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Appendix R.6-1: Recommendations for nanomaterials applicable to the Guidance on QSARs and Grouping

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Guidance on information requirements and chemical safety assessment

Appendix R.6-1: Recommendations for nanomaterials applicable to the Guidance on QSARs and Grouping

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PREFACE

This appendix to Chapter R.6 of the guidance on IR&CSA has been developed in order to provide advice to registrants preparing their registration dossiers for nanomaterials (NM).

This document intends to provide an approach on how to justify the use of hazard data between nanoforms of the same substance and it is presented as an Appendix to Chapter R.6 of the *Guidance on IR&CSA on QSARs and Grouping*[1] [1] because general concepts on grouping of chemicals are applicable to NM.

This appendix intends to provide advice specific to nanomaterials and does not preclude the applicability of the general principles given in the parent guidance. The parent Guidance applies when no specific information for nanomaterials is given in this appendix.

[1] The *Guidance on QSARs and grouping of chemicals* (Chapter R.6 of the Guidance on IR&CSA) will be called "parent guidance" in the content of this appendix.

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1. Introduction

This guidance is directed to registrant(s) who have identified several nanoforms of the same substance and need either to determine to which (nano)forms the available hazard data will be applicable and/or to design a testing strategy to fulfil the information requirements for the substance.

Additionally, the principles laid out in this document will also be useful for a registrant joining an existing registration that includes nanoforms and who needs to determine whether the hazard data available could also be applicable for the nanomaterials he is putting on the market.

2. Aim

In accordance with the European Commission Recommendation of 18 October 2011 on the definition of nanomaterial [2], a nanomaterial is a natural or manufactured material containing particles, in an unbound state or as an aggregate or agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimension is in the size range 1-100 nm.

Nanomaterials can be manufactured in different size distributions, shapes and by applying different surface treatments altering the surface chemistry of the particles. Changes in these parameters may render different nanoforms (see Appendix 4 to the *Guidance on Registration* [2]).

Nanoforms of a same substance will follow the same principle in terms of registration as forms of any other substance. This means that different forms of the same substance will normally be registered together in the same registration.

Due to changes in e.g. surface chemistry, nanoforms of the same substance[3] may potentially have different hazard profiles. Thus, the potential issue for the registrants is how to identify the potential differences in the (eco)toxicology properties of the identified nanoforms (and the non-nanoform): i.e. when additional hazard data, for a specific endpoint, will need to be generated. This document aims to provide a systematic and pragmatic approach for how to determine the impact of potential differences in the (eco)toxicology properties of nanoforms covered by the registration. The document also provides guidance on how to build a read-across justification and how to report it in the registration dossier ultimately submitted in fulfilment of registration obligations.

The approach consists of a stepwise approach (section 3) where the nanoforms are grouped based on relevant physicochemical parameters (that may vary depending on the endpoint being considered). The correct application of the strategy will allow determination of:

1) whether there are available hazard data for the nanoforms covered by the registration and whether they are applicable to the group(s) formed (for each specific endpoint)
2) what to consider in the testing strategy to ensure the applicability and relevance of available hazard data when data are not available for a group of nanoforms

The approach described in this document follows the updated OECD 2014 Guidance on Grouping of Chemicals [3].

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[2] OJ L275/38, 20.10.2011
[3] For definition of substance please see *Article 3(1) of REACH*

Moreover, the appendix further elaborates the considerations regarding physicochemical parameters.

Appendix 1: "Summary of key physicochemical parameters to be considered for grouping and read-across of nanoforms and their relevance for human health and environmental endpoints" outlines different physicochemical parameters and their potential influence on the hazard assessment depending on the endpoint being considered.

3. GROUPING APPROACH: A stepwise strategy for grouping of nanoforms for the purpose of hazard identification

This chapter outlines a stepwise approach for the grouping of and read-across between nanoforms (and the non-nanoform) of the same substance to ensure safe use. The general principles outlined in the parent guidance for grouping of chemicals [1] also apply to grouping of nanoforms. In addition, this guidance also incorporates developments in science, which addresses specific considerations needed in the context of nanoforms. For further information please see [4] where this considerations are summarised. Thus, this document focuses on nanomaterial specific considerations.

A large number of nanoforms may exist, as a consequence there may be many different ways to group these nanoforms. Therefore, the principles outlined below are intended to allow flexibility and thereby accommodate various grouping approaches.

This chapter outlines the general principles on how information on physicochemical properties and toxicological behaviour should be gathered and combined with expert judgement to provide a scientific rationale for the grouping of nanoforms, as well as guidance on how to document and justify each grouping approach. By seeking similarities in physicochemical properties and toxicological behaviour between different nanoforms, mainly using physicochemical parameters and/or *in vitro* screening methods, it may be possible to develop a robust scientific explanation, which supports the assumption of similar hazard properties within a defined group of nanoforms. Once the grouping approach has been successfully applied, available hazard information can be read-across to all nanoforms within the defined group. In all cases, the hazard information should be robust enough for the purposes of hazard identification, classification and labelling (C&L) and/or risk assessment.

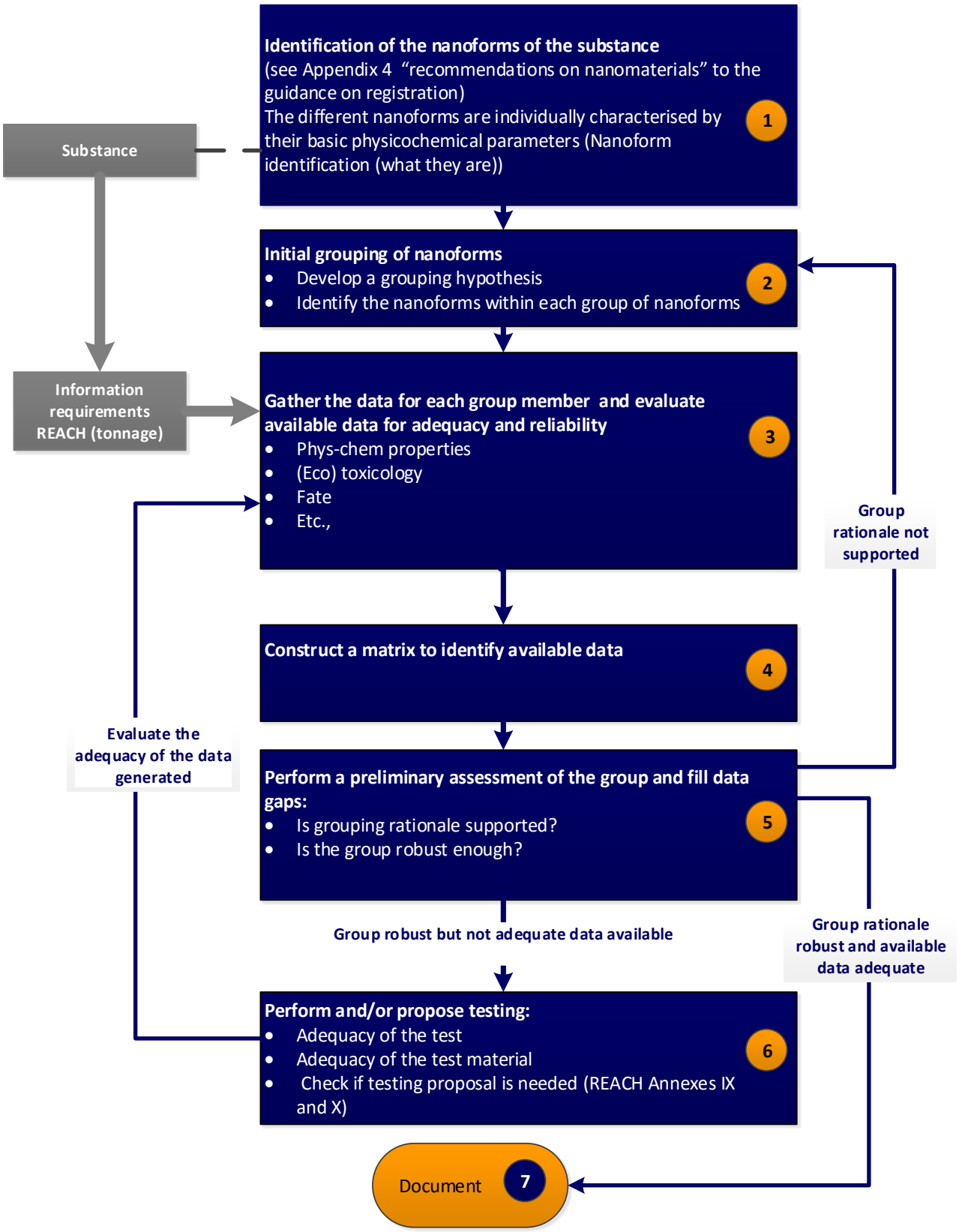
As for any chemical, grouping and read-across for nanoforms for the purpose of hazard identification are endpoint specific. A specific grouping approach must be justified endpoint by endpoint, but the same justification may be applicable to several endpoints. The decision on whether to develop the grouping strategy for one or several endpoints will depend on what scientific explanation the grouping approach is based upon. Read-across can be used as an adaptation to fill a data gap for a specific endpoint. As for any chemical, it is not the conclusion of a study (e.g. potential to be mutagenic) that is read-across, it is the results of a study conducted according to a given EU/ test method (e.g. the results of an Ames test) that are read-across from the 'source' to each 'target' nanoform within the defined group.

3.1 Stepwise strategy for grouping of nanoforms

In order to use the test results obtained with a specific nanoform to predict the properties of the other nanoforms within a given group (i.e. an analogue read-across approach), it is necessary to demonstrate that the grouping of the nanoforms and the read-across between the nanoforms is robust and justified. To facilitate data collection and a systematic and transparent documentation of the grouping and read-across approach, it is recommended to follow a stepwise approach (Figure 1) for each endpoint intended to be covered by the approach. The stepwise approach essentially follows the steps as outlined by the OECD guidance on grouping of chemicals [3].There may be alternative means to obtain the

information, thus the stepwise approach should be considered as a guide.

Figure 1: Scheme for the step-wise approach (per endpoint)

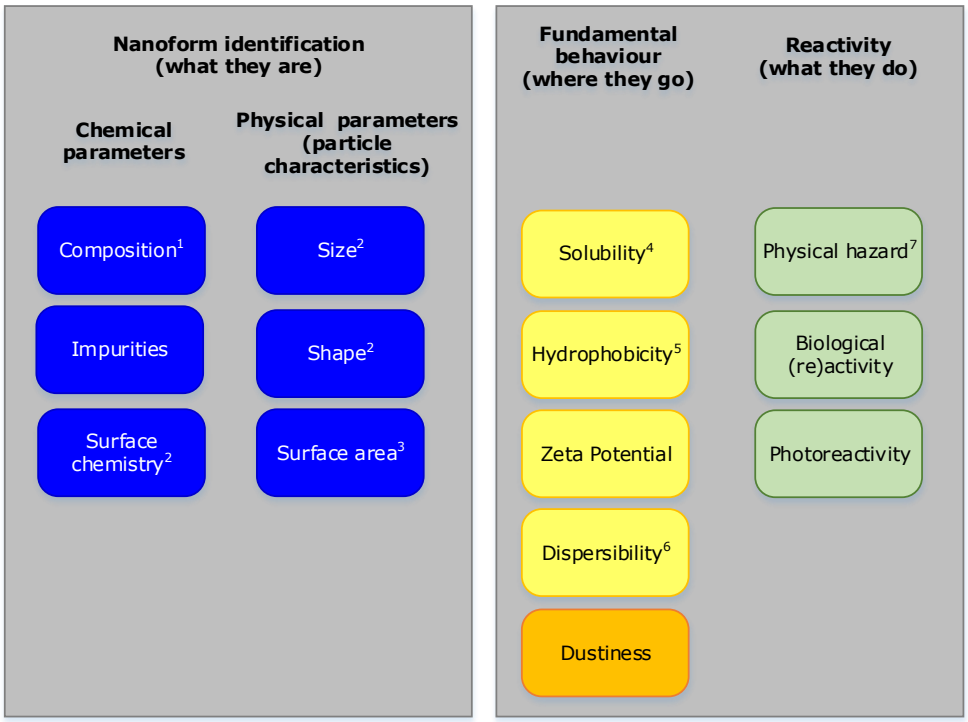


3.2 Step 1: Identification of the nanoforms

As already mentioned earlier in this guidance, a form of a substance that meets the requirements of the EC Recommendation on the definition of nanomaterial (2011/696/EU) is defined as a "nanoform". Correct and unambiguous identification of any form of a substance is a prerequisite to ensure a proper hazard assessment and thereby demonstration of safe use. Appropriate identification and characterization of form(s) of a substance includes, as a first step, consideration of the "substance identity" parameters as listed in Section 2 of Annex VI of the REACH Regulation. This includes the information on the composition of the substance, including any impurity or additive. Considerations of identification of the crystalline structure for certain types of substances such as minerals are also valid for nanoforms. Information on surface chemistry (i.e. on any surface treatment applied to the nanomaterial), must be taken into consideration for nanoforms of a substance (for further information see Appendix 4 to the *Guidance on Registration* [2]).

When dealing with nanoforms, also additional morphological parameters should be considered: these are size, surface chemistry and shape (in blue in **Figure 2**). These properties can affect exposure, toxicokinetics, fate and/or (eco)toxicological behaviour and thus the possible risk posed by nanoforms. These constitute the basic information to be considered (based on current knowledge) to implement the "stepwise strategy for grouping of nanoforms" reported above in Figure 1.

Figure 2: Key physicochemical parameters to be considered for grouping and read-across of nanoforms[4].



[4] Adapted from [4], arranged under headings from ITS-NANO [16]

[1.] Composition as reported in the dossier comprises crystalline structure and phase.
[2.] Surface chemistry includes information on surface treatments applied to the particles. Surface chemistry, size and shape are the minimum set of parameters to be considered for distinguishing one nanoform from another (e.g. as separate composition records in section 1.2 of IUCLID).
[3.] Surface area includes porosity.
[4.] Solubility includes water equilibrium solubility and rate of dissolution in relevant media.
[5.] Hydrophobicity for nanoforms is dependent on e.g. van der Waals energy, Hamaker constant, zeta potential. Analytical determination of the hydrophobicity of nanoforms is still under development, e.g. sessile drop contact angle, dye adsorption.
[6.] Dispersibility refers to the relative number or mass of particles in a suspending medium, and relates to stability [4], aggregation and agglomeration in relevant media, and is dependent on e.g. van der Waals energy, Hamaker constant, zeta potential.
[7.] Physical hazards comprise explosiveness, flammability, and autoflammability.

Size, shape and surface chemistry (i.e. surface treatment applied to the particles) are also the minimum elements that must be considered for the transparent reporting of nanoforms in section 1.2 of the IUCLID technical dossier. For details on the criteria for defining nanoforms, see the ECHA Guidance – *Appendix 4 "Recommendations for nanomaterials applicable to the Guidance on Registration* [2]".

Therefore, the parameters size, shape and surface treatment constitute the baseline for defining and characterizing a nanoform. These parameters must be taken into consideration for the identification and characterization of nanoforms of a substance, as they are fundamental in establishing the physico-chemical identity and in arriving at an understanding of "what the nanoform is".

The information on chemical parameters (e.g. different impurity profile of different manufacturing outputs) and on physical parameters (morphological parameters - "What they are") is also used for a first assessment of the possibility for bridging data gaps between - and grouping of - nanoforms of the same substance.

3.3 Step 2: Develop a grouping/read-across hypothesis, identification of group boundaries and its members

The first step in developing a group of or read-across between nanoforms is to identify the scientific basis for a robust justification. It is obvious that the starting point must include the parameters that define each nanoform in step 1. However, when developing a grouping hypothesis for the purposes of fulfilling REACH information requirements, additional parameters (other than those discussed in step 1 and reported in blue in Figure 2) that may influence the hazard properties of the nanoforms may be considered.

More specifically, the following parameters related to the fundamental behaviour of the nanoforms (respectively in yellow and green in Figure 2) must be taken into account:

- Fundamental behaviour ("where they go")
    - Solubility
    - Hydrophobicity
    - Zeta potential
    - Dispersibility
    - Dustiness
- Reactivity ("what they do")
    - Physical hazard (explosiveness, flammability, and autoflammability)
    - Biological (re)activity (redox potential, radical formation)
    - Photoreactivity.

It should be noted that differences in the morphological parameters seen when identifying the existing nanoforms may not *per se* exclude the possibility to apply read-across. Indeed, similarities in the parameters related to the fundamental behaviour e.g. solubility or those relating to their reactivity may be more important to consider when building a read-across justification.

Appendix I provides further insight on each parameter described in Figure 2, giving a particular focus on the potential influence of those parameters on hazard assessment.

The key physicochemical parameters for nanoform characterisation listed in Table 1 in Appendix 1 should be seen as a minimal set to be taken into considerations when considering grouping or read-across of nanoforms. However, some of the parameters listed above may not always be relevant for each assessment (this will depend on the nanomaterial type). For example dustiness may only apply to powders. Additional parameters (not included in **Table 1**) may need to be considered. This will depend on the specific nanomaterial type and on the endpoint considered, e.g. for acute inhalation toxicity for fibre-like materials, rigidity (stiffness) and hardness of the material may play an important role in hazard and safety assessment [5] and must therefore be considered as essential parameters for building and justifying a read-across strategy. If data is lacking for the additional physicochemical parameters that are deemed necessary for building a robust justification, additional information should be generated on a sufficient number of group members in order to demonstrate similarity across the group.

There are numerous different ways to group nanoforms of the same substance. Depending on the grouping hypothesis, different additional physicochemical parameters and/or screening methods may be needed to substantiate the grouping hypothesis and establish that the grouping has a predictive value with regard to the endpoint intended to be read-across. It is also important to consider the hazard information available on the non-nanoform(s) of the substance at an early stage of the grouping as this will provide insight into which additional parameters may be needed to establish a group of nanoforms with a predictive value for one or more endpoints.

It should be emphasised that the choice of grouping rationale will be highly case dependent. The grouping rationale may in some cases be broad and e.g. refer to a common feature of all nanoforms in a group, e.g. all nanoforms in the group have had the same surface treatment. In other cases, the grouping rationale may be more complex, e.g. based on a common surface treatment and one or more additional commonalities there may be a need to investigate other parameters e.g. dissolution rate; surface charge, induction of oxidative stress etc.). The read-across justification is in all cases endpoint specific, however, the same read-across hypothesis may apply to more than one endpoint. In general, if a grouping and read-across justification is carefully defined and robustly scientifically justified including supporting information, read-across is possible within the group and the hazard characterisation will be valid for all justified members of the group.

Some examples[5] of grouping hypothesis follow:

[5] The examples are intended to illustrate the point that different grouping strategies may be applicable to different circumstances. They are not be complete and fully exhaustive.

Example 1

- None of the nanoform(s) are surface treated;
- The non-nanoform(s) of the substance have a moderate water solubility;
- The nanoform(s) of the substance have similar water solubility to that of the non-nanoform(s).
- It is hypothesized that the hazard information available for the non-nanoform(s) is applicable also to the nanoform(s).

In this case, the amount of additional parameters needed to substantiate the hypothesis may be limited. E.g. further investigation of dissolution rates in physiological/ test media on some of the nanoforms may be sufficient in order to demonstrate possible changes in toxicokinetics behaviour can be accounted for.

Example 2

- The substance is water insoluble;
- The shape of nanoform(s) is fibre-like with a high aspect ratio;
- It is hypothesized that the substance is likely to be biopersistent and that the fibre-like structure will cause similar toxicity to that of asbestos.

In this case, it may be justified to "read across" the hazard associated with an asbestos-like fibre; or propose specific testing to further investigate the potential concern for carcinogenicity resulting from an asbestos-like fibre.

Example 3

- The non-nano form(s) of the substance have a moderate water solubility;
- The toxicity is driven by release of a toxic ion;
- The nanoform(s) are untreated or surface treated with a hydrophobic agent;
- Both oral and inhalation exposure is relevant in humans;
- It is hypothesized that two different groups can be formed based on the surface treatment: (i) a group of uncoated nanoform(s) where the toxicity is assumed to be similar to the non-nanoform(s) of the substance; and (ii) a group of nanoform(s) coated with a hydrophobic agent.

In this case, there may be different amounts and types of information needed to substantiate the grouping hypothesys. For group (i) the considerations are very much similar to that of the first example above (*i.e.* potential differences in toxicokinetics behaviour due to the particle size needs to be addressed). For group (ii) the hydrophobic nature of the surface treatment raises other questions: *e.g.* How stable is the coating?; How does the surface treatment impact the toxicokinetics of the toxic ion?; Does the surface treatment itself contribute to toxicity?; Are there route specific considerations in terms of toxicokinetics and toxicodynamics?; and How is the environmental fate of the particles affected; …

In case the nanoforms are grouped with the intention to read-across within the group based solely on similarities in physicochemical properties (what they are) and their fundamental behaviour (where they go) (Figure 2), it is essential to carefully explore the boundaries of such a group. This should include a proper characterisation of the nanoforms in the group to ensure that a justification based only on physicochemical properties is not underestimating the potential hazard of any of the group members.

In all cases the grouping and read-across hypothesis should address the following:

- Why do the similarities between the nanoforms in physicochemical properties allow for the predicting of a specific (eco)toxicological behaviour?
- A set of inclusion and/or exclusion rules that identify the ranges of applicability in terms of robust values within which reliable estimations can be made for group members for the given endpoint.

The justification should furthermore describe what information should preferably be gathered to support, or challenge, the hypothesis. It is important to note that building the hypothesis should be seen as an iterative process.

In most cases, the inclusion/exclusion rules are stringent and the group of nanoforms is limited to the nanoforms that are part of the initially formed group of nanoforms. Addition of new members to an existinggroup of nanoforms will require a consideration of the established grouping justification. It should be highlighted that also non-nanoforms of the substance may be included in the grouping approach if they display similarities in the physicochemical properties that form the basis of the grouping.

Additional questions to consider when identifying the grounds for grouping and/or read-across between nanoforms of the same substance are the following:

1. Is the nanomaterial organic or inorganic?
2. Does the nanomaterial have a coating?
3. What is the water solubility and/or dissolution rate of the nanoform?
4. Based on knowledge of the manufacturing process and/or based on analytical data, does the nanomaterial contain impurities that are of (eco)toxicological concern in relevant amounts?
5. Is there a non-nanoform of the substance? Is the non-nanoform associated with a specific hazard?
6. Is any information available about how this nanomaterial and its properties change as it ages that may be considered in (eco)toxicological and environmental fate assessment?

In some cases physicochemical similarity will not be sufficient to justify a group or a read-across. Considerations should then be given to whether additional screening methods may be needed. In (human) organisms, information on the (main) route(s) of exposure (inhalation, dermal, oral) is a first step in understanding the toxicokinetic profile of a nanomaterial. For example, for the inhalation route of exposure, specific deposition in the lungs needs to be considered. The toxicokinetic profile of a nanomaterial provides information on the absorption and subsequent exposure of target organs/tissues over time. Toxicokinetics, in a traditional sense, encompasses absorption, distribution, metabolism, and excretion (ADME). For nanomaterials toxicokinetics are further complicated by changes in the physicochemical properties of the material that may occur during these different ADME processes. The specific toxicokinetic profile of a nanomaterial depends on several different physicochemical parameters of the nanoform, e.g. composition, size, shape, agglomeration/aggregation state, surface properties (including surface charge), hydrophobicity, and dissolution
Furthermore, and parallel to the description above of the influence of environmental parameters on the fate and behaviour of a nanomaterial, the toxicokinetic profile in a (human) organism also depends on the temperature, pH and ionic strength of the biological fluid in

which the nanomaterial is taken up (e.g. serum, saliva, blood). Hence, 'system-dependent properties' (i.e. dissolution rate in biological media, surface reactivity and dispersibility), biomolecules present and interactions at the nano-bio interface[6] of cells at the target site can provide relevant information on the likelihood of distribution and potential for accumulation. Information from (available) *in vivo* studies including data on internal level of exposure and elimination over time provide further relevant information on the toxicokinetic behaviour.

Screening methods may offer a better understanding of similarities between different nanoforms in e.g. the transfer across a port of entry (skin, gastrointestinal tract, lung epithelium etc.), the deposition in the lung, tissue distribution, or clearance/persistence of the nanoforms. Such information can be obtained by a combination of certain physicochemical information (e.g. water solubility), through biophysical testing (*e.g.* dissolution rate in physiologically relevant media), *in silico* methods (e.g. multiple path particle dosimetry modelling for lung deposition), and *in vitro* testing (e.g. for skin permeability). It may also be necessary to obtain additional physicochemical parameters for selected nanoforms to be used as input for *in silico* prediction tools; e.g. information on the aggregation/ agglomeration state and aerodynamic diameter of a material in air is required for modelling to estimate lung deposition. Many of the methods have not yet been validated or are currently undergoing validation. The methods may be used at the registrant's discretion. However, the choice of methodology should be justified; this includes considerations on the constraint and limitations of the various methods used.

3.4 Step 3: Gather data for each group member and evaluate its relevance and applicability

For each nanoform that is a member of the group, all available information should be gathered and assessed for the relevance and applicability *(See Chapters R.3 and R.4 of the Guidance on IR&CSA* [6] and [7]*)* to further strengthen the justification. The data may be relating to; physical-chemical property(ies), environmental fate parameter(s), toxicological (human health) and ecotoxicity (environmental species) effect(s).

In particular, the relevance of gathered hazard data should be assessed in conjunction with a proper understanding of the characterisation of the test substance used to enable a judgement on the usability of hazard data. The requirements regarding the results of the read-across approach demand that the source study (and details of test set-up) meet all requirements for a key study used as stand-alone information to meet an information requirement under REACH (including the default REACH requirements regarding adequacy, relevance and reliability).

If there is an adequate and reliable study available within the group for the specific REACH information requirement then no additional information is needed provided that the grouping approach is robust. If however there is no adequate study available that would meet the specific REACH information requirement then additional generation of information would have to be conducted or proposed.

Test methods applied to fulfil the information requirements under REACH Annexes VII to X, cover testing in a wide variety of diverse test environments from *in vivo* tests to natural environmental samples. Nanoforms with the same substance identity may have different characteristics leading to diverse behaviour in these variable test environments. The behaviour of a specific nanoform is susceptible to change throughout its life cycle because of e.g.

[6] The 'nano–bio' interface comprises the dynamic physicochemical interactions, kinetics and thermodynamic exchanges between nanomaterial surfaces and the surfaces of biological components (for example proteins, membranes, phospholipids, endocytic vesicles, organelles, DNA and biological fluids) [17] .

exposure to other particles and/or constituents (e.g. ageing process, agglomeration, aggregation, corona formation), interactions with environmental media (e.g. dissolution, corona formation, aggregation or disaggregation, chemical reactions, transformation), and degradation/transformation (e.g. loss/modification of the coating i.e. surface chemistry). These aspects should be considered and documented, including the evaluation of available data with regards to its applicability from one nanoform for another nanoform, for different endpoints.

Differences/similarities in physicochemical parameters between nanoforms should be reflected when considering the behaviour of the nanoform in a specific environment. These physicochemical parameters are described in Table 1. Depending on the type of nanoform there might also be other additional physicochemical properties of the substance not described in this Guidance document that determine the behaviour of the substance. These aspects are advised to be reported and well described in relation to read-across justification.

Apart from physicochemical parameters of the nanoform the characteristics of the test environment should be taken into account. The role of following environmental parameters influencing the behaviour of the nanoform may be considered in justification for the read-across: temperature, pH, ionic strength (in particular, of divalent ions) and conductivity, presence and type of natural organic matter, dispersants and proteins etc.

In the environment, processes that influence transport behaviour include adsorption and desorption processes to suspended matter, aggregation and agglomeration processes, sedimentation and re-suspension, dissolution, dispersion, biodegradation (e.g. of coatings/surface chemistry), interaction with organic biomolecules at the nano-bio interface, interaction with contaminants, interaction with living organisms, and transfer via the food chain. Interactions at the nano-bio interface are clearly influenced by the type of biomolecules (proteins, exudates, etc.) that are excreted/secreted by the organism under consideration. These processes are also relevant for non-nanomaterials, but as the ecotoxicological and/or environmental fate of a nanoform depends both on its chemistry and particle characteristics the influence of these processes is particularly important for nanoforms ([8]; [4]).

Furthermore, and parallel to the description above of the influence of environmental parameters on the fate and behaviour of a nanomaterial, the toxicokinetic profile in a (human) organism also depends on the temperature, pH and ionic strength of the biological fluid in which the nanomaterial is taken up (e.g. serum, saliva, blood). Hence, 'system-dependent properties' (i.e. dissolution rate in biological media, surface reactivity and dispersibility), biomolecules present and interactions at the nano-bio interface of cells at the target site should be considered where relevant when using data from one nanoform for another nanoform. Namely, the similarities and differences between nanoforms in likelihood of distribution and potential for accumulation need to be taken into account. Furthermore, similarities and potential nano-specific differences between forms in behaviour in *in vivo* studies on internal exposure and elimination over time and at the end the effects on the toxicokinetic behaviour is to be considered.

As described above in this chapter, any change in the physicochemical parameters described in Appendix 1 may potentially affect the activity, reactivity, fate, toxicokinetics and toxicity of a nanoform in a significant way and could lead to a different behaviour. Therefore, any change in the physicochemical parameters during the life cycle may justify a careful assessment of its impact on (eco)toxicological properties to ensure that data from one nanoform may still be used for another nanoform. This may raise the questions of whether source and target nanoforms behave similarly in the environment from the moment of emission to actual exposure and inside the organism and how the specific nanoform behaves in the test medium and whether the form tested is representative for the form to which an organism or the environment is exposed. Therefore, in addition to the validity of the study itself, endpoint specific consideration on the similarities or potential differences in nanomaterial behaviour in relation to the test organism and test environment is necessary.

For reporting purposes, within the registration dossiers, gathered information e.g. studies, which are deemed relevant, should be reported as separate endpoint study records under the respective endpoint. The assessment entity in IUCLID could be used to help with a transparent reporting, further guidance is available the manual: *"How to prepare registration and PPORD dossiers"* [10].

3.5 Step 4: Construct a matrix of data availability

Once the available information has been gathered and assessed, a matrix should be constructed (endpoints covered by the grouping *vs.* members) with the group members arranged in a suitable order. The cells of the matrix should indicate whether data are available or unavailable. If possible, the cells should also indicate available reliable key study results, how these are used within the group and clearly highlight where the data gaps are.

3.6 Step 5: Perform a preliminary assessment of the group and fill data gaps

As a final step, the toxicokinetics and hazard arguments must be combined into an overall assessment of whether the gathered information is sufficient to support the proposed group/read-across and is robust enough to be used for the hazard/risk assessment. Application of available data is only deemed possible if both the toxicokinetics and hazard arguments indicate that there is no underestimation of hazard. This uncertainty should be accounted for and is not only related to the individual pieces of information but also needs to be considered in the overall assessment.

A preliminary assessment of the group should be carried out to determine whether:

- The grouping rationale is supported, i.e. the group does in fact exhibit one or more of the similarities in the physicochemical properties as postulated in step 2; and
- The group is sufficiently robust (i.e., contains sufficient, relevant and reliable information on the members) for the assessment purposes.

A preliminary assessment should be carried out for each endpoint, as the grouping approach rationale may lead to a relevant assessment for some endpoints and not for others.

If the group is sufficiently robust and the available data are adequate for the endpoint under consideration, then conclude and document your approach (step 7)

If the initial group is not sufficiently robust or justified, the following options should be considered:

- If the available data show that the group members do not have sufficiently similar properties, then the grouping hypothesis should be modified e.g. by subdivision; or
- If adequate data do not exist, but the grouping is considered robust, then testing a representative member of the group to further substantiate the justification for each information requirement would be necessary (go to step 6).
- If the proposed group cannot be adequately justified, the identified data gaps will have to be filled by either preforming the test required according to information requirements or by using the adaptions as outlined in Annex XI.

3.7 Step 6: Perform and/or propose testing

If the preliminary assessment supports the read-across or grouping rationale (i.e. similar physicochemical properties are observed for the group members), but the group does not have sufficient, relevant, and reliable information with regard to one or more endpoints, it may be necessary to perform or propose testing. Additional testing may also be needed if a new nanoform is put on the market, which has properties that result in that a group membership

cannot be granted.

Consequently, it may be that the data gap cannot be covered by developed scientific justification for the group or read-across. In these cases, the data gaps should be filled by either preforming the test in the conventional manner in accordance with Annexes VII-X for which information is missing or by the use of the adaption as laid out in Annex XI. Further guidance on how to use adaptions can be found in in *Chapter R.5 of the Guidance on IR&CSA* [9]

When performing/proposing additional testing the following aspects should be considered:

- Are the tests adequate and appropriate for the information requirement(s) in question;
- The choice of test material must be representative for what is intended to be placed on the market and the reasoning explaining why this decision does not lead to underestimation of the hazards should be documented.
- If the test is in Annex IX and X of the REACH Regulation a testing proposal is required.

3.8 Step 7: Document the finalised grouping approach and refine the grouping rationale

The finalised grouping must be documented in IUCLID by the use of Assessment Entities or under each endpoint that the group is addressing. This must be done transparently by outlining the aspects in this guidance as well as how any uncertainty has been catered for.

Appendix 1. Summary of key physicochemical parameters to be considered for grouping and read-across of nanoforms and their relevance for human health and environmental endpoints

Efforts have been made worldwide to establish a set of physicochemical parameters that would allow adequate characterisation of a nanomaterial for (regulatory) safety (risk) assessment ([8]; [10]; [11]; [12]; [13]; [14], [15]), and the topic is still under debate.

Table 1 of this Annex provides details on each of the key properties/parameters already presented in Figure 2. There is in fact a general agreement among the scientific fora that the parameters listed must be taken into considerations when dealing with safety assessment of nanomaterials. The relevance of these parameters on environmental and human health endpoints is also highlighted in the Table. A more extensive overview of the current understanding of the potential influence of these different physicochemical parameters on the toxicological properties of a nanoform can be found in the scientific literature (e.g. [8]; [4]).

Table 1: key physicochemical parameters to be considered for grouping and read-across of nanoforms and their relevance for human health and environmental endpoints

Chemical parameters (What they are)
Chemical composition, including crystalline structure Detailed information on chemical composition is fundamental for determining human health and environmental effects of nanoforms, as is the case for non-nanoforms. However, size, shape and surface characteristics of a nanoform may cause the nanoform to exhibit a different behaviour compared to the non-nanoform of a material with the same composition. Crystalline structure may for some nanoforms influence other properties of the material (e.g. reactivity, zeta potential, Hamaker constant) in a way that affects human and environmental toxicity. Decreasing size of particles may introduce crystallographic changes in the material (contraction of the crystalline lattice or deformation). Based on the present understanding of nanoparticle behaviour, changes to the crystalline structure seems particularly relevant for metals, metal-oxides or carbon based nanomaterials.
Impurities As for non-nanoforms, impurities can substantially contribute to the human and environmental toxicity of nanoforms.
Surface chemistry (coating, functionalization) Considering human health endpoints, the surface chemistry of a nanoform affects its reactivity and systemic absorption. Surface modification(s) may determine which biomolecules adhere to the nanoform, its distribution and cellular uptake, and its toxic effects. In the environment surface characteristics will influence sorption to environmental or biological media and the reactivity of a nanoform. In addition, similarly to considerations for human health endpoints, the surface chemistry of a nanoform affects its reactivity and systemic absorption. Surface modification(s) may determine the biomolecules that adhere to the nanomaterial, its distribution and cellular uptake, and its toxic effects.
Physical parameters/ Particle characteristics (What they are)
Particle size / range The size of the nanoform affects other physicochemical parameters, such as crystallinity, zeta potential and specific surface area, and may determine exposure, and whether the nanoparticle can be internalised into an organism. Once internalised, particle size may also affect the distribution within the body, and the toxicity at both the point of entry and distally. Size distribution is not a static parameter; it may also change during the course of (environmental) toxicity testing (as well as during the life cycle of the material) due to e.g. partial dissolution, interaction with test media or preferential absorption of smaller particles.

Shape Particle shape may affect the internalisation of a nanoform (e.g. the ability of a nanoform to penetrate into a cell) and its (environmental) toxicity. In inhalation studies, particle shape may influence nanoform deposition within the lungs and may also influence its persistence in the lungs and probably in other sites. Particle shape may also influence other parameters, such as zeta potential.
Surface area, including porosity The increase of relative surface area with decreasing particle size may increase the reactivity of a nanoform relative to its mass. Furthermore, as a consequence of the increased surface to volume ratio, porosity may affect the crystalline structure.
Fundamental behaviour (Where they go)
Solubility: Rate of dissolution / Equilibrium solubility The rate of dissolution depends on the chemical composition, particle size, coating, stability, manufacturing process, and biological environment; for substances that have a high rate of dissolution, the ion(s) may also dictate the toxicity of the nanoforms, which will be an important aspect of the evaluation. 'Water solubility' is an intrinsic material property, but in most cases the system-dependent property 'dissolution rate in relevant biological media' will be more relevant as this fundamentally affects the bioavailability of substances in the (biological) environment. The relevance of the different media depends on the actual route of exposure and/or the environmental compartment under evaluation.
Hydrophobicity Hydrophobicity for nanoforms is dependent on e.g. Van der Waals energy, Hamaker constant and zeta potential. Analytical determination of the hydrophobicity of nanoforms is still under development, e.g. sessile drop contact angle, dye adsorption. While these parameters can influence agglomeration and sorption, as well as 'dispersibility in biological media' and dustiness, currently the exact relationships between them are not clear
Zeta potential Surface charge may influence systemic distribution and cellular uptake of a nanoform, and ultimately its toxicity.
Dispersibility This parameter can influence the degree of environmental transport and (environmental) exposure. Furthermore, this parameter may influence the degree of internal exposure (particularly by the oral route; however particle dispersibility also affects nanomaterial mobility within the lung and hence its potential for systemic uptake).
Dustiness This parameter is mainly relevant for exposure via air (particularly by inhalation) and transport through air. In the environment this parameter is not relevant to aquatic/sediment exposures and only to a limited extent for soil exposures.
Reactivity (What they do)
Physical hazards: Flammability / Autoflammability / Explosiveness These parameters are relevant in assessing the risk of injury in occupational settings, but are not primary parameters characterised in (environmental) toxicity studies.
Biological (re)activity (or surface reactivity) The biological (re)activity or surface reactivity of a nanoform of a substance appears to generate reactive oxygen species (ROS) which induce inflammation, and thus elicit cellular toxicity.
Photoreactivity Photoreactivity may increase with decreasing particle size. In human toxicity testing, this parameter may be particularly relevant when considering dermal exposure, but it may also play a role in other exposure routes. In the environment this parameter may be particularly relevant when considering the aquatic compartment, but it may also play a role in other compartments.

For physicochemical characterisation of a nanoform, a distinction can be made between intrinsic material properties (such as chemical composition, primary particle size, shape, and water solubility) and system-dependent properties defined by the surroundings in which the nanoform is placed (e.g. dissolution rate in biological media, surface reactivity and dispersibility). The current level of knowledge does not allow deducing possible correlations between intrinsic material properties and apical toxic effects. It is therefore important to consider both the intrinsic properties of a nanoform and the available knowledge with regard to system dependent properties, biophysical interactions and in vitro effects to justify read-across.

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