

Guidance on information requirements and chemical safety assessment

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

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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 registration dossiers that cover nanoforms¹.

This document is aimed at providing an approach on how to justify the use of hazard data between nanoforms (and the non-nanoform(s)) and within groups of nanoforms of the same substance. It is presented as an Appendix to Chapter R.6 of the *Guidance on IR&CSA on QSARs and Grouping*² [1] because general concepts on grouping of chemicals are applicable to nanomaterials. Please note that no specific advice for QSARs with respect to nanomaterials is provided in this version of the guidance, as at present the state of the art does not allow provision of recommendations in this respect.

The approach and general principles provided in this document, together with the advice provided in the parent guidance, may also be useful when considering read-across between nanoforms of different substances.

This appendix is aimed at providing advice specific to nanomaterials and does not preclude the application of the general principles given in the parent guidance on the same matter.

Please note that this document does not provide endpoint specific guidance on how to meet the information requirements set out in Annexes VI to XI to the REACH Regulation. Such information is provided in Chapters R.7a, R.7b and R.7c of the *Guidance on IR&CSA* and its nanospecific Appendices on "recommendations for nanomaterials".

General information on how to meet the information requirements, such as collection and evaluation of available information, and on adaptation of information requirements is available in Chapter R.2 to R.5 of *Guidance on IR&CSA*) and is considered to also be applicable for nanomaterials.

¹ Please see *How to prepare registration dossiers that cover nanoforms: best practices* [3]

² The *Guidance on QSARs and grouping of chemicals* (Chapter R.6 of the *Guidance on IR&CSA*) will be referred to as "the parent guidance" in the content of this appendix.

Table of Contents

1. INTRODUCTION	6
2. AIM	6
3. GROUPING APPROACH: A STEPWISE STRATEGY FOR GROUPING OF NANOFORMS FOR THE PURPOSE OF HAZARD AND FATE IDENTIFICATION	7
3.1 Stepwise strategy for grouping of nanoforms	8
3.2 Step 1: Identification and Characterisation of the nanoforms of the substance	10
3.3 Step 2: Initial grouping of nanoforms – Develop a grouping and/or read-across hypothesis, identification of a group’s boundaries and its members	12
3.4 Step 3: Gather the available data for each group member and evaluate the data for adequacy and reliability	18
3.5 Step 4: Construct a matrix of data availability.....	19
3.6 Step 5: Assess the adequacy of the approach and fill data gaps within the group	19
3.7 Step 6: Perform and/or propose testing to fill the data gap for the whole group	20
3.8 Step 7: Document the finalised grouping approach and refine the grouping rationale ..	20
APPENDIX 1. SUMMARY OF KEY PHYSICOCHEMICAL PARAMETERS RELEVANT FOR GROUPING AND READ-ACROSS OF NANOFORMS AND THEIR RELEVANCE FOR HUMAN HEALTH AND ENVIRONMENTAL ENDPOINTS	21
APPENDIX 2. EXAMPLE OF TEMPLATE FOR READ-ACROSS MATRIX	24
REFERENCES	26

Table of Figures

Figure 1: Scheme for the step-wise approach.....	9
Figure 2: Key physicochemical parameters possibly relevant for grouping and read-across of nanoforms.	11

Table of Tables

Table 1. Relevance of selected environmental processes/parameters in different environmental compartments (not exclusive).	18
Table 2: Key physicochemical parameters to be considered for grouping and read-across of nanoforms and their relevance for human health and environmental endpoints	22
Table 3: Example of a read-across matrix template for nanoforms	24

1. Introduction

This guidance is directed to registrant(s) who have identified several nanoforms of the same substance³ and need either to determine to which forms (including non-nanoforms) 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 potential registrant joining an existing registration that includes (nano)forms and who needs to determine whether the hazard data available could also be applicable for the nanomaterials he is putting on the market.

The principles and approach taken to justify use of hazard data between (nano)forms of the same substance are similar to those used for read-across and grouping between different substances. Thus, in this document the read-across terminology is used, even if it is acknowledged that currently the text of Annex XI of REACH addresses read-across between substances and not read-across between forms of the same substance. Nevertheless, the rationale of read-across may be extrapolated to forms of the same substance and the terminology used in Annex XI of REACH can therefore also be relevant in these cases. Explanation on general grouping and read-across principles and explanations of general terms such as "target"⁴ and "source"⁵ can be found in the parent guidance and in the OECD 2014 Guidance on Grouping of Chemicals [2].

2. Aim

In a REACH context, a nanomaterial is a manufactured material that meets the requirements of the Commission Recommendation on the definition of nanomaterial^{6,7}.

Nanomaterials can be manufactured in different size distributions, shapes and by applying different surface treatments; the surface chemistry of the particles can be altered as well. Changes in these parameters may result in different nanoforms (for more information on nanoforms, see the document: *How to prepare registration dossiers that cover nanoforms: best practices* [3]).

Nanoforms of the same substance will follow the same principles for registration as forms of any other substance. This means that different forms of the same substance will be registered together in the same registration.

Due to differences in physicochemical parameters e.g. surface chemistry, nanoforms of the same substance may potentially have different hazard profiles. Thus, the issue for the registrants is how to determine whether there are differences in the (eco)toxicological properties of the nanoforms (and the non-nanoform): i.e. when additional hazard data, for a specific endpoint, will need to be generated.

The hazards posed by all possible forms of the substance covered by a registration, including nanoforms, must be addressed by the toxicological and ecotoxicological information provided

³ For the definition of "substance" please see *Article 3(1) of REACH*

⁴ A target chemical is one with data gap(s), for which a property or hazard is being estimated from the source chemical(s). [2]

⁵ A chemical whose properties are being used to make an estimate can be referred to as a source chemical

⁶ COMMISSION RECOMMENDATION of 18 October 2011 on the definition of nanomaterial (2011/696/EU) available at : <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:275:0038:0040:en:PDF>

⁷ Please note that the EC Recommendation of definition of a nanomaterial is currently under revision, once it is updated, ECHA will consider it and update the references to it in the ECHA Guidance, if relevant.

in the registration dossier.

This document aims to provide a systematic and pragmatic approach on how to assess whether there are differences in the (eco)toxicological properties and fate of the nanoforms (and the non-nanoform) that are 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 the data 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 principles outlined in the updated OECD 2014 Guidance on Grouping of Chemicals [2]. Moreover, in its appendix, the OECD guidance further elaborates the considerations regarding physicochemical parameters of nanomaterials.

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 and fate identification

This chapter outlines a stepwise approach for the grouping of and read-across between nanoforms (and the non-nanoform(s)) 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. Thus, this document focuses on nanomaterial specific considerations and incorporates developments in science, which address specific considerations needed in the context of nanoforms. For further information please see [4] where these considerations are summarised. The approach developed by ECETOC [5] also provides useful information for the grouping of and read-across between nanoforms (and the non-nanoform(s)). Additional information can be found in other publications, see for instance [6], [7] and [8].

A large number of nanoforms may exist. As a consequence, there may be many different ways to group nanoforms depending on the endpoint(s) to be assessed and the information available. 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, toxicokinetic and (eco)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, toxicokinetic behaviour and fate, and (eco)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 scientific basis for the grouping approach has been established and clearly justified, available hazard information (for the specific endpoint) 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 test method⁸ or otherwise internationally recognised protocol (e.g. the results of a mammalian gene mutation test) that are read-across from the 'source' to each 'target' nanoform within the defined group (for further information please see the parent guidance).

3.1 Stepwise strategy for grouping of nanoforms

In order to use the test results obtained with a specific source material to predict the properties of the other (nano)forms (targets) within a given group (i.e. read-across approach), it is necessary to demonstrate that the grouping of the nanoforms and the read-across between the source material and the target nanoforms is robust and justified⁹. 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 (see Figure 1) for each endpoint intended to be covered by the approach. The stepwise approach essentially follows the steps outlined by the OECD guidance on grouping of chemicals [2]. There may be alternative means to obtain the information, thus the stepwise approach should be considered as a recommendation.

The proposed approach (except the identification of the nanoforms) is endpoint specific. However, in practice, several endpoints can be addressed at the same time as long as it is transparently explained which endpoints are covered and why the grouping/ read-across hypothesis is applicable to all of them.

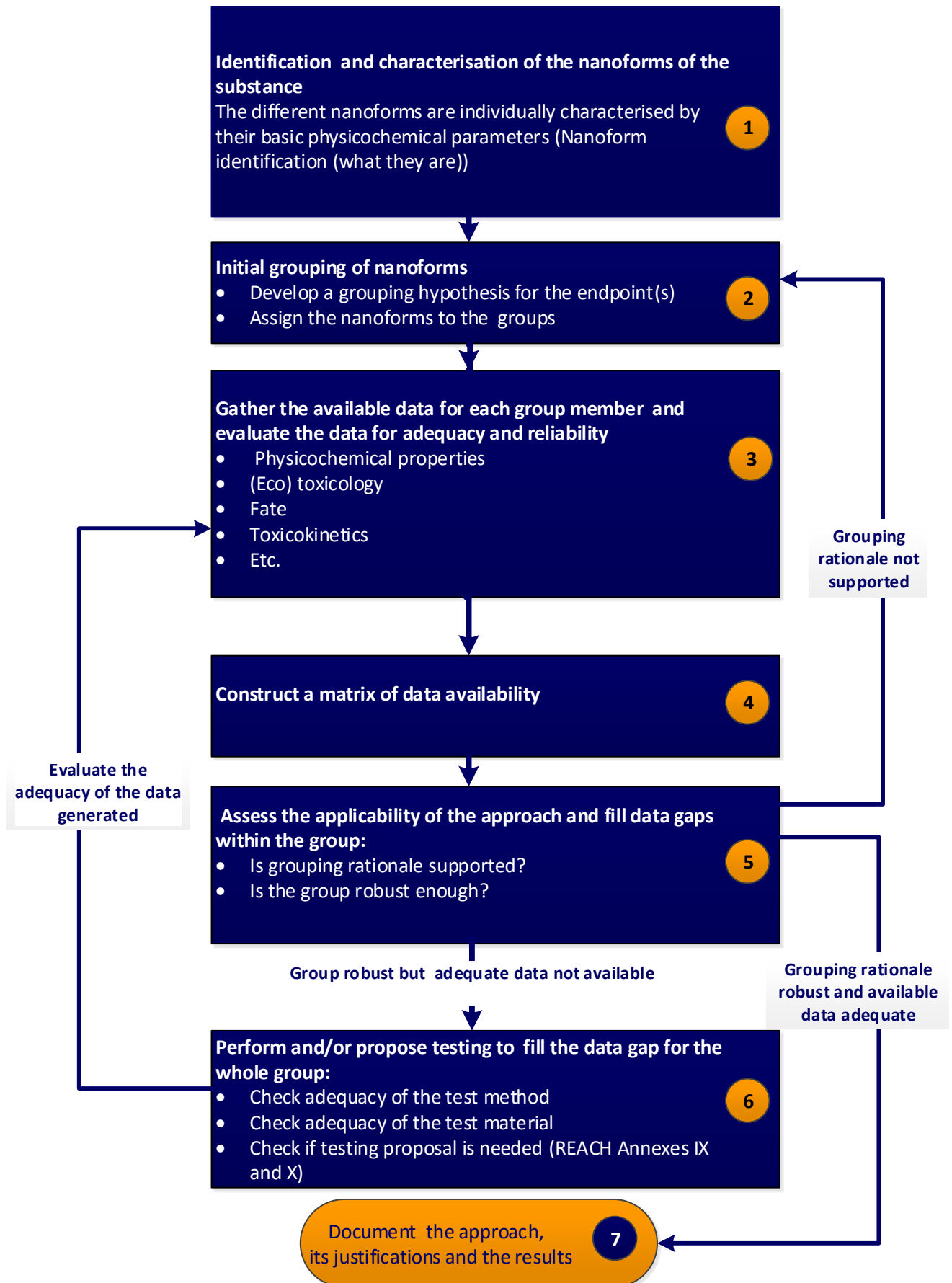
The approach can also be used by registrants joining an existing registration who want to determine whether the data available is applicable to their nanoform(s).

⁸ Please note that not all test methods have been adapted to cover nanomaterials; for advice on testing of nanomaterials in a REACH context please see *Appendices for nanomaterials to Chapters R.7a, R.7b and R.7c of the Guidance on IR&CSA*.

⁹ Section R.6.2 of the parent guidance provides further general advice on how to assess the robustness of the grouping;

General information for meeting the information requirements such as collection and evaluation of available information, and adaptation of information requirements is available in Chapter R.2 to R.5 of Guidance on IR&CSA).

Figure 1: Scheme for the step-wise approach¹⁰



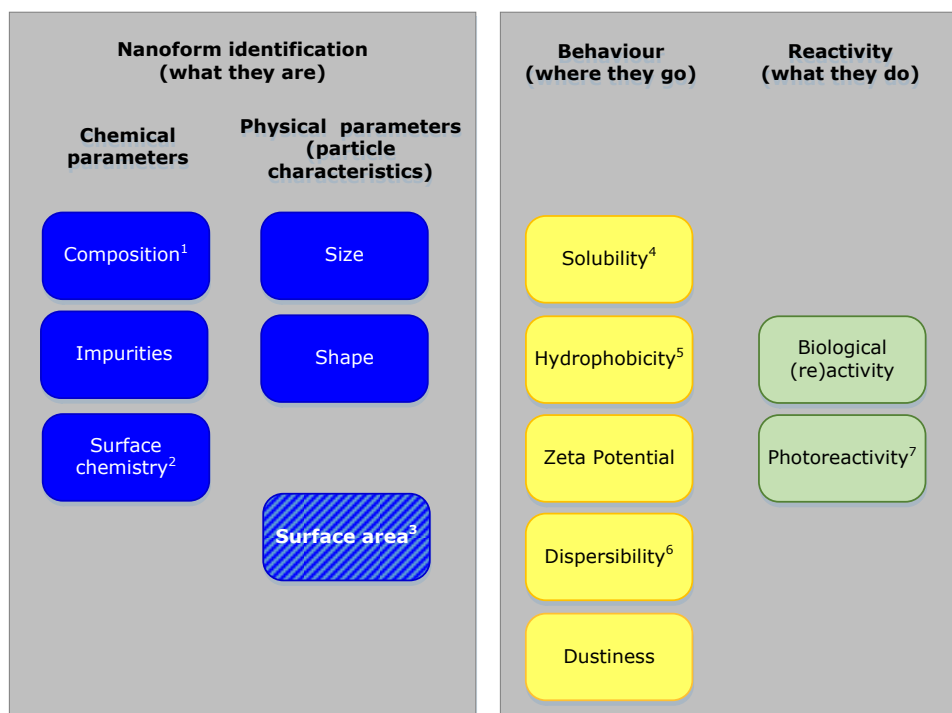
3.2 Step 1: Identification and Characterisation of the nanoforms of the substance

As already mentioned in this guidance, a substance may comprise different nanoforms due to variation in physicochemical parameters such as size, shape or surface treatments. The hazards of forms must be addressed in the registration. Correct and unambiguous characterisation of the (nano)form(s) of a substance is a prerequisite to ensure a proper hazard assessment and thereby demonstrate safe use. Appropriate identification 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 impurities or additives. As for any substances, considerations of the crystalline structure are also relevant for nanoforms. Information on surface chemistry (and in particular relating to any surface treatment applied to the nanoform) should be taken into consideration for nanoforms of a substance.

In addition to the parameters required to identify substances, when dealing with nanoforms, additional physical (morphological) parameters should also be considered: these are size, surface area and shape (in blue boxes 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 for grouping and read-across (based on current knowledge) to implement the “stepwise strategy for grouping of nanoforms” proposed above in Figure 1.

Figure 2 identifies parameters that can provide useful information to help grouping nanoforms. This includes properties that are not covered by the information requirements specified in REACH Annexes VI to X and properties for which there is a need to develop and validate suitable test methods. Given the lack of validated methods for all potentially relevant parameters, it is important that registrants include a discussion of the uncertainties in these methods and consequent results in their grouping approach as part of the justification.

¹⁰ All the steps are endpoint specific, except step 1, which does not belong strictly to the read-across and grouping approach, but which will in most cases be the starting point when using this approach in the context of REACH.

Figure 2: Key physicochemical parameters possibly relevant for grouping and read-across of nanoforms¹¹.

¹ Composition as reported in the dossier comprises chemical composition as described in the *ECHA Guidance for identification and naming of substances under REACH and CLP* [9].

² Surface chemistry includes information on, for instance, chemical coating and surface treatment(s) applied to the particles.

³ Surface area appears within the nanoform identification parameters as it can also be used to show compliance with the EC Definition [3] (it appears striped in the figure, as it is not always the case that it is used to determine whether the substance is a nanomaterial). Surface area includes porosity.

⁴ Solubility includes rate of dissolution and equilibrium solubility in relevant media.

⁵ Hydrophobicity for nanoforms is dependent on e.g. van der Waals energy and surface charge.

⁶ Dispersibility refers to the relative number or mass of particles in a suspending medium and therefore it is media-dependent. It relates to stability [8], aggregation and agglomeration in relevant media, and is dependent on e.g. van der Waals energy, Hamaker constant, zeta potential.

⁷ Photoreactivity refers to activity that enables substances to participate in or to initiate a reaction due to light. "Photo" indicates the energy source causing the activity (light). If the molecule itself becomes a radical it may easily react and be transformed. If oxygen radicals are induced (i.e. reactive oxygen species or ROS), they may easily react with other molecules, which in some cases may lead to severe effects (e.g. reaction with DNA leads to genotoxicity).

Size, shape and surface chemistry (e.g. functionalisation or coating(s) applied to the particles) may have an impact on the hazardous properties of nanoforms. However, the hazards posed by all possible forms of the substance must be addressed by the toxicological and ecotoxicological information submitted. Therefore, the above parameters should be considered to justify the use of hazard data between nanoforms. Consequently, the chemical composition and the parameters size, shape and surface treatment constitute the baseline.

¹¹ Adapted from [8]

. These parameters may be taken into consideration for the characterisation of nanoforms of a substance, when establishing their physicochemical identity and in arriving at an understanding of “what the nanoform is”.

The information on chemical parameters (e.g. different impurities) and on physical parameters (“What they are”) may also be used for grouping as well as for the assessment of the possibility of filling data gaps for nanoforms of the same substance.

The parameters related to the behaviour and reactivity of the nanoforms (respectively in yellow and green boxes in Figure 2) need to be taken into account, but may not be relevant for all assessments (for that reason these parameters are explained in the next step as they need to be considered when developing the hypothesis). The influence of these parameters on the behaviour of the nanoforms is explained in Section 3.3 and Appendix 1.

3.3 Step 2: Initial grouping of nanoforms – Develop a grouping and/or read-across hypothesis, identification of a group’s boundaries and its members

The second step for developing a group of nanoforms for which read-across may be used, defines the purpose (endpoint(s)), and develops the hypothesis and the scientific basis for a robust justification. It is obvious that the starting point must include the parameters from 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 presented in blue boxes in Figure 2) that possibly influence the hazard properties of the nanoforms may need to be considered as well to substantiate the grouping/read-across hypothesis.

The following parameters related to the behaviour and reactivity of the nanoforms (respectively in yellow and green boxes in Figure 2) are the parameters that are often relevant to substantiate the hypothesis, and should therefore be considered when relevant:

- Behaviour (“where they go”)
 - Solubility (including dissolution rate)
 - Hydrophobicity
 - Zeta potential
 - Dispersibility
 - Dustiness
- Reactivity (“what they do”)
 - Biological (re)activity (e.g. redox potential, radical formation)
 - Photoreactivity.

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

Appendix 1 provides further insight into each parameter described in Figure 2, focusing in particular on the potential influence of those parameters on hazard assessment.

The key physicochemical parameters for nanoform characterisation listed in Appendix 1 may provide useful information that can help grouping of nanoforms. However, some of the parameters listed above may not always be relevant for each assessment (this will depend on the substance and its different forms, on the endpoint, and the organism, or compartment

considered). For example, dustiness may only apply to powders. Depending on the read-across hypothesis, it could be useful to consider additional parameters (not included in Appendix 1). This will depend on the specific nanomaterial type and on the endpoint considered, e.g. for inhalation toxicity for fibre-like materials, rigidity and hardness of the material may play an important role in hazard and safety assessment [10] and must therefore be considered as essential parameters for building and justifying a read-across strategy. If there is not sufficient information on the additional physicochemical parameters for a robust conclusion, additional data may be needed in order to sufficiently demonstrate similarity across the group.

There are different ways to group (nano)forms of the same substance. 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 there are several ways to scientifically justify a specific grouping and the choice will be highly case dependent. The scientific justification may be broad in some cases and for instance 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 justification 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 such as dissolution rate, surface charge, induction of oxidative stress, etc. The hypothesis and the read-across justification are in all cases endpoint specific, however, the same read-across hypothesis may apply to more than one endpoint. A robust grouping and read-across justification needs to be carefully defined and scientifically justified. The justification should include supporting information indicating that read-across is possible within the group, such that the hazard characterization will be valid for all members of the group.

Some examples¹² of grouping hypotheses follow; see examples 1 to 3:

¹² The examples are intended to illustrate that different grouping strategies may be applicable to different circumstances. The examples do not describe a complete set of conditions to consider when justifying a read-across. Please note that the considerations made for justification need to be substantiated (e.g. if one condition is that both the non-nanoform and the nanoform have similar solubility, this needs to be substantiated).

Example 1: hypothesis based on solubility

Available information:

- The target nanoform(s) of the substance has a high dissolution rate and a similar high water solubility to the source non-nanoform of the substance; therefore it is assumed that the particle effect can be excluded in this specific case;
- The nanoform(s) are not surface treated;
- The systemic toxicity is driven by release of toxic ions.

Basis for hypothesis:

Due to the high dissolution rate and a similar water solubility, it could be claimed that the source non-nanoform and the target nanoform(s) have similar toxicokinetic behaviour and the same toxicological effects based on the hypothesis of ion driven toxicity. Therefore, the systemic toxicity for the target nanoforms(s) of the substance can be predicted from the available studies conducted with the source non-nanoform of the substance.

Further information needed to support the hypothesis:

To support such a claim, additional information is needed that demonstrates similar toxicokinetic behaviour (i.e. independent of particle size) of both the source non-nanoform and target nanoform(s), e.g. absorption studies and/or dissolution rate studies in different physiological media.

Example 2: hypothesis based on high aspect ratio

Available information:

- The substance is insoluble in water and biological media;
- The shape of both the source and target nanoform(s) is fibre-like with a high aspect ratio;

Basis for hypothesis:

It is hypothesized that both nanoforms are biopersistent and that the fibre-like structure will cause similar adverse effects via inhalation.

Further information needed to support the hypothesis:

In this case, it may be justified to "read-across" the hazard associated with the source nanoform and no further information would be needed if a similar aspect ratio can be demonstrated together with insolubility in water and biological media. In addition, rigidity and hardness of the material may play an important role in hazard and safety assessment and may need to be considered

Example 3: impact of surface treatment on grouping

Available information:

- The non-nano form(s) of the substance have a moderate water solubility;
- The toxicity is driven by release of toxic ions;
- The nanoform(s) are untreated or surface treated with a hydrophobic agent;
- The untreated nanoform shows similar water solubility to that of the non-nanoform;

Basis for hypothesis:

It is hypothesized that two different groups can be formed: (i) a group of non-treated nanoform(s) where the toxicity is assumed to be similar to that of the non-nanoform(s) of the substance; and (ii) a group of nanoform(s) surface treated with a hydrophobic agent.

Further information needed to support the hypothesis:

In this case, there may be different types of information needed to substantiate the two grouping hypotheses. For group (i) the considerations are very much similar to that of Example 1, 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 affect the toxicokinetics of the toxic ion? Does the surface treatment itself contribute to toxicity? Are there route specific considerations in terms of toxicokinetics? How is the environmental fate of the particles affected? etc. ...

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"), their reactivity ("what they do") and their 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/ differences between the nanoforms in physicochemical properties allow for predicting of a specific (eco)toxicological behaviour?
- A set of inclusion and/or exclusion rules that determine 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 the information needed to support, or challenge, the hypothesis. It is important to note that building the hypothesis should be seen as an iterative process (see Figure 1).

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. Addition of new members to an existing group of nanoforms will require a consideration of the established grouping justification. This may include an assessment of whether the new nanoform fits the existing grouping approach and, if this is not the case, reconsideration of the established approach. It should be highlighted that non-nanoforms of the substance may also be included in the grouping approach, if they display similarities in the physicochemical properties that form the

basis of the grouping.

Examples of considerations to be taken into account when identifying the basis for grouping and/or read-across between nanoforms of the same substance are the following:

1. Is there a non-nanoform of the substance? Is the non-nanoform associated with a specific hazard?
2. How do the differences in size and/or surface treatment(s) influence the assessment?
3. How would the assessment be affected by changes in water solubility and/or dissolution rate?
4. How do the variations of the composition and/or impurities /additives in the nanoforms impact the assessment?
5. How does the available information about ageing of the nanoforms impact the assessment of (eco)toxicological properties and environmental fate? [11]
6. How do the physicochemical properties of a nanoform affect the behaviour in a specific (eco)toxicological test environment?

Differences/similarities in physicochemical parameters between different (nano)forms should be assessed in view of their behaviour in various environments/media. The physicochemical parameters described in Appendix 1 and Figure 2 are only examples. However, depending on the type of nanoform, there might be other physicochemical properties that could be useful for determining the behaviour of the substance. If such properties are taken into account, these should be reported (including the methods used to determine their value) and well described in the read-across justification.

In some cases, physicochemical similarity (e.g. considering the parameters described in Figure 2) will not be sufficient to justify a group or read-across. In addition, consideration should be given to whether e.g. information on toxicokinetics (obtained e.g. by the use of additional screening methods) is needed. Information on toxicokinetic behaviour is normally useful when constructing a read-across justification. The specific toxicokinetic behaviour 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) and dissolution. Qualitative comparisons of how different nanoforms may be expected to behave in the body or in the environment may provide useful supporting arguments to justify read-across. The scientific basis for these qualitative comparisons and the associated uncertainties must be clearly explained in the justification.

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 inhalation, the potential for 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 may be 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 may depend on several different physicochemical parameters of the nanoform, e.g. composition, size, shape, agglomeration/aggregation state, surface properties (including surface charge), hydrophobicity, or dissolution. 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 interface¹³ of cells at the target site may provide

¹³ 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) [31] .

relevant information on the likelihood of distribution and potential for accumulation and excretion. Information from (available) *in vivo* studies including data on the internal level of exposure and elimination over time provide further relevant information on the toxicokinetic behaviour.

Screening methods (*in vitro*) may offer a better understanding of similarities between the behaviour of 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 and interaction with components of 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 constraints and limitations of the various methods used.

It is important to note that the behaviour of a specific nanoform in the environment is dependent on its surface chemistry and is susceptible to change throughout its life cycle because of e.g.:

- exposure to other particles and/or constituents (e.g. ageing process, (hetero)agglomeration, 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).

Therefore, apart from physicochemical parameters of the nanoform, the characteristics of the test environment/media should be taken into account. The role of the following environmental parameters influencing the behaviour of the nanoform may be considered in the 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. The list of environmental parameters is not exhaustive and, when relevant, other parameters may need to be considered.

In the environment, processes that influence transport behaviour include adsorption and desorption processes to suspended matter or soil particles, (hetero)aggregation and (hetero)agglomeration processes, sedimentation and re-suspension, dissolution, dispersion, (bio)degradation (e.g. of coatings/surface chemistry, oxidation, reduction, photodegradation), 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 depend both on its (surface)chemistry and particle characteristics the influence of these processes may be particularly important for nanoforms ([7], [12]). The relevance of specific processes in the read-across assessment is therefore always linked to both the surface chemistry of the nanoparticle and the characteristics of the receiving environment. Table 1 describes the relevance of some selected environmental compartment specific processes/parameters (in line with [12]). Described processes/parameters are not exclusive and relevance in read-across assessment is highly case specific. Further environmental endpoint specific considerations are described in *the Appendices for nanomaterials to Chapters R.7a, R.7b and R.7c of the Guidance on IR&CSA*.

Table 1. Relevance of selected environmental processes/parameters in different environmental compartments (not exclusive).

Parameter [#]	Air	Water	Sediment	Soil
Redox reactions	+	++	++	+
Dissolution/speciation	+	++	++	++
(hetero)aggregation/(hetero)agglomeration	+	++	++	++
Interactions with natural organic matter (NOM)	o	+	++	++
Nano-bio interface	o	+	+	+
Adsorption/desorption	+ ^c	+	++	++
Sedimentation	o	++	++	o
Photochemical degradation^a	++	+	o	o
(Bio)degradation^b	o	#	#	#

#: Highly dependent on chemical composition and surface chemistry of the nanomaterial.

++: High relevance

+: Relevance

o: Low relevance

^a: The parent guidance describes the challenges and limitations of the use of information on photochemical degradation in classification and chemical safety assessment.

^b: More information on transformation processes in *Appendix to Chapter R.7b of the Guidance on IR&CSA*.

^c: adsorption/desorption of other substances

Taking into account the above, any significant changes in the physicochemical parameters during the life cycle raise questions on whether the source material(s) and target nanoforms behave similarly in the environment from the moment of emission to actual exposure to organism or environment, inside the organism, and in the test medium; justifying a careful assessment of the adequacy of grouping hypothesis and read-across of (eco)toxicological properties between (nano)forms. Therefore, endpoint specific consideration should be made on the similarities or potential differences between source and target form behaviour in relation to the test organism and test environment.

3.4 Step 3: Gather the available data for each group member and evaluate the data for adequacy and reliability

For each nanoform that is a member of the group, all available information should be gathered and assessed for its relevance and applicability (*See Chapters R.3 and R.4 of the Guidance on IR&CSA* [13] and [14]) to further strengthen the hypothesis and justification. The data may relate to physicochemical property(ies), environmental fate parameter(s) and (eco)toxicological (human health and 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, test media and test conditions to enable a judgement on the usability of hazard data. For further information regarding adequacy, relevance and reliability see ECHA guidance and practical guides ([13], [14] and [15])

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 generation of additional 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* to *in vitro* tests and to

natural environmental sampling. Nanoforms with the same substance identity may have different characteristics leading to diverse behaviour in these variable test environments as described in step 2 above. These aspects should be addressed and documented, including the evaluation of available data concerning its applicability from one nanoform to another nanoform (or the non-nanoform), for different endpoints.

As described in step 2 above, any change in the physicochemical parameters described in Appendix 1 and Table 2 may potentially affect the activity, reactivity, fate, toxicokinetics and toxicity of a (nano) form in a significant way and could lead to a different behaviour. Therefore, in addition to the validity of the study itself, endpoint specific consideration of the similarities or potential differences between source and target form 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 relevant endpoint. The assessment entity in IUCLID could be used to help with a transparent reporting; further guidance on this is available in the manual: "*How to prepare registration and PPORD dossiers*" [16].

3.5 Step 4: Construct a matrix of data availability

Once the available information has been gathered and evaluated, 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 not. 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. An example of such a template for a data matrix can be found in Appendix 2.

3.6 Step 5: Assess the adequacy of the approach and fill data gaps within the group

As a final step, all relevant information gathered (e.g. the toxicokinetic data, etc.) should be combined into an overall assessment. Read-across and grouping of nanoforms is only deemed possible if the available and relevant information on the toxicokinetics, fate and (eco)toxicology support the hypothesis and that there are no indications of an underestimation of hazard and that the justification is robust [7]. The 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 made to determine whether:

- The grouping hypothesis is supported, i.e. does the group in fact exhibit sufficient similarities in the physicochemical properties based on available data as postulated in step 2 and
- The group is sufficiently robust (i.e., contains sufficient, relevant and reliable information on the members) for assessment purposes.

A preliminary assessment should be made for each endpoint, as the grouping approach rationale may lead to a situation where the scientific justification is only relevant 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 the assessment is finished. The assessment and the approach followed should be documented (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 are not available, 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 performing the test required for each nanoform having a data gap according to information requirements or by using the adaptations as outlined in Annex XI.

3.7 Step 6: Perform and/or propose testing to fill the data gap for the whole group

If the preliminary assessment supports the read-across or grouping justification (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 be needed if a new nanoform is put on the market and its properties do not allow it to be allocated to any existing group.

Consequently, in such cases, the data gap cannot be covered by using a scientific justification in accordance with this approach for the grouping or read-across. In these cases, the data gaps should be filled by either performing the test in the conventional manner in accordance with Annexes VII-X or by the use of the adaptation as laid out in Annex XI. Further guidance on how to use adaptations can be found in *Chapter R.5 of the Guidance on IR&CSA* [17].

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 of what is intended to be placed on the market. The reasoning for why this choice does not lead to underestimation of the hazards should be documented.
- If the test is in Annex IX or 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 should 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 addressed (more information with respect to uncertainty considerations can be found in Section D.5.4 of *Part D and Chapter R.19 of the Guidance on IR&CSA* ([18] and [19])). Further details on how to report nanoforms and the use of the assessment entity can be found in the ECHA manual: *How to prepare registration and PPORD dossiers* [16].

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

Efforts to establish a set of physicochemical parameters that would allow adequate characterisation of a nanomaterial for (regulatory) safety (risk) assessment have been made worldwide (see for instance, [20], [21], [22], [23], [24], [25]), and the topic is still under debate.

Table 2 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 community that the parameters listed may be taken into consideration 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 technical scientific reports (e.g. [8], [26]).

The appendix to the ECHA IR&CSA guidance (*Appendix R7-1 for nanomaterials applicable to Chapter R7a Endpoint specific guidance* [27]) provides advice on some of the parameters below, where there are specific information requirements in REACH. Additionally, the approach developed by ECETOC [5] includes as supplementary information a table that includes available analytical methods for parameters relevant for read-across and grouping of nanomaterials.

Moreover, it should be noted that when measurement of a parameter is not possible for technical reasons, qualitative considerations may be sufficient to justify the hypothesis.

For physicochemical characterisation of a nanoform, a distinction can be made between material properties (such as chemical composition, 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, as well as *in silico* data, to justify read-across [8].

Table 2: 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)
<p>Chemical composition, including crystalline structure</p> <p>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.</p> <p>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, differences in the crystalline structure may be relevant for metals, metal-oxides or carbon based nanomaterials.</p>
<p>Impurities</p> <p>As for non-nanoforms, impurities can substantially contribute to the human and environmental toxicity of nanoforms.</p>
<p>Surface chemistry (e.g. chemical coating, surface treatment)</p> <p>The term surface chemistry indicates the chemical composition at the surface of the particles as a result of chemical coating and/or surface treatment of the particle. Surface chemistry influences dissolution behaviour and agglomeration behaviour of nanoforms. Considering hazard 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 chemistry will influence sorption to environmental or biological media and the reactivity of a nanoform.</p>
Physical parameters/ Particle characteristics (What they are)
<p>Particle size / range</p> <p>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.</p>
<p>Shape</p> <p>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. For advice on characterization for shape see also <i>section 2.2.3.3 of Appendix R.7-1 to Chapter R.7a of the Guidance on IR&CSA</i>.</p>
<p>Surface area, including porosity</p> <p>The increase of relative surface area with decreasing particle size may increase the reactivity of a nanoform relative to its mass and/or volume. Furthermore, as a consequence of the increased surface to volume ratio, porosity may affect the crystalline structure.</p>

Behaviour (Where they go)**Solubility: Rate of dissolution / Equilibrium solubility**

The rate of dissolution depends on factors including, but not limited to the chemical composition, particle size, coating, surface treatment, stability, manufacturing process, and biological environment. The rate of dissolution gives information on how many ions/molecules are released from the particle over time. The ion(s)/ molecules released 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 (as represented by the Hamaker constant) and surface charge. 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. Hydrophobicity is influenced by surface chemistry of the particles. Thus, knowledge on the surface chemistry can give qualitative information about the hydrophobicity of the nanoforms.

Zeta potential

Zeta potential can be used as a proxy for surface charge and may provide information in dispersion stability, degree of agglomeration/de-agglomeration of particles in relevant media. Surface charge may influence systemic distribution and cellular uptake of a nanoform, and ultimately its toxicity.

Additionally there is evidence linking zeta potential to the inflammogenicity of nanoscale particles of metals and minerals. ([28], [29], [30]).

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). For further information, see Appendix R.7-1 to Chapter R.7a [27].

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)**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 may 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 air and aquatic compartment, but it may also play a role in other compartments. If oxygen radicals are induced (i.e. ROS), they may easily react with other molecules, which in some cases may lead to severe effects (e.g. reaction with DNA leads to genotoxicity). This parameter is relevant for nanomaterials that are photoactive.

Appendix 2.

Example of template for read-across matrix

The table below shows an example of a read-across matrix template adapted for nanoforms. The fields added for nanoforms are highlighted in grey. Please note that the list of "Additional grouping parameters" is only indicative. It does not cover all the possible parameters that may be considered when developing a read-across hypothesis, and not all the parameters that appear under the heading will always be required (see Section 3.3, step 2).

Table 3: Example of a read-across matrix template for nanoforms

EC No. (CAS No.)			
CHEMICAL NAME	[(Nano)form 1]	[(Nano)form 2]	[(Nano)form 3]
Chemical composition			
Impurities			
Nanoform identification ("what they are")			
Particle size			
Shape			
Surface chemistry			
Surface area			
Meets the EU nanomaterial definition (Y/N)			
ADDITIONAL GROUPING PARAMETERS			
<i>Behaviour ("where they go")</i>			
Water solubility			
Dissolution rate			
Hydrophobicity			
Zeta potential			
Dustiness			
...			
<i>Reactivity</i>			
Biological (re)activity			
Photoreactivity			
...			
ENVIRONMENTAL FATE and PATHWAY			
Photodegradation			
Stability in Water			
Transport and Distribution			
Aerobic Biodegradation			
...			
ENVIRONMENTAL TOXICITY			
Acute Toxicity to Fish			
Acute Toxicity to Aquatic Invertebrates			

Toxicity to Aquatic Plants			
...			
MAMMALIAN TOXICITY			
Acute Oral			
Acute Inhalation			
Acute Dermal			
Repeated dose toxicity, oral			
Repeated dose toxicity, inhalation			
Genetic Toxicity <i>in vitro</i>			
. Gene mutation			
. Chromosomal aberration			
Genetic Toxicity <i>in vivo</i>			
Reproductive Toxicity			
- Fertility			
- Developmental toxicity			
...			

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