al. (2010) and Alloy and Roberts (2010), both of which included natural organic matter in their testing, are used to support this concentration of concern. These studies are also used to support the U.S. EPA practice of requesting any aquatic testing be conducted in the presence of natural organic matter.

67. U.S. EPA conducts human health assessments in which exposures to MWCNTs are evaluated for occupational (dermal and inhalation), general population (inhalation and ingestion of water and fish) and consumer (dermal and inhalation) populations. While it is recognized that absorption of MWCNTs is expected to be poor via all routes of exposure, animal data have shown that lung overload, mutagenicity, immunotoxicity and lung cancer are concerns for inhalation exposures to MWCNTs. Furthermore, concerns about irritation and sensitization are considered for dermal exposures, whereas concerns due to the presence of residual catalysts and about systemic effects from translocation are considered for all routes of exposure.

68. Regarding MWCNT health assessments, U.S. EPA relies on available animal toxicity data from several inhalation, oral, and dermal studies. In the absence of data for a particular MWCNT, the U.S. EPA relies on read-across approaches that consist of using results from 28-day and 90-day repeated-dose inhalation studies with one (the same) MWCNT and a 90-day inhalation study with another MWCNT to read across to untested MWCNTs. For 90-day oral exposures, two studies (#82 and #83) reviewed in the OECD document *Preliminary guidance notes on Nanomaterials: Interspecies variability factors in human health risk assessment* (OECD, 2015)[18] serve as the basis for read-across to other untested MWCNTs. A single study (5-day topical exposure to a SWNCT; Murray et al., 2009) is used to assess dermal exposures (topical) to MWCNTs.

69. When U.S. EPA requests 90-day inhalation testing for MWCNTs, the test is required to be conducted using OECD Test Guideline 413. U.S. EPA generally incorporates additional measurements which are being proposed as part of the draft revision to TG 413. These include: (1) aerosol exposures with particulates with MMAD < 2 µm and GSD < 3 µm; (2) Bronchoalveolar Lavage Fluid (BALF) measurements that are biomarkers of lung injury (e.g. total protein and/or albumin, lactate dehydrogenase (LDH), cell counts and differentials for alveolar macrophages, lymphocytes, neutrophils and eosinophils); (3) post-dosing observation period to accommodate measuring pulmonary disposition (lung burden), clearance half-life (biopersistence) and translocation of test material; (4) histopathology of pulmonary and extra-pulmonary organs/tissues (cardiovascular, CNS, liver, kidney, etc.).

Presentation: Category approach for the read-across assessment of MWCNTs

Akihiko Hirose[19] (Japan)

70. It is known that MWNT-7, one of the various types of multi-wall carbon nanotubes (MWCNTs), has a potential to induce mesothelioma by intraperitoneal administration in rats and mice. A recent report[20] indicated that MWNT-7 induced lung cancer after two-year inhalation exposure in rats. However, it is unclear whether other types of MWCNTs have a carcinogenic potential or not.

[18] See Table in page 17.

[19] Division of Risk Assessment, National Institute of Health Sciences, Japan.

[20] The report is only available in Japanese http://www.mhlw.go.jp/file/05-Shingikai-11201000-Roudoukijunkyoku-Soumuka/0000089516.pdf

71. In order to estimate the carcinogenic potential of each MWCNT, rats were exposed to some types of MWCNTs (shorter, longer, thin, thick, tangled type etc.) by single intraperitoneal administration and followed for a one-year observation period. The carcinogenic potential seems to be correlated with the number of longer length fibres. However, such a chronic *in-vivo* study is not a realistic method for evaluating many types of MWCNTs.

72. As a development of the *in-vitro* evaluation method, it was found that the potency of the inflammatory cytokines (e.g. IL-1β) production in macrophage type cells (THP-1) mainly depended on the length of nanofibres. Nevertheless, the potency of cytokine induction was not always correlated with the potency of the mesothelioma induction. It is postulated that biodurability and/or some other parameters (e.g. rigidity), in addition to the fibre length, are critical for the carcinogenic potential of MWCNT. Further mechanistic research is undoubtedly important for the development of the *in-vitro* methods, and robust *in-vivo* toxicity characterization of at least one representative material among this category of materials would also be necessary.

Presentation: Safety Assessment of Nanomaterials in Cosmetics: The SCCS Opinions on Nanoforms of TiO_2, SiO_2 and ZnO

Qasim Chaudhry (Chester University)

73. Prof. Chaudhry presented his analysis of the recent opinions of the Scientific Committee on Consumer Safety (SCCS) on nanomaterials. He is a Member of the SCCS but clarified that his presentation was not on behalf of the SCCS.

74. He briefly described the composition and work of the SCCS, which is one of the two non-food independent Committees of the European Commission - charged with assessing safety of chemical and nanomaterial ingredients in cosmetics. His presentation showed that the cosmetics sector is currently one of the largest users of nanomaterials, and that the main applications on the market in Europe relate to UV filters in sunscreen products. He mentioned the difficulties in safety assessment of nanomaterials in the absence of an agreed framework for grouping and read-across, when only limited data are available on a nanomaterial but it is intended for use in a number of different variant forms. He highlighted the examples of three recently published opinions of the SCCS on nanomaterials (titanium dioxide, zinc oxide and synthetic amorphous silica).

75. In the case of TiO_2, the SCCS assessed fifteen materials in one dossier. The materials had different crystalline forms (anatase, rutile), primary particle and agglomerate sizes, surface coatings, apparent bulk densities, aspect ratios (hence particle shapes), and photo-catalytic activity. He explained that the SCCS used the available information to broadly group the materials into 3 categories on the basis of crystalline form and photo-catalytic activity. The overall conclusion on safety was based on the lack of internal exposure and the absence of local effects on dermal application. However, as inhalation toxicity of TiO_2 nanomaterials has been reported, the opinion did not recommend applications that could give rise to inhalation exposure to TiO_2 nanoparticles (such as powders or sprayable products). The opinion also proposed limitations on the acceptable levels of the anatase form and the photo-catalytic activity of the materials.

76. In relation to nano-zinc oxide, the materials submitted in the dossier were all rod-like and isometric-shaped particles, were either uncoated or coated with organosilanes, and had comparable water solubilities. ZnO also has a continuous dissolution to the ionic (Zn^{+2}) form. There was no evidence of the absorption of ZnO nanoparticles through skin or the oral route; and any trace amount absorbed was most likely in ionic form and still insignificantly small compared to the large zinc pool already present in the body. The opinion of the SCCS therefore considered the use of the assessed types of ZnO nanoparticles

safe for dermal application. Again, due to the reported inflammatory effects on the lung, the use of nano-zinc oxide in applications that might lead to inhalation exposure to ZnO nanoparticles (such as sprayable products) was not recommended.

77. The opinion on synthetic amorphous silica (SAS) materials assessed 23 materials that could be broadly grouped into hydrophilic precipitated; hydrophilic pyrogenic; hydrophobic pyrogenic (surface modified with alkyl-silylates); and colloidal form of silica. These materials differed in terms of primary particle sizes, and the available toxicological database was very patchy and incomplete. There were also large variations in Volume Specific Surface Area (VSSA), solubility and densities; the available evidence for the lack of skin penetration of silica nanoparticles/clusters was also considered insufficient and inconclusive, because it did not cover hydrophobic surface modifications that may have a role in enhancing the dermal penetration. Thus, despite the apparent categorisation of the SAS materials with the aim to use read-across to fill data gaps, there was not sufficient data on physical-chemical properties, toxicological data or exposure to allow drawing any firm conclusion either for or against the safety of the materials.

78. Prof. Chaudhry also explained that an agreed and scientifically based framework for grouping and read-across would be very helpful for both industry and regulatory bodies carrying out risk assessment. He, however, questioned the rationale behind limiting the framework to inhalation exposure in occupational settings, which would make it largely relevant to REACH but not to other regulatory frameworks, and suggested that categorisation framework should be inclusive of other exposure routes, such as dermal (due to relevance for regulatory framework on cosmetics) and oral (due to relevance for regulatory frameworks on food/feed).

Presentation: Core-shell systems: different cases

Harald Bresch (Germany)

79. Coating, stabilization layers, functionalization of particles or simple contamination are common variants of a core-shell system. For small nanoparticles this is of major importance. A particle with a 16 nm diameter and the usual surface layer (shell) of 2 nm will have the same volume for the core as for the shell. In this case, the material of the particle does not have a clear definition. If only the shell is in contact with the surroundings, the particle might be categorized according to the shell, but this is only valid if the shell is not soluble. It is common for a particle to consist of four different layers: core, shell, stabilization layer and contamination layer. Some of these layers might be very thin and the shell might cover only parts of the particle, but they can still be presumed as a layer/shell. Every layer might influence the properties and the toxicity of the particle. For example, silver particles might have a different dissolution rate for pure particles and for particles which are grown on top of a core.

80. Different solubilities or other defined properties of materials are common reasons to produce core-shell systems. Gold cores can be surrounded by silica in order to stabilize them or to ensure a defined distance between the cores. Silica might be surrounded by gold; Hollow gold shells remain, when the silica gets dissolved. Another important example of core-shell systems are quantum dots: a small core is surrounded by a different material in order to increase photoluminescence. Furthermore, a stabilization layer is needed. The smallest contribution to the volume of the final particles is given by the initial core. The photoluminescence depends on the core, but the shells have a much bigger volume and contain much more material. Categorization should take this into account.

81. Core-shell systems are not covered by most of the existing decision trees for grouping. They are either regarded as a special case or assumed to be made of an individual layer for simplicity. This put core-shell systems out of the applicability domain of the most common decision trees for grouping. There might be a very easy way to avoid this problem and even to combine some of the different decision trees.

Considering the solubility of the outer shell as first step of a decision tree and subsequently addressing the inner layers could be a pragmatic approach to solve the problem. If there is no shell, the categorization can start with a tiered approach or with the proposed "strawman" chemical categorization suggested by USA in 2014. If a shell is covering the surface, there is a need to check if the shell is stable. If it is stable, the particle can be categorized based on the shell's properties. Furthermore, grouping needs to consider that the shell might increase the particle uptake by the cells. If the shell is soluble, the released ions need to be addressed as in the classical case. If the released ions and the uptake are not critical, the decision tree can continue with addressing the next inner layer.

82. With this not-perfect but pragmatic approach, the surface layers can be addressed in a decision tree for grouping with very limited additional efforts. Most criteria are based on tabulated data for the bulk material. Including a rating system such as the Precautionary Matrix approach (proposed by Switzerland) might help to address the time and mass dependence of some parameters like solubility, ion toxicity and uptake.

Presentation: Numerical algorithms for supporting qualitative and quantitative read-across

Tomasz Puzyn & Agnieszka Gajewicz (Poland)[21]

83. The development of computational methods that support human health and environmental risk assessment of engineered nanomaterials is nowadays of high interest, because the application of these methods enables filling the existing experimental data gaps. In the context of grouping (categorization) of nanomaterials, the most promising approaches that can be applied for data filling are: (i) Qualitative or Quantitative Structure-Activity Relationships ((Q)SARs), (ii) trend analysis (iii) and read-across.

84. (Q)SAR is based on mathematical dependencies defined between the variance in molecular structures, encoded by so-called descriptors, and variance in biological activity (toxicity in this case) in a set of nanomaterials. This means that if one has calculated or experimentally measured molecular descriptors for a group of similar nanomaterials and the toxicological data are available only for a part of this group, one is able to predict the lacking data by the help of the molecular descriptors and a suitable mathematical model. The successful application of Nano-QSAR has been already demonstrated. However, there are serious limitations related to the relatively large number of nanomaterials (data points) for which the experimental data required to build the model are (>15). When some chemicals in a category have measured values and a consistent trend is observed, missing values can be estimated by simple scaling from the measured values to fill in the data gaps (trend analysis). In such a case, a smaller number of data points (> 3) can usually be used. However, when there is no observed linear trend in the category and the number of data points is rather small (1-6), either a qualitative or a quantitative read-across technique might be applied. Read-across is based on similarities between nanomaterials within the category; the predicted endpoint value for a "source chemical(s)" is used to predict the same endpoint for a sufficiently similar "target chemical(s)".

85. Unfortunately, the techniques of read-across have not been sufficiently standardized yet. In effect, very often the results of estimations with read-across are too "expert-dependent", i.e. may vary depending on the personal experience of the expert conducting the study. This is important from the regulatory perspective, because the reliability and repeatability of the results are not guaranteed.

86. Thus, an overview is presented of the algorithms currently available for read-across in the four schemes: one-to-one, one-to-many, many-to-one, and many-to-many, discussing theirs advantages and disadvantages. In addition, four novel and more suitable numerical algorithms for read-across are

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introduced, namely: (i) RA1 – qualitative read-across algorithm based on hierarchical cluster analysis; (ii) QRA1 – quantitative algorithm based on one-point-slope and two-point formula approaches; (iii) QRA2 – quantitative algorithm based on Euclidean distance as similarity measure and two-point slope formula approach; (iv) QRA3 – quantitative algorithm based on principal component scores and two-point slope formula approach. The predictions obtained by using of the algorithms have been externally validated with experimental data for nanomaterials not involved in the model development.

87. It is worth mentioning that the proposed algorithms are universal, i.e. they enable filling data gaps within established groups of nanoparticles based on the previously selected criteria of similarity. While the selection of the algorithm to be used is an important source of uncertainty in read-across, the use of algorithms should be preferred to choosing the acceptable and sufficiently standardized algorithm(s) as much transparent as possible instead of taking just the average or the most conservative values.

Presentation: Important parameters for Grouping and Read-Across

Eric Bleeker (Netherlands)

88. Grouping and read-across can serve different purposes, such as limiting the amount of necessary tests and measurements, prioritisation of materials for further scrutiny, or design of testing strategies. The purpose steers to some extent what (additional) information is necessary and what the starting point is (e.g. does a new material fit into an existing group, does a material behave similarly to another one, can we identify a representative material for a group to further test or scrutinize?).

89. The ideas of using grouping, categorisation and read-across in risk assessment of chemicals are not new. However, in contrast to chemicals, similarities in chemical structure are not sufficient to determine categories or groups for nanomaterials, nor can the justification of read-across be based on their chemical structure alone.

90. For identification as well as read-across and risk assessment purposes, information on nanomaterials is needed that determines what they are (both chemical and physical appearance). Depending on the situation, information is also needed on where they go (which part within a body or the environment is exposed) and what they do (what is their reactivity). Furthermore, a clear purpose for the grouping or read-across is essential, as this determines what specific information is most essential.

91. Recently ECHA, JRC, and RIVM developed a framework (ECHA, EU, RIVM, 2016) that describes a stepwise approach to building a justification for grouping and read-across. The strategy comprises six different steps, including (1) identification and characterisation of the nanoform(s), (2) when possible formation of initial groups of nanoforms based on physical-chemical parameters (e.g. aspect ratio, or water solubility and dissolution rate), (3) identification of available information and data gaps for each nanoform per endpoint, (4) hypothesis-driven identification of source materials for read-across, which can consider information on ‘where they go’ and ‘what they do’, (5) when necessary additional testing to substantiate the read-across, and (6) assessing the new data and remaining uncertainties to conclude on the read-across arguments. When read-across cannot be substantiated, the strategy foresees reiterating (some of) the steps, or performing appropriate testing to fulfil the information requirement(s) of the specific regulatory framework.

92. Specifically for nanomaterials, the information on where they go is influenced by the nanomaterial characteristics as well as factors in the surroundings (e.g. pH, ionic strength, temperature, constituents of the media such as proteins or dissolved solids), whereas the nanomaterial characteristics can also influence the hazard potency of a specific endpoint. The justification for read-across should take this into account. It was noted in the presentation that the chemical composition of the nanoparticle is not so

important for particle kinetics, but is important for toxicity. Furthermore, the pristine nanoparticle may undergo considerable changes due to the environment and become something different before its toxicity becomes relevant.

HUMAN HEALTH / ENVIRONMENTAL HAZARD ASSESSMENT

Break-out group 1. Human Health Hazard Assessment

Chair: Karin Aschberger (EU-JRC), Rapporteur: Ken Moss (USA)

93. 1. Attendees discussed what criteria should be considered when conducting grouping in the context of (human health) hazard assessment. They acknowledged the importance of understanding the relationship between physical-chemical properties and biological effects (endpoint effects/toxicity, which often require lengthy animal testing), and the need to justify "read-across" of data between MNs or nanoforms. It is important to understand the relationship between possible modes of action (MOAs) and the physical-chemical parameters generating them. This has to be done in a stepwise approach, with specified, validated (standardized) assays using reference materials and applying quality control; a database of reference materials would be helpful. It was agreed, that a list of parameters (intrinsic and system-dependent properties) to serve grouping and read-across of nanomaterials should be defined. The following ones were discussed during this session.

- Solubility/dissolution, biopersistence/biodegradation
- Morphology, rigidity
- Toxicity, ion release (composition)
- Dispersibility, agglomeration (aggregation)
- Surface activity, reactivity
- Cellular effects, uptake, (time course)
- Kinetics/transport

94. The change of MN properties during its life cycle need to be considered: The physical-chemical properties of MNs “as produced” may be different from those “as used”; but these are the ones to which humans and the environment will be exposed to. It was acknowledged that fundamental behaviour (where they go) and the reactivity (what they do) will have a major impact and it is important not only look at the (intrinsic) physical-chemical parameters.

95. It was important to note that particles or fibres exist as entities, functionalized or not, in various biological media, in which they are dissolved or digested, with surface activity affecting the reactivity/free radical/inflammation potential of the particle. How long particles persist in a given physical state is important because long-term disposition and toxicity (e.g. lung/liver) is of concern. Various key parameters were identified as having an important impact on kinetics, transport, cellular (uptake) and consequently biological effects, including composition, solubility, dissolution, morphology, fibre rigidity (asbestos-like), ion release potential, dispersibility and agglomeration state.

96. The purpose of grouping and read-across needs to be considered: regulatory requirement / levels of risk assessment (screening or more robust), relative risk for chemical substitution or risk communication. Should it serve to fill data gaps or inform tiered testing? Case studies were considered important to establish patterns and develop necessary assessment tools.

97. Section 6.7 (Metals and inorganic metal compounds) of the OECD Guidance on Grouping of Chemicals was cited as a good template to start the work with MNs, especially those based on metals or metal oxides. For those it is the bioavailability of the metal ion (or a redox form of this ion) at target sites that, besides the toxicity potency, will determine the occurrence and severity of the effects to be assessed. This chapter also addresses factors that could alter the basis and assumptions underlying the grouping, which are equally relevant for nanomaterials, like chemical speciation and valence, crystalline structure, particle size, surface properties, as well as other factors such as well as other factors such as presence as part of a mixture, presence of counter ions or other metal ions, and presence in the form of an organo-metallic compounds.

Break-out group 2. Environmental Hazard Assessment

Chair: Brad Fisher (Canada), Rapporteur: Kathrin Schwirn (Germany)

98. During the break-out session, the participants discussed which parameters are important for grouping and read-across in the context of environmental hazard assessment. In addition to intrinsic physical-chemical parameters, preliminary behaviour information also needs to be considered ("Where they go" or "How they behave"), for example dissolution rate and agglomeration behaviour are of relevance for nanomaterials. It was also noted that shape is an important physical-chemical parameter to be considered in environmental hazard assessment, since shape can be accompanied by physical effects and may influence uptake and depuration by organisms. Overall, physical-chemical properties influence nanomaterials in terms of their behaviour and fate in corresponding environmental and test media.

99. In turn, the medium has an influence on the behaviour and fate of nanomaterials and needs to be considered in grouping and read-across. It was recommended to identify standardised test media for testing nanomaterials. This would facilitate comparability and reproducibility of test results. However, when defining test media, it needs to be suitable for nanomaterials and the examined organism. This recommendation is also considered for the ongoing activities for developing a nanospecific OECD guidance document for testing aquatic and sediment organisms.

100. There was limited discussion within this breakout group on criteria to grouping presented in the previous sessions, since few of the presentations had an environmental purpose (grouping for ecotoxicity assessment). Most of the grouping approaches presented at the workshop focused on for human hazard, in particular inhalation toxicity: grouping for human hazard assessment and grouping for exposure assessment, etc. For environmentally relevant grouping approaches, a broader view is needed in order to take into account the complexities of 'real world' environmental conditions. It was agreed that the presented approach (Sellers et al., 2015) shows the complexity of environmental considerations. An overarching environmental grouping approach was regarded as not meaningful, and instead, the approach should be endpoint and organism specific.

101. Finally, there was a discussion on benchmarks and when different nanoforms can be considered similar or not. The participants revived aspects that were already stated at the beginning of the session. Information about where nanomaterials go and how they behave in the media (dissolution rate, agglomeration behaviour, sedimentation) is important. Since these behaviour and fate aspects will determine the exposure mechanism, environmental conditions and exposure route is needed for prediction, and comparison of test results. Determining this information will also give insight into an adequate testing strategy.

102. Participants briefly discussed grouping criteria used for comparing individual nanoparticles and the need to define similarity and stability of nanomaterials in the context of read-across. It is still unclear if a nanomaterial is considered sufficiently similar to another one if a given parameter changes.

103. Methodology of grouping and read-across presented in ENV/JM/MONO(2014)4 and other documents and implemented in various software (e.g. OECD QSAR Toolbox) uses well-established chemoiformatic and statistical techniques for supporting the process. They work independently on the problem and type of variables (i.e., criteria of similarity) we are using. Read-across must be strictly scientific-based to be useful from the regulatory point of view. If similarities are scientifically justified, elegant methods of measuring similarity developed for regular chemicals, the existing chemoinformatic techniques of read-across are applied. In case of nanoparticles we should take not only intrinsic properties of NMs, but also system-dependent properties, properties saying, where the nanoparticle goes etc.

104. Furthermore, it is still under debate which characteristics of nanomaterials fall under the term stability and how to consider them, since it is also not fully understood how characteristics change and how this influences environmental effects. Characteristics such as aging, transformation and surface coating were mentioned as influencing “Where they go” and "How they behave”.

Discussion

Chair: Andrej Kobe (EU)

105. The rapporteurs of the break-out groups 1 and 2 presented the main conclusions of each break-out group, see above. For the environment, a testing strategy is very important, as is the development of new and adaptation of existing test methods. This was already discussed at OECD (OECD, 2014a), and remains an important aspect.

OECD GUIDANCE DOCUMENT ON GROUPING OF CHEMICALS: APPLICABILITY FOR NANOMATERIALS

Break-out group 3. What adaptations of existing approaches used for conventional substances are needed for nanomaterials?

Chair: Niklas Andersson (EU-ECHA), Rapporteur: Linda Johnston (Canada)

106. The chair introduced the subject of the break-out group by outlining the general principles for read-across and asking if these principles are applicable to nanomaterials; and if not, what would the requirements be for expanding them to also address MNs. Another relevant question highlighted was whether a nanomaterial grouping concept could be fitted to the existing OECD Guidance. The chair also noted that, when using the analogue approach, while for conventional material, read-across usually focuses on two substances that are similar enough to assume they have the same effect or property so that it is possible to extrapolate from one substance to the second one. While for nanomaterials the system will be more complex due to the larger number of properties that must be considered in selecting analogues. In the category approach, the prediction of a given property (e.g. toxicity to rat) is based on more than one property of the compounds in the group (physical-chemical properties, other types of toxicity). The chair and the break-out group clarified that the principles to be developed for MNs should be generally applicable, independently from regulatory frameworks.

107. Starting with the current initial process in the OECD guidance: i) develop hypothesis, ii) identify analogues (i.e. nanoforms), iii) consider size, surface coating, composition, etc. and iv) data gathering, there seems to be no reason to deviate from this process with nanomaterials, though it may be necessary to expand some steps. It was noted that the NanoReg2 project is looking at standard methods that could be used for MNs, but many are not applicable; for example dispersion is an issue. The framework for grouping and read-across should be developed before focusing on development of new methods.

108. The next step in the OECD guidance is to assess the adequacy of available data and applied methods, and whether additional testing would be needed, including what to test and how. It was noted that for chemicals, the key is similarity (analogues). However, grouping will have to be done in a different way for MNs, since more descriptors (not just chemical composition) will be needed. The concept of "similarity" will also need to be developed; for example, are two MNs with the same size but a different composition part of the same group or not (e.g. TiO_2 vs TiO_2/ZnO), and how would this be addressed in a framework for MNs?

109. There was a general agreement that the existing framework for grouping can be retained, but details will be different: In case of usual chemicals there is always discussion on the grouping criteria. The only issue in case of NMs is that we should take into account system-dependent behaviour as additional criterion (criteria). The framework may be the same but will have to be expanded with additional nano-related information, and actual grouping will always have to be defined on a case-by-case basis, using a practical approach based on what is known about/available for specific MNs.

110. Looking at the next steps in process, the existing data is identified and then assessed with regard to its reliability and adequacy. Furthermore, the data gaps are identified. Then a read-across hypothesis is developed that needs to be substantiated, and it is still open which methods are needed to substantiate the hypothesis. For chemicals, read-across involves looking for analogues, in the case of analogue approach. For MNs, it is not clear what criteria should be used, and if it is possible to merge results from different sources (and at what level), e.g. databases. The comparability of the data may also be an issue, as frequently the approaches are not consistent. Generally, it is possible to draw qualitative conclusions, but not quantitative ones. Tools addressing this are under development, and for example the European Commission has started to develop databases of (validated) studies performed in EU projects. Thus, data measured with standard or other methods are becoming available.

111. Regarding the identity of the materials for grouping, for chemicals the molecular structure is available. For MNs, the basic nomenclature is missing. A multi-dimensional data set (including relevant equations for determining the properties) for MNs is needed, capturing composition, size, shape, specific surface area/volume ratio etc. and including an answer to the following question: "how big a change can be tolerated before one can no longer consider the two materials to be the same nanoform?" For example, are 20 nm and 40 nm particles (of a specific nanomaterial) the same nanoform? Guidance should be developed for defining identity for MNs, addressing, among other questions: What constitutes a new substance when dealing with similar MNs (i.e. similar composition or size or others)? Is there an *in-vitro* test to address this point? The break-out group noted that toxicity of particles is very different from that of dissolved chemicals, even if some molecules can also be very complex, and it is difficult to identify the property that drives toxicity. Furthermore, a database with source nanomaterials' properties for specific purposes and for each mode of action would be very useful, though very challenging to develop; as such a database could need as many as 100 entries. In this context, the OECD materials and NIST reference materials were noted. They may be very pure and well-characterized, but possibly not very applicable for assessing real-world materials.

112. Filling identified data gaps was discussed, including the appropriate method to fill a gap. Furthermore, after developing a new test method and obtaining new data, it may be necessary to re-evaluate the initial grouping. The group discussed how to substantiate the read-across hypothesis and link it to the data and relevant endpoint. Current guidance lists the elements that should be considered but is not very specific; flexible grouping will be required, and also a description of how to deal with bulk vs. nano issues, as the bulk material can be used as an analogue in some cases. In any case, the selection of analogues should be justified. Another issue in the bulk vs. nano comparison is that molecules have a defined structure, whereas MNs have multiple forms. Will a definition be required for each specific nanoform?

113. The breakout group concluded that the principles of existing guidance are adequate after additional nano-specific details are added. Prioritization of parameters is crucial, so that method development can address the key priority parameters, and this needs further work including a roadmap. Methodology development is needed, for which solubility (dissolution rate), surface reactivity (including hydrophobicity) and surface coating are priority areas; composition is more straightforward. The quality and availability of data is an issue. Terminology and standardized formats for grouping are needed, as well as reference materials. Furthermore, for the practical application of grouping for nanomaterials, it is important to consider the application to industrial materials and not only well-characterized (laboratory) reference materials. Guidance on methods and tools is needed, including a consistent and harmonised data reporting format. Ideally, the guidance would also include examples as well as endpoint specific physical-chemical parameters. In addition, grouping has to be justified on a scientific basis and to define the limits for specific cases. The time line envisaged is several years.

Break-out group 4. What criteria should be considered when conducting read-across in the context of hazard assessment, and what data of what quality are required?

Chair: Tala Henry (US), Rapporteur: Paula Jantunen (EU-JRC)

114. The Chair opened the discussion of break-out group 4 by stating the basic question: are we ready to update the section 6.9 of the OECD Guidance on the Grouping of Chemicals for read-across on nanomaterials (OECD, 2014b), which currently consists of a few paragraphs about ongoing research, with elements for appropriate justification for such read-across, and what are the criteria for data and computing approaches of good enough quality for read-across? The Chair also proposed section 6.7, concerning read-across on metals and inorganic metal compounds, as a potential generic model for shaping up the content of section 6.9.

115. It was suggested that data required for filling in data gaps via grouping and read-across for hazard assessment need to be experimentally produced, well documented and validated. Data on the toxicokinetics of MNs was considered particularly important. The point was raised that such data should concern nanomaterials in industrially relevant forms rather than very pure nanomaterials, although it was also argued that impurities of industrial nanomaterials, not necessarily of nanosize themselves, may also be significantly toxic and therefore confuse the hazard assessment of MNs. As with UVCB[22] substances, the real-life variation of industrial MNs may call for careful consideration of what can be used as a representative MN sample for testing for hazard assessment.

116. The quality of the data really needed for grouping and read-across for hazard assessment was discussed. It is known that nanomaterials may interfere with many standardized test methods (through e.g. reactivity, catalytic activity or fluorescence), which may result in either false negative or false positive test results. Such interference should always be checked for before using a test method for a specific MN. General standardized methods and descriptors need to be validated for MNs, and case studies were seen as important. Where specific standardized methods or guidance for testing either intrinsic properties or toxicity of MNs exist (including e.g. guidance for sample preparation), they should be followed, but very few nanospecific standardized methods are yet in existence. The position of new screening tools that are being developed for nanomaterials in regulatory hazard assessment before formal regulatory recognition was pointed out as unclear, although such tools may be necessary for producing the data needed in specific cases. The level of confidence in test results should also always be considered and reported, as confidence may be high when testing established and well-known types of MNs, but much lower when testing novel types of MNs for which the suitability of the method is unknown. Moreover, attention should be paid to templates for reporting test results, as these templates tend not to be harmonized yet. For the sake of comparability and usability of reported test results, similar reporting practices should be aimed for in guidance. For example, the particle size of the MN tested should be reported as a particle size distribution of sufficient frequency rather than as a simple one size parameter.

117. Since there are no known good predictors for toxicological parameters of nanomaterials among physical-chemical parameters yet, "in between" functional parameters whose relationship with toxicological parameters is better established need to be used for read-across. The justification for read-across must present a proper hypothesis about the relationship of the relevant parameters and also address uncertainty. Screening by biotic and abiotic methods in order to strengthen grouping arguments was pointed out as a possibility, and the hope that such practices could be recognized by regulatory frameworks was expressed. The question whether the criteria of the Read-Across Assessment Framework published in by ECHA (ECHA, 2015), addressing the human health hazard assessment of chemicals, is applicable also to nanomaterials was made but not addressed.

[22] Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials.

118. Criteria for the similarity of nanoforms for grouping and therefore grouping and read-across were discussed at length. Compared to traditional chemicals, thorough (beyond physical-chemical) characterization and identification of the nanoforms for grouping was considered essential. However, relevant metrics (possibly entirely new ones) vary by endpoint, and while characterization requirements are largely set by regulators, fit-for-purpose read-across where only the parameters relevant for determining sufficient similarity (with regard to the endpoint in question) need to be determined was called for. It was also pointed out that the required quality of data used for determining whether two nanomaterials are similar enough to be placed in the same group should consider both the margin of error of the relevant parameter(s) and the required degree of similarity. While OECD testing guidelines generally include standards for the validity of the test results, it is unclear whether such standards cover the testing of MNs, and this should be addressed when testing guidance is adapted for MNs.

119. As it has been argued that no nanospecific mechanisms of toxicity as such seem to exist (e.g. Gebel et al., 2014), the fundamental difference in the hazard assessment between traditional chemicals and that of MNs was discussed. Compared to traditional chemicals, MNs were described as "entities with chemical composition" or "chemicals whose physical form defines their (toxic) action"; their toxicity may have both physical and chemical elements, and the relevance of each element varies by case and context. It was pointed out that such a difference is not restricted to nanosize entities, but that e.g. fibres and particles in the micrometre size range have similar toxicological characteristics of both chemical and physical nature. The effect of the physical form on toxicity was seen to be largely defined by the size and shape of the (nano) particles. As with larger-size particles, separate criteria should probably be used for the hazard assessment of MNs of different shape, e.g. "fibre-like" vs. "particle-like" MNs. Particle size was suggested to have a modulating effect on MN toxicity but not to drastically change its mechanism or potency. Particularly for fibre-shaped MNs, the current hazard classification of asbestos and glass fibres according to e.g. chemical composition, fibre dimensions and biodurability was suggested as a precedent and model for grouping for hazard assessment.

120. The updating of current OECD Guidance for read-across on nanomaterials was seen as possible. It was however pointed out that outside of some very generic guidelines, such guidance would soon have to be updated again in order to keep up with the developments of the relevant methodology in the scientific literature.

Discussion

Chair: Andrej Kobe (EU)

121. The rapporteurs of the break-out groups 3 and 4 presented the main conclusions of each break-out group, see above. Taking the two sessions together, one recommendation to the WPMN is to open the section 6.9 of the guidance document to review after making an outline of the possible update. Furthermore, the content of section 6.7 (read-across on metals and inorganic metal compounds) could be used as a model for the update of section 6.9, but a final recommendation can only be given once a firmer update proposal is available.

122. It is also highly important to continue the method development, to ensure that the predictive value of grouping is as high as possible. Furthermore, the new section could include case studies.

CONCLUSIONS AND RECOMMENDATIONS

Chair of the Meeting: Juan Riego Sintes (EU-JRC), Rapporteur: Kirsten Rasmussen (EU-JRC)

123. The chair of the meeting summarised the conclusions and recommendations of the expert meeting in the form of bullet points, which are listed below:

- Grouping and read-across are necessary and appropriate tools for filling data gaps in the hazard assessment of manufactured nanomaterials.

- This meeting confirms the outcome of the workshop on the Categorisation of Manufactured Nanomaterials, where it was agreed that definitions and terminologies need to be clarified and consistently applied.

- The general parameters that have been presented by different schemes are considered generally as the core elements to initiate grouping and read-across; however, depending on the material, endpoint of concern being addressed, the list of parameters will need to be adapted.

- The general scheme for building and justifying groups and the read-across seems acceptable for nanomaterials. However, there is need to develop guidance that addresses the specificities of MNs.

- A possible first step forward is to develop an outline for section 6.9 on the specificities of nanomaterials and assess where existing guidance is sufficient and where additional guidance could be provided. It was re-iterated that the full Guidance on Grouping of Chemicals [OECD, 2014b] is not to be reviewed. The focus on possible revisions is only on nanomaterials (i.e. Section 6.9, and using section 6.7 as an indicative model for the contents and format of the possible revised chapter).

- Generic categories based on the ones presented at the workshop on the Categorisation of Manufactured Nanomaterials (OECD, 2016) and the ones based on solubility, shape and Mode of Action (i.e. US NIOSH) can be starting points for building the hypothesis for grouping and read-across.

- Tiered approaches should be developed to address the specific data needs for the material and concerns being considered.

- This requires evaluating the available screening methods to assess their relevance and reliability.

- There is a need to obtain high-quality data and make them available (both already existing data and/or new generated data) particularly to build and substantiate the hypothesis.

- Data management procedures need to be put in place in order to facilitate sharing and accessibility to information. In particular, reporting templates for results should be harmonized to improve comparability. It was noted that some initiatives are already well under way[23].

- There is a need to produce and use Reference Materials and benchmark materials.

- There is still a need for reliable Testing Methods: Development of such methods is a main concern and it should be further prioritised. It was noted that some OECD Test Guidelines and Guidance Documents are under development (e.g. Dissolution)[24].

- There is a need to further develop the knowledge on how to connect the parameters with the effects intended to be predicted in the grouping and read-across exercise. Practical experience in real cases is necessary.

- Having this in mind, and to illustrate their practical application, frameworks should be tested with case studies both for environmental and human health hazard assessment.

- The uncertainty of the approaches needs to be addressed.

- It would be interesting to further develop a compilation of grouping methods presented/available and compare them to see the differences and common elements.

- For (eco)toxicological screening purposes, High Throughput Systems, and –omics methods should be explored, including development of additional such methods.

[23] As an example, the JRC reporting templates developed within NANoREG in collaboration with e-NanoMapper can be found here: http://www.nanoreg.eu/media-and-downloads/templates/269-templates-for-experimental-data-logging

[24] Test Guidelines and Guidance Documents under development are made available for public comments at: http://www.oecd.org/env/ehs/testing/chemicalstestingdraftoecdguidelinesforthetestingofchemicals-sections1-5.htm

REFERENCES

Arts et al., 2015. A decision-making framework for the grouping and testing of MMNs (DF4nanoGrouping) Regulatory. Toxicology and Pharmacology, 71, S1-S27.

ECHA, 2015. Read-Across Assessment Framework (RAAF) ECHA-15-R-07-EN, European Chemicals Agency (ECHA), Helsinki, Finland. Available at: http://echa.europa.eu/documents/10162/13628/raaf_en.pdf.

ECHA, EU, RIVM, 2016. Usage of (eco)toxicological data for bridging data gaps between and grouping of nanoforms of the same substance: elements to consider. This paper was jointly prepared by the European Chemicals Agency, Joint Research Centre, Dutch National Institute for Public Health and the Environment.

Gebel T et al., 2014. Manufactured nanomaterials: categorization and approaches to hazard assessment. Arch. Toxicol, 88, 2191-2211.

OECD, 2014a. Expert Meeting on Ecotoxicology and Environmental Fate of Manufactured Nanomaterials [ENV/JM/MONO(2014)1].

OECD, 2014b. Guidance on grouping of chemicals, second edition, [ENV/JM/MONO(2014)4].

OECD, 2016. Categorisation of Manufactured Nanomaterials; Workshop Report [ENV/JM/MONO(2016)9].

Oomen et al., 2015. Grouping and Read-Across Approaches for Risk Assessment of Nanomaterials. Int. J. Environ. Res. Public Health 2015, 12(10), 13415-13434; http://www.mdpi.com/1660-4601/12/10/13415.

Sellers K. et al., 2015. Grouping Nanomaterials - A strategy towards grouping and read-across. RIVM Report 2015-0061, National Institute for Public Health and the Environment (RIVM) & Arcadis, Bilthoven, the Netherlands. Available at: http://www.rivm.nl/bibliotheek/rapporten/2015-0061.html.