

Guidance on information requirements and chemical safety assessment

Appendix R.6-1 for nanoforms 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 (or sets of nanoforms).

This document aims at providing an approach on how to justify the use of hazard data between nanoforms and/or sets of nanoforms, and the non-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 nanoforms. Please note that no specific advice for QSARs with respect to nanoforms is provided in this version of the guidance.

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 of studies between nanoforms and/or sets of nanoforms, of different substances.

This appendix aims at providing advice specific for nanoforms but 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 for nanoforms.

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 nanoforms.

¹ Please see *Appendix for nanoforms to the Guidance on Registration and the Guidance on substance identification* [2]

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.

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1. Introduction

This guidance is directed to registrant(s) who have identified nanoforms (or sets of nanoforms) of the same substance³. In such cases, it is necessary to determine to which forms (including non-nanoforms) the available hazard data is applicable; and/or to design a testing strategy to fulfil the specific information requirements. The principles laid out in this document will also be useful for a potential registrant joining an existing registration that includes nanoforms (or sets of nanoforms) creating a need to determine whether the hazard data available could also be applicable for the additional nanoforms which are put on the market.

The principles and approaches taken to justify the use of hazard data between nanoforms (or sets of nanoforms) of the same substance are similar to those used for read-across between different substances. Explanation on general principles for grouping and read-across including explanations of general terms such as "target"⁴ and "source"⁵ can be found in the parent guidance .

According to Annex VI of REACH: *"Where technically and scientifically justified, the methodologies set out in Annex XI.1.5 shall be used within a registration dossier when two or more forms of a substance are "grouped" for the purposes of one, more or possibly all the information requirements."*

The revised Annex VI of REACH introduces the concepts of "nanoform" and sets of nanoforms. It requires manufacturers and/or importers to submit the necessary information on intrinsic properties for each nanoform or sets of nanoforms of the substance.

A "nanoform" is a form of a natural or manufactured substance that meets the requirements of the Commission Recommendation on the definition of nanomaterial^{6,7}. Each nanoform must be characterised in accordance with Annex VI section 2.4 of REACH. A substance may have one or more, different nanoforms, based on changes in the parameters given under points 2.4.2 to 2.4.5 (size distribution, shape, surface treatment and functionalisation and specific surface area of the particles). Due to the differences in physicochemical parameters (e.g. surface chemistry) nanoforms, of the same substance, may also have different hazard profiles.

A "set of similar nanoforms" can be created, when it is possible to conclude that the hazard assessment, exposure assessment and risk assessment of these nanoforms (with clearly defined boundaries in the Annex VI parameters) can be performed jointly. The *Appendix for nanoforms to the Guidance on Registration and the Guidance on substance identification* [2] explains how to create sets of different nanoforms as well as provide further details on the characterisation and reporting requirements when registering such sets of nanoforms.

Both the development of a set of nanoforms and of a read-across approach involve "grouping" of chemicals for the purpose of predicting their properties. However, as explained below, this guidance does not deal with the creation of sets of nanoforms. This guidance exclusively refers to read-across. In this context, read-across is a technique for predicting endpoint information for one nanoform or set of nanoforms identified in the dossier under Annex VI (target), by using data on the same endpoint from another nanoform also identified in the dossier under

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). [8]

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.

Annex VI (source).

2. Aim

According to Annex XI 1.5 *“When nanoforms [or sets of nanoforms] are covered by the registration the [read-across approach] shall address the nanoforms [or sets of nanoforms] separately. For grouping different nanoforms [or sets of nanoforms] of the same substance the molecular structural similarities alone cannot serve as a justification.*

This guidance aims at providing support for registrants in complying with the legal obligations requiring safe use for each nanoform (or sets of nanoforms) to be transparently demonstrated and reported. In particular, this guidance offers advice on how to bridge data gaps on specific nanoforms (or sets of nanoforms) by the use of data on another nanoform (or sets of nanoforms) of the same substance.

The starting point for a read-across approach is well characterised nanoforms (or sets of nanoforms) which are reported in the dossier in accordance with Annex VI of REACH, following the *Appendix for nanoforms to the Guidance on Registration and the Guidance on substance identification* [2].

Furthermore, a read-across approach aims at meeting a specific information requirement applicable to a nanoform (or set of nanoforms). In contrast, a “set of nanoforms” is reported as an entity in the registration dossier in accordance with Annex VI of REACH.

A set of nanoforms aims at grouping several nanoforms for which the hazard assessment, exposure assessment and risk assessment can be performed jointly.

In this respect, it should be noted that the development of a set of nanoforms cannot replace the development of a read-across approach between nanoforms. If a registrant can demonstrate that the hazard assessment is valid for several nanoforms based on a justification that applies generically to all the endpoints, he can create a set of nanoforms. If, however, to demonstrate that a hazard assessment is valid for several nanoforms, a registrant needs different hypothesis for different endpoints, he has to report the nanoforms separately. However, this does not mean that the registrant has to develop different data sets for each nanoform. Instead, this can be addressed via read-across between those nanoforms in accordance with Section 1.5 of Annex XI of REACH.

Reference to “read-across of studies between (sets of) nanoforms” in the present guidance should be understood as a read-across of studies between nanoforms, sets of nanoforms and forms of the substance that are not nanoforms, as appropriate in specific cases.

This document aims to provide a systematic and pragmatic approach on whether the same study or same set of studies are relevant for identifying the (eco)toxicological properties and fate of several nanoforms (or sets of nanoforms) 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 specific information requirements for the nanoforms (or sets of nanoforms) are read-across based on relevant physicochemical parameters (that may vary depending on which endpoint is being considered) and appropriate justification. The correct application of the strategy will allow determination of:

- 1) whether available hazard data specific to a (sets of) nanoform reported in the registration dossier is also relevant for other nanoforms (or sets of nanoforms) (for each specific information requirement)

- 2) when considering the testing strategy, it is important to ensure the applicability and relevance of all available hazard data for existing nanoforms (or sets of nanoforms).

Appendix 1 of this Guidance states: "Summary of key physicochemical parameters to be considered for grouping and read-across of nanoforms and their relevance for human health and environmental endpoints". This 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 and read across of nanoforms for the purpose of hazard and fate identification

This chapter outlines a stepwise approach for the read-across between nanoforms/sets of 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 the read-across between several nanoforms. Thus, this document focuses on specific considerations on nanoforms and incorporates developments in science, which address specific considerations needed in this context. For further information please see [3] where these considerations are summarised. The approach developed by ECETOC [4] also provides useful information for the read-across of studies between nanoforms. Additional information can be found in other publications, see for instance [5], [6] and [7].

A large number of nanoforms and sets of nanoforms may exist. As a consequence, there may be many different ways to read-across between them 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 approaches.

This chapter outlines the general principles on how information on physicochemical properties, toxicokinetic and (eco)toxicological behaviour can be used with expert judgement to provide a scientific rationale for a read-across approach applicable to several (sets of) nanoforms. It also provides guidance on how to document and justify a read-across 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 between different (sets of) nanoforms. Once this scientific basis has been established and clearly justified, available hazard information (for the specific endpoint) can be read-across to all the (sets of) nanoforms within the defined group. In all cases, the hazard information must be robust enough for the purposes of hazard identification, classification and labelling (C&L) and/or risk assessment.

As for any chemical, read-across for (sets of) nanoforms for the purpose of hazard identification is endpoint specific. A specific read-across approach must be justified endpoint by endpoint, but the same justification may be applicable to several endpoints. The decision on whether to develop the read-across strategy for one or several endpoints will depend on what scientific explanation the 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 *in vitro*) that are read-across from the 'source' to

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*.

each 'target' nanoform within the defined group (for further information please see the parent guidance).

3.1 Stepwise strategy for grouping of (sets of) nanoforms

A read-across approach enables to use the test results obtained with a specific source material to predict the properties of the other (sets of) nanoforms (targets). For this approach to be valid, it is necessary to demonstrate that the selection of the target nanoforms/sets and the read-across of studies between the source material and the target nanoforms/sets is robust and justified⁹. To facilitate data collection and a systematic and transparent documentation of the 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 [8]. There may be alternative means to obtain the information, thus the stepwise approach should be considered as a recommendation.

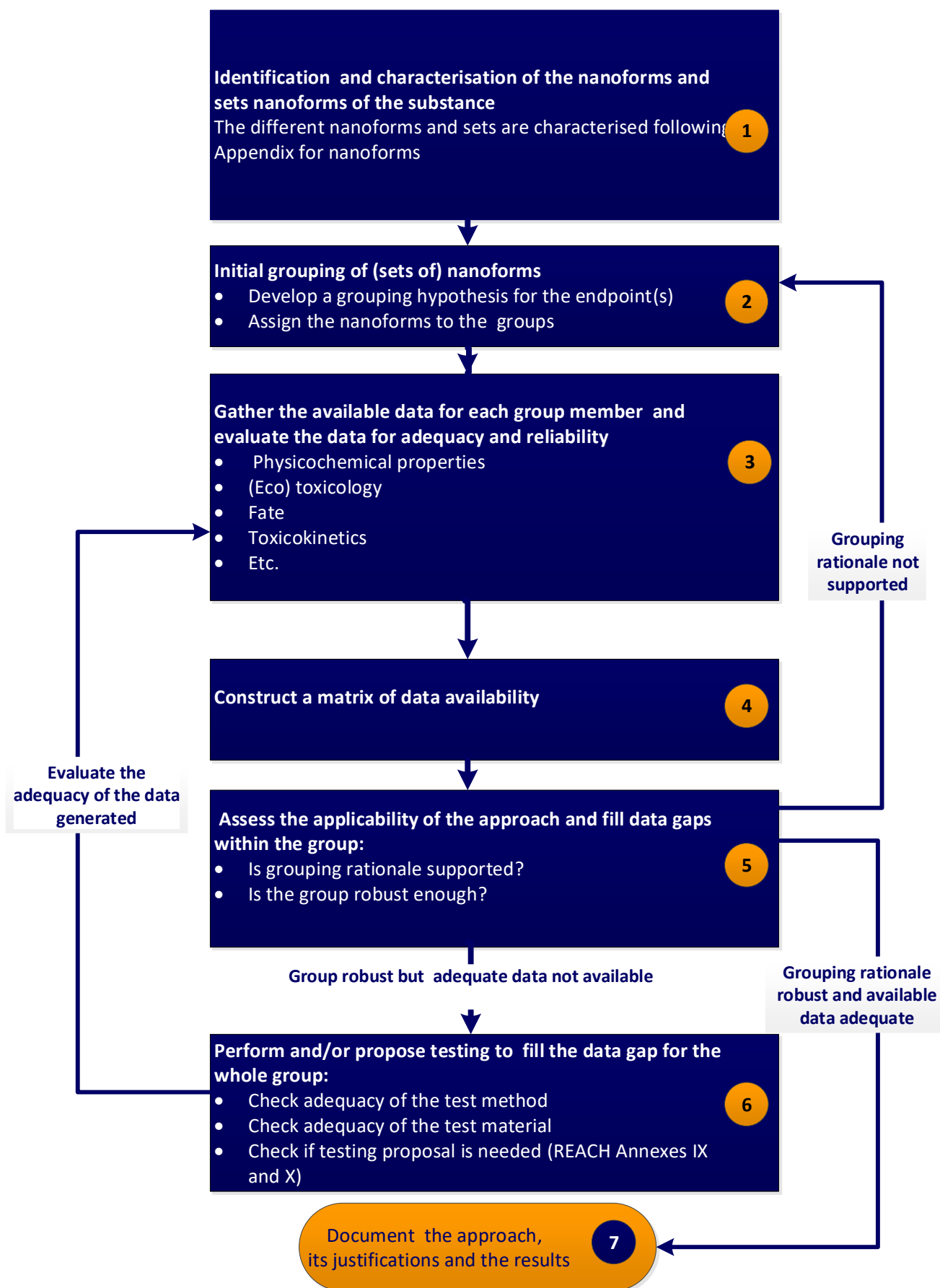
The proposed approach (except the identification of the nanoforms and sets of nanoforms) is endpoint specific. However, in practice, several endpoints can be addressed at the same time as long as it is transparently explained which endpoints are covered and why the read-across hypothesis is applicable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

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).

1 **Figure 1: Scheme for the step-wise approach¹⁰**



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

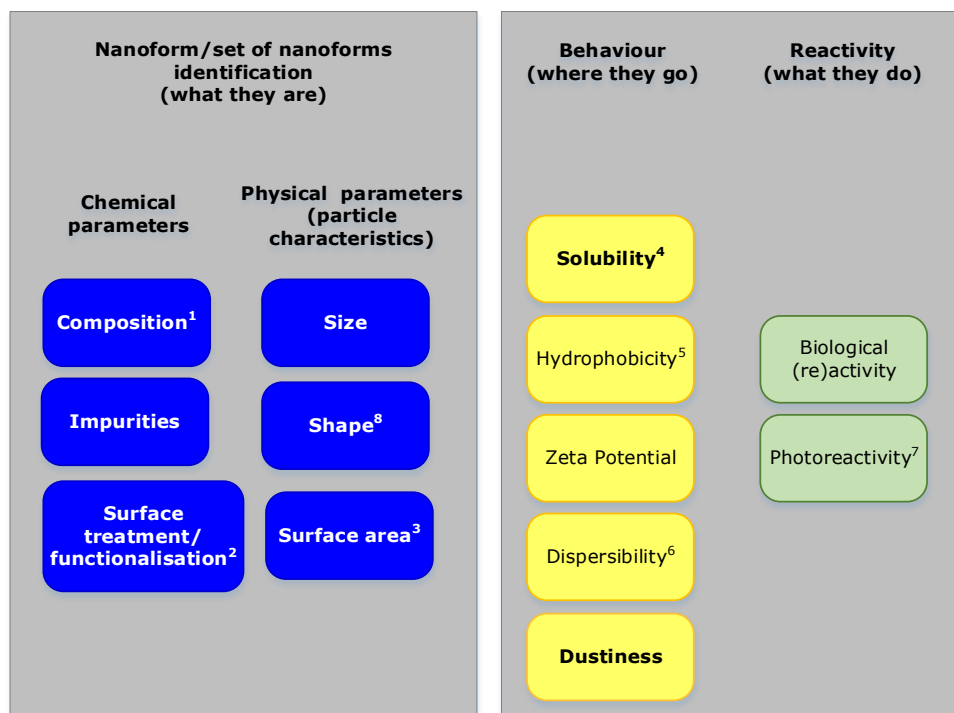
As already mentioned in this guidance, a substance may comprise different (sets of) nanoforms due to variation in physicochemical parameters such as size, shape or surface treatments. In principle, the hazards of each nanoforms must be addressed specifically in the registration dossier. Correct and unambiguous characterisation of each (set of) nanoforms of a substance is a prerequisite to ensure a proper hazard assessment and thereby demonstrate safe use. Appropriate identification of nanoforms 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.

In addition to the information on composition and impurities required to identify substances, for (sets of) nanoforms Annex VI of REACH requires the identification and characterisation of parameters in section 2.4.2 to 2.4.5. These are size distribution, shape and crystallinity, surface treatment and functionalisation and specific surface area of the particles of the nanoform (in blue boxes in Figure 2). These parameters 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. All these properties are required as part of the information on substance identification for nanoforms and sets of nanoforms .

Figure 2 identifies parameters that can provide useful information to help the read-across of studies between (sets of) nanoforms. This includes some 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 (properties that are REACH information requirements for nanoforms are in bold font). 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 nanoforms and sets 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 treatment/functionalisation includes information on, for instance, chemical coating and surface treatment(s) applied to the particles.

³. Surface area is one of the nanoform identification parameters and it can also be used to show compliance with the EC Definition [2] (. 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 [7], 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).

⁸. Shape includes: Shape, aspect ratio and other morphological characterisation: crystallinity, information on assembly structure including e.g. shell-like structures or hollow structures, if appropriate

Size, shape and crystallinity, surface. functionalisation and treatment as well as specific surface area may have an impact on the hazardous properties of nanoforms..

¹¹ Adapted from [7]

These parameters must be taken into consideration for the characterisation of nanoforms and sets of nanoforms of a substance, and are required by REACH Annex VI.

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 (sets of) nanoforms – Develop a read-across hypothesis, identification of a group's boundaries and its members

The second step for developing a read-across between (sets of) nanoforms defines the endpoint(s) concerned, 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 read-across 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 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 (sets of) 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 read-across studies between (sets 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 source and target (set of) nanoforms.

There are different ways to read-across studies between (sets of) nanoforms of the same substance. Different additional physicochemical parameters and/or screening methods may be needed to substantiate the read-across hypothesis and establish that the hypothesis 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 of nanoforms/sets as this will provide insight into which additional parameters may be needed to determine the 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 of (sets of) nanoforms for read-across 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 (sets of) nanoforms in a group, e.g. all these (sets of) nanoforms have the same surface treatment. In other cases, the read-across 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 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 of nanoforms/sets, such that the hazard characterization will be valid for all of the (sets of) nanoforms.

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).

1 Example 1: hypothesis based on solubility for systemic toxicity

Available information:

- The target (set of) 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 (set of) 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/sets (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 (set of) 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.

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Example 2: hypothesis based on high aspect ratio for inhalation effects

Available information:

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

Basis for hypothesis:

It is hypothesized that both (sets of) 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

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In case where studies are read-across between (sets of) nanoforms 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 the group of nanoforms/sets to which the read-across hypothesis applies. This should include a

proper characterisation of the nanoforms/sets in the group to ensure that a justification based only on physicochemical properties is not underestimating the potential hazard of any of the (sets of) nanoforms.

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

- Why do the similarities/ differences between the (sets of) 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 of nanoforms/sets 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/sets of nanoforms is limited to the nanoforms/sets of nanoforms that are part of the initially formed group. Addition of new members to an existing group of nanoforms/sets of nanoforms will require a consideration of the established grouping justification. This may include an assessment of whether the new nanoform/sets of nanoforms fits the existing read-across 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 read-across approach, if they display similarities in the physicochemical properties that form the basis of the grouping of nanoforms/sets of nanoforms.

Examples of considerations to be taken into account when identifying the basis for reading-across studies between (sets of) 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 in the composition and/or impurities /additives of 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 nanoforms 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 read-across approach. 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 nanoform 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

1 behave in the body or in the environment may provide useful supporting arguments to justify
2 read-across. The scientific basis for these qualitative comparisons and the associated
3 uncertainties must be clearly explained in the justification.

4
5 Information on the (main) route(s) of exposure (inhalation, dermal, oral) is a first step in
6 understanding the toxicokinetic profile of a nanoform. For example, for inhalation, the potential
7 for deposition in the lungs needs to be considered. The toxicokinetic profile of a nanoform
8 provides information on the absorption and subsequent exposure of target organs/tissues over
9 time. Toxicokinetics, in a traditional sense, encompasses absorption, distribution, metabolism,
10 and excretion (ADME). Toxicokinetics may be further complicated by changes in the
11 physicochemical properties of the nanoform that may occur during these different ADME
12 processes. The specific toxicokinetic profile may depend on several different physicochemical
13 parameters of the nanoform, e.g. composition, size, shape, agglomeration/aggregation state,
14 surface properties (including surface charge), hydrophobicity, or dissolution. The toxicokinetic
15 profile in a (human) organism also depends on the temperature, pH and ionic strength of the
16 biological fluid in which the nanoform is taken up (e.g. serum, saliva, blood). Hence, 'system-
17 dependent properties' (i.e. dissolution rate in biological media, surface reactivity and
18 dispersibility), biomolecules present and interactions at the nano-bio interface¹³ of cells at the
19 target site may provide relevant information on the likelihood of distribution and potential for
20 accumulation and excretion. Information from (available) *in vivo* studies including data on the
21 internal level of exposure and elimination over time provide further relevant information on the
22 toxicokinetic behaviour.

23
24 Screening methods (*in vitro*) may offer a better understanding of similarities between the
25 behaviour of different nanoforms in e.g. the transfer across a port of entry (skin,
26 gastrointestinal tract, lung epithelium etc.), the deposition in the lung, tissue distribution, or
27 clearance/persistence of the nanoforms. Such information can be obtained by a combination of
28 certain physicochemical information (e.g. water solubility), through biophysical testing (e.g.
29 dissolution rate and interaction with components of physiologically relevant media), *in silico*
30 methods (e.g. multiple path particle dosimetry modelling for lung deposition), and *in vitro*
31 testing (e.g. for skin permeability). It may also be necessary to obtain additional
32 physicochemical parameters for selected nanoforms to be used as input for *in silico* prediction
33 tools; e.g. information on the aggregation/agglomeration state and aerodynamic diameter of a
34 material in air is required for modelling to estimate lung deposition. Many of the methods have
35 not yet been validated or are currently undergoing validation. The methods may be used at the
36 registrant's discretion. However, the choice of methodology should be justified; this includes
37 considerations on the constraints and limitations of the various methods used.

38
39 It is important to note that the behaviour of a specific nanoform in the environment is
40 dependent on its surface chemistry and is susceptible to change throughout its life cycle
41 because of e.g.:

- 42 • exposure to other particles and/or constituents (e.g. ageing process,
43 (hetero)agglomeration, corona formation),
- 44 • interactions with environmental media (e.g. dissolution, corona formation, aggregation or
45 disaggregation, chemical reactions, transformation), and
- 46 • degradation/transformation (e.g. loss/modification of the coating i.e. surface chemistry).

47
48 Therefore, apart from physicochemical parameters of the (sets of) nanoform the characteristics
49 of the test environment/media should be taken into account. The role of the following
50 environmental parameters influencing the behaviour of nanoforms may be considered in the
51 justification for the read-across: temperature, pH, ionic strength (in particular, of divalent

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] .

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-nanoforms, 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 treatment/functionalisation 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)	0	+	++	++
Nano-bio interface	0	+	+	+
Adsorption/desorption	+ ^c	+	++	++
Sedimentation	0	++	++	0
Photochemical degradation^a	++	+	0	0
(Bio)degradation^b	0	#	#	#

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

++: High relevance

+: Relevance

0: 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 (sets of) 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 (sets of) nanoforms 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 (set of) nanoform(s) 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 of nanoforms/sets of nanoforms for the specific REACH information requirement then no additional information is needed, provided that the read-across 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. (Sets of) 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 (set of) nanoform(s) to another (set of) nanoform(s)(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 nanoform 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 (set of) nanoform(s) 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 of nanoforms/sets of nanoforms 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 of nanoforms/sets of nanoforms 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 of nanoforms/sets of nanoforms

As a final step, all relevant information gathered (e.g. the toxicokinetic data, etc.) should be combined into an overall assessment. Read-across of (sets 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 [6]. 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 of nanoforms/sets of nanoforms should be made to determine whether:

- The read-across hypothesis is supported, i.e. does the group of nanoforms/sets of nanoforms in fact exhibit sufficient similarities in the physicochemical properties based on available data as postulated in step 2 and
- The read-across hypothesis 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 read-across approach rationale may lead to a situation where the scientific justification is only relevant for some endpoints and not for others.

If the read-across hypothesis 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 read-across hypothesis is not sufficiently robust or justified, the following options should be considered:

- If the available data show that the (sets of) nanoforms in the group do not have sufficiently similar properties, then the read-across hypothesis must be modified e.g. by subdivision; or
- If adequate data are not available, but the read-across hypothesis is considered robust, then testing a representative nanoform of the group to further substantiate the justification for each information requirement would be necessary (go to step 6).
- If the read-across hypothesis cannot be adequately justified, the identified data gaps will have to be filled by either performing the test required for each (set of) nanoform(s) having a data gap according to information requirements or by using another adaption as outlined in Annex XI.

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

If the preliminary assessment supports the read-across justification (i.e. similar physicochemical properties are observed for the (sets of) nanoforms in the group), but the read-across hypothesis 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 registered and its characterisation does not allow it to be covered by the read-across approach.

Consequently, in such cases, the data gap cannot be covered by using a scientific justification in accordance with this approach for the 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 read-across approach 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 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 nanomaterial can be found in technical scientific reports (e.g. [7], [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 [4] 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 [7].

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)	
Chemical composition	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.
Impurities	As for non-nanoforms, impurities can substantially contribute to the human and environmental toxicity of nanoforms.
Surface treatment/ functionalization	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. Required by REACH (Annex VI). See [2]
Physical parameters/ Particle characteristics (What they are)	
Particle size / range	The size of the particles 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. Required by REACH (Annex VI). See [2]
Shape and crystallinity	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. Crystal 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 crystal lattice or deformation). Based on the present understanding of nanoparticle behaviour, differences in the crystal structure may be relevant for metals, metal-oxides or carbon based nanomaterials. Required by REACH (Annex VI). See [2]
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 and/or volume. Furthermore, as a consequence of the increased surface to volume ratio, porosity may affect the crystalline structure. Required by REACH (Annex VI). See [2]

Behaviour (Where they go)
<p>Solubility: Rate of dissolution / Equilibrium solubility</p> <p>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. Required by REACH (Annex VII).</p>
<p>Hydrophobicity</p> <p>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.</p>
<p>Zeta potential</p> <p>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.</p> <p>Additionally there is evidence linking zeta potential to the inflammogenicity of nanoscale particles of metals and minerals. ([28], [29], [30]).</p>
<p>Dispersibility</p> <p>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].</p>
<p>Dustiness</p> <p>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. Required by REACH (Annex VII).</p>
Reactivity (What they do)
<p>Biological (re)activity (or surface reactivity)</p> <p>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.</p>
<p>Photoreactivity</p> <p>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.</p>

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 (incl. crystallinity etc)			
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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