A common European approach to the regulatory testing of nanomaterials

NANoREG

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Report on identification and setting of categorization, read-across, and extra/intrapolation criteria

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Table of Content

1 Description of task

The description of task 5.1 is taken from the description of work (DoW) and is included below for convenience.

From DoW:

There is a need to understand how far different MNMs can be categorized, and how far data and results can be used for read-across from the bulk-material of the same chemical composition, as well as extrapolation from and interpolation between different nanoparticle sizes of a nanomaterial of the same chemical composition. Previous initiatives (including RIP-oN 1: REACH Implementation Project on Substance Identification of Nanomaterials) show that there is no consensus on this topic (which is sometimes also referred to as the sameness principle). The results of WP2 on characterisation and categorisation of nanomaterials for regulatory purposes and results of WP4 on the toxicity of nanomaterials will help to focus on the (physico-chemical) properties of nanomaterials that can be linked to their behaviour in the environment and in the human body. This task will develop a system for the categorization of nanomaterials from a risk assessment point of view based on expected biological, ecological and/or toxicological effects. In addition criteria for read-across, extrapolation and interpolation will be developed. The recommendations for categorisation and criteria for read-across, extrapolation and interpolation can be used in the development of the decision tree developed (Task 5.7) and regulatory framework/toolbox (Task 1.4).

Here, research will focus on:

1. Evaluation of other research on this topic: Several research projects have investigated this issue (BSI, 2007; IFA, 2009; NanoSafetyVision Report, 2012). An overview of the results of these projects will be made as a starting point of this task.

2. Identification of key properties that influence the behaviour of nanomaterials in the environment and the human body which can be used for categorization of nanomaterials, including secondary properties such as reactive oxygen species production.

3. Identification of key properties in support of read-across between bulk material and nanomaterials, including solubility (see task 5.2).

4. Identification of key properties in support of extrapolation from and interpolation between different particle sizes of a nanomaterial of a given chemical composition

5. Development of recommendations for scientifically based categorisation based on similar biological, ecological and/or toxicological effects, read-across, and extrapolation and interpolation criteria.

6. Experimental verification of scientifically based categorisation, read-across, and extrapolation and interpolation criteria with respect to differences in one or two characteristics (e.g. size and/or surface treatment).

7. Development of rapid characterisation/screening methodology in support of categorisation, read-across, and extrapolation and interpolation.

8. Assessment of data-modelling integrity and predictive capability of (Q)SAR algorithms.

2 Description of work & main achievements

2.1 Executive summary

2.1.1 Aims and goals

With the increasing use and availability of a large number and variety of nanomaterials, the need to develop more efficient ways to evaluate potential adverse effects, such as grouping and read across, is becoming more important.

Both in discussions on nanomaterials and in discussion on grouping and read across, terminology is often somewhat ambiguous. Within NANoREG a terminology document has been developed and those terms are used in this document (Deliverable D1.10). As a consequence, the term grouping is preferred over the term categorisation in this document.

Grouping and read across can be done with different aims. One of those aims is to screen or prioritise different nanomaterials with respect to one or more potential harmful effects. Another goal is to fill data gaps by using grouping to support read across. The latter can be done for the whole spectrum of the REACH and CLP required endpoints, as well as for other nano-specific endpoints not yet included in REACH. While most examples of grouping and read across link physico-chemical properties to toxicological endpoints, it can also be used to link physico-chemical properties to other physico-chemical properties, exposure or toxicokinetics. Because, the physico-chemical properties of nanomaterials can be modified in each step of the life cycle, change in the physico-chemical parameters during the life cycle should be taken into account, which may lead to different grouping and read across approaches of the same nanomaterial within the different parts of its life cycle.

2.1.2 Limitations of the regulatory context

NANoREG, as "testing the test" project, and being regulatory focused on REACH, defines some boundaries and poses pre-requisites that a NANoREG approach and/or recommendations have to comply with. Also, the philosophy of NANoREG and the expected results, have to be taken into account when developing a NANoREG approach and/or recommendations. First of all the regulatory focus of NANoREG is REACH. This implies that the REACH information requirements should be taken into account when considering which data gaps should be filled and which type of information will be available from analogue materials. In addition, before a specific grouping or read across proposal will be regulatory accepted, its scientific validity needs to be assured and it should be accompanied by clear explanations on how to use it for making decisions.

2.1.3 Previous initiatives

There have been many initiatives and proposals for grouping of nanomaterials. Only a few of these link physico-chemical properties to the environmental and human toxicity of the nanomaterials. For an effective grouping aimed at screening or read across, benchmarks are needed to determine the similarity among the different nanomaterials within a group. With the exception of some (Q)SARs, most approaches do not contain such benchmarks. It is difficult to identify benchmarks for all relevant properties, or rather all relevant combinations of properties. The need for a multidimensional approach with several criteria is acknowledged by several institutions and working groups. Those approaches that include benchmarks have usually not been validated because there is not enough high quality scientific data to facilitate such validation. Based on the different initiatives and proposals for grouping and read across of nanomaterials, the most important properties that need to be considered as well as a step wise approach have been identified.

2.1.4 Properties for human and environmental behaviour

The behaviour and effects of a nanomaterial are influenced by a combination/interaction of several physico-chemical properties as well as the surrounding medium. These properties can tentatively be placed in four categories:

- Substance identity, including chemical composition, crystal structure, surface coating, functionalization and capping agents, impurities, all of which influence surface charge and reactivity;
- Particle characteristics, including size (distribution), surface area (which depends on particle size and porosity), surface roughness, shape and aspect ratio, all of which generally influence mobility and transport;
- Transport behaviour, which reflects characteristics of the nanoparticle that are (partly) influenced by the surrounding medium, such as solubility/dispersibility (rate of dissolution and equilibrium concentration, both size-related), surface charge, tendency to agglomerate, dustiness.
- Activity and reactivity, including redox potential.

The properties above can influence not only the toxicity and ecotoxicity of a nanomaterial, but also the interactions between the nanoparticle and the environment, whether external or within an organism. On the basis of the available literature and study reports, it appears that the toxicity of nanomaterials is not a generic response to a particular property; rather, multiple characteristics affect toxicity including size, composition, shape/aspect ratios, crystalline structure, surface area, surface coating/ functionalization, surface charge, solubility and reactivity. However, additional properties play a crucial role in determining the toxicity of nanomaterials, such as dustiness, and the surrounding medium. Therefore, a fundamental understanding of the physico-chemical properties and biological interactions of nanomaterials with cells, proteins, tissues, and living organs as well as in the environment is vital to the development of grouping and read across as well as for future design of safe nanotechnologies.

2.1.5 A stepwise approach for using data between (nano)forms

Two main goals of grouping are identified. The first is initial grouping for screening purposes and the second is grouping for the purpose of read-across to fill data gaps. In a working document on read across between nanomaterials by ECHA, RIVM and JRC, the following stepwise approach for using data between (nano)forms is described:

1. ***Identification of the nanoform.*** This involves the identification of the nanoform, based on the basic physicochemical parameters of the nanoform, for which potentially no or insufficient information is available for hazard characterisation (unassessed nanoform).

2. ***Initial grouping of nanoforms.*** Based on similarities in chemical identity, particle characteristics, fundamental behaviour (i.e. solubility, hydrophobicity, dispersibility and dustiness) and reactivity (i.e. flammability, explosiveness, biological (re)activity, surface reactivity and photoreactivity) initial grouping of nanoforms may be considered. Such initial grouping should be justified by similarities in the behaviour of the (nano)forms and the boundaries of a group should be clearly defined.
3. ***Identification of available data and data gaps.*** This involves making an inventory of the information available per endpoint required under REACH for this nanoform, and consequently identifying data gaps for REACH Compliance.
4. ***Identification of potential source materials.*** For each data gap, this involves the identification of source materials, e.g. other (groups of) (nano)forms, from which information may be used for read-across. This also involves (hypothesis based) justification of the appropriateness of the identified source material.
5. ***Substantiate hypothesis.*** This involves information gathering to substantiate the hypothesis for read-across. When groups of (nano)forms are considered in read-across it may be necessary to re-evaluate the initial grouping. If applicable, a testing strategy can be build that may (partly) cover multiple data gaps.
6. ***Assess any new data for the impact on the hypothesis.*** In an iterative process, interpret the information that becomes available to evaluate if the information sufficiently substantiates the hypothesis and builds justification for read-across (or not).

For (initial) grouping for screening purposes, the first two steps of this procedure may suffice.

For nano-forms, examples of read-across used within regulatory risk assessment are only available for specific narrow groups of nanomaterials for a specific route of exposure and/or endpoint. Examples are the use of read across between high-aspect-ratio CNTs and asbestos (which is currently done in many control banding tools), the SCCS evaluation of TiO_2 in sunscreens and the NIOSH RELs for CNTs and TiO_2.

2.1.6 Conclusions and recommendations

The most important physico-chemical properties that need to be considered for read-across and grouping, as well as a stepwise approach to come to a justified grouping or read-across, have been identified. However, further development is still needed to establish justified values of specific physico-chemical properties that set the boundaries of a group, i.e. benchmarks that determine whether a nanomaterial belongs within a specific group or not. For the development of more predictive groups to support read across, a better fundamental understanding of the physico-chemical properties and biological interactions of nanomaterials with cells, proteins, tissues, and living organs as well as in the environment is needed. This can only be obtained with more high quality data on carefully chosen sets of nanomaterials in well-defined media. The tools to generate and collect high quality data to support these benchmarks are developed within other task of the NANoREG project. For some groups of nanomaterials (e.g. ion release from certain metal-based nanomaterials) sufficient data of high quality appear to be available to justify the boundaries of a group. Nevertheless, for other nanomaterials further work is needed to justify the (boundaries for) grouping for a certain information requirement under REACH (or other legislation).

3 Scope and readership

In the NANoREG report 'Report on a Virtual Workshop to identify, formulate and prioritize issues/questions' (Deliverable D1.1) a number of regulatory issues and questions in the area of regulatory toxicology and risk assessment of nanomaterials have been compiled. These questions are to be addressed by the NANoREG

project. In table 5[1] of D1.1 report all 18 key questions from a regulatory perspective are presented. To ensure that all questions are addressed by NANoREG each work package has assessed the relevance of the 18 questions for various tasks. For Task 5.1 eight questions (Table 1) where deemed to be relevant. Especially question 5 (Extrapolation and grouping) and 8 (Kinetics and fate, extrapolation) where considered to have main relevance, whereas questions 1, 2, 3, 4, 7 and 9 are related to the task.

Table 1. Questions relevant for Task 5.1 (taken from D1.1[1])

Q	Questions with main relevance for Task 5.1
5	**Extrapolation and grouping:** What guidance can be provided on how to decide when information from different forms of MNMs (or from the bulk material) can be "re-used" in the sense of read-across, categorisation and grouping? Should / could guidance be based exclusively on physico-chemical properties or could exposure related (eco)toxicological and mechanistic information (as Mode of Action) be used as well and how? Take into account the relation with the following questions.
8	**Kinetics and fate, extrapolation:** How and when can information on kinetics and fate be used to justify grouping / read across or testing triggering / waiving and for building knowledge on the relationship between physico-chemical properties and toxicity? In other words: to what extent are the kinetics and fate of MNMs (e.g. environmental distribution or deposition and biodistribution in the lung) different from the bulk material? Are there ways to extrapolate this information from the bulk material or from several forms (size, shape, coating, etc.) of the same chemical and how should this extrapolation be made?
	Questions related to Task 5.1
1	**Measurement and characterization - Identification**: How can MNMs be identified according to the EC recommendation for a definition of MNMs and for regulatory purposes (i.e. the implementation of the EC definition in e.g. REACH, CLP, cosmetics, novel food, etc.), including other jurisdictions (global harmonisation)? Can we develop robust measurement protocols which enable assessment of whether a NM falls under, or not, the EC definition? Are there robust measurement protocols available (and for which matrices) that enable identification?
2	**Measurement and characterization**: Could an "intelligent characterisation strategy" be defined? What is a minimal set of physical (and/or chemical) characteristics that should be available for risk assessors within the context of regulatory toxicology? What are the relevant features to characterise MNMs, e.g. size, form, aspect ratio, rigidity, flexibility and coating? What methods (SOPs) should be developed / used to determine the physic-chemical characteristics of MNMs throughout their different life cycle stages within the context of regulatory toxicology? These questions (closely related to Q1) refer to developing cost effective standard methods, detailed protocols and reference materials both for calibration and analysis of both pristine materials and materials in relevant media or complex matrices throughout the complete life cycle of the nanomaterial. they also refer to whether different categories of characterisation methods (varying e.g. in precision and accuracy) can be defined: Could an "intelligent characterisation strategy" be defined?
3	**Characterisation/Transformation:** What testing should be performed to identify surface modifications that occur once a MNM has been released into the environment or taken up into the body? How can transformation, including agglomeration surface modification, dissolution and incineration, be determined and considered in the exposure and hazard assessment and how do they change the intrinsic toxic properties and biodistribution Do we need to know the details of such surface modifications or of what is bound, or do we need some simple test systems that actually determine the behaviour and transformation of MNM in relevant media throughout all life cycle stages? Is a nano-derived material still nano when it becomes agglomerated? Take into account relationship with questions 7-9.
4	**Metrology and dose metrics:** Which metrics (metrology) should be used for MNMs in regulatory toxicology? As recommended by several committees and guidance, notwithstanding e.g. the OECD GSPD, NANoREG should use mass, particle numbers and surface area (as far as possible) to characterise dose. The data generated within the project will contribute to the development of a body of comparative data (e.g. shape and aspect ratio should be examined when appropriate for the MNM). Using this comparative data, NANoREG should examine which metrics are the most appropriate depending on the different types of materials and media involved, as well as the (eco)toxicological effects and exposure to be assessed in the Risk Assessment process.
7	**Kinetics and fate, determination:** How and when should information on absorption from the various routes of exposure, on deposition (e.g. lung burden), on biodistribution, on potential persistence and bioaccumulation, and on internal exposure (taking into account dose, duration, coating and interaction with biological systems) be generated and used? Relate the information with, for instance, the following objectives:

[1] Deliverable 1.1: table 5 on page 15 of the document 'NANoREG D1.1 2013-07-15 JRS plus annexes.pdf' in CIRCABC (Library > C-Consortium > 03 Deliverables uploaded to EC)

	• To perform more accurate risk assessment • To decrease uncertainty (safety factors), • To select, if needed, a second route for acute toxicity testing, • To design additional tests – that are 'affordable' – or to relate to studies that involve exposed workers, such as in the silica industry, • To decide on a strategy for further testing (carcinogenicity, reproductive toxicity, etc.).
9	**Mode of action:** What are the physical and chemical properties driving exposure and (eco)toxicity of MNMs at all stages of their life cycle? How is MNM interaction with biological systems affected? What are critical characteristics of MNMs that need to be considered and included / excluded when developing MNMs to ensure they are safe and which materials have a known increased toxicity in the nanoform vs. the bulk form, and why? How will this facilitate the regulatory safety assessment of new nanomaterials?

The partners in Task 5.1 have aimed to address the issues raised in the eight questions in order to discuss the **identification and setting of grouping, read-across, and extra/intrapolation criteria** for nanomaterials. Based on discussions among Task 5.1 partners on the DoW description and the relevance of the eight questions, this report has been prepared. Due to limited resources, two tasks were not fully executed in the report. The two tasks were the experimental verification of scientifically based categorisation, read-across, and extrapolation and interpolation criteria with respect to differences in one or two characteristics (e.g. size and/or surface treatment) and development of rapid characterisation/screening methodology in support of categorisation, read-across, and extrapolation and interpolation.

3.1.1 Readership

Following the NANoREG DoW, this report is for restricted use. It will have to be discussed with the Partners/Consortium Management Committee if a public version will be made available.

4 Introduction

Nanomaterials broadly refer to materials in the 1 to 100 nm size ranges that may exhibit additional or different properties and behaviour as compared to macroscopic materials with the same chemical composition. Not only size-dependent properties but also surface properties make nanomaterials promising candidates for various applications. A large number of nanomaterials have been developed including carbon-based nanomaterials, e.g., fullerenes, organic nanoparticles e.g. dendimers, nanocapsules, and lipids, metal and metal oxide nanomaterials e.g. gold, silver, silica, titanium dioxide, and semiconductor nanoparticles or quantum dots such as cadmium sulfide. Figure 1 shows the number of publications that has been published between 2000 and 2014 in public and scientific domains. The data extracted by searching with a keyword “nanomaterials” as a “title” in Web of Sciences resulted more than 6000 articles, while using the same keyword as a topic, resulted more than 28000 publications.

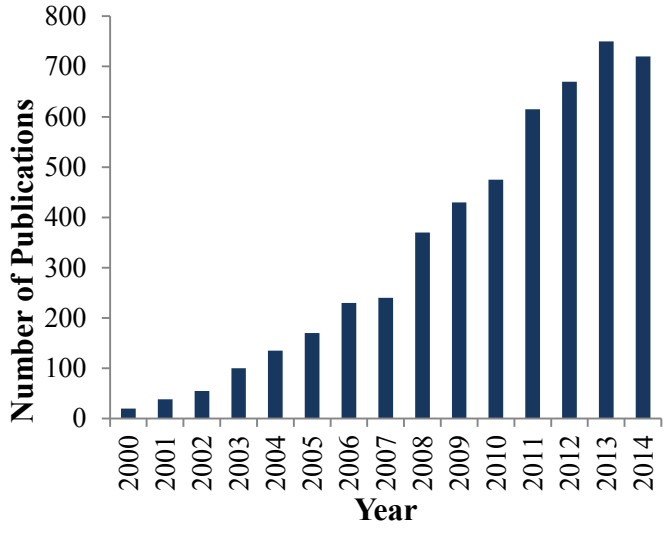


Figure 1: Number of articles published / year between 2000 –2014, searched with a key word “Nanomaterials” in Web of Sciences.

It is clear that the technology for making nanomaterials has already pushed the boundaries and leads to combining various nano-objects and nanostructures for creating nano-devices with distinct physical and chemical properties. One important aspect with nanomaterials is the formation of smart nanocomposites with desired functionality obtained by mixing of various organic or inorganic materials that can show superior properties as compared to their pure forms. Many combinations of nanocomposites or nanostructures created with an unlimited set of known or unknown properties. Examples are the incorporation or doping of organic functionalities with specific binding sites on an inorganic substrate with superior optical, electronic, magnetic or thermal properties.

Over the past few years, development of advanced materials with distinct properties has led manufactured nanomaterials (MNMs) to play an important role in the industrial development for making nanoscale devices and products that contain nanomaterials. A large number of nanomaterials and their nanocomposites are already claimed to be present in the consumer products with the number being more than 1800+ products (http://www.nanotechproject.org/cpi/, 2014.).[1] However, it should be noted that these numbers are not scientifically validated. These examples include MNMs in cosmetics (sunscreens, skin creams, oral hygiene products), paints, fabrics, electronics, automotive and construction parts.[2] The specific case of the nanomaterials include for ex: TiO_2 and ZnO which are used in sunscreens because they are known to reflect and scatter UV light, and protect skin against adverse effects of UV light, including skin cancers. Carbon black, an intense cosmetic colorant, can be used in the nano-form and is a good example of how reducing the pigment particle-size can alter the strength and opacity of colour.[3] Other MNMs *include* SiO_2, *Ag,* TiO_2 *and ZnO* used extensively in paint industries to enhance their performance.[4] Carbon nanotubes and Fullerenes are carbon nanomaterials that are increasingly used in consumer electronics.[5]

The European commission has set an action plan for immediate implementation of safe (see definition of nanomaterial doc), integrated and responsible approach for nanoscience and nanotechnologies. Since, 2008 EU has spanned out number of projects aiming to define legislation for nanomaterials including ENNSATOX, NANOMMUNE, ENPRA, EuroNanoTox, HINAMOX, InLiveTox, INSTANT, ITS-NANO, MARINA, MembranenanoPart, MODERN, NanoPolyTox, NanoPUZZLES, NanosafePACK, NanoStair, NanoSustain, NanoValid, Nanogenotox, NANoREG, etc. to name a few. Some of them are completed, and some of them are still under progress.[3]

In addition to peer-reviewed articles, an overwhelming number of documents were published by the European Commission that describes nanomaterials in REACH,[4] such as guidance on information requirements and chemical safety assessment,[5] physico-chemical properties of manufactured nanomaterials, identification and naming of substances under REACH and CLP,[6] scientific and technical support on assessment of nanomaterials in REACH registration dossiers and adequacy of available information, EU commission recommendation on the definition of nanomaterial,[7] scientific basis for the definition of the term ”nanomaterial” risk assessment of products of nanotechnologies, REACH implementation projects:[8,9] substance identification of nanomaterials, specific advice on fulfilling information requirements for nanomaterials under REACH, specific advice on exposure assessment and hazard/risk characterization for nanomaterials under REACH, considerations on a definition of nanomaterial for regulatory purposes,[10] nanomaterials impact assessment report etc... to name a few. However, for materials registration and regulatory risk assessment, challenges arise due to the diversity in composition, introduction of morphological variations of known compounds of functionalization etc, which can result in different physicochemical and biological properties.

However, there are still concerns, and clarity is needed on specific issues such as definition of nanomaterial, nanoscale size limits, natural vs man-made nanomaterials, classification and grouping of nanomaterials, information requirements for substance identification and reporting of nanomaterials, naming a substance contain nanoscale properties, etc.

To fully enjoy the benefits of nanomaterials, a clear and a concise investigation of their health and environmental effect is necessary. Studies show that many of manufactured nanomaterials are potentially toxic to either humans by means of dermal, inhalation, and oral exposure or to the environment by means of release and accumulation. As shown in Figure 1, several physical and chemical properties influence the toxicity of nanomaterials to human and environment.

Release of MNMs may come from point sources such as production facilities, landfills or wastewater treatment plants or from nonpoint sources such as wear from materials containing MNMs. The release of MNMs can be either intentional (e.g. for remediation, agricultural, or water purification purposes), or unintentional, e.g. release at point sources, such as wastewater treatment plants, waste incineration plants, manufacturing facilities, and possibly, hospitals or clinics, where there is a complete lack of information

regarding the source and concentration of nanomaterials found in these locations.[6] Accidental release during production or transport is also possible. Clearly the most common NMs in the environment are SiO_2, TiO_2 and FeO_x since they have higher production volumes as well as higher environmental concentrations.[6] Whether the particles are released directly into water/soil or the atmosphere, they all end up in soil or water, either directly or indirectly for instance, via sewage treatment plants, waste handling or aerial deposition. In the environment the formation of aggregates and therefore of larger particles that are trapped or eliminated through sedimentation affects the concentrations of free NP. Humans can be either directly influenced by NP through exposure to air, soil or water or indirectly by consuming plants or animals which have accumulated NP. Aggregated or adsorbed NP will be less mobile, but uptake by sediment-dwelling animals or filter feeders is still possible.

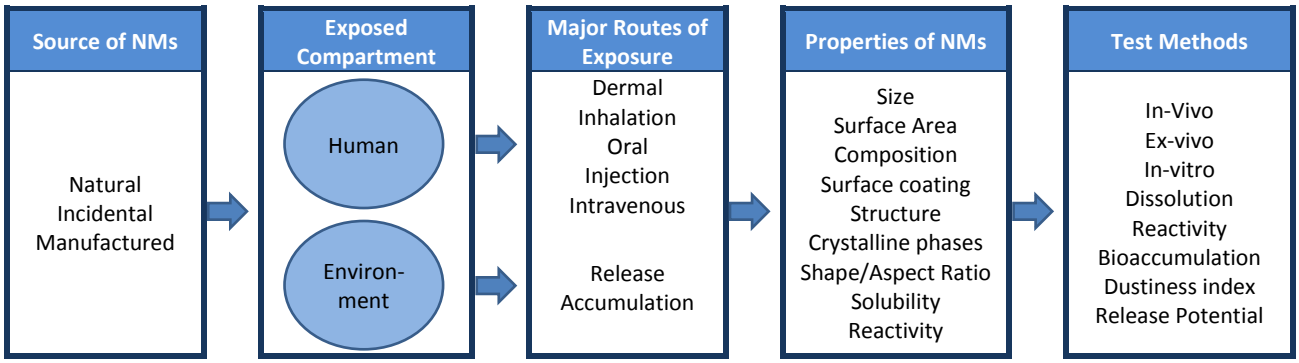


Figure 1: Overview of Nanomaterial properties and their link to exposure to environment and health.

The use of NM in paints and coatings is an important application area. The NMs are used as biocides and additives for protection against microbial, physical and chemical deterioration. With regard to NMs containing paint, TiO_2, SiO_2 and Ag NMs may be released from exterior facades of paints into surface waters.[7] Paint debris containing SiO_2 nanoparticles release a limited amount of Si into the environment, and with adjusting the properties of the binder in combination with common pigments it is possible to reduce the release of SiO_2 nanoparticles. Similarly, TiO_2 nanoparticles are released from buildings into the aquatic environment.[8] The nanosized TiO_2 in food products and personal care products may release as much as 16 mg of nanosized TiO_2 per individual per day to waste water.[9] For nano TiO_2 used in cosmetics, the expected scenario anticipates an end up in waste water, surface water, exposure of landfills, and in soils and surface water after sewage treatment. The consequences in these cases are not yet totally assessed. In addition, there is a significant gap between the available data on the nanomaterials production and toxicity evaluations, which can prohibit the safe use of nanomaterials.

REACH was developed to address Chemical Safety Assessment, assuring safe use of all chemicals used in the EU market. Under previous legislation, including existing substances (Regulation 793/93/EEC) and new substances (Dangerous Substance Directive, 67/548/EEC), the procedures to assess the safety were not homogeneous, and the data requirements were different. Therefore, only a fraction of chemicals marketed in EU were effectively evaluated and classified for their hazard. In REACH, to cover the data gaps for the "existing" chemicals (i.e. to generate data with sufficient quality), and also for the chemicals not yet assessed only via experimental work, would mean to spend a lot of resources and time. Therefore, different ways to estimate different substances characteristics (e.g. phys-chem properties, ecotoxicity, biodegradation) through models were developed and implemented in legislation. Some examples are QSAR models, read-across (extrapolation, and interpolation), trend analysis etc. The basis for an effective application of these tools is the categorization of chemicals, which means to identify common traits, i.e. similarities, between different properties of substances that are undergoing the grouping exercise, and define a category of chemicals.

Read-across can be defined as ''the use of hazard specific information for one substance (''source'') to predict the same hazard for another substance (''target''), which is considered to have similar physical–chemical environmental fate and/or (eco)toxicological properties'' (EC, 2009). Various applications of read-across have become particularly useful for filling data gaps and meeting new regulatory demands within REACH. *Similarity* is the key word and is usually based on structural and/or physicochemical properties; for chemicals often common functional groups, common constituents, or the likelihood of common precursors and/or breakdown products (OECD, 2014). Read-across may be performed in a qualitative or quantitative manner. In qualitative read-across, the presence (or absence) of a property for the target substance is inferred from the presence (or absence) of the same property for one or more source substances. Qualitative read-across gives thus a "binary" or "yes/no" answer. Quantitative read-across is instead used to obtain a

quantitative value for an endpoint, such as a dose-response relationship (e.g., NO(A)EL and LO(A)EL). The known value of a specific property/endpoint for one or more source substances is used to estimate the unknown value of the same property for the target chemical (OECD, 2014). In mathematical terms, this is called extrapolation (if the estimated value is based on extending a known sequence of values or facts beyond the area that is certainly known) or interpolation (if the estimated value is lies within two known values in a sequence of values).

Conventional chemicals are fully defined by their chemical composition and molecular structure, and in principle from a SMILE description it is possible to put a chemical in a specific group, with similar characteristics. So, the "similarity" concept, its interpretation, and its implementation, is very relevant for grouping. For conventional chemicals there are statistical metrics that are able to define the similarity of two or more chemicals (see OECD QSAR Toolbox).

The process of developing an *analogue* or *category* starts with the identification of one or more similarity rationales. This lead to the overall hypothesis that defines the applicability domain of the analogue or category to ensure that read across is appropriate for the endpoints of interest. If, for example, the hypothesis is based on structure and mode of action, then it may be valid for certain aspects of mammalian toxicology, but not hold for environmental endpoints. Once a hypothesis has been developed it then needs to be examined using the available data (evidence) to see if the hypothesis is verified for the intended endpoint, a process called "read-across justification". Possibly, additional data need to be provided in order to support the analogue/category. It should be noted that the results of read-across may be used for different purposes, from screening of particular concern, which may be endpoint specific, to classification/labelling as well as risk assessment.

However, MNM are not defined only by chemical composition, but also by size, shape, aspect ratio, solubility, and so on (see chapter 2). Therefore, similarity is not linked only to the presence of e.g. functional groups, specific chemical bonds, specific chemical elements, or other properties that can be derived from this information. This multidimensional approach to MNM grouping is acknowledged by several institutions and working groups, suggesting using more than one criteria to justify similarity, including not only chemical composition, but also other MNM properties.

4.1 Terminology

Within NANoREG, Task 1.4 has compiled an annex to deliverable D1.10 that is a Collection of existing definitions for the key terms used in the NANoREG Framework to be towards a harmonised terminology in NANoREG. The current document has aimed to use the terms and definitions proposed from Task 1.4. A few terms are discussed below for simplicity of reading the document.

4.1.1 A definition of "Nanomaterial"

Materials exist in different forms and sizes depending on the source and manufacturing process. Nature has a large number of nano-sized materials with properties that are distinct and relatively comparable to man-made nanomaterials. Recently discovered carbon based nanoparticles such as Buckminsterfullerene and graphene are typical examples of it. Man-made nanomaterials are defined as either manufactured nanomaterials or engineered nanomaterials or synthetic nanomaterials or modified nanomaterials. Either bottom-up or top-down process has been developed for their synthesis. Monodisperse particles with spherical shape are simple to define under the term "nanomaterial." However, in reality the particles do not exist in free form and in many cases the particles are not in uniform shape, particularly when the materials are scaled up. Particles do agglomerate and aggregate and have different size ranges depending on the use and the environment. [1]Therefore, there is a challenge to the research community, risk assessors and policy makers to introduce a meaningful single definition for the term "nanomaterial." As a result ISO has published a large set of definitions that describe the different forms in the 'nanoscale'[1], although 'nanoscale' is not clearly defined, i.e. "size range from approximately 1 nm to 100 nm" [1]. Where this may be suitable for research purposes, for regulatory purposes a definition of 'nanomaterial' should ideally fulfil the requirements of being a single definition that is broadly applicable in different (EU) legislation and policies, that is legally clear and unambiguous, enforceable through agreed measurement techniques and procedures, and in line with other approaches worldwide[2]. Overviews of different definitions are given in several reports, e.g. by JRC[2] and RIVM[3].

In the EU discussions resulted in a 'recommendation on the definition of nanomaterial'[4] that was mainly based on scientific insights from JRC[2] and SCENIHR[5]. The EU definition is primarily intended to provide clear criteria to identify materials for which special legal provisions may apply. EU explicitly stated that

nanomaterials should not be seen as implicitly hazardous[4]. For legal clarity the definition is solely based on size.

Recently US-EPA published a proposal to gain information on different discrete nanoforms within one chemical substance. Similar to regulations in other jurisdictions, nanomaterials are primarly defined based on a size between 1 and 100 nm. Different forms of the same chemical are further distinguished “based on a combination of three factors: (1) a change in process to affect a change in size and/or a change in properties of the chemical substances manufactured at the nanoscale; (2) a change in mean particle size of 10% or greater; and (3) the measured change in at least one of the following properties, zeta potential, specific surface area, dispersion stability, or surface reactivity, is greater than 7 times the standard deviation of the measured values (+/− 7 times the standard deviation).”[6] The combination of these 3 factors should prevent that each different production batch will be seen as a separate form.

Overall, it is clear that there is no ideal definition for nanomaterials. Though a size based definition is prevalent, proposed definitions are not fully coherent between the international organizations. One challenge remains with the policy makers to introduce a meaningful definition of nanomaterial, which is clear, concise and in particular understandable by end users. In a recent report (March 2014),[13] “Towards a review of the EC recommendation for a definition of the term nanomaterial," JRC presented the information on scientific-technical issues considered when reviewing the current EC nanomaterial definition. While a second report on the issue was published in October 2014[7], the third and final report was published in 2015. The review of the definition by the EC that is scheduled in the recommendation[4] is delayed.

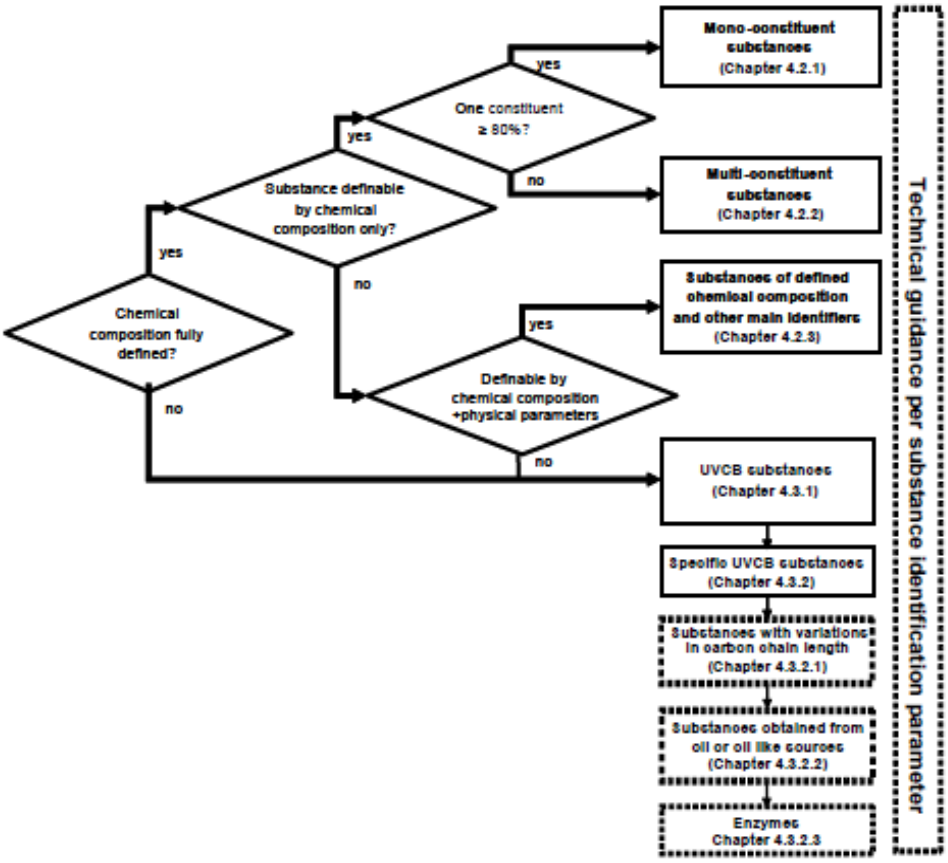
5 Nanomaterials under REACH

The European Union has been active for issuing policies that regulate substances at nanoscale and issued different advisory reports. However, there are some uncertainties and unclear guidelines for issues such as the definition of nanomaterial, nanoscale size limits, natural vs. man-made nanomaterials, classification and grouping of nanomaterials, information requirements for substance identification and reporting of nanomaterials, naming a substance with nanoscale properties, to name a few. This paragraph gives a concise summary of developments made in the last few years on substance identity and information requirements of nanomaterials. The other issues mentioned above are described in more detail in D2.5 report. This paragraph is developed based on a review of existing scientific publications, and guidelines published by various international organizations.

5.1 Substance identification: Substances at Nanoscale

Under the REACH legislation, regulations of nanomaterials are covered by EU legislation (covered by the definition of “substance”) even though there is no explicit reference to nanomaterial and guidance documents apply to nanomaterials (Scheme 4). Under REACH and CLP (ECHA 2007, 2012, and 2014) guidance,[6,21] a substance is usually identified by its chemical composition, the chemical identity and the content of each constituent in the substance. Well-defined substances can be mono- or multi-constituent depending on the concentration range of constituent.

A substance that has at least 80% of a constituent by weight is considered as mono-constituent substance, while one or more constituents present at greater than 10% and less than 80% is considered to be a multi-constituent substance. A mono-constituent substance is named after the main constituent while the multi-constituent substance is named as a reaction mass of the main constituent of a substance (i.e., not the starting materials needed to produce the substance). On the other hand, substances of Unknown or Variable Composition, Complex reaction products or Biological materials, are grouped under UVCB substances. UVCB substances are define by their manufacturing process or reactants used for predicting their final composition. It’s argued that for regular substances, the constituent weight % determines whether it is the main constituent or an impurity. Nevertheless, 80/20 weight % argument is not a good measure to identify nanomaterials and their nanocomposites. For example, surface hydrophobization of silica nanoparticles at as low as 1.5 % by weight of methyl groups, is enough[22] to get distinct dispersion properties compared to bare silica. W/ w% will not give any information on the surface coverage of nanoparticles as it does not take into account of surface properties. Therefore, special provisions for substance identification are needed for substances at nanoscale.



Scheme 4: Specific guidance for the substance identification of various types of substances. (Reprinted from the guidance document REACH and CLP, 2014).

In 2010, the EU commission initiated several REACH implementation projects on Nanomaterials (RIP-oNs)[8,9] to evaluate the applicability and validation of the existing REACH guidance to nanomaterials, and if needed develop specific advice on how the guidance could be updated considering substances contains nano-objects. In the proposal RIP-oN 1, substance identification of nanomaterials was evaluated towards the existing guidance documents and highlighted few important identifiers for substances contain nano-objects. Various identifiers have been proposed such as size and surface treatments as the main identifiers, and other potential identifiers such as surface area and aspect ratio. There was a strong disagreement between the experts in relation to whether these parameters should be identifiers or characteristics to describe the nano-form of a substance. Consensus could not be reached on these issues among the experts and further policy advice on the issues before developing a specific guidance was suggested. A common scheme for substance identification may not work for all nanomaterials. Size could be a primary identifier that distinguishes nanomaterial from a non-nanomaterial, but a clear, and a concise additional identifier must also be used to indicate the specificity of the nanomaterial.

5.2 Information requirements for risk assessment of substances in REACH

EU commission has proposed a mandatory reporting of substances at the nanoscale, depending on the tonnage and hazard class. Since 1 June 2008, substances at the nanoscale (non-phase-in substances) which are manufactured or imported in quantities of 1 ton/year or more need to be registered before manufacturing or importing. According to REACH, reporting schemes and deadlines as follows,

- 31st December 2008 - end of **Pre-registration**
- 1st December 2010 - end of **Registration** (> 1,000 tonnes/year)
- 1st December 2013 - end of **Registration** (> 1,00 tonnes/year)
- 1st December 2018 - end of **Registration** (> 1 tonnes/year)

Standard information requirements for substances manufactured or imported in quantities of 1 ton or more are covered in Annex VII (REACH). It includes reporting of several properties such as composition (incl. purity), melting/freezing point, boiling point, relative density, vapor pressure, surface tension, water solubility, partition coefficient n-octanol /water, flash point, flammability, explosive properties, self-ignition temperature, oxidizing properties, granulometry, adsorption /desorption, solubility in organic solvent and degradation production, dissociation constant and viscosity. An updated guidance for reporting of substances at nanoscale included two new sections describing SHAPE (R.7.1.19) and SURFACE AREA (R.7.1.20).[25,26] These additional requirements were set based on the advice report,[9] RIP-oN2. RIP-oN 2 also advices to report few additional physicochemical properties such as Surface energy, Surface chemistry, Surface charge, Redox potential, Cell-free ROS/RNS, production capacity, State of dispersion and State of agglomeration. However, these were not included in the recent version of the REACH guidance documents. It is important to note that, REACH regulation exempts for products or substances imported or manufactured at above 1 ton per annum also for a limited period under the scheme of product and process orientated research and development (PPORD), to facilitate innovation and exclude less burden for SMEs. However, at this stage it is unclear if the rule applies for the substances at nanoscale. On the other hand, critical information requirements for higher tonnage levels such as TOXICOLOGICAL INFORMATION and ECOTOXICOLOGICAL INFORMATION vary in addition to the physico-chemical properties of the substance and special provisions have been set at those levels (Annex VIII, IX and X). These differences in information requirements between different tonnage levels also implies that read across will only need to be performed for those endpoints that are required at the specific tonnage level. (Maybe also include an example here).

5.3 Other information requirements for risk assessment of nanomaterials

The Canadian Department of the Environment (CDE) has set case-by-case information requirements for various nanomaterials; examples include multiwall carbon nanotubes, aluminate, magnesium and vanadate, and potassium titanate. Under this provision, multiwall carbon nanotubes have special dimensions set for their reporting as:

“Substance” means short tangled multi-walled carbon nanotubes having the following characteristics:

- (a) at least 90% of the substance composed of elemental carbon;
- (b) the nanotubes measure from 0.09 to 10 micrometres in length, with a 1.1 micrometre average; and
- (c) the diameter of the nanotubes measures from 5 to 25 nanometres, with a 12 nanometre average.

OECD has recently released a guidance document (July 2013), which deals with the information requirements on active substances and biocidal products.[28] However, the guidance on nanomaterials is still pending, and OECD is reviewing all existing methodologies in order to identify and implement the necessary changes need for their application to nanomaterials. In a recent OECD expert meeting (July 2014) on the physical–chemical properties of manufactured nanomaterials and test guidelines,[29] the experts focused the discussion on selected endpoints including 1) State of Dispersion, Aggregation and Agglomeration of Nanomaterials, 2) Size (and Size Distribution) of Nanoparticles, 3) Surface Area and Porosity, and 4) Surface Reactivity. Therefore, it is assumed that OECD guidance document may consider these properties to be included in the reporting documents for substances at nanoscale.

Danish EPA (Environmental Protection Agency) recently has summarized information from existing projects/reports/references and proposed a stepwise information requirement scheme for nanomaterials.[30] They recommend new information requirements compared to information normally required for chemicals such as primary particle size distribution, Agglomeration/aggregation, Specific surface area and morphology/shape/aspect ratio.

United Kingdom has drafted a supplementary guide for voluntary reporting scheme.[31] The report includes information on the key physical characteristics of the nanomaterials such as the size, shape, structure, solubility, and surface area, along with details of the measurement technique used. The scheme aims for developing reasonable and responsible approaches for managing the risks from nanomaterials and hence does not consider tonnage level as a base for registering criteria.

Many peer-reviewed articles were also published to address the issues related to legislation of nanomaterials. Hansen et al. proposed a scheme for categorization of nanomaterials and proposed 9 properties being the important for estimating the toxicity of nanomaterials such as: 1) Chemical composition, 2) Size, 3) Shape, 4) Crystal structure, 5) Surface area, 6) Surface chemistry, 7) Surface charge, 8) Solubility, and 9) Adhesion. A total of 428 studies were reviewed based on the above categorization method

and recommended that the location of the nanoscale structure in the system as well as the nine relevant physical and chemical properties must be reported in the data sheets. Recently, Pettitt et al. proposed the minimum physicochemical parameters required to describe nanomaterials adequately for regulatory purposes including size, shape, surface charge, surface modification, crystalline from, and oxidation state. They argued that nanoforms with the same core chemistry but distinctive differences in the physicochemical characteristics should be treated as separate entities for regulatory purposes. In addition, use of multiple methods to describe a given physicochemical parameter is strongly recommended.

Overall, it is clear that particle size, shape and surface area seem to be the dominant parameters to consider when reporting substances at nanoscale, as recommended by all the organizations. However, there are uncertainties rely on reporting a bunch of physical and chemical parameters, and different organizations have different information requirements. This may lead to registration problems between the countries and may show considerable impact in the international mobility of the nanotechnology industries.

6 Existing and proposed approaches for grouping of nanomaterials

Nanomaterials exist in many different forms (i.e., compositions), characteristics (e.g. size) and properties (e.g. surface area). Development of nanomaterials towards commercial aspects has been taking shape and high number of nanomaterials and their nanocomposites are already in the consumer products. Unfortunately, there is no systematic approach in classification or classifying various manufactured nanomaterials by their composition, properties, application, or risk assessment. Many combinations of nanomaterials can be crated with an unlimited set of known or unknown properties. Examples are the incorporation or doping of organic functionalities with specific binding sites on an inorganic substrate with superior optical, electronic, magnetic or thermal properties. Making a validated method for classifying nanomaterials is very tricky and needs careful choice of wording and a systematic procedure.

ISO proposed “nano-tree,” a globally harmonized methodology for classifying various nanomaterials. The document (ISO/TR 11360:2010) is published as Nanotechnologies – Methodology for the classification and categorization of nanomaterials.[14] The classification system utilizes 1) special dimension, and 2) quantum confinement as the basis for classifying wide range of nanomaterials including nano-objects, nanostructures and nanocomposites. Special dimension is defined as any external dimension of the material in the nanoscale between 1 nm and 100 nm while the quantum confinement uses the size of a solid material comparable to the wavelength of the particles that interact with the system. The dimension approach is used as the preferred method for the classification system than the quantum confinement. It is because of the high technical information requirement such as electron wave function that is not readily accessible by the majority of industrialists and scientific community.

Within the technical report ISO/TR 11360:2010, the “nano-tree” based on the dimension approach consists of four parts that describe a nanomaterial including Dimension, Internal/external structure and type of nanomaterials, Chemical Nature/identity and Properties/behaviour. A simplified sketch of the nano-tree based on dimension (D) approach is shown in Figure 2. Accordingly, 1D, 2D and 3Ds described if any one, two or three of the external dimension of the material is less than 100 nm, respectively. Each dimension is subdivided by their composition and structure into single-, multi-component or nanostructured material containing a discrete nanoscale feature. More complex nano-objects and nanostructures are further subdivided based on their chemical identity/nature, highlighting the effect of chemical nature or bonding on the overall properties of nanomaterials. One of the key characteristics when downsizing a material is the change in their “functional” properties in terms of their physical, chemical, mechanical, biological properties. The fourth part of the “nano-tree” includes those properties to aid the classification system. The aforementioned properties are subsequently further broken down based on characteristics of each nanomaterial.

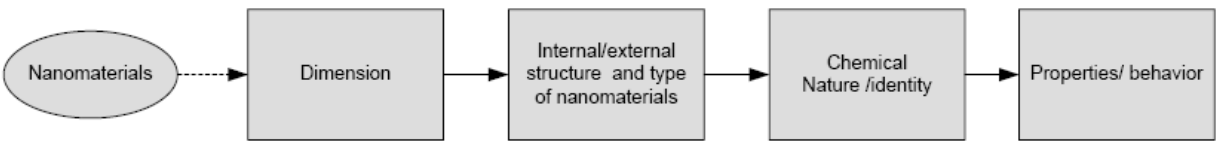


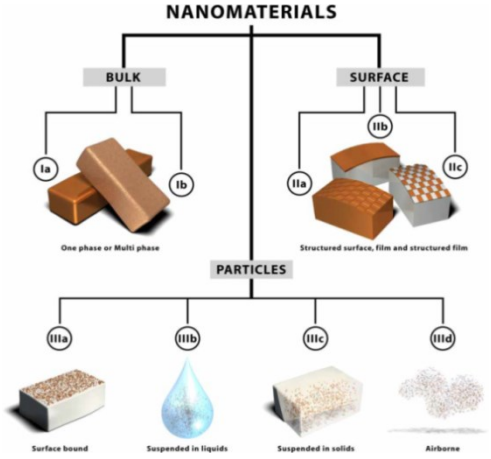
Figure 2: A simplified sketch of the “nano-tree” based on dimension approach for classification and categorization of nanomaterials proposed by ISO.

The ISO methodology serves an effective way to classify various nanomaterials by their *dimension* and associated properties. The report considers size as the base for the classification, but the extent of components (e.g., surface treating agents) in a multicomponent nano-object or nanostructures or nanocomposites is not defined. In addition, morphology at nanoscale is an important parameter that was not considered, for example, spherical spheres vs. faceted spheroids. Nanomaterials may have different shapes under different manufacturing conditions but still possess the same composition. According to ISO, for example, silica forms colloidal nanoparticles, monodispersed nanoparticles, rod-shaped nanoparticles or faceted nanoparticles, goes under different classification system. Consideration of chemical identity/nature as a parameter for classifying nanomaterials might be difficult for complex nanocomposites as they contain more than three different types of nano-objects and exhibit multiple characteristics under different environments. Under the method, properties/behaviour column does not give any information about the properties that distinguishes nanomaterials vs. non-nanomaterials. In addition, the categorization method does not link the properties for evaluation of toxicity of nanomaterials. Categorization of nanomaterials with the hazard identification may render great benefit for manufacturers to end users.

In a recent publication, Hansen et al.[15] proposed a new categorization system based on the *location of the nanoscale structure* in the system/material. They suggested a hazard identification scheme that combines the categorization framework with the inherent physical and chemical properties, relevant for each particular category of nanomaterials. The three main categories in the classification system and their subcategories shown schematically in the Scheme 1. The main category-I contain nanostructures in the bulk of the material while, in category-II nanostructures present on the surface of the material. Category-III contains nanoparticles that are nanosized in at-least two dimensions.

Tervonen et al.[16] proposed a decision support system for classifying nanomaterials into different risk categories. The classification system was based on a set of *performance metrics* that measure both the toxicity and physico-chemical characteristics of the original materials, as well as the expected environmental impacts through the product life cycle. Stochastic multi-criteria acceptability analysis (SMAA-TRI), a formal decision analysis method, was used as the foundation for this task. They demonstrated the application of the framework by classifying five nanomaterials such as C60 (fullerene), multi-walled carbon nanotube (MWCNT), CdSe, silver nanoparticles, and aluminium nanoparticles. Particle size was used as the main quantitative criterion. The results show some uncertainty for the nanoparticles in terms of the risk posed by these particles.

As an outcome of NANODEFINE project, JRC has recently proposed a material classification system by taking into account REACH naming and identification guidance documents. As per their scheme, the first criterion is to know if the material to analyse is a mono-constituent substance, a multi-constituent substance or a mixture (with other types of particles/substances), or an article or a commercial product (embedded in a solid or liquid/gel matrix). Unfortunately, the 80/20 weight% criteria for mono-constituent may not be suitable for MNMs, as the properties change drastically at the level of 20% minority components. 80/20 weight % argument is not a good measure for well-defined substances, in particular to identify nanomaterials and their derivatives. For example, surface hydrophobization of silica nanoparticles at as low as 1.5 % by weight of methyl groups, is enough to get distinct dispersion properties compared to bare silica. Therefore, the scheme is of concern for MNMs, when the impurity level or minority components below 20% in a substance.



Scheme 1: Categorization of nanomaterials according to the location of the nanostructure in the material, proposed by Hansen et al.

Hallock and colleagues[17] suggested a classification system to aid waste treatment and disposal mode of nanomaterials. Accordingly, nanomaterials are grouped by *product matrix* as pure nanomaterials, items contaminated with nanomaterials, liquid suspensions, and solid matrices.

The US National Institute of Occupation Safety and Handling (NIOSH) suggested grouping of nanomaterials by physical state to improve safe handling and reduce worker exposure as

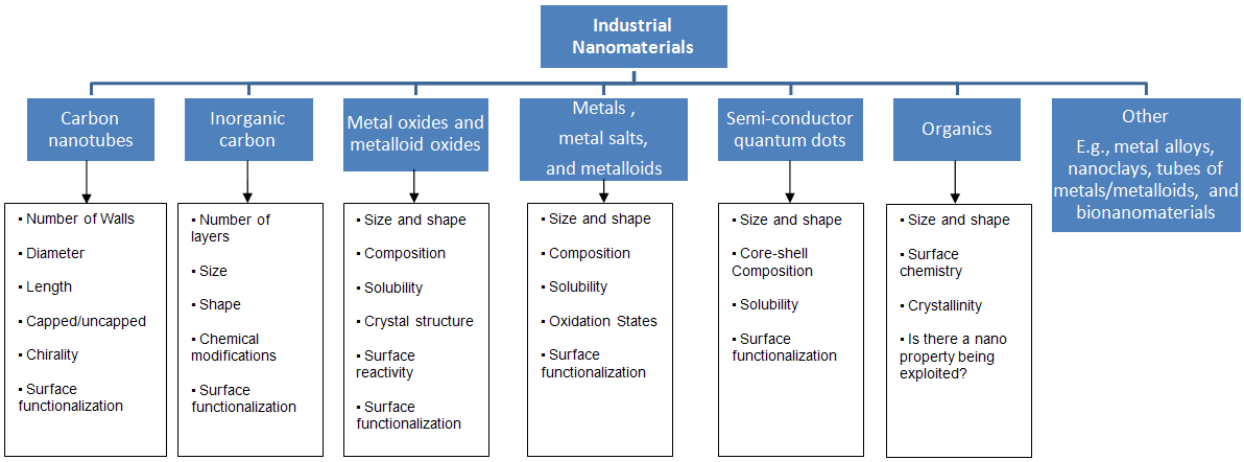
(a) bound of fixed nanostructures (polymer matrix);

(b) liquid suspension, liquid dispersion;

(c) dry dispersible nanomaterials and agglomerates; and

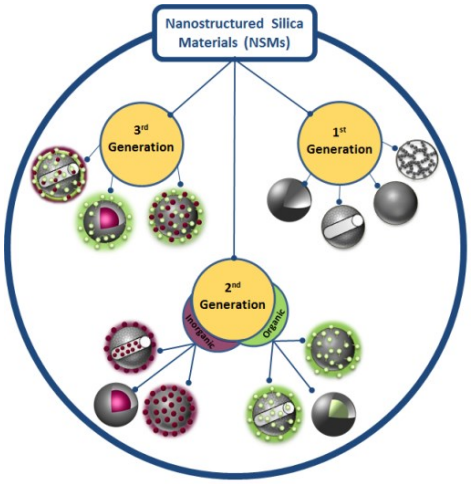
(d) nano aerosols and gas phase synthesis (on substrate).

Stone et al.[41] (2010) suggested decision trees (schemes) that could be used for the classification of nanomaterials, for strategies to assess their toxicity, and/or strategies to inform safe handling of nanomaterials. The classification schemes for nanomaterials are based on *size and similarities in chemical composition* from an environmental perspective. Depending on the complexity of the nanomaterial, the decision tree uses dimensions, shape, and composition as starting points. Neither scheme is complete, but instead provides an indication of the types of formats and content a scheme might include; clear definitions for each term would be required. The advantage of such an approach is that it provides clear guidelines, for non-experts, industry and regulators as well as scientists with respect to how to handle a new nanomaterial for characterization, hazard and exposure assessment purposes. However, the difficult part of making it as a practical approach is the lack of knowledge regarding which physicochemical characteristics are most key and what decisions should be derived on the basis of such knowledge.



Scheme 2: Proposed classification scheme of RCC based on similarities in chemical composition.

Similarly, nanotechnology initiatives program by the Regulatory Cooperation Council (RCC) of Canada and USA proposed a classification scheme (Scheme 2) based *on similarities in chemical composition*. Nanomaterials are considered as new substances, and regulated in Canada and the US under the Canadian Environmental Protection Act (CEPA) and Toxic Substances Control Act (TSCA), respectively. The classification scheme represents the *intrinsic* physicochemical parameters which are similar between two nanomaterials for them to be considered for read-across or analogue information. Depending on the composition of nanomaterials, various physicochemical parameters are considered for example, carbon nanotubes are grouped by the number of walls, dimensions, chirality and surface functionalization while metal oxides and metalloid oxides consider size and shape, composition, solubility, oxidation states and surface face functionalization. Though a large number of nanomaterials fall in the classification Scheme, some important nanomaterials cannot fit in the scheme, for example, graphite and graphene.



Scheme 3: *Illustration of the proposed classification paradigm for nanostructured silica materials (NSMs) with sub-groups illustrating non-porous and porous types and presence of surface-treating agents as organic and/or inorganic as their counterparts.*

In our (Atluri et al., 2015) recent work on nanostructured silica materials (NSMs),[20] we reviewed various forms of NSMs and recommend a classification system (Scheme 3) by their composition and structural complexity. Though, size being the dominant property of the classification, structural complexity of NSMs is indeed considered as an important parameter. The structural complexity derived by the location and the extent of surface treating agents including organic or/and inorganic compounds. Bare silica nanoparticles are grouped to 1st generation NSMs (1G-NSMs), nanocomposites of silica with organic or inorganic as their secondary phase are in the 2nd Generation NSMs (2G-NSMs) and finally, nanocomposites of silica comprising both organic and inorganic as their counterparts as 3rd Generation NSMs (3G-NSMs). The proposed classification enables to identify various NSMs by their complexity and helps to define the requirements for risk and hazard assessment. However, this approach does not link the properties for evaluation of toxicity of nanomaterials.

There are several initiatives and proposals which do link physicochemical properties to environmental and/or human toxicity of nanomaterials. Some of these are only applicable to certain types of nanomaterial, such as metal oxides (Zhang et al., 2012; Gaiewicz et al., 2015). Others are specific for a certain route of exposure (Arts et al., 2015; Gebel et al., 2014; BSI, 2007; IFA, 2009; Broekhuizen et al., 2012) or endpoint (Gaiewicz et al., 2015). Unfortunately, many endpoint specific approaches usually concern in vitro assays, which are generally not predictive for endpoints included in the information requirements under REACH. However, they may still be useful for (initial) grouping for screening purposes. Further development, especially of those approaches that mainly concentrate on intrinsic properties of the MNMs are needed, as there is increasing knowledge showing that the (ultimate) toxicological effect depends on a combination of surface properties in physical and biological environment (Nell et al., 2012, Huk et al., 2015). Approaches which are more generally applicable (Oomen et al., 2015; Sellers et al., 2015; Godwin et al., 2015; ECHA, 2015)), usually only describe the most important physicochemical properties and a general procedure or strategy, but do not contain specific benchmarks, boundaries or cut-of points.

Considering all the different positions together, the result is that in general the multidimensional approach to MNM grouping is acknowledged by several institutions and working groups, suggesting using more than one criteria to justify similarity, including not only chemical composition, but also other MNM properties. Some considered it premature to develop guidance for MNM grouping (OECD, 2014). However, based on the current knowledge, recommendations in terms of relevant (combinations of) parameters and maybe also some benchmarks can be formulated. In some cases, exposure parameters (e.g. exposure directness and production volume) are considered an important factor for categorisation supporting the identification of information requirements. Also, grouping can be really relevant for substances that have many different nanoforms, in terms of e.g. size, shape, coating, functionalization, and also uses. In the occupational safety field, MNM are consistently categorized in four classes, determined by solubility, bio-persistence with low and high toxicity, and high aspect ratio.

The multidimensional aspect of MNM grouping is well captured by several research projects on nanosafety that are developing proposals for MNM grouping. In general the grouping is done by linking physico-chemical properties with hazard, but in some cases also exposure is considered, as well as biokinetics.

Besides the multidimensional aspect linked to the physico-chemical properties of MNM, there is also a multidimensional aspect linked to the MNM life cycle. MNM are more and more used in several industrial sectors, ending up in different phases of the production process, and in consumer products, which are then disposed of. In each step of the life cycle an MNM can be modified, both intentionally (e.g. being incorporated in a matrix) and unintentionally (e.g. biodegradation of coating in natural water). Therefore, considering only the substance at the beginning of the life cycle may lead to a grouping that is not justifiable in the other portions of the life cycle. Overall, many initiatives and proposals for grouping of nanomaterials exist, but only a few of these link physico-chemical properties to the environmental and/or human toxicity of the nanomaterials. For an effective grouping, a group needs to be well-defined to determine which nanomaterials belong or do not belong within a certain group. Groups are generally defined by benchmarks, i.e. a certain value for a specific physico-chemical property that sets a boundary of the group. For nanomaterials, such benchmarks need often be a combination of several different physico-chemical properties. Especially for nanomaterials, the need for setting multidimensional groups with several criteria is acknowledged by several institutions and working groups, but lack of data often hampers setting benchmarks for grouping approaches. (Note: As part of NanoReg and deliverable D.2.5, one of the main activities is to recommend a categorization paradigm of different manufactured nanomaterials (MNM) by their physico-chemical properties. Please refer to D.2.5 report for NANoREG view on this topic).

7 Possibilities and limitations of using (Q)SARs for grouping and categorisation of nanomaterials[2]

7.1 What is (Q)SAR?

Another way to develop categories of nanomaterials is by using (Q)SARs. (Q)SAR is a collective term that refers to both Structure-Activity Relationships (SAR) and Quantitative Structure-Activity Relationships (QSAR). Both approaches are designed to predict “biological activity” (e.g. toxicity) from “descriptor values” characterizing the physicochemical properties of compounds. As the name suggests, QSAR is a quantitative (i.e. regression) method for relating the structural and compositional features of a compound to its toxicity, while SAR concept is supposed to be qualitative in nature. The descriptors used as an input in (Q)SAR models are the numerical representation of the physicochemical (e.g. surface coating, surface charge), geometrical (e.g. molecular volume, molecular surface area), topological (e.g. substructure-based descriptors, atom and bond counts, connectivity and kappa indices, molecular symmetry) and electronic properties (e.g. HOMO and LUMO energies, dipole moment, bond orders, partial atomic charges) of the structures. There are thousands of molecular descriptors most of which are derived from empirical and quantum-mechanical computations.

The nano(Q)SAR modelling process begins with the acquisition of experimental data on the biological activity of a range of nano-compounds. The next step is the measurement and/or computation of the molecular characteristics that are going to be used as the descriptors of the physicochemical features and the predictors of the observed biological activity. In the data pre-processing step, the data should be trimmed and normalised in order to remove non-physical values and bring the variables into alignment. Depending on the nature of the collected data, different data mining algorithms can be employed to develop classification- or regression-based (Q)SAR models. After the model construction phase, the validity of the derived model should be checked both internally (i.e. performance on the sample used to develop the model) and externally (i.e. performance on a different population). Finally, the model’s applicability domain and the uncertainties in the constructed (Q)SAR model should be clearly and transparently reported by the model builder.

The predictive capacity of (Q)SAR model has meant that is a powerful platform for use in categorisation of chemicals. In chemicals, a (Q)SAR model is created using a data set referred to as the training set e.g. chemicals of known activity**.** Then the model is constructed by using classical chemo-informatic methods, such as classical linear methods (e.g. partial least squares and multiple linear regression), and non-linear methods (e.g. Support vector machine and artificial neural networks) [8-12]. Modellers also sometimes employ principal component analysis (PCA) to aid the process of designing (Q)SARs [13, 14] e.g. for searching for structural similarity patterns and thus predefining new categories of the studied chemicals. In

[2] References for section 7 are presented separately in the reference list.

SAR, as there is no attempt to derive a quantitative model; classification algorithms such as decision trees and discriminant analysis are used instead [15].

It is not the intent of this report to delve into the details of the processes involved in (Q)SAR, as they have been covered in great details elsewhere [16-19]. However, the most crucial steps in (Q)SAR modelling are to: demonstrate the robustness of the model (validation) and set the boundaries of the validated model that define the domain of applicability. This helps to check if external chemicals (or nanomaterials) can be predicted using the built model. Furthermore, it will help to establish how the model will perform when faced with compounds that were not included in the training or test set.

The use of (Q)SARs to categorise chemicals is quite advanced; this in turn have allowed past workers to create a broad hazard identification profile, prioritise chemicals and estimate values for untested chemicals, thus promoting the safe use of chemicals [20-28]. Furthermore, the development of chemical categories have also been used to: identify hazards (associated with safe storage, handling and disposal of waste) [29-32], predict physicochemical properties or toxic/biochemical effects [33-36] and provide information about mechanism of action [37, 38].

Compared to chemicals, the use of (Q)SAR in relation to nanomaterial (i.e. nano(Q)SAR) categorisation (especially in relation to hazard) is limited. No clear strategy is in place to categorise nanomaterial but several suggestions have been made. Glotzer and Solomon [39] suggest that nanomaterials can be categorised through the use of eight orthogonal dimensions (surface coverage, aspect ratio, faceting, pattern quantization, branching, chemical ordering, shape gradient, and roughness) in order to describe the key attributes of nanomaterials. Hansen and Stone suggest the use of a chemistry based categorisation system as a starting point [40, 41]. Although little work has been done in relation to the use of (Q)SAR to categorise nanomaterial, the approach (of using such models much in the same way as in chemicals) would be attractive, if proven possible.

There have been several comprehensive reviews surrounding nano(Q)SAR exists, which seems to indicate that this potentially useful tool is gaining population in the field of nanotechnology [17, 42] [43, 44]. Several studies reported correlation between toxicity and the physicochemical properties of the nanomaterials [45-47], which led researchers to query whether it is possible to build a predictive model from such correlations. If possible, then the impact will be large, as the ability to do so will mean a reduction in nanomaterial testing (as in chemicals), resulting in the ability to efficiently assess risk of nanomaterial and contribute guidance for safe/better design of future products [48, 49]. However, several authors have already pointed out some limitations surrounding the use of such models for accurate predictions. For example, there is a need to have sufficiently large dataset, as well as the need to have some understanding on the underlying mechanism associated with the toxicological response [43, 50]. Although these hold true with nanomaterial, arguably such limitations are also applicable in the case of chemicals. However, nanomaterials are not chemicals and a better understanding on the differences, with regards to such limitations, should be highlighted.

In this section, our aim is to understand some of the issues associated with nano(Q)SAR and the gaps in establishing data quality requirements necessary for QSAR modelling. Primarily, our focus is on data quality and what it means in order to develop reliable nanomaterial categories. We will also evaluate other limitations associated with uptake of nano(Q)SAR and discuss implications of future work, for successful uptake.

7.2 Issue: Data quality and quantity in nano-(Q)SAR

The predictive power of nano(Q)SAR models can be affected by many factors such as the quality of input data and the selection of the data pre-processing or mining algorithms to be used for model development. The collection of empirical data can be considered to be one of the most critical components for the successful application of (Q)SAR methodologies, as no data-driven model can be built without adequate data input.

The rapid growth of nanotoxicology research in recent years, has significantly increased the amount of the nanotoxicity data available in the literature. However, the vast majority of the existing nanotoxicity-related studies are very limited in nature, especially in terms of sample sizes. In other words, these studies are usually focused on a small number of nanomaterials (e.g. fewer than ten) that are poorly defined and incompletely characterised. The nano(Q)SAR approach, however, requires a large set of systematically gathered data on the biological activity of a diverse collection of nanoparticles.

Unlike chemicals, measuring the physicochemical characteristics of nanomaterials is not straightforward with current instrumentations. From a scientific perspective, nanomaterials cannot be considered as a homogeneous group and subsequently this means that getting reliable data is not easy to achieve.

Potentially, this leads to a situation in which experimental data gets reported without proper understanding of the associated errors and subsequently the propagation of such errors through the model.

Sources of experimental errors may arise from a number of factors including polydispersity of the nanomaterial, inappropriate techniques/methods employed and the complex environment that the nanomaterial is dispersed in. In relation to the polydispersity of nanomaterial, it has been argued by Baalousha and Lead [51] that most nanomaterials tested are too polydisperse. Materials close to monodispersity are needed in order to have better reliability of result findings associated with studying environmental behaviour, dose, structure–activity relationships and mechanisms of toxicity. Although there is great effort in the scientific community to develop test/reference materials e.g. OECD Working Party on Manufactured Nanomaterials [52], there is a need to assess the suitability of such polydisperse materials for testing (and subsequently the quality of data generated). If monodisperse and homogeneous nanomaterial sample is needed, then only a handful of nanomaterials in existence have been certified and sold under the banner of reference nanomaterials, to include National Institute of Standards and Technology (NIST) gold nanoparticle reference materials (10, 30 and 60 nm). The use of such materials are ideal for use in nanotoxicology studies and recently they been shown suitable to act as negative controls for nanoparticle genotoxicity studies [53].

The main issue with having a highly polydisperse sample is the lack of analytical techniques that can measure the properties accurately. Anderson et al. [54] show that complex particle size distributions i.e. away from the simple monomodal distribution, will result in large data variability. In particular, light scattering based Particle Tracking Analysis and Dynamic Light Scattering platforms were only able to detect a single population of particles corresponding either the largest or smallest particles in a multimodal sample. Clearly, the inadequacy of the instrumental methods to characterise nanomaterials is a huge barrier in this field, as previously echoed by several workers [51, 55].

In addition to polydispersity issue, nanomaterial dispersed in complex biological matrix can also pose problems where measurement is concerned. Nanomaterial-media interactions can be dynamic in nature and thus physicochemical properties measured may not being directly associated with the observed biological effects. Furthermore, nanomaterial dispersed in complex medium may be unstable, potentially resulting in agglomeration and sedimentation; this may pose further difficulties for the instrument to measure the sample under such conditions. Due to the analytical challenges posed on measuring complex and unstable sample, some studies have characterised nanomaterials in their “pristine” state i.e. absence of the actual biological test media. In fact, few studies have assessed the potential transformation of nanomaterials in an environmental or mammalian system [56, 57].

In addition to issues associated with the accurate measurement of physicochemical properties, measurements of biological endpoints may also be problematic. In *in vitro* measurements, several biological endpoints can reflect changes associated with cell activity, which may be employed to indicate toxicity e.g. evidence of appreciable cell death relative to suitable control experiments, growth retardation and cell membrane damage. Several bioassay tests that can measure these biological endpoints exist, but these are not always reliable. Again potential sources of errors may arise, leading to false interpretation. These include:

a) the presence of endotoxin (LPS, lipopolysaccharide) contamination in the nanomaterial [58].

b) the choice of inappropriate end-points for the nanomaterial. There is a need to define a standard set of biological assays (and protocols) clearly in order to evaluate the overall *in vitro (and in vivo)* response of the tested nanoparticles. The assay chosen should be indicative of key activity, property or toxicological effects caused by these nanomaterials [59].

c) the interference by the nanomaterial in the assay readout e.g. tetrazolium based assays, in which the formzan salts can interact with nanomaterials [60, 61].

d) variations on how researchers disperse their nanomaterial in liquid media. Sources of data variability arising from the dispersion step can include: differences in the amount, source and pre-treatment of serum proteins used (affecting particle size distribution and agglomerates/aggregates population, etc.) [62, 63].

The problems identified so far imply the need to develop better research techniques/ methods exclusively associated with nanomaterials. There is thus a need to ensure the reliability of analytical data, in which methods developed must be validated. Eurachem [64] clearly states that *analytical measurements should be made using methods and equipment which have been tested to ensure that they are fit for purpose*. The current state of toxicological research in nanomaterials is that methods developed are rarely validated. Once

the conditions of the method validation are met, only then can researchers consider a higher metrological standard of measurement, conduct uncertainty analyses to estimate uncertainty and the propagation of uncertainty.

Finally, it is also critical to have universally agreed and standardised data reporting formats in nanotoxicology to support database development and facilitate data collection for modelling purposes.

7.3 Issue: Generation and selection of molecular descriptors in nano-(Q)SAR

Molecular descriptors can be determined either from experimental data or theoretical calculations. However, a certain amount of uncertainty exists in both descriptor types. The first step in the computation of theoretical descriptors is the representation of the molecular structure, which shows how the atoms and bonds are aligned. This symbolic representation enables the computation of the predicted values of physicochemical properties. However, the full structure of the nano-substances cannot be simply represented with the use of traditional techniques, mainly because of the complexity and the non-uniformity of the molecular architecture of nanomaterials. In other words, the majority of the existing theoretical descriptors are not directly applicable to nanomaterial due to the difficulties in expressing nanostructures in a 'valid' representation. Therefore, the translation of the physical and structural features of nanostructure into the novel and interpretable descriptors is one of the most important research needs in the field of computational nanotoxicology. More research is needed to develop a new format and notations for the appropriate transformation of the nanostructures into a language for computer representation. Once the issues related to the generation of nano-specific descriptors are resolved, the adaptation of (Q)SAR approach to nanomaterials will be less problematic.

The molecular descriptors (and fingerprints) can be obtained both through experimentation and theoretical calculation. The uncertainties which exist in both descriptor types are more significant for the complex nano-systems. The computation of the theoretical predictors includes two main steps: representation of the molecular structure and calculation of the descriptors transforming these symbolic representations into numerical quantities. The main issue that makes the generation of theoretical descriptors for nano-compounds challenging is the inherent complexity of nanostructures. As the nano-systems are very large to be studied by the majority of the existing computational chemistry methods, it is required to (reasonably) simplify the whole structure of the nanomaterial in order to reduce the computational time of evaluating the nano-structures. In the absence of the "logical" representation of the nanostructure, the nano-(Q)SAR investigations must rely on experimental descriptors, which could prevent (Q)SAR from reaching its full potential.

Overall, having an agreement on suitable descriptors is important, as past workers found that the choice of descriptors can affect the quality, significance and interpretation of nano-(Q)SAR model [17, 65]. It may be that descriptors can be selected or adapted from traditional chemical descriptors, but it is highly likely that descriptors specifically developed for nanomaterials are needed. For example, Borders et al have shown that new types of defects in carbon nanotubes can be represented as a new descriptor in the prediction of its mechanical properties [66].

Selecting the most influential descriptor is not trivial and Wang et al. [67] have used Principal Component Analysis (PCA) as a tool to identify suitable descriptors. Through the use of PCA, they identified: particle charge, aspect ratio and metal content as potential descriptors. Although PCA can provide insights into the relative importance of the physicochemical properties for toxicity, it is not an automatic feature selection method. There are a wide range of methods, such as stepwise procedures (forward selection and backward elimination), genetic algorithms, random forest and clustering, that have been commonly used for the purpose of variable selection prior to (Q)SAR model construction. In summary, much attention must be given to the selection of trial descriptor sets when developing nano(Q)SAR models, as they play a pivotal role in predictive quality of the model.

7.4 Issue: Model validation in nano-(Q)SAR

A pre-requisite for the uptake of (Q)SAR [19] and subsequently nano(Q)SAR, is for the model itself to be validated. Irrespective of the method used to construct the (Q)SAR models, the validity of the outcomes of the predictive models should be evaluated both internally and externally. Internal validation is the process of evaluating the prediction accuracy of (Q)SAR models based on the dataset used in the modelling process. The most common internal validation techniques used in (Q)SAR studies are least squares fit (R^2), chi-squared (χ^2), root-mean squared error (RMSE), leave-one-out or leave-many-out cross-validation, bootstrapping and Y-randomization. It is always beneficial to use more than one validation metric to quantitatively measure the accuracy of the model prediction.

It states clearly that the principles for validation should include: a defined endpoint, an unambiguous algorithm, a defined domain of applicability, appropriate measures of goodness-of-fit, robustness, predictivity and a mechanistic interpretation, if possible. Validation is vital to ensure that the predictive ability of the model is not due to chance factors.

Validation for nano(Q)SAR is not straightforward due to the following reasons:

The paucity of data available for nano(Q)SAR. Since nano(Q)SARs are developed with statistical methods (MLR, neural networks etc.), the variance in the group of nanomaterial needs to be sufficiently represented in the training and validation sets of particles. It is commonly accepted that the ratio between the number of descriptors and compounds in the training set should be at least as 1 to 5 [68, 69].

The lack of standardized validation metrics. The approach used for validation needs to be defined, for example whether to employ variants of cross validation or external test set validation [70]. Ideally, validation should be done externally i.e. by an external predication set, in which the test data set has to be independent not only from model building but also from model selection [71]. However, this step may not be always possible and often if additional testing is not feasible then an internal validation can sometimes be carried out e.g. using the "leave one out" cross validation (CV) method; CV is carried out by omitting a point and then calculating the value of this location using the remaining points [72]. Having said this, Tropsha et al. [73] demonstrated that leave-one-out cross validation and external test set metrics do not correlate [74] and stated that a *"high value of leave-one-out cross-validated R^2 appears to be a necessary but not sufficient condition for a model to have a high predictive power*". Hence, it should be noted that the model's predictivity can only be confirmed with external validation, without which the (Q)SAR modelling procedure is incomplete.

Moreover, ensuring the reliability of (Q)SAR models is also very critical to gain the acceptance and confidence of potential end-users including regulatory bodies.

7.5 Issue: Industry uptake and regulatory acceptance of nano-(Q)SAR

In relation to chemicals, there seems to be a clear regulatory application of (Q)SAR in the US but less so in Europe [75]. The wider acceptance of (Q)SAR in the US is attributed to the need to evaluate chemical substances within a relatively short period of time. The US Environmental Protection Agency (EPA) in particular is keen to promote the use of (Q)SAR to develop chemical category in hazard and risk assessment [76]. In the European Union, regulatory acceptance of (Q)SARs is limited, even though there is a push by REACH, through activities arising from the existing OECD chemicals programme [19, 77], to promote the use of (Q)SAR as an alternative method to evaluate chemicals. However, the fact that the uptake of (Q)SAR in relation to chemicals pose its own challenges, this would indicate that there is a long way to go in relation to nano(Q)SAR, before it can be accepted. In order to implement nano(Q)SAR it is vital to demonstrate to regulators, and industry, that nano(Q)SAR is scientifically valid and that clear explanations on how to use such models for making decisions are made [78]. Once this is achieved, our next step is to "harmonise activities" e.g. by forging internationally agreed document standards and guidelines. Guidelines of relevance should include the provision of detailed guidance in relation to the practicalities on the use of nano-(Q)SAR e.g. detailing how to identify acceptability criteria, how to generate adequate and relevant descriptors [79-81].

8 Properties for human and environmental behaviour

In this paragraph, we will discuss the properties of nanomaterials and their influence to human and environmental fate with emphasis on currently available toxicology data. Characteristic parameters of nanomaterials such as size, surface area, composition, surface coating/functionalisation, structure, shape/aspect ratio, surface charge, reactivity, and solubility/dissolution, and their influence on the biological interaction of nanoparticles are discussed in detail.

8.1 Properties of NMs[10]

8.1.1 Particle Size

OECD has defined particle size as "*The physical dimensions of the smallest discrete form of a substance under specified measurement conditions. If a group of particles are of differing sizes they may be described by a particle size distribution*"[62]. SCENIHR concluded that particle size is the only physical parameter that clearly distinguishes a nanomaterial from their non-nanoform (bulk).[61] This is in agreement with nanomaterials (NMs) being generally defined by having small dimensions in the nanoscale, i.e., between ca. 1 and 100 nm. Therefore, for regulatory purposes significant efforts have been put forward by different

governmental bodies and policy makers to correctly define the size limits of a nano-object.[12] As the size of a particle decreases, the proportion of atoms on the surface of the particle increase and, consequently, the physicochemical properties will be different from the properties known for non-nanomaterials.[11]

Measurements of primary particle size are difficult for two reasons. One is more technical in nature and related to the shape of the materials. In many cases the particles are not in uniform shape, particularly when the materials are scaled up[17], which hampers accurate measurements of particle sizes as these measurements are often based on two-dimensional images.

Another reason that hampers particle size measurements is the tendency of nanoparticles to form agglomerates/aggregates, resulting in different size ranges depending on the use and the environment. In many synthetic processes for nanoparticles, especially surfactant-free chemical reactions, aggregation or agglomeration occurs immediately as particles are generated. Agglomerates or aggregates make it especially difficult to explore the properties and hence their toxicological effects. Aggregation of NPs reduces the surface area to volume effects on MNM reactivity. This increase in aggregate size in turn affects their transport in porous media, sedimentation, reactivity, uptake by organisms, and toxicity. It was shown that agglomerated CNTs have more adverse effects than well-dispersed CNTs and they changed the morphology and performance of a mesothelioma cell line.[18] However, improved dispersion of the CNTs also caused both aspiration of smaller structures as well as their easy entrance into the alveolar walls, and enhanced pulmonary interstitial fibrosis.[19]

Over time, aggregation of NPs into clusters is inevitable without engineered or incidental coatings to decrease aggregation. Aggregation may take on two forms: homoaggregation between the same NMs, or heteroaggregation between a MNM and another particle in the environment. In most cases, the (much) higher concentration of environmental particles compared to MNMs will result in heteroaggregation. Where aggregation occurs, the number concentration of MNMs in the suspension decreases, with a concomitant increase in their effective (aggregate) size. For example, 30−70 nm diameter Fe(0)NPs rapidly aggregate in water to form micrometre-sized aggregates, greatly decreasing their mobility in the subsurface and likely pathways of exposure to sensitive receptors[6] Heteroaggregation between MNMs and comparatively larger particles (e.g., clay) could change NM behaviour if the NM−clay heteroaggregates ultimately move more like a clay particle than like a NM.[6] Release of metallic silver nanoparticles (Ag-NPs) from a paint used for outdoor applications indicate that after a period of one year, more than 30% of the Ag-NPs were released into the environment. The particles were mostly <15 nm and were released as composite colloids attached to the organic binder of the paint.[20]

A review of nanoparticle functionality and toxicity on the central nerves system shows that not only nanoparticles of small sizes but also metal ions released from their core structures can cross through many cell barriers and cause serious damage to the cells.[16] Such ion release may also influence particle size. In addition, size may be treated as a barrier for nanomaterials that prevents penetration of organisms or organs and induce significant toxicity.[14] A size-dependent in vivo toxicity study of PEG-coated gold nanoparticles shows that 5 nm and 10 nm particles mainly accumulate in the liver, and that the 30 nm particles preferentially accumulate in the spleen. However, the 60 nm particles had a wider distribution, with limited accumulation in the organs. The metabolism of these particles will be more important issues for medical applications of gold-based nanomaterials in future.

Overall, it is likely that the most appropriate means of expressing size related toxicity for engineered nanomaterials must be determined on the basis of individual case by considering various parameters including dose, exposure route, aggregation/agglomeration index, toxicity test method, etc.

8.1.2 Surface Area

A distinct characteristic of nanomaterials to their non-nanoform is the quantity of accessible surface, described by surface area.Due to their large surface-to-volume ratio, nanomaterials are highly reactive, and therefore, lead to a lot of new properties stemming from quantum effects and surface/interface effects. For applications requiring a large relative surface area, such as hydrogen storage for vehicles, chemical sensing, light harvesting, and for catalysts, nanomaterials or nanostructured materials are promising candidates. A typical example are metal-organic frameworks (MOFs), displaying ~7000 m^2/g of surface area.[21] To put it another way, just a few grams of nanoparticles is equivalent to the size of a football field.

Several well established methods exist to determine surface area (e.g. BET)[63], and hence the EU recommends a complementary definition to distinguish nanomaterials from non-nanomaterials by volume

specific surface area (VSSA). The recommended VSSA (i.e. greater than 60 m^2/cm^3) corresponds to a 100 nm sphere.[12][23]

Relative surface area is related to particle size, shape and porosity. As the size of a particle decreases, the ratio of surface area to volume increases or, in other words, the proportion of the atoms on the surface of the particle increases. Due to this high density of surface atoms, the reactivity of the nanomaterials towards itself or surrounding environment increases. Nanoparticles of metal oxide or metals are more susceptible to oxidation and dissolution, which could further contribute to their toxic activity. It has been shown that silver nanoparticles of 20 nm in size produced much higher toxicity compared to bigger nanoparticles of 80 and 113 nm in size.

Particle surface area can be an important parameter to consider when comparing the results of studies with differently sized particles. In some cases in which different behaviours were observed for different sized particles, the apparent difference disappeared when the results were normalised to surface area. For instance, the apparent difference in toxicity of 20 and 250 nm anatase TiO_2 particles (where the 20 nm particles appeared to be more toxic per unit mass) was eliminated when the results were compared based on the specific surface area of the particles[64]. Similarly, while it appeared that 7 nm CeO_2 nanoparticles induced stronger oxidative stress and damage to DNA *in vitro* than did 300 nm CeO_2 particles, no significant difference existed once the data were normalised to surface area[65].

8.1.3 Chemical composition

The chemical composition of a nanomaterial refers to entities of which the material is composed. The chemical composition is the key to classifying various nanomaterials, e.g. carbon based nanomaterials, metal and metal oxides, nanocomposites, etc. The function of nanomaterials is influenced by the chemical composition and hence different physical, chemical, mechanical and biological properties. Though a very large number of complex nanomaterials have been developed, OECD has listed 13 important nanomaterials due to their high relevance in consumer products or experiencing clear growth. They include fullerenes (C_{60}), carbon nanotubes (SWCNTs and MWCNTs), silver nanoparticles, iron nanoparticles, titanium dioxide, aluminium oxide, cerium oxide, zinc oxide, silicon dioxide, dendrimers, nanoclay, and gold nanoparticles.

It has been suggested that size or surface area may be more important than chemical composition in nanomaterials toxicity. Nevertheless, similar to non-nanomaterials, chemical composition cannot be ruled out as a factor of influence. Harper et al.[24] evaluated the toxicity of 11 metal oxide nanoparticles with similar particle sizes in an embryonic zebrafish model. Approximately, half of the nanoparticles show significant mortality after a 5 day continuous waterborne exposure. In another study,[25] the toxicity of similarly sized silver, copper, aluminium, nickel, cobalt and titanium dioxide NPs and their corresponding soluble salts were studied on various sea organisms. The authors found that nanosilver, nanocopper, and their soluble forms caused toxicity in all organisms tested; however, titanium dioxide did not show any toxicity. Although the NPs were of similar size but different surface charges, the chemical composition of nanoparticles appeared to be the most important factor in toxicity.

8.1.4 Surface coating and functionalisation

Surface treatment or surface modification or functionalisation or doping of nanomaterials induces distinct chemical and physical properties compared to their pristine form. For example, the surface treatment of silica with methyl groups is an effective way to disperse the silica nanoparticles in a wide range of organic solvents in contrast with the pristine form. Functionalisation has been used to conjugate drug molecules, polymers and organic groups to NPs. It has been demonstrated that non-covalent attachment of polyethyleneimine (PEI) polymers to the silica surface not only increases cellular uptake but also generates a cationic surface to which DNA and siRNA constructs could be attached.[26] In another case, functionalisation has also been shown to protect NPs against agglomeration and render them compatible in other phases. Silica coating on semiconductor materials such as CdS nanoparticles improves the stability of the particles as well as prevents coagulation during the chemical or electronic processing.[27][28][29][30][31] Therefore, surface functionalisation of NPs is rather necessary to render specific functionality over the core NPs. As such, addition of this new function to nanomaterials in the form of doping or functionalisation poses distinct toxicological/ ecotoxicological problems relative to their pristine form. Surface coating is not necessarily uniform; the degree of coating can vary from particle to particle within a batch of manufactured nanomaterials or between batches of nanomaterials.

The adverse effects of NPs may be migrated or eliminated by a selective surface coating. Their behaviour can point to important mechanisms, especially accumulation of nanoparticles in the environment,

permeability of NPs through cell walls, absorption level of NPs in lungs, etc. For example, quantum dots (QDs) coated with negatively charged serum protein albumin showed higher liver uptake and faster blood clearance relative to the QDs without protein coating.[33] Coating of nanoparticles with biologically compatible polymers often shows significant levels of toxicity. As ruled by Berry et al.[34], dextran coated magnetite (Fe_3O_4) nanoparticles cause cell death and reduced proliferation similar to uncoated iron oxide particles, which is due to the breakdown of the dextran shell exposing the cellular components to chains or aggregates of iron oxide nanoparticles.

Metallic silver NPs tend to oxidise and may become sulphidised in the environment. Sulphidation of the particles changes their aggregation state, surface chemistry and charge, as well as their ability to release toxic Ag^+ ions and therefore their persistence and toxicity (Renata et al. 2013). Once released into the environment, the mobility, bioavailability and toxicity of silver nanoparticles in any ecosystem is largely determined by colloidal stability. Colloidal stability is a function of many factors including the type of capping agent, the characteristics of the surrounding environment such as the pH, ionic strength, presence/absence of humic acids and other ligands, and the background electrolyte composition (Römer et al. 2012). An extensive number of capping agents have been investigated to enhance the ability of nanoparticles to stay suspended in solution. Capping agents are chemicals that are used in the synthesis of silver nanoparticles to prevent their aggregation through electrostatic repulsion, steric repulsion or both. In the case of silver, the most prevalent capping agents are citrate, sodium borohydride ($NaBH_4$) and polyvinylpyrrolidone (PVP). The mechanism and functional groups involved in colloid stabilisation differ with capping agents, which may lead to varying particle sizes and stability. Colloidal interactions, mobility and toxicity may differ.[35]

Overall, the studies show that surface coating/functionalisation can alter the toxicity, pharmacokinetics, bio-distribution, and accumulation of nanoparticles.

8.1.5 Crystal Structure

Crystal structure describes how the molecules of an inorganic substance are physically arranged in space. Many materials with the same chemical composition can have different lattice structures and consequently exhibit different physico-chemical properties. Several aspects of the crystallinity of metals and metal oxides may vary with particle size[66][67]. It is evident from the current toxicological literature that the toxicological effects of engineered nanomaterials can vary considerably depending on the structural properties.[36] For example, the composition of silica is stoichiometrically similar, but various forms of silica differ in their physicochemical and toxicological properties. Silica exists in crystalline and amorphous states that show long and short range order, respectively. It is well known that inhalation of crystalline silica induces serious adverse effects among workers in the form of increased lung cancer and has been classified as a human lung carcinogen. However, synthetic amorphous silica (SAS) shows no adverse effects because of its amorphous state.[37] Similar distinctions between rutile and anatase phases of titanium oxide can be made.

Nanomaterials are commonly produced with an organic capping agent or stabiliser, often a small anion or polymer. Transformations of the material can therefore affect the core material, the capping agent, or both. For example, the simple coordination of ZnS nanoparticles (NPs) with water molecules can alter their crystalline phase and properties.[38]

Hence it is important to clearly identify the crystalline phase of the NMs in light of their different behaviour related to toxic properties both in human and environmental conditions.

8.1.6 Shape and Aspect Ratio

Depending on the synthesis conditions and composition, nanoparticles may take different shapes: spherical, triangular, dendritic, or needle-like, for example. The term “aspect ratio” refers to the ratio between a particle’s length and width. This parameter can be relevant to the toxicity of carbon nanotubes, nanowires, and other “needle shaped” particles. Its relevance is perhaps best understood by analogy to the well-known inhalation toxicity of asbestos. This is further discussed below.

In contrast with non-nanomaterials (bulk), the thermodynamic and surface energy considerations at nanoscale are more complicated by the high surface area to volume ratio. For a material with a perfect symmetric sphere, the total surface energy is lowered by decreasing the amount of surface area corresponding to a given volume. On the other hand, faceted nanoparticles show a high number of reactive and high atom density facets that may influence the properties such as dissolution, aggregation, and reactivity. Bottom-up methods are used for producing a large variety of nanomaterials under controlled conditions for a desired shape.

Particularly for nanomedicine applications, the shape of nanoparticles has recently been identified as a key factor influencing circulation time, bio-distribution, cellular uptake, as well as targeted drug delivery.[39] However, shape effect studies show considerable toxicity to human cells and question the health and environmental fate of nanoparticles.

It was shown that wire-shaped silver nanoparticles induced a stronger cytotoxicity to human cells (A549) than spherical silver nanoparticles.[40] Similarly rod-shaped ZnO nanoparticles were shown to be more toxic to marine algae than spherical ZnO nanoparticles.[41] Also a recent study based on fluorescent mesoporous silica nanoparticles (MSN-FITC) of different shape (aspect ratios: 1, 1.5, 2, 5) shows potential concern on the toxicity (Figure 2) [42]. The organ distributions show that short-rod MSNs are easily trapped in the liver, while long-rod MSNs distribute in the spleen. MSNs with both aspect ratios have a higher content in the lung after PEG modification. The MSNs are excreted by urine and faeces, and the clearance rate of MSNs is primarily dependent on the particle shape, where short-rod MSNs have a more rapid clearance rate than long-rod MSNs in both excretion routes. Clearly, shape is also an important parameter when considering the fate of nanoparticles.

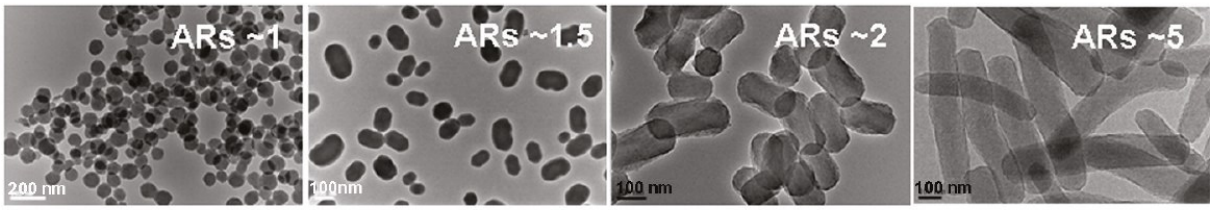


Figure 2: TEM images of different shaped fluorescent mesoporous silica nanoparticles (MSN-FITC) and their aspect ratios (ARs).

8.1.7 Surface Charge

Surface charge influences the fate and transport of nanoparticles. Any surface charge on nanoparticles causes electrostatic repulsion between particles of like charge, which can counter the tendency to agglomerate. This was shown in a study that investigated the impact of capping agents and environmental conditions (pH, ionic strength, and background electrolytes) on surface charge and aggregation potential of silver nanoparticles (AgNPs) suspensions[43] The results in this study reveal that the suspension stabilisation through the use of capping agents can dramatically influence the surface changing behaviour and aggregated particle size of AgNPs. The type of stabilising mechanism has a profound effect on the aggregation potential of AgNPs. Thus, the potential fate and transport of AgNPs are more closely associated with the chemistry of the capping agent. This information may provide an insight into the potential mobility of AgNPs in natural and engineered environments. Positively charged AgNPs would most likely have limited mobility in soils, groundwater, and other environments where negative charged clay minerals and/or metal oxides are present. Sterically stabilised AgNPs may have the greatest potential for mobility and transport.

8.1.8 Solubility/Dispersibility

Water solubility/dispersibility refers to the mass proportion of a given sample of nanomaterial which is held in water solution or as a colloidal suspension in water as a function of time or where the sample of nanomaterial loses its particulate character as it changes from a particle form to a molecular form[62]. It must be recognised that solubility and dispersibility are not identical though the distinction can be difficult to recognise with nanomaterials.

Water solubility can depend upon particle size. Briefly, the rate of dissolution of soluble materials increases with decreasing particle size, and the Ostwald-Freundlich equation predicts that equilibrium solubility should increase with decreasing particle size (although experimentally, this is often not the case due to non-ideal behaviour)[66]. Water solubility also depends upon the solution characteristics and can depend on the particle coating.

Dispersibility refers to the relative number (or mass) of primary particles in a suspending medium. This property characterises the way in which nanoparticles can form colloidal suspensions that differ from solutions of dissolved substances. The pH and ionic strength of the aqueous phase can affect a nanomaterial's dispersibility[62]. The introduction of an insoluble or very sparingly soluble nanomaterial to a liquid or other aqueous medium with the intention of making a stock "solution" will involve dispersion. Some metal nanoparticles may release ions from the surface into the surrounding water (corrosion/degradation) and it is therefore possible that these nanomaterials will eventually degrade completely. Because of the

particle size of many nanomaterials, it can be difficult to distinguish between when a nanomaterial is dispersed and when it is dissolved[68].

Interactions between the nanoparticles and natural and anthropogenic chemicals in the system, along with biological and abiotic processes, are expected to control the transport of nanoparticles in aqueous environments.[44] The solubility of industrially-produced nanostructured TiO_2 in aqueous sodium chloride solutions of pH 1 to 13 has been investigated in one typical study.[44] The study showed that, in general, an amorphous particle with a highly hydroxylated surface will have a higher solubility than a highly-crystalline particle.[45]

Dissolution time, primary particle size, and hydroxylation and hydration of the solid phase are parameters that may impact solubility and hence should be reported in solubility studies[46]. A study on nano-TiO_2 in water and transport in porous media showed that pH and aggregate size control the interactions between the TiO_2 aggregates and other nanoparticles or water. Approximately 80 percent of suspended particles and aggregates were mobile in the pH range of 1 to 12.[47] In another study the particle size of TiO_2 nanoparticles increases due to the ionic strength of the solution. The same study also showed that varying the solution pH resulted in a significant change in the particle surface charge.[46]

The effects of other environmental factors were also clearly shown in studies with silver nanoparticles under environmentally relevant conditions. Again importance of pH and ionic strength was shown. Silver nanoparticles could have long residence times in aquatic systems in the presence of humic substances, potentially resulting in increased bioavailability.(47)[48]

8.1.9 Reactivity

Reduction and oxidation are coupled processes in natural systems and involve the transfer of electrons to and from chemical moieties. A number of NMs may be composed of or contain constituents that undergo reduction, oxidation, or both in aquatic and terrestrial environments. These include elemental metal NMs such as silver and iron. The redox potential of dispersed metallic compounds and nanoparticles shows a strong relationship between the chemical stability and their in vitro toxicity. Reports suggest that chemically stable metallic nanoparticles have no significant cellular toxicity, whereas nanoparticles able to be oxidised, reduced or dissolved are cytotoxic and even genotoxic for cellular organisms.[49] In a recent study, two types of nanoceria with the same distribution of primary size (3–5 nm), but different redox activity, were synthesised by precipitation (Ceria-p) and hydrothermal route (Ceria-h). Both Ceria-p and Ceria-h induced oxidative stress, inflammatory responses and cytotoxicity in mice, but their toxicological profiles were quite different. Ceria-h had a higher reactivity to catalysing the generation of reactive oxygen species.[50]

In the environment for example: C_{60} could oxidise via photoactivation (UV/sunlight) and ozonation,[51][52][53] to form poly-oxygenated/hydroxylated C_{60} derivatives (e.g. fullerols) – species that are more hydrophilic than C_{60} (water solubility ≥ 50 mg/L).[54][55] Aside from this transformation, natural organic matter (NOM), which is ubiquitous in soil, could modify the C_{60} surface by adsorbing to its outer shell via hydrophobic or pi-pi interactions[56] to form species that may behave differently from bare C_{60}. These changes and modifications will have significant implications on the behaviour and eventual fate of fullerenes in the terrestrial environment[57], to some extent analogous to the intentional surface modifications discussed above.

Ceria NPs can contain both Ce(III) and Ce(IV) and subsequent sorption of macromolecules can alter the ratio of Ce(III)/Ce(IV) on the NP surface. In some cases, oxidation may result in the accumulation of a relatively insoluble oxide surface coating on the NP that passivates the surface and reduces subsequent oxidation, while also forming metal-oxide phases with a high capacity for binding ions from solution. In other cases, (e.g., AgNPs), oxidation of Ag(0) to Ag(I) is required to dissolve and release bactericidal Ag^+. Natural waters and aerated soils are predominantly oxidising environments, while carbon-rich sediments and groundwater may be depleted of oxygen and result in NM reduction.[58] The oxidation of iron oxides such as magnetite may change the magnetic properties and, thus, the magnetic forces between particles, which influence the aggregation behaviour of the material. Oxidation, therefore, could lead to decreased aggregation rates of magnetite NPs and a potential enhancement of their colloidal stability. In addition, the oxidation and reduction of iron oxide NPs may have a large impact on the retention and release of sorbed contaminants such as arsenic.[6]

It has been shown that the dispersion properties of TiO_2 and CNTs were improved by addition of dipalmitoyl phosphatidylcholine in PBS buffer, but they show significant increase in the inflammatory response of rats after intratracheal instillation.[60]

8.1.10 Other Properties

Contrary to the physico-chemical properties stated above, other properties such as surface roughness, redox potential, dustiness, and NPs medium, also play a critical role in determining the outcome of their interactions with cells and living organisms.

Excessive levels of dust emissions during the handling and transport processes of powder materials can cause adverse health effects on workers. In a recent review, a significant respirable dustiness was observed, suggesting that workplace procedures may result in inhaled airborne dust, a significant fraction of which may be capable of reaching a worker's deep lung.[59].

As with non-nanomaterials, effects may sometimes be solely attributed to impurities from the production process. It has been shown that high aspect ratio nanomaterials (HARN) such as CNTs show considerable toxicity especially because of catalyst metal contaminants such as the transitional metals and metal oxides, introduced during production and purification process. The toxicity of CNTs is due to the release of metal contaminants and their ability to cross the cell membrane.[32]

8.2 Conclusions on properties of NM

The behaviour and effects of a nanomaterial are influenced by a combination/interaction of several physico-chemical properties. These properties can tentatively be placed in four categories:

- Substance identity, including chemical composition, crystal structure, surface coating, functionalisation and capping agents, impurities, all of which influence surface charge and reactivity;
- Particle characteristics, including size (distribution), surface area (which depends on particle size and porosity), surface roughness, shape and aspect ratio, all of which generally influence mobility and transport;
- Transport behaviour, which reflects characteristics of the nanoparticle that are (partly) influenced by the surrounding medium, such as solubility/dispersibility (rate of dissolution and equilibrium concentration, both size-related), surface charge, tendency to agglomerate, dustiness.
- Activity and reactivity, including redox potential.

It is important to note that these parameters can influence not only the toxicity and ecotoxicity of a nanomaterial, but also the interactions between the nanoparticle and the environment, whether external or within an organism. Therefore, a fundamental understanding of the physico-chemical properties and biological interactions of NPs with cells, proteins, tissues, and living organs as well as in the environment is vital to the future design of safe nanotechnologies.

9 Grouping, read-across, extra- and intrapolation within NANoREG

In this paragraph, building on the ongoing work and existing approaches and proposals as described in section 2.5, the aims of MNM grouping will be defined, taking into account the multidimensional nature of MNM. For each identified purpose, a different combination of parameters to evaluate the similarities of the MNM can be used. The parameters can be used in parallel, by weighing the parameters equally, or in a stepwise fashion, by assigning different parameters to different tiers with different roles. A strategy stepwise approach is usually applied in the literature, but to apply the same for all grouping can be difficult at this time for lack of information. It will be verified for few examples if it can be possible to apply a stepwise approach. If achievable, recommendations in terms of decision trees and benchmarks (e.g. parameters value related to reference materials) will be formulated.

9.1 Aims and goals

Grouping and read across can be done with different aims. One of those aims is to screen or prioritize different nanomaterials with respect to one or more potential harmful effects. Another goal of grouping approaches is to support read-across and in general to support the filling of data gaps. In principle, this can be done for the whole spectrum of the REACH and CLP required endpoints, as well as for other nano-specific endpoints not yet included in REACH.

Grouping can for example be done to infer skin sensitization concentration/effect, assess persistence in the environment, or extrapolate the negative effects on algae of a MNM. The grouping can also be done in different steps of a MNM life cycle (e.g. to infer the impacts of a MNM in the end-of-life phase taking into account the release patterns from the disposed product). Different groups can be established by using

similar parameters in different ways, and at the same time, the same MNM can be in different groups. The integration of the different grouping results will lead to the identification of MNMs with a similar/equal level of safety concern, whatever their chemical composition, shape, size, etc.

In line with the NANoREG orientation, the grouping approach should also consider exposure-related parameters as one of the first steps, to identify definite MNM of concern (i.e. MNM fixed in matrices and that are never release along the life cycle, can be categorized as of low concern) and the best testing strategy on the basis of the potential exposure pathways and targets. The integration with hazard/toxicity parameters can lead to the final grouping.

In general terms, grouping can be aimed at physico-chemical properties, exposure potential, or (eco)toxicological effects, fate and transport. For each of these aims, a different combination of parameters to evaluate the similarities of the MNM can be used. In the next sections, the relevant parameters for each of these aims are described.

9.1.1 Physico-chemical grouping

The purpose of physic-chemical grouping is to provide an indication of the general hazardousness of MNM in relation to inherent properties. This grouping should be independent from exposure, target, and environmental compartments. In some way it is a substance identification approach, linking inherent properties to hazard characteristics as defined in CLP. The main result should be the identification of very hazardous MNM, MNM of no concern, and all the others in between. More detailed groups can be created if it is possible to link the considered physico-chemical properties to higher level characteristics (persistence, bioaccumulability, reactivity, explosivity, etc.). Examples of parameters can include high-aspect-ratio, chemicals inherent toxicity, reactivity, redox potential, solubility rate, (bio)degradability. Data from non-nano-chemicals can be used in this stage for some parameters (a degradable chemical is likely degradable in every form, non-nano or nano). Benchmarks can be available for some parameters, as provided by CLP and literature.

9.1.2 Exposure grouping

The exposure grouping is linked to the need to evaluate the bioavailability of the MNM (how likely it is that the MNM reaches its toxicological target). Therefore, it includes both external (e.g. concentration in the environment) and internal (i.e. ADME) exposure, from the point of release to the point of action. The scope of this grouping could be to estimate the target MNM environmental behaviour, the exposure pathway or the likelihood of release from a product. All these endpoints are the result of the interaction between the MNM and the surrounding environment.

Parameters to be considered in this case includes environmental behaviour (aggregation, solubility, biodegradability), consumer exposure (MNM status in the product), life cycle information (MNM transformation along the life cycle), and occupational exposure. Production volume, in the scope of the exposure grouping, is too generic to be useful. It can be a parameter to be included in the physico-chemical grouping, to rank the “all the other” MNM.

Specific exposure-related parameters can be: dustiness, chemical/physical stability, (bio)persistence, (bio)accumulability, release potential (from products, during production steps, during life cycle steps).

9.1.3 (Eco)Toxicity grouping

The (eco)toxicity grouping goal is to use relevant physico-chemical parameters to identify or estimate toxicological endpoints and general toxic mode of action of target MNM. For chemicals, it is sometimes possible to estimate the effect concentration for some endpoints, such as for example ecotoxicity effects, through specific models built on chemical properties.

For nanomaterials, the same scheme can be applied, by identifying the relevant physico-chemical parameters. The grouping can be based on a similar mode of toxic action (MoA), such as for example: inflammation, genotoxicity, protein denaturation, altered cell cycle alteration, cytotoxicity, ROS generation, cellular uptake. The physico-chemical parameters accompanied by relevant benchmarks can allow a preliminary grouping, which then should be demonstrated via appropriate testing, and testing results comparison. The testing has to be linked to the target organ, which in turns can be influenced by the exposure route.

An example of the linking between MoA and physico-chemistry is the RIP-oN2 report (http://ec.europa.eu/environment/chemicals/nanotech/pdf/report_ripon2.pdf) defining a testing strategy linked to chemical composition and shape of MNM.

To identify the MoA, basic physico-chemistry should be linked to higher level parameters (e.g. reactivity, cellular uptake, interaction with proteins), or higher level parameters (with respect to the basic physico-chemistry as required by REACH) should be directly used among the grouping criteria.

For an effective grouping, it should be possibly to measure the similarity among the components of a group. For traditional chemicals, benchmarks are sometimes measured via statistical approaches (e.g. cluster analysis), or through a clear-cut value, a function linking the distance from the nearest neighbour to a property (or set of properties), or qualitative, based on literature findings about the effects of change of some properties. It is difficult to identify benchmarks for all relevant MNM properties, but for parameters where this is possible, it should be implemented already.

To demonstrate and support the grouping concepts, few examples among best characterized and studied MNM will be selected. The strategy can follow both the analogue approach (i.e. compare target MNM with one source MNM), or a categorization approach (i.e. defining a group with homogeneous properties linked to the target MNM). Another approach could be to identify a class of MNM (e.g. CNT, TiO_2), identify all (or several) different forms in the literature, and try to find similarities or differences for selected endpoints by using relevant measured properties.

9.2 Prerequisite of grouping in NANoREG

NANoREG, as “testing the test” project, and being regulatory focused, defines some boundaries and poses prerequisites that the planned tools and recommendations have to comply with. Also, the philosophy of NANoREG and the expected results have to be taken into account when developing the different tools. There are generic prerequisites, and specific prerequisites about grouping. This paragraph will describe and justify the boundaries of the grouping approach.

Grouping has to comply with current regulations (basic requirements). This point covers not only the legal text, but also the technical guidelines. The reference regulation of NANoREG is REACH, which will represent the main reference. However, this does not exclude that other requirements linked to other regulations could be taken into account. An example could be food regulations and internal exposure as a parameter to assess risk of food nanosized ingredients.

The grouping could be applicable to the whole life cycle steps of the MNM. This could mean that a worst-case approach is taken into consideration (e.g. grouping based on the critical life cycle steps), or that for each life cycle step a different grouping is done (e.g. the same MNM is categorized as inactive when fixed to the polymer, and ecotoxic in the end-of-life phase). In general, grouping should be nanoform and use-specific to be effective for safety assessment.

9.3 Stepwise procedure for read across of nanomaterials

Specific regulatory guidance on the grouping of MNMs has not yet been published in the European Union (or elsewhere). Also the second edition of the OECD guidance specifically excludes recommendations on the grouping of MNMs: “At present, it seems premature to develop guidance on grouping specifically for NMs.” In a working document by ECHA, RIVM and JRC on read-across between nanoforms (ECHA et al., 2015), the following stepwise procedure was proposed for using data between (nano)forms:

1. ***Identification of the nanoform.*** This involves the identification of the nanoform, based on the basic physicochemical parameters of the nanoform, for which potentially no or insufficient information is available for hazard characterisation (unassessed nanoform).
2. ***Initial grouping of nanoforms.*** Based on similarities in chemical identity, particle characteristics, fundamental behaviour (i.e. solubility, hydrophobicity, dispersibility and dustiness) and reactivity (i.e. flammability, explosiveness, biological (re)activity, surface reactivity and photoreactivity) initial grouping of nanoforms may be considered. Such initial grouping should be justified by similarities in the behaviour of the (nano)forms and the boundaries of a group should be clearly defined.
3. ***Identification of available data and data gaps.*** This involves making an inventory of the information available per endpoint required under REACH for this nanoform, and consequently identifying data gaps for REACH compliance.

4. ***Identification of potential source materials.*** For each data gap, this involves the identification of source materials, e.g. other (groups of) (nano)forms, from which information may be used for read-across. This also involves (hypothesis based) justification of the appropriateness of the identified source material.
5. ***Substantiate hypothesis.*** This involves information gathering to substantiate the hypothesis for read-across. When groups of (nano)forms are considered in read-across it may be necessary to re-evaluate the initial grouping. If applicable, a testing strategy can be build that may (partly) cover multiple data gaps.
6. ***Assess any new data for the impact on the hypothesis.*** In an iterative process, interpret the information that becomes available to evaluate if the information sufficiently substantiates the hypothesis and builds justification for read-across (or not).

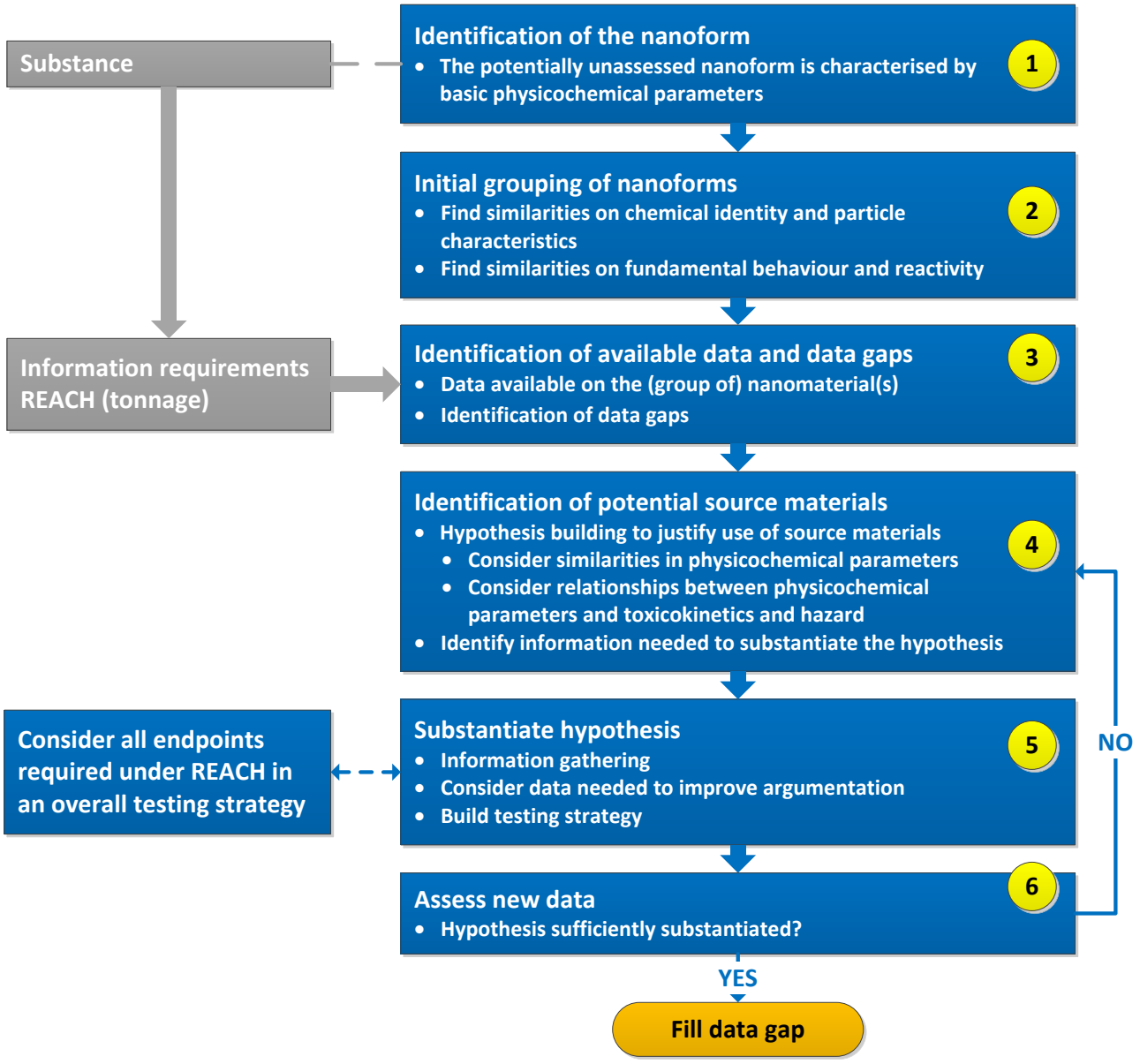


Figure 3: Proposed strategy or stepwise procedure for using data between (nano)forms (adapted from ECHA et al, 2015).

In this working document (ECHA et al., 2015), some further considerations are given for each step which may support building a transparent and scientifically sound justification of using data from one (nano)form to another. This working document is based on work already done in ECHA such as the Read Across Assessment Framework but also RIVM's own expertise as well as outputs from ECETOC and ongoing FP7 discussions. The document is aiming at capturing the current understanding for how and when data on one (nano)form may be used to cover a data gap on another nanoform(s), in other words read-across between (nano)forms. It is not an ECHA guidance document on read across between (nano)forms, nor is it a finished document but a thought-starter for discussion. The document will provide input for future discussions within ECHA, as well as for the ongoing work at OECD and existing FP7 projects on how to use read across between (nano)forms. It does not give suggestions for benchmarks or boundaries to be used, but it does refer to other documents in which benchmarks are proposed (e.g. Arts et al., 2015). Furthermore, the working document will be amended with some case studies which might give some more details on its implementation. Unfortunately, a description of these case studies will not be available in time for this report to take them into account.

For grouping for screening purposes, the first two steps of this procedure may already suffice.

9.4 Examples of grouping of nanomaterials

At present, there is are only a few examples of the use of grouping or read-across for nanomaterials in regulatory risk assessments. As described in chapter 6, several examples of grouping for screening purposes (or initial grouping) have been suggested (Arts et al., 2015, Gajewicz et al., 2015, Zhang et al. 2012, etc.). One of such initial groups are HARNs (High Aspect Ratio Nanomaterials). Although this group of nanomaterials are still under investigation and discussion, this is one of the few examples for which there seems to be consensus about their potential to cause major adverse health effects in humans after inhalation. For non-nanoforms (conventional chemicals) there are several examples of read-across. Below, two examples which might be relevant for nanomaterials are described. For nanoforms, an example on a risk assessment in which grouping/read across from available data was used is the SCCS evaluation of TiO_2 in sunscreens. The SCCS concluded that the use of TiO_2 nanomaterials with certain characteristics (as discussed below), at a concentration up to 25% as a UV-filter in sunscreens, can be considered not to pose any risk of adverse effects in humans after application on healthy, intact or sunburnt skin (SCCS, 2014). Based on this SCCS opinion, read-across of a hypothetical TiO_2 nanomaterial using the data of this group of TiO_2 nanomaterials in sunscreens, is described. Finally, another example of read-across of a hypothetical silver nanomaterial is described.

9.4.1 Initial grouping based on shape and solubility: HARNs

High aspect ratio, or fibre-shaped, nanoparticles (HARNs) are increasingly produced in society and grouping/read-across possibilities has been suggested based on the "fibre paradigm". It is today well known that inhalation exposure to asbestos and naturally occurring high aspect ratio fibres can lead to a number of diseases that affect the lungs (e.g., fibrosis and bronchogenic carcinoma) or the pleura (e.g., mesothelioma, pleural effusion and pleural fibrosis). The fibre pathogenicity paradigm is a structure/toxicity model for fibres that highlights width, length and biopersistence in dictating whether or not a fibre will be pathogenic upon inhalation. A pathogenic fibre is thin enough to penetrate the lung, long enough to cause frustrated phagocytosis (exact length not totally clear, around 10-20 µm) and biopersistent (so that it remains in the lung). An important question is thus to understand whether possible carcinogenic effects of nanofibres, such as CNTs, can be estimated based on the fibre paradigm i.e. using read-across from known pathogenic fibres. Indeed, Poland et al showed in 2008 for the first time that multiwalled carbon nanotubes can produce asbestos-like, length-dependent inflammogenic and fibrotic responses (using a peritoneal model of mesothelium exposure). Even though more knowledge is still needed (quantitative data for biopersistence, cut-off for length etc), it is likely that the fibre paradigm can be useful for grouping of nanofibres, such as CNTs, which is already done so in several control banding tools.

9.4.2 Read-across of non-nanoforms based on solubility/metal ion release: Nickel compounds

In the case of metal-containing substances, it can often be the case that the metal ion is the responsible entity for the observed toxicity of the compounds. Thus, bioavailability data can be used to perform read-across assessments for metal substances. Bioavailability, defined as the fraction of the dose that reaches systemic circulation, is then the key for determining toxicity and can thus be the basis for read across. Ideally, information on the bioavailability of the metal substances is derived from *in vivo* toxicokinetic/ toxicological tests, but when such data is lacking, the amount of ions available for absorption may be

estimated using *in vitro* methods. Determination of released metal ions in synthetic fluids (sometimes termed ‘‘bioaccessibility’’) can provide a surrogate measure of the amount of a substance (e.g., metal ion) available for absorption. For nickel compounds, Henderson et al (2012) developed a read-across paradigm based on the correlation between gastric bioaccessibility and *in vivo* acute oral toxicity. Bioaccessibility data (Ni^{2+} release) in synthetic gastric fluid (2 hours) were gathered from 12 different nickel compounds. Acute oral toxicity data testing (LD50) was then performed and a clear correlation was observed between Ni^{2+} release in gastric fluid and acute oral toxicity. Grouping was then performed and the hazard classifications (European Regulation on Classification, Labelling and Packaging of Chemical Substances and Mixtures) for all oral systemic endpoints (including reproductive toxicity and repeated dose oral toxicity) were evaluated based on read-across from three source nickel compounds (sulfate, subsulfide, oxide).

9.4.3 Read-across of non-nanoforms based on the fibre paradigm: Refractory ceramic fibres

Another example of fibre read across has been used for refractory ceramic fibres (RCF), also termed aluminosilicate wools (ASW). These are amorphous fibres from a class of materials called synthetic vitreous fibres (SVFs), which also includes e.g. glass wool and rock (stone) wool. The possible carcinogenicity of RCF with relevance to setting occupational exposure limits (OELs) was recently reviewed and evaluated by Greim et al (2014). Chronic nose-only inhalation bioassays have shown that RCF exposure in rats increased the incidence of lung cancer and similar exposures resulted in mesothelioma in hamsters, but the authors argue that these studies may have been compromised by overload and other issues related to the aerosol generation. RCF and rock wool have similar airborne fibre dimensions and biopersistence. Therefore, the authors argue, it is likely that these fibres have similar toxicology. Traditional rock wool has been the subject of numerous cohort and case control studies. For rock wool, IARC (2002) concluded that the epidemiological studies did not provide evidence of carcinogenicity. Based on analogies with rock wool (read across), the authors consider that it is reasonable to believe that increases in lung cancer or any mesotheliomas are unlikely to be found in the RCF-exposed cohort (Greim et al, 2014). The same conclusion was reached by Boffetta et al (2014) regarding the risk of mesothelioma among workers exposed to SVF. The low risk was suggested to be due to the low biopersistence of SVFs, typically with a half-life in rat studies of tens of days compared to amphibole asbestos which has a half-life of 400-500 days.

9.4.4 Read across/grouping of nanoforms based on chemical identity and photocatalytic activity: TiO_2 nanoparticles

In 2014, the SCCS (Scientific Committee on Consumer Safety) published an opinion on titanium dioxide (TiO_2) (nano form). Based on the data and information provided on the potential hazards, common characteristics were identified in the evaluated TiO_2 nanomaterials to form a “group” of TiO_2 nanomaterials. The use of these TiO_2 nanomaterials as a UV filter in sunscreens at a concentration up to 25% was considered safe for humans after application on a healthy, intact or sunburnt skin. Using the data presented in this SCCS opinion, the step-wise approach as presented in Section 9.3 can be applied to a hypothetical TiO_2 nanomaterial (the numbers below refer to the different steps). As the SCCS opinion is limited to the human health risks of the use of TiO_2 nanomaterials in sunscreens, this example will focus on filling data gaps on human toxicity only.

Step 1. The specific hypothetical nanoform defined for the purpose of this example consists of TiO_2 nanoparticles (97% rutile and 3% anatase), with a mean particle size of 62 nm in dispersion (primary particle size 18 nm), specific surface area of 260 m^2/cm^3 and aspect ratio of 2.4. The TiO_2 nanoparticles have a purity of $\geq$ 99% and a stable silica coating. The particles are photo stable and have a photo-catalytic activity of 2%. This is further referred to as target material.

Step 2. Other nanoforms of TiO_2 (like the “group” of TiO_2 nanomaterials evaluated by the SCCS) are considered as initial grouping, since the behaviour and toxicity is expected to be dependent on the chemical composition and photocatalytic activity of the nanoparticles.

Step 3. Most physico-chemical data on the target material are known (i.e. chemical composition, particle size, degree of agglomeration, surface area, aspect ratio and surface treatment), but some others are not (surface charge, water solubility, reactivity, etc.). In REACH testing data on the following human health endpoints are required for the target material:

- acute toxicity,
- skin irritation,
- eye irritation,
- skin sensitisation,

- mutagenicity and
- repeated dose toxicity (90 days via relevant exposure route)

Depending on the results of these studies carcinogenicity and reproductive/developmental toxicity testing may also be required for the target material. However, none of these toxicity data are available for the target material.

Step 4. As a null hypothesis it is assumed that the target material exhibits a unique behaviour for all human health endpoints. The (nano)forms (source materials) from which information may be used to fill the data gaps consists of the "group" of TiO_2 nanomaterials evaluated by the SCCS. Based on the data described in the SCCS opinion, it was concluded that skin exposure was found to be unlikely to lead to:

- Skin penetration and thus systemic exposure
- Acute toxicity via dermal application and oral ingestion
- Skin irritation, eye irritation or skin sensitization when applied on healthy skin
- Reproductive effects when applied on healthy skin

However, the source materials have shown to lead to:

- Inhalation toxicity
- Carcinogenic effects after inhalation
- Inconclusive results with respect to potential genotoxicity

Step 5. The first hypothesis to test is whether the physicochemical properties of the target material are similar to those of the source materials. The physicochemical properties of the "group" of TiO2 nanomaterials evaluated by the SCCS (source materials) from which information may be used to fill the data gaps consists of rutile (or rutile with up to 5% anatase) titanium dioxide particles, with a mean particles size >30 nm in the cosmetic formulation, specific surface area up to 460 m2/cm3 and aspect ratio from 1.0 to 4.5. The particles are photo stable and have no (or <10%) photo-catalytic activity. They have a purity of $\geq$ 99% and a stable coating that does not affect the particle properties related to behaviour and/or effects. Data on the chemical composition, size (in dispersion), specific surface area, aspect ratio, surface coating, photo stability and photocatalytic activity of the target material support the use of the source materials for read-across for the evaluation of human health effects after application on healthy, intact or sunburnt skin. This, however, does not apply to applications that might lead to inhalation exposure to TiO_2 nanoparticles (such as powders or sprayable products), since these have been explicitly excluded in the conclusions of the SCCS opinion.

Step 6. This example was based on the scientific evidence described in the SCCS opinion which showed an overall lack of dermal absorption of TiO_2 nanoparticles. If any new evidence shows that TiO_2 nanoparticles can penetrate skin, the conclusion may need to be revised. In addition, more information on the long term stability of coatings may require a separate evaluation for the uncoated nanomaterial as well as the coating material.

9.4.5 Read across/grouping of nanoforms based on particle size, shape and surface treatment: Silver nanoparticles

In the recent report by Sellers et al. (2015) a case study on silver is presented as a hypothetical test case for the approach they developed. Based on the data presented in the report by Sellers et al., the step-wise approach as presented in Section 9.3 can be applied (the numbers below refer to the different steps). Although both human health and ecotoxicological endpoints are described, only the ecotoxicological endpoints are used here. As the report only summarises short term ecotoxicity data, here focus will be on filling data gaps on short term ecotoxicity only.

Step 1. The specific nanoform under consideration consists of triangle shaped particles (20-40 nm) in a liquid suspension with citrate. This is further referred to as target material.

Step 2. Other nanoforms of silver are considered as initial grouping, since the toxicity is expected to be dependent on the solubility and dispersibility of particles.

Step 3. Some physico-chemical data on the target material are known (i.e. particle size and shape, and a surface treatment with citrate), but many others are not (aggregation/agglomeration state, water solubility, aqueous dissolution rate, etc.). In REACH short-term ecotoxicity testing is required for algae, invertebrates and fish, but for the target material these data are not available.

Step 4. As a null hypothesis it is assumed that the target material exhibits a unique behaviour under ecotoxicity testing. As data are available for other nanosilver forms, these forms are considered as source materials.

Step 5. First hypothesis to test is whether the physicochemical properties of the target material are similar to those of the source materials, in particular when taking the test conditions in the short-term toxicity tests into account. Data on the solubility (rate of dissolution and equilibrium solubility) and the dispersibility of the target material are essential for the interpretation and comparison of data on aquatic species and to support the use of potential read-across substance(s). As these are not known for the target substance and they cannot be estimated otherwise, testing for these physicochemical parameters is essential. Data from the other nanoforms found in literature can only be used if this first tier indicates that similar rate of dissolution (and equilibrium solubility) can be reached, which will also depend on the dispersion method used.

Step 6. It can be expected that, because of the citrate coating, the solubility will be limited relative to the solubility of uncoated nanosilver or non-nanoparticles. Therefore, the soluble silver compounds (the non-nanoforms like $AgNO_3$) cannot be used as a possible read-across substance (or only in a worst case approach, if the nanosilver particles do not contribute significantly to the aquatic toxicity in comparison with silver ions). If results from this first tier indicate that size (and agglomeration state) and solubility are not similar to those found in the literature cited above, then read-across between those substances is not possible and either a worst case approach (if possible) or ecotoxicity testing on the nanomaterial of interest should be performed. The choice for either next step (i.e. using data on ions or ecotoxicity testing) should be argued and justified. For example, to enable the use of data on silver ions as a worst case, it should be argued that the acute toxicity is mainly caused by external exposure of silver ions, and not via uptake and subsequent release of ions within a certain part/organ of the organism. This assumption should be supported by data to justify it.

9.4.6 9.4.6. Read-across/grouping of CNTs based on the pathogenic fibre paradigm

As already mentioned, it is likely that the fibre paradigm and read-across between various CNTs can be useful for grouping and filling data gaps for various CNTs. The step-wise approach can be applied to a hypothetical MWCNT according to below.

Step 1. The specific hypothetical CNT defined for the purpose of this example is a pristine MWCNT with a purity of >95%, a diameter of 58 nm and a length of 5-10 μm. This is further referred to as target material.

Step 2. Other CNTs are considered as initial grouping, since the behaviour and toxicity is expected to be dependent on the diameter, length (aspect ratio) and impurities of the CNTs.

Step 3. Many physico-chemical data on the target material are known, i.e. particle diameter and length (aspect ratio), impurities, surface area and surface treatment, but some others are not (biopersistence, water solubility of the impurities, reactivity, etc.). In REACH testing data on the following human health endpoints are required for the target material: acute toxicity, skin irritation, eye irritation, skin sensitisation, mutagenicity and repeated dose toxicity (90 days via relevant exposure route). Furthermore, depending on the results of these studies carcinogenicity and reproductive/developmental toxicity testing may also be required for the target material. Available data on the target material show weak genotoxic activity but no mutagenicity. Carcinogenicity data was considered to be required.

Step 4. As a null hypothesis it is assumed that the target material exhibits a unique behaviour for carcinogenicity. CNTs from which information may be used to fill the data gaps consists of the published data on carcinogenicity for other CNTs.

Step 5. The first hypothesis to test is whether the physicochemical properties of the target material are similar to any of the source materials. Several MWCNTs with similar properties in terms of length (around 1–20 μm) and diameter (40–170 nm) have shown to cause mesotheliomas in male and female rats, and inhalation of MWCNT-7 (length 6 μm and diameter 74 nm) promoted bronchioloalveolar adenoma and carcinoma in male mice. Impurities in the source materials were not higher than in the target material. The similar properties of the source material and the target material support the use of the source materials for read-across for the evaluation of carcinogenicity.

Step 6. It was concluded that the target material should be considered as carcinogenic based on read-across for similar materials. This example was based the fibre paradigm and on the carcinogenicity of MWCNTs with similar properties as the target material.

9.5 Conclusions on grouping, read-across, extra- and intrapolation within NANoREG

Two main goals of grouping are identified. The first is initial grouping for screening purposes and the second is grouping for the purpose of read-across to fill data gaps. In a working document of ECHA, RIVM and JRC on read-across between nanoforms (ECHA et al., 2015), a stepwise procedure was proposed for using data between (nano)forms, For grouping for screening purposes, the first two steps of this procedure may already suffice.

From the examples of grouping and read across, it becomes clear that initial grouping for screening purposes is already possible for some limited nanomaterials, route of exposures and endpoints. For nanoforms, examples of read-across used within regulatory risk assessment are only available for specific narrow groups of nanomaterials for a specific route of exposure and/or endpoint. Examples are the use of read across between high-aspect-ratio CNTs and asbestos (which is currently done in many control banding tools), the SCCS evaluation of TiO_2 in sunscreens and the NIOSH RELs for CNTs and TiO_2). Applying a stepwise approach to two hypothetical nanomaterials showed that detailed information on the physicochemical characteristics is already needed to support and justify read-across for specific exposure routes and/or endpoints.

10 Conclusions and recommendations

10.1 Existing or proposed approaches

There are many initiatives and proposals for grouping of nanomaterials, but only a few of these link physico-chemical properties to the environmental and/or human toxicity of the nanomaterials. For an effective grouping, a group needs to be well-defined to determine which nanomaterials belong or do not belong within a certain group. Groups are generally defined by benchmarks, i.e. a certain value for a specific physico-chemical property that sets a boundary of the group. For nanomaterials, such benchmarks need often be a combination of several different physico-chemical properties. Especially for nanomaterials, the need for setting multidimensional groups with several criteria is acknowledged by several institutions and working groups, but lack of data often hampers setting benchmarks for grouping approaches.

10.2 Data quality and predictive capacity of QSAR

With the increasing use of nanomaterials for commercial purposes, human and environmental exposure to these materials has become more likely. However, toxicological evaluation of nanomaterials still involves many difficulties, such as the availability of a large number and variety of nanomaterials, the difficulties in categorizing nanomaterials for toxicological considerations, and the fact that even a slight variation in the characteristics of nanomaterial may also be reflected in the biological response, that dramatically increase the effort required to evaluate the potential adverse effects of nanomaterials. Therefore, there is a widespread regulatory and scientific interest in developing intelligent and cost effective testing. In particular REACH is promoting the use of alternative methods such as (Q)SAR for the purpose of categorising chemicals. As the name suggests, (Q)SAR is a computational technique that attempts to predict the biological activity of a compound by relating this activity to a set of structural and compositional properties, such as particle size, size distribution, particle shape, surface area, zeta potential, and crystal structure. The basic idea behind this approach is that different types of toxic effects (e.g., cytotoxic, genotoxic, and inflammatory effects) can be related to measurable or calculable physicochemical descriptors. QSAR models offers the advantages of higher speed and lower costs, having been seen as “an enabler” in bringing new chemicals to commercialisation. The actual accuracy of predictions however is still a critical problem because there are still several barriers that need to be overcome to establish predictive, reliable, and legally acceptable nano(Q)SAR models.

The current toxicity measurement methods used for bulk materials are not always fully adequate to examine nanomaterials and would, in any case, have to be used with due attention to the material tested. The WPMN launched a series of expert meetings to review the applicability of the OECD test guidelines to nanomaterials and to identify gaps in availability of test guidelines, resulting in a number of proposals to the OECD Test Guidelines Programme for updating existing guidelines and adding new ones with a view to better address the testing needs of nanomaterials.

One of the main issues that complicates adaptation of computational toxicity approaches to nanotoxicology is the scarcity of consistent and high-quality experimental data, which hinders the development of robust and predictive nano(Q)SAR models. The scarcity of such data is mainly caused by lack of standardized nanotoxicity testing procedures and characterization conditions for physicochemical properties, reflecting

that the scientific community is still learning to test nanomaterials. The establishment of standard protocols is essential for enabling accurate measurement of the physicochemical and biological properties of nanomaterials. There is a need to choose realistic characterization media/conditions and appropriate toxicity endpoints for nanomaterials, which will subsequently makes accurate measurement of physicochemical and biological properties possible.

The lack of knowledge about the interactions of nanomaterials with biological systems brings into question the effects of several factors, such as aggregation and coating, on the toxicity of nanomaterials. If the particle size is an important factor that directly affects the biological activity of nanomaterials, the size of aggregates in biological systems should also be considered in nanotoxicity modelling. However, there is still no clear consensus on how to characterize aggregation in relevant media.

The remaining problems in the characterization of nanomaterials for toxicity testing are directly related to the establishment of the relationship between physicochemical characteristics and the toxicological response. Therefore, the development of reliable nano(Q)SAR models requires in situ and careful characterization of nanomaterials in a relevant biological environment by taking into account the possible effect of nano-bio interactions (i.e., interactions of nanomaterials with biological components) on the basic properties (i.e., particle size, aggregation state, and coating). To be able to draw specific conclusions about the properties influencing the toxicity, it is critical to adequately define time- and media-dependent nanocharacteristics. However, whether some types of interactions and aggregation mechanisms should be included in the nano(Q)SAR modelling process is still unclear

Another issue that makes the accurate measurement of nanocharacteristics difficult is the high polydispersity of nanomaterials. To increase the quality of experimental characterization data, new analytical methods/instruments need to be developed that can deal with the polydispersity and heterogeneity of nanomaterial samples. The complex and dynamic nature of nanomaterial–media interactions should be carefully taken into account during characterisation to ensure that the measured properties are directly associated with the toxicological response.

The recent application of (Q)SAR to nanomaterials shows that researchers are starting to use such models in order to categorise nanomaterials in the same way as chemicals. This may be beneficial as it aims to improve the efficiency of hazard and risk assessment. This may be is useful in market categories where there is a restriction on *in vivo* testing. However, it is clear from this review that the valid implementation of nano-(Q)SAR is a long way off, as past experience associated even with chemicals shows the considerable amount of time and effort needed to implement the use of such tools at an internationally acceptable level.

Several issues have been highlighted in this report, which cast serious doubts over the reliability of such models to support nano-regulation:

If nano-(Q)SAR is to be implemented, then issues associated with experimental data quality used to develop the model in the first place must be tackled. We have identified the need for better analytical techniques to deal with polydispersity in a sample and when the nanomaterial is dispersed in complex media. In addition, there is a need to have validated methods and more ideal test/reference materials.

In relation to the validation of the model itself, an external validation with independent series of data should be used

Another problem that complicates the development of nano(Q)SAR is the heterogeneity of the nanomaterial family. There is a need to develop separate models that are specific to nanomaterial types and thus properties. Due to large number of nanomaterial types that can be engineered (and subsequently different mechanisms of toxicity), then individual classes of nanomaterials should be modelled separately.

In relation to the development of the model itself, there is a need to generate practical guidance e.g. how to identify relevant descriptors for nanomaterials.

In addition to guidance on what data to measure, and how and where to measure the data, it is also important to continue the development of standardized data reporting formats in nanotoxicology to facilitate consistent reporting of the outcomes of nanotoxicity studies, which will greatly facilitate data collection, database development, data mining, and resource integration efforts in the field of nanotoxicology.

Finally, the existing challenges are not only scientific but also related to insufficient communication and integration between different scientific disciplines, which lead to unnecessary overlapping of studies. Once the issues associated with reliability have been tackled, the next step is to ensure better co-ordination between the scientific community with industry and regulatory authorities. More focused research, integrated

processes, and more dialogue are required, which, in part, is currently addressed by a growing number of European projects and international efforts focusing on various areas of nanotoxicity.

10.3 Properties for human and environmental behaviour

The behaviour and effects of a nanomaterial are influenced by a combination/interaction of several physico-chemical properties. These properties can tentatively be placed in four categories:

- Substance identity, including chemical composition, crystal structure, surface coating, functionalisation and capping agents, impurities, all of which influence surface charge and reactivity;
- Particle characteristics, including size (distribution), surface area (which depends on particle size and porosity), surface roughness, shape and aspect ratio, all of which generally influence mobility and transport;
- Transport behaviour, which reflects characteristics of the nanoparticle that are (partly) influenced by the surrounding medium, such as solubility/dispersibility (rate of dissolution and equilibrium concentration, both size-related), surface charge, tendency to agglomerate, dustiness.
- Activity and reactivity, including redox potential.

It is important to note that these parameters can influence not only the toxicity and ecotoxicity of a nanomaterial, but also the interactions between the nanoparticle and the environment, whether external or within an organism. Therefore, a fundamental understanding of the physico-chemical properties and biological interactions of NPs with cells, proteins, tissues, and living organs as well as in the environment is vital to the future design of safe nanotechnologies.

10.4 Conclusions on Grouping, read-across, extra- and intrapolation within NANoREG

Two main goals of grouping are identified. The first is initial grouping for screening purposes and the second is grouping for the purpose of read-across to fill data gaps. In a working document of ECHA, RIVM and JRC on read-across between nanoforms (ECHA et al., 2015), a stepwise procedure was proposed for using data between (nano)forms, For grouping for screening purposes, the first two steps of this procedure may already suffice.

From the examples of grouping and read across, it becomes clear that initial grouping for screening purposes is already possible for some limited nanomaterials, route of exposures and endpoints. For nanoforms, examples of read-across used within regulatory risk assessment are only available for specific narrow groups of nanomaterials for a specific route of exposure and/or endpoint. Examples are the use of read across between high-aspect-ratio CNTs and asbestos (which is currently done in many control banding tools), the SCCS evaluation of TiO_2 in sunscreens and the NIOSH RELs for CNTs and TiO_2). Applying a stepwise approach to two hypothetical nanomaterials showed that detailed information on the physicochemical characteristics is already needed to support and justify read-across for specific exposure routes and/or endpoints.

11 Overall assessment/conclusions

The most important physico-chemical properties that need to be considered for read-across and grouping, as well as a stepwise approach to come to a justified grouping or read-across, have been identified. However, further development is still needed to establish justified values of specific physico-chemical properties that set the boundaries of a group, i.e. benchmarks that determine whether a nanomaterial belongs within a specific group or not. The tools to generate and collect high quality data to support these benchmarks are developed, and for some groups of nanomaterials (e.g. ion release from certain metal-based nanomaterials) sufficient data of high quality appear to be available to justify the boundaries of a group. Nevertheless, for other nanomaterials further work is needed to justify the (boundaries for) grouping for a certain information requirement under REACH (or other legislation).

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