Usage of (eco)toxicological data for bridging data gaps between and grouping of nanoforms of the same substance

Elements to consider

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Nanomaterials based solutions have a lot to offer for a wide range of industry sectors from pharmaceuticals to surface technologies. Nanomaterials may be manufactured in several different shapes, sizes and/or with a wide range of surface treatments. Changes made to the particles may not only provide a new function for the nanomaterial but also influence its (eco) toxicological behaviour.

Therefore, a need was identified in 2014 to develop a pragmatic approach for how to ensure safe use of the potentially numerous nanoforms of the same substance under REACH. A nanoform is defined in this document as a composition of a substance that meets the requirements of the EC definition of a nanomaterial. Different nanoforms may differ with regard to size distributions, shape and/or surface chemistry.

Together with RIVM and JRC, ECHA initiated and led a project over 2015 to develop a scientific reference paper to explore the scientific aspects of justifying when and how to use test data from an (eco)toxicity study on one nanoform to cover other nanoforms of the same substance.

This work resulted in this scientific reference paper consolidating the current state of play in science across several FP7 projects. In the process of drafting the paper, ECHA Nanomaterial Working Group, was consulted twice as well as industry. This scientific reference paper will be a cornerstone in future works in the field in the EU as well as a contribution to international discussions at OECD.

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1. Introduction

1.1 Aim

In general, application of grouping of substances and read-across between substances is recognised as a valuable approach in regulatory frameworks e.g. to fill potential data gaps in the hazard characterisation, based on availability of adequate data from similar substances. Grouping and read-across, together with e.g. weight of evidence approaches, are considered as 'adaptations', i.e. alternative ways to minimise e.g. animal testing to meet the regulatory information requirements, while fulfilling the ultimate aim of REACH (Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals) (EC, 2006), which is to protect human health and the environment thus ensuring safe use of chemicals. It is expected that also for nanomaterials grouping and read-across approaches will be an important means of addressing identified data gaps.

The European Commission issued a Recommendation on the definition of the term 'nanomaterial' (EU, 2011), which is the definition that will be used in this document; of special importance is Article 2 stating that: *"'Nanomaterial' means a natural, incidental or manufactured material containing particles [...] one or more external dimensions is in the size range 1 nm-100 nm."*.

The term 'nanoform' is used in this document to distinguish forms of a substance that fulfil the EC Recommendation on the definition of the term 'nanomaterial' but differ with regard to size distributions, shape and/or surface chemistry.

Due to the numerous possible nanoforms with the same chemical identity (i.e. covered by the same registration dossier) but with differences regarding other physicochemical properties (e.g. surface modification, size distribution and particle shape), there is a need for alternative approaches that would allow predicting hazard properties by reading across between nanoforms and/or from non-nanoforms to nanoforms of the same substance to minimise testing, in particular, on animals. Under REACH, each nanoform may be seen as being manufactured/imported for a specific use of that registered substance, and thus the data submitted to fulfil the information requirements for the substance should be demonstrated to be representative also for each nanoform.

The aim of this document is to consolidate existing information and develop approaches that a registrant can use to scientifically justify that certain (eco)toxicological studies undertaken on one nanoform of a substance (or the non-nanoform) can be used to predict the hazard properties of (an)other form(s) of the same substance. The approaches will form a cornerstone in further discussions with the European Commission, Member States, Industry and Non-Governmental Organisations whose outcome will form a basis for ECHA's processes for developing guidance.

Work on this subject has already been done for conventional substances under REACH such as the development by ECHA of "Guidance on information requirements and chemical safety assessment. Chapter R.6: QSARs and grouping of chemicals" (ECHA, 2008) and "Read-Across Assessment Framework" (ECHA, 2015). Earlier initiatives included ECHA's "Background paper An Introduction to the Assessment of Read-Across" in ECHA's Experts Workshop on Read-Across Assessment with the

active support of Cefic LRI[1]. 2-3 October 2012"[2]; and ECHA "Summary of Workshop on the Read-Across Assessment Framework (RAAF) (2-3 October 2014)"[3]. Furthermore, the OECD revised its guidance on grouping of chemicals (OECD, 2014a), whose first edition dates back to 2007.

For nanomaterials, Industry (ECETOC) (e.g. Arts et al., 2014; Arts et al., 2015a; Arts et al., 2015b) and Member States (e.g. RIVM-Arcadis project, see Sellers et al., 2015) as well as several research projects in FP7 (e.g. MARINA (Oomen et al., 2015), NanoREG and GUIDEnano[4]) and individual researchers (e.g. Gebel et al., 2014; Walser and Studer, 2015) have started proposing methodologies for grouping and read-across and illustrating their ideas with case studies.

The OECD guidance on grouping of chemicals (OECD, 2014a) also mentions nanomaterials, however noting that *"Principles and guidance for grouping nanomaterials for the purpose of assessing their (eco)toxicological and fate properties are under development."* No concrete guidance is provided for nanomaterials in that document, as accepted principles are not established in scientific literature yet. To facilitate the discussion on this subject, OECD organised an "Expert Meeting on Categorization of Manufactured Nanomaterials" in Washington in September 2014 (OECD, 2016) and is organising a second event under the heading "Expert Meeting on Grouping and Read Across for the Hazard Assessment of Manufactured Nanomaterials" in Brussels in April 2016.

It is crucial to harvest the outcome from these studies to integrate the important experiences gained and have a broad basis. Furthermore, emphasis should be put on finding a workable balance between scientific uncertainty and regulatory needs.

Fulfilling the standard information requirements under REACH by means of read-across encompasses predicting the unknown properties of a certain substance using test data generated on a substance that is considered to be similar based on structural considerations or mode of action. This is different from predicting the unknown properties of one or more nanoform(s) of the same substance from known properties of (an)other nanoform(s). In particular, the type and amount of supporting evidence needed to support a claim of 'similarity' between nanoforms of the same substance may differ. Therefore, the main challenge is to devise an approach that enables an acceptable level of confidence that test data generated on one or more nanoform(s) (or on non-nanoforms) apply to other nanoform(s) without compromising the hazard assessment.

Finally, the aim of this document is to illustrate a number of core principles (i.e. elements to consider), which are based on the current, albeit incomplete, scientific understanding. This would lead to a structured approach to guide registrants and regulators on how to apply grouping and read-across concepts to nanoforms. The guidance should provide some generic guidelines, which should be combined with the fact that the specific physicochemical and (eco)toxicological properties of a substance and its nanoforms affect how filling data gap(s) may be scientifically justified on a

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1 LRI: Long Range Initiative

2 Available at: http://echa.europa.eu/documents/10162/5649897/ws_raa_20121003_background_paper_an_introduction_to_the_assessment_of_read-across_in_echa_en.pdf

3 Available at: https://echa.europa.eu/documents/10162/13628/workshop_summary_raaf_en.pdf.

4 More information on these projects can be found on their respective websites: www.marina-fp7.eu, www.nanoreg.eu, and www.guidenano.eu.

case-by-case basis. The decision on whether a data gap exists for a specific nanoform as well as whether the data gap can be filled by using information from another nanoform, and consequently the scientific justification for it, remains the responsibility of the registrant.

1.2 Legal basis

The objectives of REACH are set out in its preamble, including recitals 1 and 38, and in Article 13, which all underline the need to generate data for hazard assessment by means other than tests whenever this is possible. Annex XI, Section 1.5 of REACH sets the conditions/criteria for using grouping and read-across approaches to fulfil the information requirements for substances.

If the read-across approach is adequate, testing to fulfil the information requirement for that endpoint is unnecessary. A read-across approach and subsequent development of a robust scientific justification may also support a conclusion for a certain hazard endpoint by using a weight-of-evidence approach.

1.2.1 What is grouping of substances?

Grouping describes the general approach to assessing more than one chemical at the same time (ECHA, 2008; OECD, 2014a). Substances that are structurally similar and have physicochemical, toxicological, ecotoxicological and/or environmental fate properties, which are likely to be similar or to follow a regular pattern, may be considered as a group of substances. These similarities may be based on a number of factors in accordance with REACH Annex XI, section 1.5:

- Common functional group (i.e. chemical similarity within the group);
- Common precursors and likely common breakdown products via physical and/or biological processes which result in structurally-similar chemicals;
- A constant pattern in the changing of the potency of the properties across the group (i.e. of physicochemical parameter and/or biological properties).

Usually, the terms category approach and analogue approach are used to describe techniques for grouping chemicals (ECHA, 2008; OECD, 2014a). The term analogue approach is used when the grouping is based on a very limited number of chemicals, where trends in properties are not apparent (ECHA, 2008; OECD, 2014a). In a category approach, more members are generally present, enabling the detection of trends across endpoints (ECHA, 2008; OECD, 2014a).

For registration of a substance under REACH, the information requirements have to be met. Within a group of substances, a data gap might be filled in by using several techniques including read-across (as described in section 3.1).

Grouping of nanomaterials often addresses different forms of the same substance rather than grouping nanoforms of different substances. Nevertheless, approaches proposing the grouping of nanoforms of different substances (i.e. different nanomaterials) have also been published (Arts et al., 2014; 2015a).

1.2.2 What is read-across between substances?

Read-across is an established technique for predicting endpoint information from one or more source substance(s) to a target substance by using data concerning the same endpoint. This prediction should be based on a robust scientific justification (OECD, 2014a). Consequently, the read-across approach has to be considered on an endpoint by endpoint basis due to the different complexities (e.g. key parameters, biological targets) of each endpoint.

In an analogue approach, read-across is employed within a group of a very limited number of substances for which trends are not apparent: i.e. the simplest case is read-across from one source substance to one target substance.

In a category approach, read-across is used within a group of a number of substances for which trends are apparent.

In all cases, read-across must be justified scientifically and documented thoroughly. Several pieces of evidence may be used to justify the read-across, with the aim of strengthening the case.

When comparing with the grouping approach presented above, it is seen that read-across between nanomaterials differs from read-across between substances. Target and source nanomaterials used in read-across are generally different forms of the same substance rather than different substances.

1.2.3 Consideration for whenever read-across is used

Annex XI, Section 1.5 of REACH requires that whenever read-across is used all of the following conditions should be fulfilled:

a. "*Be adequate for the purpose of classification and labelling and/or risk assessment"* – If the read-across data on the source substance is used as a key study, the data shall be adequate, reliable and robust enough to enable the registrant and the evaluator to decide on the appropriate classification and labelling for the target chemical. Similarly, if the data from the source substance is used as a key study, it shall provide a sufficiently reliable dose descriptor, i.e. point of departure for the risk assessment.

b. "*Have adequate and reliable coverage of the key parameters addressed in the corresponding test method referred to in Article 13(3)*" – Test methods referred to in Article 13(3) are regularly revised to reflect progress in science, and thus, the revised test methods may include investigations on additional important parameters. Covering these key parameters is essential to ensure that the level of information gathered by testing the source substance is equivalent to that expected from a new study performed according to the most current test method.

c. "*Cover an exposure duration comparable or longer than the corresponding test method referred to in Article 13(3) if exposure is a relevant parameter*" – As an example: a sub-chronic repeated dose toxicity (90-day) study can be used to cover the information requirements for a sub-acute repeated dose toxicity (28-day) study but not vice versa.

d. "*Adequate and reliable documentation of the applied method shall be provided.*" – The documentation provided must be sufficient to allow an independent assessment of the

adequacy and the scientific validity of the read-across approach (see also section 3.1.). The following elements are considered essential to adequately document a read-across approach:

1) a read-across hypothesis;
2) a justification for the read-across hypothesis;
3) a list of all the substances included in the approach;
4) detailed substance identity information of all substances included in the approach;
5) a list of the endpoints that are to be read-across;
6) a data matrix;
7) a conclusion on the applicability of the proposed read-across approach.

1.3 Differences between "non-nanoforms" and nanoforms

REACH is based on the principle *"that industry should manufacture, import or use substances or place them on the market with such responsibility and care as may be required to ensure that, under reasonably foreseeable conditions, human health and the environment are not adversely affected"*.

This means that all forms of a substance shall be covered by the hazard information submitted to demonstrate safe use of the registered substance.

The question of how to register nanoforms under REACH is currently under discussion. In principle, there are two scenarios to consider:

i. A nanoform that has a corresponding non-nanoform, i.e. a form of the substance with a particle size distribution that does not meet the requirements of the EC Recommendation on the definition of the term 'nanomaterial' (EU, 2011) (e.g. silver ingots vs. silver nanoparticles). Multiple different nanoforms and non-nanoforms may exist for a given substance, depending on variations in key physicochemical parameters (e.g. surface treatment, shape).

ii. A nanoform that has no corresponding non-nanoform (e.g. single walled carbon nanotubes). Multiple different nanoforms may exist for a given substance, depending on variations in key physicochemical parameters (e.g. surface treatment, shape)

Even for substances that do not exist in nanoforms, it is acknowledged that the differences in particle size, shape and surface treatment, as well as other physicochemical parameters may lead to differences in the hazard properties specified in the REACH standard information requirements of Annexes VII-X. Therefore, the different nanoforms should be assessed individually in order to determine whether the difference(s) in e.g. size, shape and/or chemical composition due to surface treatment will result in different hazard profiles. Although this need for a separate assessment of nanoforms is not stated explicitly in REACH, ECHA considers this to be implicit in the legal text. This practice of performing a separate assessment of each individual nanoform in a registration dossier covering multiple form(s) of a substance, should serve as a general guiding principle in this document.

The general rules for adaptation of the standard information requirements specified in Annex XI of REACH also apply to nanoforms of a registered substance. As described in Section 1.2.3, read-across according to REACH Annex XI, Section 1.5. is regarded as a technique for predicting endpoint (hazard) information for one target substance by using data from the same endpoint from another substance(s) (source substance(s)). These conditions will be slightly different for nanoforms, as all nanoforms of a substance are normally considered to be the same substance for REACH registration purposes. Grouping of nanoforms based on similarity and read-across between nanoforms within a registration dossier may be a useful tool for demonstrating safe use for all nanoforms of a registered substance.

By analogy to REACH Annex XI, Section 1.5, the application of the grouping concept to different nanoforms of the same substance requires that (eco)toxicological effects can be predicted by interpolation of data from source nanoform(s) (and/or non-nanoform(s)) of a substance to target nanoform(s) of that same substance. Based on the same principle, the relevant (eco)toxicological properties of the target nanoform(s) in a group could be predicted from the properties of the source nanoform(s) within that group. This prediction may be a result of observing a constant pattern of change of properties across each group.

Generally, the correct application of REACH Annex XI, Section 1.5 requires sufficient knowledge about the identity of the source and target substance(s). Insufficient knowledge of substance identity (e.g. inadequate knowledge of the chemical composition) can lead to challenges for regulators in assessing and thus accepting a proposed grouping (category) and/or read-across approach. By analogy, when applying grouping and read-across approaches to nanoforms within the same registration dossier, there is a need to have a sufficiently clear description of the identities of the source and target nanoform(s) so that both industry and regulators know “what the nanoform is”. However, adequate knowledge of the “what a nanoform is” may require additional information that is not part of the standard identity information for substances. To apply the principles outlined in REACH Annex XI, Section 1.5, adequate information on “what the nanoform is” should also be given in the registration dossier.

Therefore, the document at hand also presents details on the type of information needed to determine “what the nanoform is” (see below in Section 1). It is not only a prerequisite for successful use of the principles outlined in REACH Annex XI, Section 1.5, but also the basis for scientifically sound grouping of nanoforms.

Decreasing the size and surface modification(s) of a particle can change its characteristics in sometimes startling ways, affecting fundamental behaviours such as solubility, reactivity, environmental transport, and toxicokinetics, which in turn can affect its (eco)toxicological effects and the fate in the environment. In consequence, the Group Assessing Already Registered Nanomaterials (GAARN) has noted that (ECHA, 2013)

> *“When considering reading across to another nanoform or a counterpart bulk material, a solid scientific justification should be provided in the IUCLID dossier of the registered substance. It is insufficient to justify the use of data for read-across based only on the chemical composition of a nanomaterial, and further physicochemical parameters such as aspect ratio, shape, form, solubility, surface area, charge, surface treatment etc. should*

provide a reliable dataset to support a sound scientific interpretation of the similarities or differences among (nano)forms."

Of note, in the "Guidance for identification and naming of substances under REACH and CLP" (ECHA, 2014) shape and surface area are indicated as 'other' identification parameters, particularly for minerals. In addition, a revision of the REACH Annexes and related standard information requirements for substances that addresses possible amendments for nanomaterials is currently (2016) on-going.

2. Nanospecific considerations for read-across and grouping

As described in Chapter 1, application of read-across requires the proper characterisation of each nanoform. The spectrum of physicochemical parameters to be considered is provided in Section 2.1 Subsequently, Section 2.2 summarises the current state of knowledge on how the specific characteristics of nanoforms/nanomaterials may influence their (eco)toxicological and/or environmental fate properties.

Section 2.3 discusses the transformation of nanomaterials throughout their life cycle. As for any chemical, an important recognition is that relevant physicochemical properties of a nanomaterial may change during its life cycle or in different steps of its biological pathways (Arts et al., 2014; Oomen et al., 2014; Arts et al., 2015a). The OECD published a "Guidance Manual towards the Integration of Risk Assessment into Life Cycle Assessment of Nano-enabled Applications" (OECD, 2015) that may provide relevant background considerations in this context.

Furthermore, when considering possible trends in behaviour, it should be realised that several physicochemical parameters are closely related and may directly impact each other. Physicochemical parameters may work together enhancing their "individual" effects (e.g. reducing size and increasing relative surface area may both lead to a higher reactivity), or they may counteract changing the overall impact on the behaviour of a nanoform (e.g. reducing size may lead to higher reactivity but also to a higher agglomeration rate, which may reduce exposure and may result in a lower toxicity). Some physicochemical parameters may also influence the toxicokinetic behaviour of a nanomaterial whereas other parameters impact aspects of its toxicity.

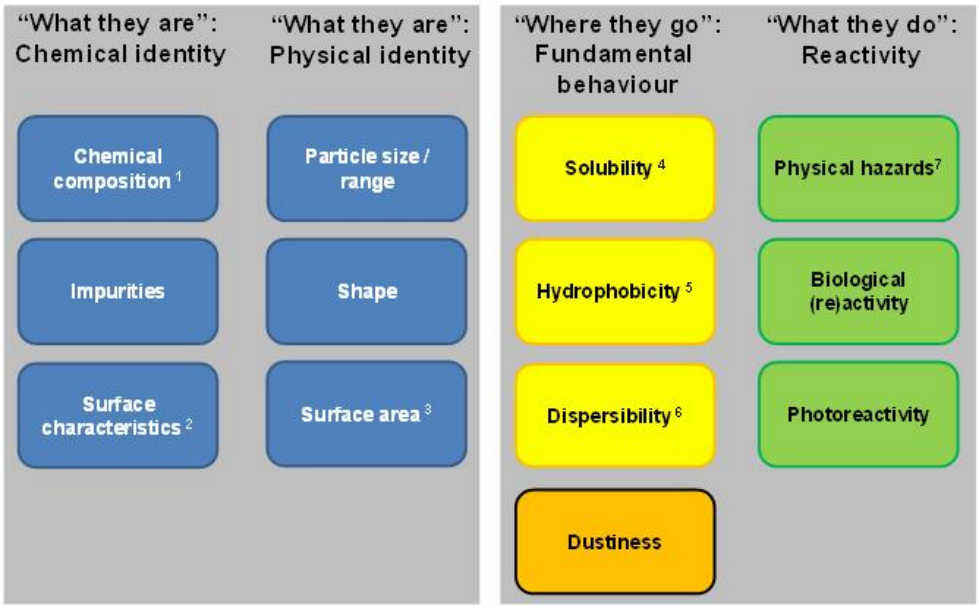
2.1 Physicochemical characterisation of different (nano)forms

The information currently required under REACH in Annex VI[5] may in some cases not be sufficient to identify and characterise nanoforms (SCENIHR, 2009; JRC, 2011) thus additional information on particle characteristics is essential to enable proper hazard assessment of nanoforms.

Efforts have been made worldwide to establish a set of physicochemical endpoints that would allow adequate characterisation of a nanomaterial for (regulatory) safety (risk) assessment (Oberdörster et al., 2005; SCENIHR, 2009; OECD, 2010; Stone et al., 2010; JRC, 2011; OECD, 2012, 2014b) and it is still subject to some discussion. There is general agreement that, in addition to the standard data requirements for physicochemical properties, some further properties to be routinely considered for

[5] Based on consolidated version of 22 August 2014, available at: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02006R1907-20140822.

studies of nanoforms include: specific surface area, particle size and particle size distribution, surface chemistry, agglomeration and aggregation, crystalline phase, shape and aspect ratio, photo-catalytic properties, porosity and pour density, dustiness, dispersibility, zeta potential and reactivity (redox potential, radical formation). The relevance of these properties would depend on the individual nanomaterial.



1. Chemical composition comprises crystal structure and crystalline phase.
2. Surface characteristics, which include coating chemistry, functionalisation (e.g. capping agents), surface charge (e.g. zeta potential).
3. Surface area includes porosity.
4. Solubility includes water equilibrium solubility and rate of dissolution in relevant media.
5. Hydrophobicity for nanoforms is dependent on e.g. van der Waals energy, Hamaker constant, zeta potential. Analytical determination of the hydrophobicity of nanoforms is still under development, e.g. sessile drop contact angle, dye adsorption.
6. Dispersibility refers to the relative number or mass of particles in a suspending medium, and relates to stability (Sellers et al., 2015), aggregation and agglomeration in relevant media, and is dependent on e.g. van der Waals energy, Hamaker constant, zeta potential.
7. Physical hazards comprise explosiveness, flammability, and autoflammability.

Figure 1. Key properties that characterise a nanoform (adapted from Sellers et al., 2015), arranged under headings from ITS-NANO (Stone et al., 2014). 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 needed (based on current knowledge) to implement the assessments described in Figure 3. The information on the chemical and physical identity ("What they are") can be used for a first assessment on the possibility to apply read-across for a given nanoform[6].

For physicochemical characterisation of a nanoform, a distinction can be made between intrinsic material properties (such as chemical composition, primary particle size, shape, and water solubility) and system-dependent properties defined by the surroundings in which the nanoform is placed (e.g.

[6] Note that i) some properties may not always be relevant for each read-across case, for example dustiness may only apply to powders; ii) some properties (for example dispersibility and solubility) are system-dependent, iii) the key physicochemical properties are derived mostly from studying nanoforms of metals, metal oxides or carbon based materials, and iv) differences in "what they are" may not have to exclude read-across per se, as "where they go" and "what they do" may be more important in read-across justification.

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, bio-physical interactions and *in vitro* effects to justify read-across. In this context, detailed information on the nanoform and its production process may also be valuable to better understand the behaviour and evaluate the validity of read-across.

The key physicochemical parameters for nanoform characterisation (that includes both REACH requirements for substances and additional nanospecific endpoints) listed in Figure 1 should be seen as a minimal set to be addressed when considering grouping or read-across of nanoforms.

2.2 Physicochemical parameters and their influence on (eco)toxicological and/or environmental fate

As described in Section 2.1, for nanoforms in particular both the intrinsic properties as well as system dependent properties affect (eco)toxicological and/or environmental fate. Table 1 includes a summary of the different parameters presented in Figure 1 and their relevance for environmental and human health endpoints. A more extensive overview of the current understanding of the potential influence of these different physicochemical parameters on the toxicological properties of a nanoform can be found in the scientific literature (e.g. Oberdörster et al., 2005; Sellers et al., 2015). On a case-by-case basis, additional parameters not included here may be relevant for certain nanomaterial and endpoints, e.g. for acute inhalation toxicity of fibre-like materials, rigidity (stiffness), hardness and aspect ratio of the material may play an important role in hazard and safety assessment (Tran et al., 2008).

Table 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

What they are (Chemical identity)
Chemical composition, including crystalline structure Detailed information on chemical composition is fundamental for determining human health and environmental effects of nanoforms, as is the case for non-nanoforms. However, size, shape and surface characteristics of a nanoform may cause the nanoform to exhibit a different behaviour compared to the non-nanoform of a material with the same composition. Crystalline structure may for some nanoforms influence other properties of the material (e.g. reactivity, zeta potential, Hamaker constant) in a way that affects human and environmental toxicity. Decreasing size of particles may introduce crystallographic changes in the material (contraction of the crystalline lattice or deformation). Based on the present understanding of nanoparticle behaviour, changes to the crystalline structure seems particularly relevant for metals, metal-oxides or carbon based nanomaterials.
Impurities As for non-nanoforms, impurities can substantially contribute to the human and environmental toxicity of nanoforms.

Surface characteristics, including coating chemistry, functionalisation (e.g. capping agents), surface charge density (e.g. zeta potential)

Considering human health endpoints, the surface chemistry of a nanoform affects its reactivity and systemic absorption. Surface modification(s) may determine which biomolecules adhere to the nanoform, its distribution and cellular uptake, and its toxic effects. Surface charge may influence systemic distribution and cellular uptake of a nanoform, and ultimately its toxicity.
In the environment surface characteristics will influence sorption to environmental or biological media and the reactivity of a nanoform. In addition, similar to considerations for human health endpoints, the surface chemistry of a nanoform affects its reactivity and systemic absorption. Surface modification(s) may determine the biomolecules that adhere to the nanomaterial, its distribution and cellular uptake, and its toxic effects. Surface charge may influence systemic distribution and cellular uptake of a nanomaterial, and ultimately its toxicity.

What they are (Particle characteristics)

Particle size / range

The size of the nanoform affects other physicochemical parameters, such as crystallinity, zeta potential and specific surface area, and may determine exposure, and whether the nanoparticle can be internalised into an organism. Once internalised, particle size may also affect the distribution within the body, and the toxicity at both the point of entry and distally. Size distribution is not a static parameter; it may also change during the course of (environmental) toxicity testing (as well as during the life cycle of the material) due to e.g. partial dissolution, interaction with test media or preferential absorption of smaller particles.

Shape

Particle shape may affect the internalisation of a nanoform (e.g. the ability of a nanoform to penetrate into a cell) and its (environmental) toxicity. In inhalation studies, particle shape may influence nanoform deposition within the lungs and may also influence its persistence in the lungs and probably in other sites. Shape may also influence mode-of-action like for high aspect ratio materials, as well as other parameters, such as zeta potential.

Surface area, including porosity

The increase of relative surface area with decreasing particle size may increase the reactivity of a nanoform relative to its mass. Furthermore, as a consequence of the increased surface to volume ratio, porosity may affect the crystalline structure.

Where they go (Fundamental behaviour)

Solubility: Rate of dissolution / Equilibrium solubility

The rate of dissolution depends on the chemical composition, particle size, coating, stability, manufacturing process, and biological environment; for substances that have a high rate of dissolution, the ion(s) may be dictating the toxicity also 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 is depending on the actual route of exposure and/or the environmental compartment under evaluation.

Hydrophobicity

Hydrophobicity for nanoforms is dependent on e.g. Van der Waals energy, Hamaker constant and zeta potential. Analytical determination of the hydrophobicity of nanoforms is still under development, e.g. sessile drop contact angle, dye adsorption. While these parameters can influence agglomeration and sorption, as well as ‘dispersibility in biological media’ and dustiness, currently the exact relationships between them are not clear

Dispersibility

This parameter can influence the degree of environmental transport and (environmental) exposure. Furthermore, this parameter may influence the degree of internal exposure (particularly by the oral route; however particle dispersibility also affects nanomaterial mobility within the lung and hence its potential for systemic uptake).

Dustiness This parameter is mainly relevant for exposure via air (particularly by inhalation) and transport through air. In the environment this parameter is not relevant to aquatic/sediment exposures and only to a limited extent for soil exposures.
What they do (reactivity)
Physical hazards: Flammability / Autoflammability / Explosiveness These parameters are relevant in assessing the risk of injury in occupational settings, but are not primary parameters characterised in (environmental) toxicity studies.
Biological (re)activity (or surface reactivity) The biological (re)activity or surface reactivity of a nanoform of a substance appears to generate reactive oxygen species (ROS) which induce inflammation, and thus elicit cellular toxicity.
Photoreactivity Photoreactivity may increase with decreasing particle size. In human toxicity testing, this parameter may be particularly relevant when considering dermal exposure, but it may also play a role in other exposure routes. In the environment this parameter may be particularly relevant when considering the aquatic compartment, but it may also play a role in other compartments.

In the environment, processes that influence the transport behaviour include adsorption and desorption processes to suspended matter, aggregation and agglomeration processes, sedimentation and re-suspension, dissolution, dispersion, biodegradation (of coatings), interaction with organic biomolecules at the nano-bio interface[7], interaction with contaminants, interaction with living organisms, and transfer via the food chain. Apart from physicochemical parameters of the nanomaterial these processes are influenced by environmental parameters, including temperature, pH, ionic strength (in particular, of divalent ions) and conductivity, presence and type of natural organic matter, dispersants and proteins. Interactions at the nano-bio interface are clearly influenced by the type of biomolecules (proteins, exudates, etc.) that are excreted/secreted by the organism under consideration. These processes are also relevant for non-nanomaterials, but as the ecotoxicological and/or environmental fate of a nanoform depends both on its chemistry and particle characteristics the influence of these processes are particularly important for nanoforms (Oberdörster et al., 2005; Sellers et al., 2015). In particular, the dispersion method and the composition of the aqueous media can influence the results when determining ecotoxicological endpoints for nanoforms (see section 2.3).

In (human) organisms, information on the (main) route(s) of exposure (inhalation, dermal, oral) is a first step in understanding the toxicokinetic profile of a nanomaterial. For example, for the inhalation route of exposure, specific deposition in the lungs needs to be considered. The toxicokinetic profile of a nanomaterial provides information on the absorption and subsequent exposure of target organs/tissues over time. Toxicokinetics, in a traditional sense, encompasses absorption, distribution, metabolism, and excretion (ADME). For nanomaterials toxicokinetics are further complicated by changes in the physicochemical properties of the material that may occur

[7] 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) (Nel et al., 2009).

during these different ADME processes. The specific toxicokinetic profile of a nanomaterial depends on several different physicochemical parameters of the nanoform, e.g. composition, size, shape, agglomeration/aggregation state, surface properties (including surface charge), hydrophobicity, and dissolution.

Furthermore, and parallel to the description above of the influence of environmental parameters on the fate and behaviour of a nanomaterial, the toxicokinetic profile in a (human) organism also depends on the temperature, pH and ionic strength of the biological fluid in which the nanomaterial is taken up (e.g. serum, saliva, blood). Hence, 'system-dependent properties' (i.e. dissolution rate in biological media, surface reactivity and dispersibility), biomolecules present and interactions at the nano-bio interface of cells at the target site can provide relevant information on the likelihood of distribution and potential for accumulation. Information from (available) *in vivo* studies including data on internal exposure and elimination over time provide further relevant information on the toxicokinetic behaviour.

2.3 Changes through the life cycle of a nanomaterial

While considering available data and evaluating the applicability of data from one nanoform for another nanoform, it is important to realise that throughout its life cycle, a nanoparticle is due to change because of e.g. encounters with other particles and constituents (e.g. ageing process, agglomeration, aggregation, corona formation), interaction with environmental media (e.g. dissolution, corona formation, aggregation or disaggregation, chemical reactions, transformation), and degradation (e.g. loss of the coating). This implies that nanoforms can change during their life cycle. Any change may affect one or more of the physicochemical parameters described in Table 1 and may potentially affect the activity, reactivity, fate, toxicokinetics and toxicity in a significant way, which could lead to a different behaviour. Therefore, any change in the physicochemical parameters during the life cycle may justify a careful assessment of its impact on (eco)toxicological properties to ensure that data from one nanoform may still be used for another nanoform. This may raise the question on whether source and target nanoforms behave similarly in the environment from the moment of emission to actual exposure and inside the organism. It also impacts on the way data on a form are obtained, raising questions on how the specific nanoform behaves in the test medium and whether the form tested is representative for the form to which an organism or the environment is exposed.

Similar processes of change may take place inside organisms as well as during the absorption, distribution, metabolism and excretion processes (ADME).

3. Justifying grouping and read-across of nanoforms

This chapter presents different steps to group nanoforms and to decide on read-across of data between nanoforms taking into account the provisions and definitions summarised in Chapter 1. Most importantly, the use of hazard data from a source nanoform to the target nanoform must be justified and scientifically substantiated. Therefore, the present chapter addresses the issues relevant for building and substantiating a hypothesis for such justification specifically for nanoforms. It should be noted that a different hypothesis may apply and may have to be developed per information requirement under REACH for which a data gap is identified.

Guidance on the approach for read-across under REACH is developed for chemicals and outlined in ECHA Guidance Chapter R.6 (ECHA, 2008). Further nanospecific considerations are indicated in the current document and ECHA Guidance (ECHA, 2012a, b, c). A matrix could serve to obtain a comprehensive overview of the available data and how data gaps have been addressed, either by generating new data or by using adaptations such as read-across. In the case of a registration dossier under REACH including nanoforms, the matrix should identify the different forms covered by that specific registration dossier. Where relevant, the matrix could furthermore illustrate the possible grouping of nanoforms, and could support in the justification used to define the boundaries of each group.

Taking note of the ECETOC decision-making framework for the grouping of nanomaterials (Arts et al., 2015a), information on the water solubility, dissolution rate, the aspect ratio, biophysical interactions and cellular effects, likelihood of biopersistency, and the reactivity of the nanoform may support a first formation of group(s) of nanoforms with a similar chemical identity for the purpose of read-across. A hypothesis for read-across may accordingly be developed for these group(s) of nanoforms rather than for each individual nanoform. During the development of this hypothesis, one may come to the conclusion that i) the initial grouping is well suited to address the data gap(s) identified, or ii) there is reason to redefine the group(s) (possibly into subgroups) to address the data gap(s) or iii) no grouping is possible.

3.1 A strategy for using data between nanoforms

This document focuses on a strategy for read-across, i.e. for using data from one or more source nanoforms to a target nanoform of the same substance to fill gaps in the hazard data.

The strategy is building on six different steps, which are further clarified in sections 3.1.1–3.1.7:

1. ***Identification of the nanoforms.*** This involves the identification of the nanoforms based on their basic physicochemical parameters (see “what they are” in Figure 1).
2. ***Initial grouping of the nanoforms.*** Based on similarities in those physicochemical properties that influence “what they are” and “where they go” (Figure 1), grouping of the identified nanoforms in the dossier may be considered. The boundaries of each group should be clearly defined.
3. ***Identification of available data and data gaps.*** This involves making an inventory (possibly in matrix form as described above) of the information available per hazard endpoint required under REACH for the nanoforms in each group, and consequently identifying where the data gaps are i.e. the target nanoform(s) and the endpoint(s) that need to addressed via read-across.
4. ***Identification of potential source nanoforms.*** For each data gap, this involves the identification of source nanoforms in the group from which information may be used for read-across to the target nanoform(s). This also involves (hypothesis based) scientific justification of the appropriateness of the identified source nanoforms.
5. ***Substantiate hypothesis.*** This involves information gathering to substantiate the hypothesis for read-across. When a group of nanoforms is considered for read-across it may be necessary

to re-evaluate the initial grouping. If applicable, a testing strategy can be built that may (partly) cover multiple data gaps.

6. ***Assess any new data for the impact on the hypothesis.*** In an iterative process, interpret the information that becomes available to evaluate if it sufficiently substantiates the hypothesis and builds justification for read-across (or not).

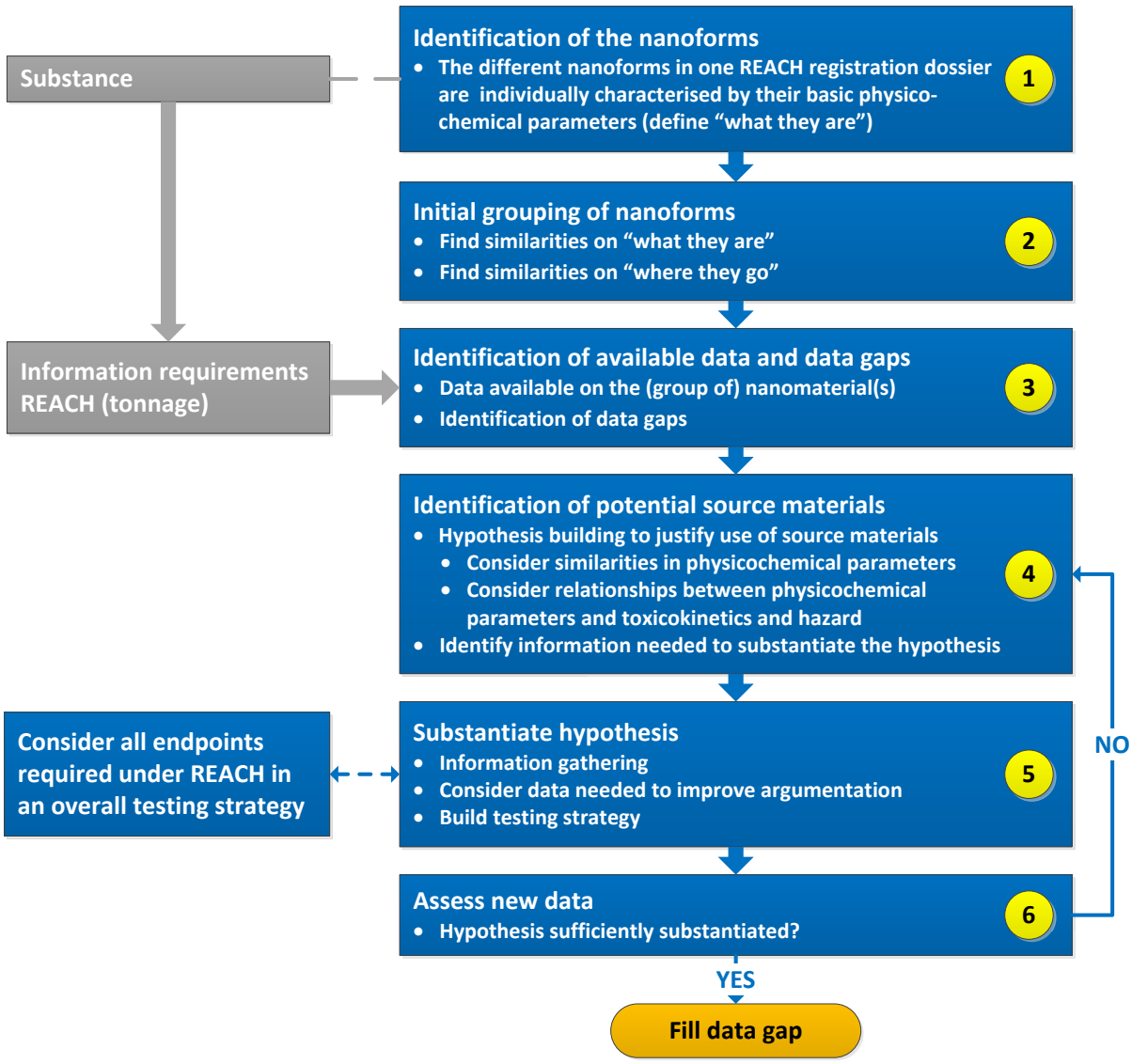


Figure 2. Strategy for using data between nanoforms.

3.1.1 Step 1: Identification of the nanoforms

The starting point is well characterised nanoforms that are uniquely defined by chemical and physical properties but share the same substance identity within one REACH registration dossier. When necessary, additional parameters may be needed to provide a proper characterisation of the identified nanoforms. In particular the chemical and physical parameters that define the nanoforms (i.e. chemical and physical identity parameters under the heading "what they are" in Figure 1) should be used as a starting point in identifying nanoforms.

3.1.2 Step 2: Initial grouping of the nanoforms

As with non-nanoforms, in case some nanoforms are grouped based on similarities in those physicochemical properties that influence “what they are” and “where they go” (see Figure 1), it is essential to characterise such a group and provide clear boundaries to define the scope of the group. This may include characterising every single nanoform in the group, or a (scientific) justification that the group can be characterised differently (cf. Arts et al., 2014; Arts et al., 2015a; Oomen et al., 2015), for example by characterising the output of a well-defined production process. The boundaries of a group should be defined by (a set of) specific physicochemical properties, e.g. by a detailed description of the production process, by the water equilibrium solubility of the nanoform that is similar to that of the non-nanoform, or by a specific (high) aspect ratio (cf. Arts et al., 2015a). It should be noted that there may be a limit to the diversity of nanoforms within a group, as a too diverse group might reduce the possibilities for read-across or strongly hamper a clear scientific justification for read-across.

As outlined above in the introduction of Chapter 3, in further steps in the read-across, additional gathering of data may initiate reconsideration of group boundaries, or subgroups of the initial group may need to be defined.

3.1.3 Step 3: Identification of available data and data gaps

Information requirements under REACH also apply to nanoforms. As nanoforms with the same substance identity may behave differently, there is a need to carefully assess, for each data requirement under REACH, if the data available on the substance are also representative for the registered nanoforms. Data gaps need to be identified and addressed. Motivated grouping of nanoforms (as addressed above in Section 3.1.2) may optimise the use of available data.

3.1.4 Step 4: Identification of potential source nanoforms

When a data gap is identified for a target nanoform[8], the first step in read-across is to assess whether available data for (an)other (nano)form(s) in the same group could be used to fill this data gap. This evaluation should be based on a hypothesis, meaning that hypothesis formulation and source material identification are two closely connected processes. The hypothesis should be fit to guide justification on which material(s) may be used as a source (non-)nanoform(s) to fill the data gap of the target nanoform, under which conditions or assumptions the identified data on source form(s) might be used, and which information (if any) should be generated to substantiate the hypothesis and validate the actual use of this data. A group approach (see Section 3.1.2) may provide a useful starting point for such hypothesis formulation, as well as e.g. knowledge about the production process, the mode-of-action, and the functionality of the nanoform or its uses. Further, the hypothesis should combine the chemical and physical identity (i.e. physical and chemical identity parameters described in Figure 1 and Section 3.1.1) with information regarding fundamental behaviour and reactivity to identify potential source form that are sufficiently similar to the target nanoform to be used for read-across.

[8] See description of read-across (Annex I) for further explanation of target and source forms.

Obviously, multiple data gaps may be present per nanoform or per group of nanoforms. To address these data gaps, hazard data from the same source nanoform may not be available. There are different possibilities to evaluate and scientifically justify the use of data from a source nanoform to fill a data gap.

Justifying read-across should aim at arguing that the source and target nanoforms are sufficiently similar to share data for a given endpoint under REACH. This, however, also implies developing a rationale for a scenario that maximises safety:

1. the target nanoform is equally or less hazardous than the source nanoform (hazard argument), and
2. very similar or smaller amounts of the target nanoform reach the target site compared to the source nanoform (toxicokinetics/environmental fate argument).

This argumentation can be based on available knowledge on relationships between physicochemical parameters, toxicokinetics and hazard characterisation.

In general, it is assumed that the more similarities are identified between the physicochemical parameters of source and target nanoforms, the more likely it is that read-across can be scientifically justified.

Consideration of source forms preferably include the assessment of benchmark materials[9] to establish when “similar” is “similar enough” for the purpose of read-across. However, the current state of knowledge does not yet allow the establishment of benchmark materials. Systematic testing and the evaluation of testing results should aim for a better understanding of nanoforms and especially the potential correlation between physicochemical characteristics and toxicity, understanding of toxicokinetic behaviour and fate, which may eventually result in the identification of benchmark materials. Arts et al. (2014; 2015a) presents first thoughts on a possible approach towards benchmark materials, which may inspire further thinking on how to evaluate “similar” for the different elements presented in Figure 1 in the evaluation of read-across potential and the identification of source materials.

When drafting a hypothesis, the following questions may further aid in the identification of potential source forms[10]:

- Is it a single nanoform under consideration or multiple nanoforms of the same substance that may be placed in one group?
- Is the nanomaterial organic or inorganic?
- Does the nanomaterial have a coating?
- What is the water solubility and/or dissolution rate of the nanoform?

[9] Benchmark materials are source (nano)materials, which have been tested and evaluated according to standard criteria and to which target materials may reliably be compared for grouping purposes.

[10] This list of questions is not intended as an exhaustive list.

- Based on knowledge of the manufacturing process and/or based on analytical data, does the nanomaterial contain impurities that are of (eco)toxicological concern in relevant amounts?
- Is there a non-nanoform of the material?
- Does the manufacturer make any claims regarding special properties of this material that are related to its purpose but may also be relevant in a read-across or grouping approach (e.g. transparency, reactivity, antibacterial activity)?
- Is any information available about how this nanomaterial and its properties change as it ages that may be considered in (eco)toxicological and environmental fate assessment?

For the potential source materials the following information should be available:

- Hazard and/or toxicokinetic data related to the specific endpoint
- Sufficient information to assess the data quality and reliability (see Section 3.1.6)

Currently, it is considered necessary that source nanoform hazard data are obtained via the same exposure route as the identified data gap for the target nanoform. This is based on the fact that the different routes of exposure may correspond to different exposure media that affect the toxicokinetic behaviour of nanomaterials. The specific effect of the exposure media on toxicokinetic behaviour of nanoforms, however, is not yet sufficiently understood to allow the extrapolation for nanoforms of data from one route to another, which sometimes can currently be applied for non-nanoforms provided that it can be justified.

The justification for using the data from the source material for the target material should then describe the evidence and assumptions feeding into the rationale for using the same data also for the target nanoform. The justification should furthermore describe what information should preferably be gathered to support, or falsify, the hypothesis. It is important to note that building the hypothesis should be seen as an iterative process. Based on the development of further insight in the potential to use data of the source material for the target material, the original hypothesis may have to be adjusted or another source material has to be identified.

3.1.5 Step 5: Substantiate hypothesis

Based on the hypothesis and the information needed to substantiate or falsify the hypothesis the required information should be gathered and/or a testing strategy should be developed. This strategy may follow a tiered approach, focussing first on information that could be obtained based on the physicochemical parameters, i.e. *in silico* or general insights. If needed, a second tier for substantiating read-across could then include *in vitro* testing. *In vivo* testing should be considered as the highest tier and should (currently) only be used to generate the REACH relevant data when read-across cannot be substantiated based on *in silico* or *in vitro* data.

This approach on information gathering is usual for read-across applied to chemicals, and the first steps in the tiered approach proposed by Arts et al. (2014; 2015a) are in line with it where Tier 1 involves testing of the intrinsic material (i.e. ‘what they are’ in Figure 1), and Tier 2, the ‘system-dependent properties’ (i.e. ‘where they go’ in Figure 1). Based on the outcome of their Tiers 1 and 2 Arts et al. (2015a) foresee further *in vivo* testing in Tier 3. When *in vivo* testing is considered

necessary to fill a data gap, this should be done for the REACH relevant endpoint, preferably in such a way that results can be used for a broader group of nanoforms.

Similar to building the hypothesis, it should be noted that the testing strategy potentially has to be adjusted after each test result obtained which may involve reconsidering the applicability of the source material.

Also a hypothesis based on assuring that less of the target nanoform reaches the target site (toxicokinetics argument), and the target nanoform is less hazardous (hazard argument), requires scientific substantiation. This is illustrated in Figure 3 and further explained below.

For toxicokinetics (i.e. 'where they go' in Figure 1), it may be possible to scientifically substantiate the argument that less of the target material is transferred across a portal of entry (skin, gastrointestinal tract, lung epithelium), deposited in the lung or distributed to target tissues, or that the target nanoform is better cleared/less persistent and thus less likely to accumulate over time. Such information can be obtained by physicochemical information (e.g. solubility) or biophysical testing (e.g. dissolution rate in physiologically relevant media), *in silico* (e.g. multiple path particle dosimetry modelling for lung deposition) and *in vitro* testing (e.g. skin permeation). It may also be necessary to obtain physicochemical parameters as input for e.g. *in silico* tools. For example, information on the aggregation/agglomeration state and aerodynamic diameter of a material in air is needed for modelling to estimate lung deposition. Obviously, the level of confidence that can be derived from the data of these different approaches to substantiate the hypothesis is different and this needs to be taken into consideration.

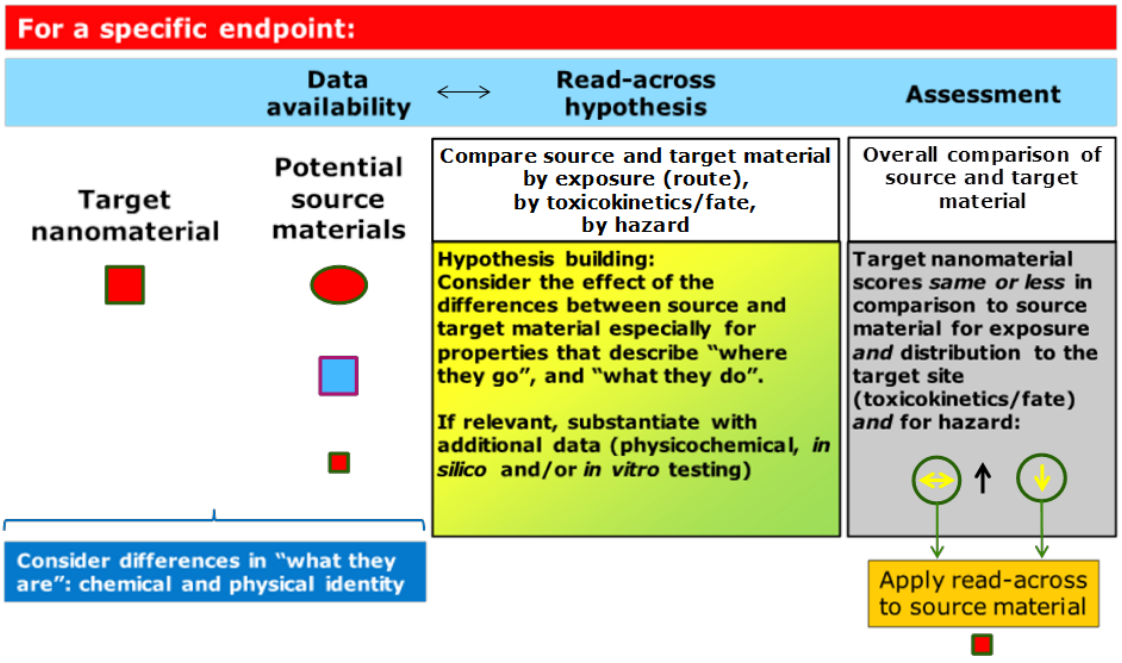


Figure 3. Different aspects of hypothesis substantiation per endpoint based on assuring that less of the target material reaches the target site (toxicokinetics argument), and the target material is less hazardous (hazard argument) (adapted from Oomen et al., 2015).

For hazard characterisation (i.e. 'what they do' in Figure 1), it may be possible to scientifically substantiate the argument that the target nanoform is equally or less hazardous than the source nanoform, e.g. by *in silico* methods and *in vitro* testing (e.g. reactivity, formation of reactive oxygen species). Knowledge on the mechanism of toxicity can help to select suitable tests and build the argumentation. The *in silico* methods such as quantitative structure-activity relationships are in the initial phases of development, see e.g. Burello and Worth (2011).

For both *in vitro* and *in vivo* toxicokinetic and hazard data, the testing conditions should be considered, and especially their relevance for the target nanoform. For example, it should be evaluated if the testing medium and dispersion method used to obtain the *in vivo* data of the source material are also relevant for the target nanoform; and if it can be anticipated that the testing conditions result in differences in behaviour between target and source nanoform, e.g. that one nanoform may aggregate whereas the other does not.

3.1.6 Step 6: Assess new data

As a final step, the toxicokinetics and hazard arguments are combined into an overall assessment of whether the data of a source nanoform can be used for hazard/risk assessment of the target nanoform. Application of source data is only deemed possible if both the toxicokinetics and hazard arguments show that less or similar amounts of the target material reaches the target site and is less or equally hazardous. The uncertainty related to the different pieces of information needs to be considered in the overall assessment.

Ultimately, the information gathered in Step 5 should be used to scientifically justify the read-across hypothesis. This justification should at least address the potential influences of physicochemical parameters and any differences between the source and target nanoforms as illustrated in chapter 1. This approach is illustrated in Figure 3.

In summary, any nanoform needs to be properly characterised by chemical and physical composition to enable identification (cf. Section 2.1 including Figure 1 and Table 1 as well as Section 3.1.1). Ideally, there should be no relevant differences in the impurity profile between the actual test material and the form with an identified data gap. If any differences are identified, their impact on the prediction should be assessed.

It is important that a hazard characterisation of nanomaterial(s)/nanoform(s) contains sufficient (eco)toxicological data, including scientifically sound justification for grouping and read-across and supporting information to enable adequate risk assessment. All hazard data should be presented in conjunction with a specific argument to what extent these data support the hazard characterisation of that nanomaterial.

The generation of an overview matrix of available data and data gaps identified by the registrant (cf. Section 3.1.3) may be especially helpful to organise the information on the different forms covered by the registration dossier under REACH, and to illustrate how these have been grouped and the justification used to delimit each group (cf. Sections 3.1.2, 3.1.4 and 3.1.5). The approach used for read-across should follow the one developed for chemicals as outlined in ECHA Guidance Chapter R.6 (ECHA, 2008) with nanospecific considerations as indicated in the current document and ECHA Guidance (ECHA, 2012a, b, c).

All supporting information that is relevant to the assessment of the nanoform(s) under evaluation should be reported. This includes any additional studies conducted to support the grouping and read-across approach. All data provided must also be accompanied with a detailed description of the materials (i.e. full characterisation, cf. Sections 2.1 and 3.1.1) and of the methods used, as well as appropriate statistical indicators of the quality and reliability of the results.

An analysis of the source study used for the prediction of a property needs to be conducted. The requirements regarding the results of the read-across method demands that the source study (and details of test set-up) meet all requirements for a key study used as stand-alone information to meet an information requirement under REACH (including the default REACH requirements regarding adequacy, relevance and reliability).

3.1.7 Final considerations

The underlying aim of this read-across assessment is to minimise testing as much as possible whilst maintaining a high level of protection of man and the environment, through compliance with REACH. When building the testing strategy, the overall goal of REACH, which is to protect human health and the environment thus ensuring safe use of chemicals, should be fulfilled.

At present, the knowledge on the interplay between physicochemical parameters, (eco)toxicological properties and environmental fate is rapidly developing. This provides an avenue for better understanding of nanomaterials' specific properties and behaviour and can subsequently support progress in development of guidance to enhance the current risk assessment framework.

Nevertheless, the present approach for read-across between nanoforms as outlined above, points towards the need for data on physicochemical parameters of each nanoform as the crucial starting point to obtaining a better understanding on the behaviour, fate, toxicokinetics and toxicity of nanomaterials, which is the cornerstone in developing a scientific, robust justification for grouping or the use of data for read-across. Furthermore, the data quality is critical and monitoring of physicochemical parameters during testing is therefore a key element. This also requires harmonisation and standardisation of regulatory test methods for physicochemical, toxicokinetics and hazardous endpoints.

The approach in this document uses physicochemical parameters of each nanoform as the crucial starting point, and is limited to read-across for hazard endpoints. In the future it may be possible to explore the possibilities for read-across for physicochemical properties as well.

Each potential read-across option, including additional (time, money and animals for) potential testing needed for its scientific justification, should thus be weighed against the actual testing of the target material for REACH relevant endpoints.

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Annex I: Glossary of commonly used terms

CLP	Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures.
Nanoform	The term 'nanoform' is used in this document to distinguish forms of a substance that fulfil the EC Recommendation on the definition of the term 'nanomaterial' but differ with regard to size distributions, shape and/or surface chemistry.
Group (or category)	Under REACH, substances that are structurally similar with physicochemical, toxicological, ecotoxicological and/or environmental fate properties that are likely to be similar or to follow a regular pattern may be considered as a group of substances. Within a group of substances, a data gap might be filled by read-across, as described below.
Read-across	Under REACH, read-across is a technique for predicting endpoint information for one substance (target substance), by using data from the same endpoint from (an)other substance(s), (source substance(s)). Consequently, the read-across approach has to be considered on an endpoint-by endpoint basis.
REACH	Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Toxicokinetics	The relationship between systemic exposure of a substance/nanoform and its toxicity. Four main processes exist: absorption, distribution, metabolism and excretion (ADME).

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