ECHA
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GUIDANCE

Appendix for nanoforms applicable to the Guidance on Registration and Substance Identification

Draft (Public) Version 1.0 June 2019

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Reference:
ISBN:
Cat. Number:
DOI:
Publ. Date: xxx
Language: EN

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Version	Changes	Date
Version 1.0	First edition	xxx 2019

PREFACE

This appendix for nanomaterials has been developed in order to provide guidance to registrants preparing registration dossiers that cover "nanoforms". The advice provided covers nanospecific issues related with registration and characterisation of nanoforms.

This appendix does not preclude the applicability of the general principles given in the *Guidance on Registration* [1] and the *Guidance on Substance Identification* [2]. The parent guidance documents apply when no specific information for nanoforms has been given in this appendix.

The aim of this document is to provide guidance on how to interpret the term "nanoform" for registration purposes and provide advice on how to create "sets of nanoforms" in a registration dossier. It also outlines what is expected in terms of characterisation of the nanoforms and sets of nanoforms in the registration dossier.

This guidance does not aim to give potential registrants advice on how to fulfil their information requirements for the substances they are registering. This is addressed in other guidance material (See [3], [4], [5], [6]).

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1. Introduction

This guidance has been developed to provide advice to registrants preparing registration dossiers that cover "nanoforms".

Section 2 of the guidance explains general requirements regarding the registration of nanoforms.

Section 3 explains the concept of nanoform, how to distinguish one nanoform from another and the characterisation requirements when registering individual nanoforms.

Section 4 focuses on how to create and justify sets of different nanoforms and details the characterisation and reporting requirements when registering sets of nanoforms instead of individual nanoforms.

Section 5 illustrates the concepts of nanoforms and sets of nanoforms in the context of a joint submission.

2. General considerations

The Guidance on Registration outlines the steps that potential registrants need to follow. These include:

- Determining their registration obligations, including establishing the identity of the substance and considering joint submissions with other registrants where relevant, and
- collecting/generating of relevant Annex VII-XI data,
- ultimately submitting this information in technical dossiers to ECHA.

This document will not repeat this information, as registrations that cover nanomaterials will follow the same principles as for registrations that cover a variation in compositions of the substance registered, and/or in any other relevant parameters. For additional information, see ECHA Guidance for identification and naming of substances under REACH and CLP [2].

This Guidance gives in the first place advice on how to fulfil the REACH Annex VI requirements, i.e. requirements applicable to each individual registrant in a joint registration. However, the general principles regarding the creation of sets of nanoforms are applicable at joint submission level. Appendix III of the ECHA Guidance for identification and naming of substances under REACH and CLP [2] gives advice on how to apply substance identification principles when collectively defining the identity and scope of the substance covered by a registration in a format of a substance identity profile (SIP). For further details see also section 5 that provides an overview on how the identification of nanoforms and the creation of sets of nanoforms fits into a joint submission.

This document provides additional advice for potential registrants to assist them in understanding what nanoforms are and how to characterise them for registration purposes. It also provides advice on how to build sets of nanoforms and how to report the identified nanoforms and sets of nanoforms in section 1.2 of the registration dossier consistently and clearly.

2.1 Registration obligations

The Commission Regulation ((EU) 2018/1881) of 3 December 2018 amending REACH to address nanoforms of substances makes it explicit that nanoforms of a substance need to be

covered by the registration dossier. Annex VI defines the terms "nanoform" and "set of similar nanoforms" and establishes the requirements for characterisation of the identified nanoforms/sets of similar nanoforms of the substance. The parent guidance on registration [1] explains in section 4.1.1 the minimum information that the registrant has to provide on the intrinsic properties of the substance. These requirements depend on the manufacturing tonnage of the substance. For nanoforms, REACH Annexes VII-XI include some specific information requirements (e.g. dustiness) or modifications to the existing ones in the form of adaptations or limitations of waiving possibilities.

Once the registration obligation is triggered for a substance, any of its nanoforms manufactured or imported must be reported in the registration dossier of the substance. Otherwise, such nanoform will be in breach of the REACH regulation

The tonnage trigger requirements apply, as explained in the Guidance on Registration [1], in the same way as they apply to any different compositions of the same substance.

This means that the tonnage trigger for registration applies to the total tonnage of a substance manufactured or imported by a single registrant [7]. Thus, for registrants of non-nanoforms and nanoforms of the same substance, it is the total volume that will determine the need for registration and the information requirements for the registered substance.

The registrants must ensure that the information provided to fulfil the information requirements for the registered substances with nanoforms, is adequate for assessing all the nanoforms covered by the registration.

According to Annex VI of REACH, "*More than one dataset may be required for one or more information requirements whenever there are significant differences in the properties relevant for the hazard, exposure and risk assessment and management of nanoforms*".

3. Nanoforms

The revised Annex VI of REACH introduces the concept of "nanoform" into the Regulation. It establishes the principles that all the nanoforms of the substance that are covered by the registration have to be reported in the registration dossier. By derogation to this principle, the revised Annex VI enables registrants to report several nanoforms together if certain conditions are met. The following sections will explain the criteria and conditions to report nanoforms (section 3.1) and sets of nanoforms (section 4).

3.1 Nanoform concept

According to Annex VI of the REACH Regulation, a "nanoform" is a form of a natural or manufactured substance[1] containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm, including also by derogation fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm. The concepts and terms used for nanoform in this guidance follow the concepts and terms used in the European Commission's definition of nanomaterial [8] as

[1] Please note that some substances may not require a registration. For further information on substances exempted from the REACH Regulation, exempted from registration or regarded as already registered see sections 2.2.2, 2.2.3 and 2.2.4 of the *Guidance on registration*.

outlined and explained in the Joint Research Centre (JRC) Report 'An overview of concepts and terms used in the European Commission's definition of nanomaterial' [9].

A 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 differences in the parameters in points 2.4.2 to 2.4.5 (size distribution, shape, surface treatment and functionalisation and specific surface area of the particles).

Variation of one or several of the characterisers defined in section 2.4.2-2.4.5 results in a different nanoform, unless such variation results from a batch-to-batch variability. A batch-to-batch variability only results from the variation of parameters inherent to a manufacturing process that is defined by a series of process parameters (e.g. starting materials, solvents, temperature, order of manufacturing steps, purification steps, etc.). In this context, the process parameters can be modified only to minimise the batch-to-batch variations. Any other modification in process parameters results in a different nanoform.

Different processes may result in almost identical characterisers. These different nanoforms can be registered as part of a set of different nanoforms. In such cases, the creation of a set of nanoforms will be simple as the variation of the different characterisers will be small (see section iv. The smaller the variation the easier the justification to cover different nanoforms in the same set.

Sections 3.1.1 to 3.1.4 below provide explanations on the determination of nanoforms in practice for each parameter set out in section 2.4.2-2.4.5 of the revised Annex VI of REACH. Each of the sections explaining how nanoforms are identified includes a subsection on the characterisation requirements for an individual nanoform for the parameter described. For the sake of clarity, the explanations are given for each specific parameter. However, when considering what constitutes a different nanoform, the four parameters need to be considered jointly.

3.1.1 Particle size distribution and number fraction of constituent particles

REACH Annex VI section 2.4.2 requires the reporting of the number based particle size distribution with indication of the number fraction of constituent particles in the size range 1 nm to 100 nm. When the Guidance refers to 'particle size distribution', it refers to the number based particle size distribution in line with the JRC Report [9]. When the Guidance refers to number fraction (of constituent particles or of nanoparticles) it refers to the number fraction of constituent particles in the size range 1 nm to 100 nm.

3.1.1.1 Distinguishing one nanoform from another

Each single nanoform has a specific particle size distribution where the variability in the distribution is within the batch-to-batch variability. Any variability in the particle size distribution beyond batch-to-batch variability creates another nanoform. The range of the values to be reported as described in the section 3.1.1.2.1 reflects the batch-to-batch variability.

3.1.1.2 Requirements for measurement or calculation method

The measurement or calculation method to determine the particle size distribution and the number fraction of constituent particles needs to be scientifically sound. When selecting the most suitable measurement or calculation method(s), the registrant needs to keep in mind that not all methods are suitable for nanoforms, and some methods are suitable only for certain nanoforms. For example, shape, size range as well as the chemical and physical nature of the particles need to be taken into consideration when the method is selected [10], [11], [12]. The registrant is recommended to use at least one electron microscopy technique to measure the particle size distribution and the number fraction of constituent particles. The

electron microscopy techniques can also provide essential information for reporting the length of the elongated particles and the two lateral dimensions (orthogonal external dimensions other than thickness) of the platelets.

The particle size distribution should be measured on the nanoform as manufactured. Where the particles are surface-treated or functionalised, the method(s) to measure the particle size distribution should be selected in such way that results provide information on the size of the particles in accordance with the nanomaterial definition [8], [9]. This may require use of more than one method providing complementary results.

3.1.1.2.1 Reporting in the dossier

The registrant needs to provide in the dossier the particle size distribution of the external dimension of the particles of the nanoform following the concepts defined in the JRC Report [9] as a histogram with a table showing values on which the histogram is based. In addition, the registrant needs to provide the number fraction of constituent particles with at least one of the external dimensions in the size range 1 nm to 100 nm as a value between 50 % and 100 %[2]. In the case of high-aspect ratio particles and platelets, the external dimensions are the width and the thickness, respectively. In the context of reporting the particle size distribution, a d_{10}[3], d_{50}[4] and d_{90}[5] value each with a range reflecting the batch-to-batch variability must be reported. For the determination of the number fraction of the constituent particles, all the measured particles of the nanoform must be taken into consideration.

The registrant must describe the method(s) used and provide all the relevant bibliographical references in the dossier. The description of the method(s) needs to include the description of sample preparation, instrument parameters, functions and calculations applied, as appropriate, as well as the measurand or precise name of the external dimension of the particles used in the measurement (e.g. minimum Feret diameter or maximum inscribed circle diameter) and the corresponding measurement uncertainty. The measurement uncertainty needs to be expressed in line with principles outlined in the document JCGM 100:2008 [13]

3.1.2 Shape, aspect ratio and other morphological characterisation

According to section 2.4.4 of Annex VI of the REACH Regulation information on "Shape, aspect ratio and other morphological characterisation: crystallinity, information on assembly structure including e.g. shell-like structures or hollow structures, if appropriate", must be assigned to each nanoform.

Morphological characterisation of a nanoform requires information on the shape of the particles (including information on the aspect ratio and assembly structure), and information on crystallinity of the constituent(s) of the nanoform. In this document, shape (including aspect ratio and assembly structure) is discussed in a separate section (Section 3.1.2.1) from crystallinity (see section 3.1.2.2).

While shape and crystallinity are in different sections in this document, a registrant must take into account both parameters when deciding whether to distinguish between nanoforms.

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[2] For a nanoform, the value for the number fraction needs to be 50 % or more. If a registrant manufactures or imports a form were the number fraction is below 50 %, the registrant should still maintain the information on the particle size distribution of those forms as an evidence for any possible enforcement actions.
[3] Size for which 10 % of the particles have size less than this value
[4] Median size of the particles
[5] Size for which 90 % of the particles have size less than this value

3.1.2.1 Shape, including aspect ratio and assembly structure

3.1.2.1.1 Distinguishing one nanoform from another

Solid particles can exist in a wide variety of shapes, such as spheres, cubes, tubes, wires, plates, etc. Each nanoform, as a result of a defined manufacturing process, can consist of particles of the same shape (e.g. cubic) or particles with different shapes can be present simultaneously (e.g. 30% spheres and 70% cubes). Any variability in the shape of the particles beyond batch-to-batch variability defines a different nanoform. When assessing batch-to-batch variability for shape, several descriptors/parameters need to be considered, e.g. aspect ratio and assembly structure.

When defining a particular nanoform, registrants should first see if any variability beyond the batch-to-batch variability occurs in size distribution (e.g. variation in the width for high-aspect ratio nanoforms). If no variations occur in width but changes in length occur (and consequently a different aspect ratio value is obtained), a different nanoform is defined.

Regarding assembly structure (e.g. multi-walled carbon nanotubes or nano-onions), variations in the characteristics of the assembly structure (e.g. number of walls or of concentric layers formed), will likely be captured by other parameters such as size distribution, and the result will in that case be the creation of a different nanoform. If such variations in assembly structure that go beyond the batch-to-batch variability are not already captured by the parameter size, the registrant must consider these variations separately.

The batch-to-batch variability is reflected by the range of values to be reported as described in the section 3.1.2.1.3.

3.1.2.1.2. Requirements for measurement or calculation method

In support to the description of the shape of the particles that constitute a nanoform, the registrant must always provide representative electron microscopy image(s) with a scale bar and the size in pixels (e.g. 2000 px x 3000 px) and the resolution in nm/px (e.g. 2 nm/px) of the image, accompanied by a description of the sample preparation method (e.g. dispersion medium and energy, temperature, etc.) and a reference to the standards and reference materials used. Electron microscopy techniques that can be typically applied for the analysis of the morphology of the particles are Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). Atomic force microscopy (AFM) is a microscopic technique that can be used for obtaining topological pictures of the surface of nanoparticles fixed on a flat substrate. The registrant must select, based on the material properties, the most appropriate technique for determining the morphology of the particles. The representativeness of the sample used for the measurements is fundamental. The issue of sample preparation and representativeness is extensively discussed in the documents ISO/TR 16196:2016 [14], OECD/ENV/JM/MONO(2012)40 [15] and ISO 14488:2007 [16]. Specific protocols for preparation of nanoparticles-containing products for microscopy methods are available within the Nanodefine project deliverables [17].

3.1.2.1.3. Reporting in the dossier

In order to characterise the shape (including aspect ratio and assembly structure) of the particles that constitute a nanoform, registrants must provide in the dossier, at first instance, an electron microscopy image that allows visualising the shape of a representative number of the particles that constitute the nanoform. A qualitative description of the shape of the particles must also be provided.

As the number of possible particle shapes for nanoforms is very large, for organisation purposes, four broad *categories of shapes* are defined and reported below:

- **Spheroidal:** this category includes particles with aspect ratio up to 3:1 and thus this is a category for approximately "equiaxial" particles. Examples of shapes included in this category are spherical, pyramidal, cubic, 3D star-shaped particles orthorhombic, polyhedral, etc.

- **Elongated**: this category includes particles with two similar external dimensions and a significantly larger third dimension (aspect ratio larger than or equal to 3:1). Examples of shapes included under the elongated category are tubes (particles with hollow structures), rods (solid, non-hollow particles), wires (electrically conducting or semi-conducting particles), etc.

- **Platelets**: this category includes particles with one external dimension significantly smaller than the other two external dimensions. The smaller external dimension is the thickness of the particle. Examples of shapes covered under this category are discs, plates, etc.

- **Multimodal shapes**: this fourth category includes particles whose shapes belong to different shape categories (e.g. 60% spheroidal and 40% elongated). A nanoform consisting of particles with multimodal shapes is the outcome of a manufacturing process and it is therefore by definition not obtained by mixing particles of different shapes.

Particles with irregular shapes are covered under the categories reported above and must be assigned to one of those categories based on their aspect ratio and on having one, two or three similar external dimensions.

These four categories of shape are illustrated in Figure 1.

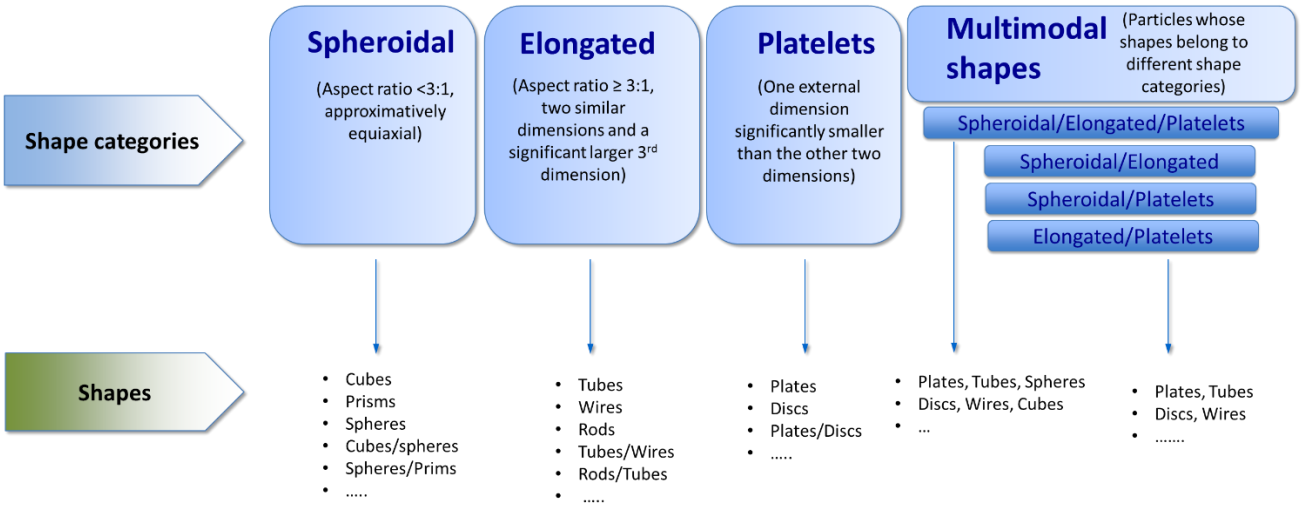

Figure 1: Schematic representation of shape categories and examples of some shapes for the categories a) spheroidal, b) elongated, c) platelets and d) multimodal shapes.

i. In order to qualitatively describe the shape of particles constituting a certain nanoform, at first instance the registrant must identify under which of the four shape categories (spheroidal, elongated, platelets, multimodal shapes) the specific nanoform would fall. The shape of the particles that constitute a nanoform will be allocated to one of the shape categories for reporting purposes. However, it should be noted that particles originating from distinct manufacturing processes resulting in different shapes falling

within a same category (e.g. spherical and cubical) are to be considered as different nanoforms.

ii. Within such generic categories of shape, a more precise description of the shape of the particles must also be provided by registrants (e.g. spherical particles with regular shape, for nanoforms that fall within the category spheroidal).

iii. Further specific information must be reported in the situations explained below:

i. For nanoforms made of particles falling under the elongated shape category (i.e. aspect ratio ≥ 3:1) and for platelets the aspect ratio must be provided. The **aspect ratio** is a geometrical shape descriptor defined as the length (or longest dimension) to width ratio of a particle. It is obtained from particle size measurements performed on the nanoform: by measuring the length/ lateral dimension (or longest dimension) and the width (or the smallest dimension perpendicular to the length dimension) of individual particles in the nanoform [18]. Where the nanoform in question contains elongated particles or platelets, the registrant should report the average aspect ratio with an indication of the variation (as a range), as well as the length/ lateral dimension (longest dimension of the particle), in addition to the width/ thickness of the particle (as also specified in 3.1.1.2). This information concerns specifically nanoforms consisting of elongated particles or platelets.

ii. For nanoforms made of particles with an **assembly structure**, specific information on the assembly structure must also be provided. Examples of assembly structures are those found in high aspect ratio nanoparticles with hollow structures such as nanotubes, or nano-onion spherical nanoparticles with concentric multiple shell structure, as described in ISO/TS 80004-2 [19, 20]. Another example is the one of the multi-layers formed in platelets, for example in graphene-based materials that consist of multi-layers rather than mono-layers. For these materials, information on the number of multiple walls/shells/layers formed will need to be provided.

iii. For elongated particles and for platelets, registrants are recommended to provide information on (flexural) **rigidity**. Rigidity, in the context of this Guidance, is the ability of an elongated particle or platelet to retain its shape, without damage, when subject to mechanical (bending) forces. The rigidity, together with aspect ratio, is known to influence the toxicity of all high aspect ratio nanoparticles (HARN) [21]. While there is currently no agreed measurement method for a parameter "rigidity", an indication of the rigidity of particles can be provided for example based on electron microscopy images (e.g. coiled/tangled versus straight particles), based on the particle width (covered by the requirement under section 2.4.2 of Annex VI of REACH) and length, number of walls (for particles with an assembly structure), etc.

iv. For nanoforms with multimodal shapes, details on the reporting are provided in the summary below.

Summary of reporting for shape

To summarize, when reporting information on shape for a single nanoform, the registrant must provide:

- The shape category under which the nanoform falls (e.g. spheroidal)
- The specific shape of the nanoform (e.g. cubic)
- An indication of the (average) number of walls or layers for particles with an assembly structure (e.g. nanotubes, nano-onions) with an indication of the variation (as a range)
- Electron microscopy image(s)

In addition to the above,

For a **nanoform** made of **elongated particles** the registrant:

- Must provide the average length (longest dimension) of the particles, the range reflecting the batch-to-batch variability and the supporting analytical data.
- Must provide the value of the average aspect ratio with an indication of the variation (as a range)
- Is recommended to provide an indication of the rigidity: the registrant is recommended to indicate in the dossier if the particles that constitute the nanoform are rigid or not

For **platelets,** the registrant**:**

- Must provide the average value of the lateral dimensions (two orthogonal external dimensions other than thickness, which is already covered under the requirement under REACH Annex VI section 2.4.2) of the platelets, the range reflecting the batch-to-batch variability and the supporting analytical data.
- Must provide the value of the average aspect ratio with an indication of the variation (as a range)
- Is recommended to provide an indication of the rigidity: the registrant is recommended to indicate in the dossier if the platelets are rigid or not

For a **nanoform containing particles with different shapes falling under a same category,** the registrant must provide:

- The shape category (e.g. spheroidal)
- An indicative composition in terms of specific shapes of the individual nanoform (e.g. 30% spherical and 70% cubic particles or 90% spherical and 10% cubic particles) and the range reflecting the batch-to-batch variability
- Reporting of particle size according to the selected shape category: for spheroidal particles reporting of size distribution as described under 3.1.1, for elongated additional reporting of length and aspect ratio and for platelets reporting of thickness, lateral dimensions and aspect ratio, as described above.

For a **nanoform containing particles with multimodal shapes (the shapes fall under different shape categories),** the registrant must provide:

- The shape categories and the specific shapes of the particles
- An indicative composition in terms of specific shapes of the individual nanoform e.g. 30% spherical particles and 70% nanotubes or 90% spherical particles and 10% nanotubes) and the range reflecting the batch-to-batch variability
- Reporting of particle size according to the shape categories. This means that if a nanoform is made of 70% cubic particles and 30% nanotubes, the dimensions related to the two different shapes (following the rules described above), should be reported separately.

3.1.2.2 Crystallinity

According to section, 2.4.4 of Annex VI of the REACH Regulation information on crystallinity must be assigned to each nanoform. Nanoforms can consist of atoms organized in periodic arrays (crystalline nanoform) or of atoms arranged in random assemblies without long-range atomic/molecular periodicity (amorphous nanoform). Moreover, in case of crystalline nanoforms of a substance, different crystal structures may (co-)exist.

3.1.2.2.1 Distinguishing one nanoform from another

Each nanoform of a substance has a specific amorphous or crystalline structure or a mix of the two. Any change in the structure beyond batch-to-batch variability creates another nanoform.

It must be noted that certain nanoforms may consist of particles with different crystal structures present simultaneously. This kind of nanoforms are not obtained by physically mixing particles of two different crystal structures, but are rather manufactured by specific processes that result in powders containing particles with different crystal structures. An example is that of a titanium dioxide powder, where anatase and rutile particles are present in the powder [22]. When a variation on the proportion of the different crystal structures occurs that goes beyond the batch-to-batch variability, a different nanoform is defined.

3.1.2.2.2 Requirements for measurements or calculation method

Information on crystallinity can be obtained through electron diffraction or (more often) through X-ray diffraction (XRD) analysis of the material. XRD can provide information on crystal structure (e.g. symmetry of the atoms in the unit cell and unit cell size); it can allow identification and indicative quantification of the crystal structures contained in a mixture. Different experiments or diffracting/scattering techniques may be used (e.g. small or wide-angle diffraction/scattering) depending on the type of structural information that one wants to gain [23].

For the characterisation of amorphous or partially amorphous nanoforms the interplay of more than one technique (e.g. XRD and X-ray absorption spectroscopy (XAS)) may be needed to obtain a complete picture of amorphous and crystalline fractions of nanoforms) [24]. A quantitative analysis using the Rietveld method can be performed on an X-ray diffraction pattern. The method involves fitting the diffraction pattern with calculated profiles and backgrounds to obtain precise quantitative analysis of a form containing particles with different crystalline and/or amorphous structures [25]. High-resolution TEM images may also be needed to demonstrate the amorphous nature of nanoforms.

3.1.2.2.3 Reporting in the dossier

When reporting in the dossier information on crystallinity of an individual nanoform, the registrant must specifically provide:

- Analytical data proving the amorphous/crystalline nature of the nanoform
- A description of the analytical method(s) used (including information on reference material), the functions and calculation method(s) used, as well as a description of the method uncertainties. The description should be given in such detail that the method can be reproduced.
- For crystalline nanoforms the registrant must report the name of the crystal structure (e.g. rutile) or the related crystallographic parameters (crystal system, Bravais lattice parameters)

In addition to the above, the registrant must clearly report in the dossier:

For **crystalline nanoforms** consisting of particles with more than **one crystal structure**:

- The percentage and type of each different crystalline structure present (e.g. 20% (w/w) rutile, 80% (w/w) anatase) and the range reflecting the batch-to-batch variability.

For **partially crystalline nanoforms**:

- The percentage and type of crystalline structure(s), the percentage of amorphous fraction (e.g. 20% (w/w) rutile, 70% (w/w) anatase, 10% (w/w) amorphous titanium dioxide) and the ranges reflecting the batch-to-batch variability.

3.1.3 Surface functionalisation or treatment and identification of each agent including IUPAC name and CAS or EC number

According to section 2.4.3.of Annex VI of the REACH Regulation, characterisation of a nanoform of a substance must include a "*Description of surface functionalisation or treatment and identification of each agent including IUPAC name and CAS or EC number*".

3.1.3.1 Distinguishing one nanoform from another

Surface functionalisation or treatment can be defined as a reaction between the functional groups on the surface of a particle and a substance called surface treating substance. The surface of particles can be modified by single or multiple surface treatments and the treatment(s) can fully or only partially cover the surface of the particles.

Particles can be extensively modified with the addition of various agents to their surfaces (e.g. inorganic treatment, organic treatment) or modification of their surface functionalities (e.g. oxidative treatment, reductive treatment). For example, particles of synthetic amorphous silica can be functionalised with very different surface treating agents (e.g. alumina, trichloromethylsilane, low silanol group density, high silanol group density, etc.).

Surface functionalisation/treatment can be applied to control particle properties like dispersibility in specific solvents (water, organic, polymers, etc.), reactivity (e.g. enhance catalytic activity or switch it off completely), solubility/dissolution rate (e.g. treatment of calcium carbonate, silver, ZnO, etc.), etc.

Surface treatment can refer to organic surface treatment (e.g. silica particle surfaces modified with alkylsilane), inorganic surface treatment (e.g. TiO_2 particle surfaces modified with alumina, zirconia, silica, etc.) or sequential inorganic and organic treatments to a given particle core (e.g. TiO_2 particle surfaces modified sequentially with zirconia, alumina, silica and alkylsilane giving layers of different chemistries with the alkylsilane as the last/outer layer).

A good schematic of possible types of surface treatments/functionalisations is provided in the DaNA website at the following link: https://nanopartikel.info/en/nanoinfo/cross-cutting/993-coatings-cross-cutting-section [26].

Any variation beyond the batch-to-batch variability on the surface treating agent applied, of the reaction conditions, of the molar ratio of surface treating agent applied generates a different nanoform.

3.1.3.2 Requirements for measurement or calculation method

The registrant must select the most appropriate analytical method(s) that allow obtaining a full picture on the overall composition of the nanoform (the composition of the particle as a whole, including its surface treatment). The registrant is also recommended to provide, when feasible, analytical data that would support specifically the identification of the functionalities/treatment layer(s) formed on the particle's surface. Based on the nature of the treating agent (e.g. inorganic or organic), different types of analytical techniques (e.g. IR,

NMR, TGA, ICP-MS, XRF, XPS, EDX, etc.) may be used for both the identification and the quantification of the surface treatment. Specific protocols have been developed for quantitative analysis of both inorganic and organic surface coatings within the context of NANOREG [27] and by ISO [28]

3.1.3.3 Reporting in the dossier

When reporting information on surface treatment/functionalisation of a nanoform, the registrant must report the following:

- IUPAC name and CAS or EC number of each agent used for surface functionalisation/treatment
- Description of the main features of the process: a description of the type of process/reaction (hydrolysis, oxygen treatment, acid washing, etc.), together with relevant ranges of process parameters such as reaction conditions (pH, temperature) and any purification step applied
- Molar ratio of each surface treating agent used
- A description of the functionalities introduced by the treatment (e.g. carboxyl, amino, hydroxyl groups)
- Information on the indicative weight-by-weight contribution of the surface treating agent(s) over the total weight of the particle
- When possible, an indication of the percentage of coverage of the particle's surface. Weight-by-weight contribution and indicative percentage of coverage of the particle's surface can be provided based on knowledge of the type of reaction occurring, amount of starting materials used, purification steps, combined with information achieved by using standard analytical techniques, such as ICP, XRF, IR, elemental analysis of C, H, N, O and S (as part of the determination of the overall composition of the nanoform)
- A description of the analytical method(s) used for determining the overall composition of the nanoform, including its surface treatment. The description of the methods must be given at a level of details that would allow the methods to be reproduced.

Schematics of the functionalisation/treatment can also be provided to visually describe the treatment, including the functionalities formed on the surface of the particles that constitute (a) certain nanoform(s).

For example, organosilanes are important coupling agents used to modify surface chemistry [29]. An illustrative example of an organosilane coupling chemistry is given in Figure 2.

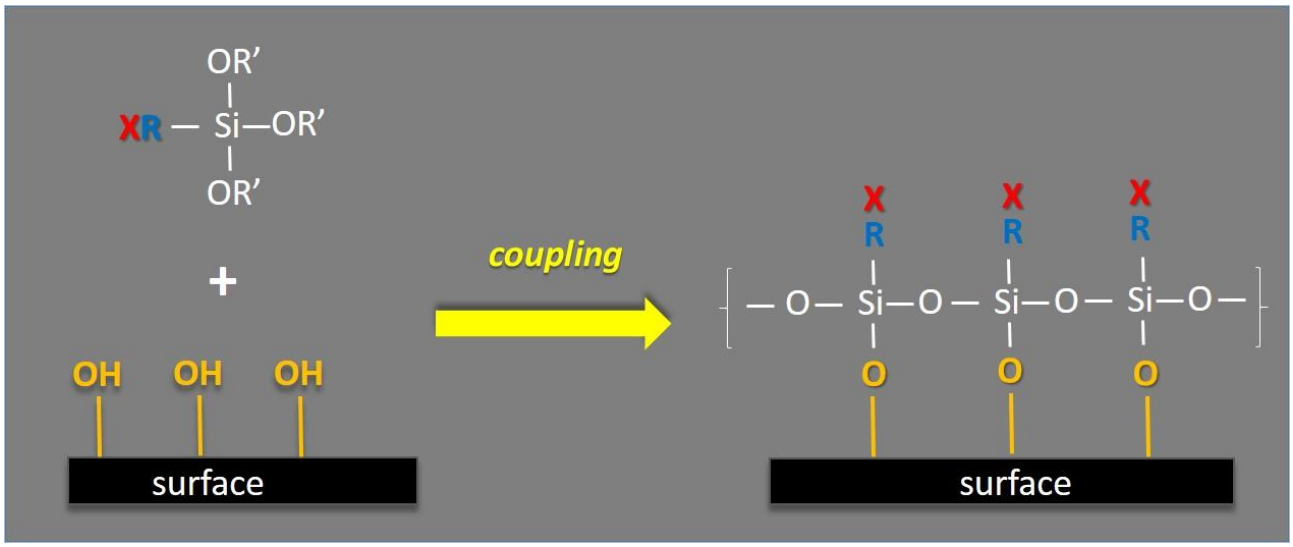

Figure 2: Schematic of an organosilane surface treating agent XR-Si-$(OR')_3$ and the chemistry it

imparts to the surface of the particle after surface treatment.

The alkoxysilane groups -Si-(OR')3 react via hydrolysis and condensation reactions with the surface hydroxyl groups to covalently bond functional polysiloxanes to the surface. Note that the chemistries of the agent and the treated surface are different. X-R-Si(OR')3 is an organosilane molecule where X = a non-hydrolyzable organic moiety e.g. vinyl, OR' = a hydrolysable group like e.g. an alkoxy group that can react with various forms of hydroxyl groups. R is a spacer that can be for example a linear alkyl chain.

Multiple/sequential surface treatments

When sequential surface treatments are applied to a nanoform multiple layers can be formed (see Figure 3) that can either fully or partially cover the particle's surface.

When multiple layers are formed, information on surface functionalisation/treatment as described above must be provided for each different surface layer. The registrant must therefore provide identification of each agent used for each sequential surface functionalisation/treatment, including IUPAC name and CAS or EC number.

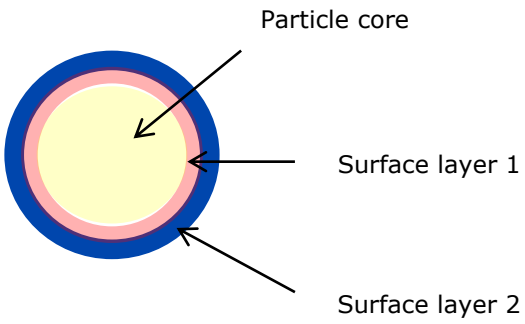

Figure 3: Idealised schematic representation of a nanoform whose surface has been modified by sequential surface treatments.

The registrant must provide the weight-by-weight contribution of each surface treating agent and, when possible, an indication of the percentage of coverage of the particle's surface for each individual layer.

When incomplete/not homogeneous coverage is obtained on the surface of the particles, the registrant is recommended to provide an indication (e.g. as a scheme) of the distribution and amount of the different surface treatment components on the surface of the particles.

3.1.4 Surface area (specific surface area by volume, specific surface area by mass or both)

In accordance with Annex VI, Section 2.4.5 of the REACH regulation, information on surface area (specific surface area by volume, specific surface area by mass or both) is required for

nanoforms of a substance.

The surface area of a material may also be a useful metric in deciding whether the particular material meets the definition of a nanomaterial. According to the current EC recommendation for the definition of a nanomaterial, materials with a volume specific surface area > 60 m^2/cm^3 are nanomaterials. Further information on role, as well as challenges of using the surface area to determine whether a material is a nanomaterial can be found in the JRC report "An overview of concepts and terms used in the European Commission's definition of nanomaterial" [8], as well as the NanoDefine methods manual [9].

3.1.4.1 Distinguishing one nanoform from another

For nanoforms, the specific surface area represents one of the characterisation parameters required by the regulation. Each nanoform will have a defined (specific) surface area with batch-to-batch variability. Any variability in the specific surface area beyond batch-to-batch variability creates another nanoform. The batch-to-batch variability is reflected by the range of the values to be reported as described in the section 3.1.4.3.

As the specific surface area in principle is related to the size of the particles (with smaller particles in general having relatively larger specific surface areas, and vice versa, all other things including shape and porosity being equal), the particle size and specific surface area of any particular nanoform are linked together. Therefore, because deliberate changes to particle size distribution result in new nanoforms (as described in the section on particle size distribution), this will in most cases be accompanied with changes to the specific surface area of the (new) nanoform.

3.1.4.2 Requirement of measurement or calculation method

The surface area is measured as the total surface of the substance, including both the internal and external surface of the substance. The information can represent the total surface area of the nanoform per unit mass (specific surface area by mass, in units of m^2/g), or alternatively the surface area of the nanoform per unit volume (specific surface area by volume, in units of m^2/cm^3).

The specific surface area of a nanoform is generally measured via gas adsorption using the Brunauer-Emmett-Teller (BET) isotherm. In this technique, an inert gas, typically nitrogen, is used as an adsorbate. It should be noted that the identity of the adsorbate gas used in the measurement can impact the results obtained. The measurement of the specific surface area by volume using BET requires information on the density of the substance in question.

The principle of the method is to measure the adsorbate that is adsorbed to the surface of the material as a monolayer. The technique measures that amount of the adsorbed gas as a function of pressure, while holding the temperature constant, and this adsorbed amount is plotted against the relative pressure in order to obtain an adsorption isotherm. The adsorption isotherm is used then to calculate the area of the monolayer equivalent with the amount of adsorbed gas by applying the BET equation. The ISO method ISO 9277:2010 [30] provides a standardised method for the determination of the specific surface area of solids by gas adsorption-BET[6].However, the BET method is not applicable to all materials, and the ISO standard above is only applicable to adsorption isotherms of type II and type IV. Annex C of the ISO standard provides a strategy for the determination of specific surface area of materials

[6] According to the JRC Report on "Requirements on measurements for the implementation of the European Commission definition of the term "nanomaterial", nanomaterials must be a powder or a suspension of particles. JRC Report EUR 29647 EN, 2019

with a type I isotherm. Further information regarding the application of gas physisorption to the evaluation of surface area can be found from the IUPAC Technical Report on this subject. [31]

The calculation of a volume specific surface area via the BET method requires information about the density of the substance in question. Information on **relative** density is an information requirement under the REACH regulation Annex VII, 7.4, and detailed information on how to measure and report relative density can be found under the relevant ECHA guidance [32]. However, some important distinctions need to be taken into account in order to derive a correct value for volume specific surface area.

- The term density, as well as relative density can refer to different values/concepts. The relative density represents the density of a substance in relation to the density of water, and this is a dimensionless value (see Chapter R.7a of the Guidance on IR&CSA) [32]. Nevertheless, in order to report relative density, information on true density is needed. Furthermore, density often can refer to different values, including: bulk density, tap density, and skeletal density.

The measurement of these different values is done using different methods. In order to calculate volume specific surface area, information on **skeletal density** is needed, whereas information on bulk or tap density are inappropriate for the purposes of calculating volume specific surface area. Density is the quotient of the mass m and its volume V. The skeletal density is obtained when the volume measurement excludes measurement of void space between particles, and pore space within a particle. Skeletal density is usually measured using gas pycnometry (e.g. using ISO standard ISO 12154:2014). The current draft OECD Test Guideline on the measurement of surface area using the BET method provides further information on the appropriate measurement of density for the purpose of converting mass specific surface area to volume specific surface area.

3.1.4.3 Reporting in the dossier

When reporting information on individual nanoforms, registrants must report the following for each nanoform:

- The specific surface area of the nanoform (either by weight, volume, or both).
- The range of values for a single nanoform, reflecting batch to batch variability
- A description of the method used to determine the surface area
- When reporting volume specific surface area derived from BET measurements, the registrant must also submit information on the skeletal density that is necessary for determination of the volume specific surface area.

4. Sets of nanoforms

According to Annex VI of REACH: *A 'set of similar nanoforms' is a group of nanoforms characterised in accordance with section 2.4 where the clearly defined boundaries in the parameters in the points 2.4.2 to 2.4.5 of the individual nanoforms within the set still allow to conclude that the hazard assessment, exposure assessment and risk assessment of these nanoforms can be performed jointly. A justification shall be provided to demonstrate that a variation within these boundaries does not affect the hazard assessment, exposure assessment*

and risk assessment of the similar nanoforms in the set. A nanoform can only belong to one set of similar nanoforms[7].

Thus, registrant(s) can identify and characterise nanoforms in the form of *"sets of similar nanoforms"*, subject to explicit conditions:

1) boundaries for the parameters in 2.4.2-2.4.5 must be clearly defined. The variations will in this case arise from merging of information on different nanoforms (i.e. parameters such as shape, particle size distribution, surface treatment, surface area, are different, see section 3, for further information on what situations create different nanoforms).

2) A justification must be provided as to:

- Why the hazard assessment can be performed jointly, i.e. why the hazard profile of all the nanoforms within the set is the same. Some small variability is allowed as long as the hazard assessment is conservative and a single hazard conclusion can be reached for the whole set. For instance, when considering particle size distribution: gradual changes in hazard when reducing particle size may be covered within the same set. This may be justified by an adequate choice of testing material.

  The development of a set of nanoforms must not 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. If, however, to demonstrate that a hazard assessment is valid for several nanoforms, a registrant needs specific 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 per nanoform. Instead, this can be addressed via read across between those nanoforms in accordance with Section 1.5 of Annex XI of REACH.

  The justification should always be accompanied by the data supporting it, and it may include proposals for the testing to support the hypothesis.

- Why the exposure and risk assessment can also be performed jointly for the set of nanoforms. In practice if the same hazard profile is applicable and a common conclusion on exposure assessment can be reached for the set, the risk assessment should also cover the set.

  The assessment of the hazards of nanoforms and the evaluation of the exposure serve as a basis for the risk assessment. The developments mentioned below focus on the conditions under which the hazard assessment of the nanoforms in a set can be performed jointly.

  Regarding the exposure assessment for the nanoforms or the sets of nanoforms: it is not required to create different nanoforms or sets only because the individual nanoforms have different uses. However, the set of nanoforms needs to detail the complete list of uses (and corresponding contributing activities) for all the individual nanoforms. Where relevant, the identified uses need to be assessed and demonstrated to be safe. Such assessment must be relevant to all nanoforms, even if in practice a specific nanoform does not have a specific use (yet).

[7] In this document often the term "set of nanoforms" is used instead of "set of similar nanoforms", for simplicity, but it should be always interpreted the "set of similar nanoforms" as defined in Annex VI

In order to facilitate the building of a set of nanoforms this guidance provides for each parameter principles clarifying the boundaries of a set of nanoforms. These principles explain when differences in the characterisation parameters in 2.4.2 to 2.4.5 in Annex VI may trigger the need to build a different set of nanoforms. The guidance also provides advice on the information to be submitted for justifying each set of nanoforms.

In the same way as for the identification of nanoforms (see section 3), the explanations on how to build a set of nanoforms are given per individual parameter, for clarity. However, when building a set, variability of all the characterisation parameters in 2.4.2 to 2.4.5 in Annex VI needs to be taken into account together with chemical composition.

Where the registrant constructs a set of nanoforms, the information reported must be applicable to the entire set. The principles of reporting defined in section 3 for individual nanoforms should be applied to report the characteristics of the nanoforms defining the boundaries of the set.

A nanoform can only belong to one set of nanoforms.

4.1 Particle size distribution and number fraction of constituent particles

4.1.1 Principles on the boundaries of sets of nanoforms

If existing scientific knowledge shows that for a certain substance there is a threshold particle size within the range 1-100 nm, which induces a specific effect for particles with size below/above that size, the registrant must define two different sets of nanoforms. If a certain nanoform contains particles with size below and above the threshold, the registrant may consider, upon justification, where to allocate the nanoform (e.g. including such a nanoform to a set based on worst-case scenario considerations). The threshold size is substance dependent and the impact on some properties can be more or less significant in each specific case. The threshold may be related to quantum confinement or to other properties affecting hazard (e.g. rigidity). The registrant must assess based on available information whether a threshold effect exists for the nanoforms included in the set. The Registrant must include this assessment in the justification.

Given the impact of the particle size on the properties of the substance, including the hazard of the substance, the registrant must take into account the impact of particle size distribution when constructing any sets. The registrant must justify why the particle size distribution of the different nanoforms included within the set does not change the hazard assessment, exposure assessment, and risk assessment of those nanoforms. The registrant's justification must address as a minimum the following:

- How does the particle size of the different nanoforms impact the dissolution rate and solubility of the set members?
- How does the particle size of the different nanoforms within the set impact the toxicokinetic behaviour as well as fate and (bio)availability of the set members?
- How does the particle size of the different nanoforms within the set impact the (eco)toxicity of the set members? Is there a direct relationship between the particle size and the (eco)toxicity?

4.1.2 Reporting in the dossier

As a minimum and in accordance with the requirements under section 3.1.1.2.1 for a single nanoform, a registrant reporting a set of nanoforms must provide the particle size distribution and the number fraction of constituent particles of the nanoforms included in the set with the smallest and largest d_{10}, d_{50}, and d_{90} value. The registrant must also report the boundaries for

the set of nanoforms defined by smallest d_{10} and largest d_{90} value.

The registrant must submit a justification demonstrating that the hazards of the nanoforms covered by the set can be assessed jointly. This justification must include the minimum elements specified in the previous section. The registrant must also report the scientific information on which this justification is based or indicate if this justification is in the form of a hypothesis not based on scientific information.

4.2 Shape, aspect ratio and other morphological characterisation

4.2.1 Shape, including aspect ratio and information on assembly structure

4.2.1.1 Principles on the boundaries of sets of nanoforms

Particle shape can influence the mechanism of interaction of a nanoform with a cell (e.g. shape is an important factor that determines internalisation of nanoparticles) [33] and may affect the kinetics of deposition and absorption in the body [34]. For example, particle shape can influence the deposition of nanomaterials in the lungs upon inhalation [34].

Given the impact that the shape of the particles can have on the (eco)toxicological properties of nanoforms, differences in the shape of the particles must always be considered when building sets of nanoforms. If nanoforms of the registered substance fall under different shape categories (spheroidal, elongated, platelets or multimodal shapes as defined in section 3.1.2.1.3), those nanoforms must a priori not be part of a same set of nanoforms. The registrant may consider including nanoforms in a same set (e.g. spheroidal and elongated), if no significant differences in aspect ratio exist (e.g. nanoforms with aspect ratio of 3:1 and nanoform with aspect ratio of 4:1), however a justification must be provided.

Spheroidal nanoforms

Nanoforms with particles with different shapes all falling into the category of spheroidal particles (e.g. spherical and pyramidal nanoforms) may or may not have a different hazard profile. Separate reporting in different sets may be necessary if scientific publications/toxicological tests indicate that the difference in the shape of the particles leads to a difference in the toxicological profile. Therefore, if the registrant decides to report in a same set nanoforms with particles with different shapes all falling into the category of spheroidal particles, the registrant must justify why the differences in shape do not affect the hazard profile of the different nanoforms. For instance, this can be demonstrated by providing supporting literature demonstrating that the difference in shape of a nanoform does not affect the hazard profile or following criteria from available frameworks on grouping, see for instance the framework develop by ECETOC applicable for inhalation toxicity [35].

Platelets

The specific shape (plates, discs, etc.) and the thickness and lateral dimensions of the platelets can vary. The registrant must justify how these parameters will affect the toxicological profile of the different nanoforms. When different nanoforms are reported together, the registrant must justify why the variations do not affect the hazard profile.

Elongated nanoforms

Nanoforms with particles with different shapes (e.g. nanotubes, nanowires, nanorods) all falling into the category of elongated particles are likely to have different properties and a different hazard profile. As a principle, they should not be included in the same set.

Moreover, for elongated particles and especially for high aspect ratio particles, different

parameters can have an influence on their toxicity. The registrant will first need to consider the variation in width (i.e. cross sectional diameter).

The width, together with length, is considered as a critical parameter that can be used as an indication of the rigidity of these nanoforms. Consideration on rigidity is therefore linked to the requirement on particle size distribution in point 2.4.2 of Annex VI of REACH and the registrant must justify how the variation in width of the particles of the different forms will affect the rigidity of the particles and consequently the toxicological profile of the different nanoforms. When there is a variability in the width of the particles constituting the nanoforms covered by the set, the registrant must provide a justification demonstrating that this variation does not affect the joint hazard assessment of these nanoforms.

The registrant must also take into account variations in the length and aspect ratio of elongated particles when building the set of nanoforms. When there is a variation in length and/or aspect ratio of the particles of the nanoforms covered by the set, the registrant must provide a justification demonstrating that this variation does not affect the joint hazard assessment of these nanoforms.

Therefore, the registrant needs to decide whether to create additional sets based on these additional parameters and justify the choices made in the registration dossier. In cases where threshold values in length are known (e.g. from literature or from tests) to trigger a different behaviour, e.g. are linked to the carcinogenic potential typical of fibre-like materials, the registrant must create sets taking into account these thresholds. This means that if a different hazard is foreseen when length is higher than e.g. 15 µm, and some nanoforms have length above and others below 15 µm, two different sets must be created. If a certain nanoform contains particles having values of length below and above the threshold, the registrant may consider, upon justification, where to allocate the nanoform (e.g. including such a nanoform to a set based on worst-case scenario considerations).

Multimodal shapes

In the situation that a nanoform consists of particles with shapes that fall into different shape categories (e.g. of spheres and wires), as a principle this nanoform should be reported on its own (i.e. a new set should be defined). The registrant may still consider including such a nanoform in a set where the particles of the other nanoforms fall into one of these shape categories, but this decision must be justified, based on the grounds identified above for the respective shapes.

For instance, it may be known that a form with high aspect ratio particles has a higher toxicity than the nanoform with particles with other shapes, and therefore the nanoform with particles with other shapes can be included in a set of nanoforms with high aspect ratio particles by justification via worst-case scenario. It must be highlighted that the justification shall cover all different endpoints, i.e. the registrant shall be able to justify that the specific shape has a lower toxicity for all endpoints.

4.2.1.2 Reporting in the dossier

When reporting a set of nanoforms, the registrant must always provide:

- The shape category of the set (e.g. spheroidal)
- A list of the specific shapes covered under a certain set (e.g. spherical, cubic, pyramidal)
- The range of number of walls or of layers for particles with an assembly structure (e.g. nanotubes, nano-onions). The range must reflect the variation between the nanoforms

that are part of the set.

- An electron microscopy image for each nanoform with a different shape included within the set (i.e. one for the spherical, one for cubic) or for each nanoform with a different combination of different shapes. This practically means that if a set includes two nanoforms consisting of 100% spherical particles, two nanoforms consisting of 100% cubic particles and three nanoforms with different concentrations of both cubic and spherical particles, three electron microscopy images must be provided in total (one for the 100% spherical, one for the 100% cubic and a representative image for the nanoforms with the spherical/cubic combination of shapes).

In addition to the above, for a set of **elongated nanoforms** the registrant must provide:

- The range of the aspect ratios of the different nanoforms covered under the set
- The maximum and minimum length of the nanoforms that are part of the set.
- Where relevant (e.g. when rigidity is a part of the justification), an indication of the rigidity of the nanoforms that are part of the set (e.g. based on the cross sectional diameters/widths)

For a set of nanoforms consisting of **platelets** the registrant must provide:

- The range of the aspect ratios of the different nanoforms covered under the set
- The boundaries of the set for what concern the lateral dimensions (i.e. the two orthogonal dimensions, other than thickness): the maximum and minimum value of the lateral dimensions of the nanoforms that are part of the set
- Where relevant (e.g. when rigidity is a part of the justification), an indication of the rigidity of the nanoforms that are part of the set

For **a set including nanoforms that consist of particles with different shapes that fall under a same shape category** the registrant must provide:

- The shape category of the nanoforms included in the set (e.g. spheroidal)
- The range (as number based %) of the shapes covered under the set (e.g. the set includes nanoforms consisting of 20-40% spherical and 80-60% cubic particles)
- Reporting of particle size ranges according to the shape categories

For **a set including nanoforms that consist of particles with different shapes that fall under different shape categories (multimodal shapes)** the registrant must provide:

- The shape categories of the different nanoforms that are part of the set
- The range (as number based %) of the shapes covered under the set (e.g. the set includes nanoforms consisting of 20-40% spherical and 80-60% plates)
- Reporting of particle size ranges according to the shape categories

Based on the principles on the boundaries described above, a justification must be submitted to demonstrate that the hazards of the nanoforms covered by the set can be assessed jointly. The registrant must report the scientific information on which this justification is based or indicate if this justification is in the form of a hypothesis not based on scientific information.

4.2.2 Crystallinity

4.2.2.1 Principles on the boundaries of sets of nanoforms

Crystallinity may affect the behaviour and (eco)toxicity of nanoforms. Amorphous and crystalline forms (e.g. amorphous versus crystalline silica) can have a different hazard profile

and the same may apply to different crystal structures of the same substance.

Therefore, fully amorphous and fully crystalline nanoforms must a priori not be part of a same set of nanoforms.

In the same way, nanoforms with different crystal structure (e.g. a rutile nanoform and an anatase nanoform) must a priori not be part of a same set of nanoforms.

Upon justification nanoforms with different crystalline structure could be grouped in the same set. For instance, when there is existing scientific knowledge showing no difference in hazard for two structures or where the nanoforms are readily soluble.

In relation to nanoforms of mixed crystallinity, the following situations are possible:

1. Nanoform that consists of amorphous particles and particles with one precise crystal structure (e.g. 30% (w/w) amorphous TiO_2 and 70% (w/w) rutile)

2. Nanoform that consists of amorphous particles and particles with more than one crystal structure (e.g. 20% (w/w) amorphous TiO_2, 30% (w/w) rutile, 50% (w/w) anatase)

3. Nanoform that consists of particles with two or more precise crystal structures (e.g. 70% (w/w) rutile, 30% (w/w) anatase)

The number of combinations increases rapidly when more than two crystalline forms are possible.

All these different nanoforms must be reported separately from nanoforms that are uniquely crystalline or uniquely amorphous, unless one crystal structure is widely known to be more toxic and therefore considerations based on worst-case scenarios may be possible when creating the sets.

It must be highlighted that information on crystallinity obtained by XRD analysis performed on the nanoform(s) will also be used in combination with other techniques (e.g. ICP, TGA, etc.) to derive the complete chemical composition of the nanoform(s) (concentration ranges of the constituents/impurities/additives).

4.2.2.2 Reporting in the dossier

When reporting in the dossier information on the crystallinity of a set of nanoforms, the registrant must specifically provide:

For a **set including amorphous nanoforms**:

- A representative analysis (e.g. XRD) proving the amorphous nature of the nanoform(s) covered within the set
- A description of the analytical method(s) used
- A clear indication that the set includes only amorphous nanoforms

For a **set including crystalline nanoforms with precise crystal structure**:

- The name of the specific crystal structure covered (e.g. rutile)
- A typical diffraction pattern
- A description of the analytical method(s) used

- A clear indication that the set includes nanoforms made of particles with only specific crystal structure (e.g. rutile)

For a **set including crystalline nanoforms where the individual nanoforms** consist of particles **with more than one different crystal structure**:

- The names and the ranges (as w/w percentage) of different crystal structures covered by the set (e.g. 20-40% (w/w) of crystal structure 1, 80-60% (w/w) of crystal structure 2).
- Typical diffraction patterns recorded on nanoforms representing the boundaries of the set.
- A description of the analytical method(s) used.

For a set including **partially crystalline nanoforms**:

- The range(s) (as w/w percentage) and the name of different crystal structure(s) and the range of amorphous fraction (e.g. 20-40% (w/w) rutile, 60-10% (w/w) anatase, 20-50% (w/w) amorphous titanium dioxide) covered by the set.
- A typical diffraction pattern recorded on nanoforms representing the boundaries of the set.
- A description of the analytical method(s) used.

Based on the principles on the boundaries described above, a justification must be submitted to demonstrate that the hazards of the nanoforms covered by the set can be assessed jointly. The registrant must report the scientific information on which this justification is based or indicate if this justification is in the form of a hypothesis not based on scientific information.

4.3 Surface functionalisation or treatment

4.3.1 Principles on the boundaries of sets of nanoforms

Due to the high specific surface area of nanomaterials, the surface chemistry of a nanoform can have a profound influence on its properties ([36], [37], [38]).

Where both surface-treated and non-surface-treated nanoforms are covered by a registration, surface treated and non-surface-treated nanoforms must a priori not be included in one unique set of nanoforms. The registrant must rather create, as a minimum, two sets of nanoforms; one for the non-surface-treated nanoforms and one for the surface treated nanoforms (assuming other parameters remain the same).

Any difference in the surface treating agent(s) applied and/or in reaction conditions is likely to result in a different surface chemistry of the resulting nanoform. Consequently, the resulting different surface chemistries can result in a nanoform with a different hazard profile.

Accordingly, in principle, when a nanoform of a substance is subject to different surface treatments, each different surface treatment must result in the reporting of a separate nanoform in section 1.2 of the registration dossier.

Alternatively, the registrant may decide to group different surface treated nanoforms under one set of similar nanoforms, but only if each of the following conditions is met:

1) The surface treating agents used are chemically similar (common functional groups, similar alkyl chains, etc.)

2) The surface chemistry resulting from the treatment is similar in terms of the specific

functionalities formed at the surface of the particles and the overall composition of the particle surface.

3) No significant variability is expected in the percentage of coverage of the particle surface.

4) There is no difference in the (eco)toxicity of the surface treating agent used

The registrant must explain and justify in the dossier how all the points mentioned above are met for the nanoforms with different surface treatments that are part of the set.

Where sequential surface treatments are applied and multiple layers are formed, the different order of the layers must be taken into account, and not only the nature/composition of the most external layer, when/if a set of nanoforms is built.

4.3.2 Reporting in the dossier

When reporting information on surface chemistry for a set of nanoforms, a registrant must provide:

- A list of all the agents used for surface treatment of all the nanoforms covered under a set (e.g. list of IUPAC names, CAS and EC numbers)
- A description of the common type of reaction/treatment applied and of the functionalities introduced by the chemical treatment(s). Schematics may be provided to visually describe the functionalisation/treatment of the nanoform(s) included in the set.
- A description of the functionalities introduced by the treatment(s) (e.g. carboxyl, amino, hydroxyl groups)
- An indication of the upper and lower percentage of coverage of the particle's surface for the nanoforms that are part of the set and the relative weight-by-weight contribution and surface treating agent linked to those
- Representative analytical data for determining the overall composition of the nanoform(s) that are part of the set, including their surface treatment and a description of the analytical methods used

Based on the principles on the boundaries described above, a justification must be submitted to demonstrate that the hazards of the nanoforms covered by the set can be assessed jointly. The registrant must report the scientific information on which this justification is based or indicate if this justification is in the form of a hypothesis not based on scientific information.

4.4 Surface area (specific surface area by volume, specific surface area by mass or both) for sets of nanoforms

4.4.1 Principles on the boundaries of sets of nanoforms

The surface area of nanoforms may have an influence on the hazard assessment of a particular nanoform. Higher surface area materials, all other things being equal, exhibit higher reactivity on the surface of the nanoform[8]. This in turn may impact properties such as water solubility,

[8] The reactivity can be normalised per unit surface area. The reactivity per unit surface area may remain constant as the surface area is increased, although the total reactivity will increase.

as well as toxicity and ecotoxicity.

Given the impact of the surface area on other properties of the substance, including the hazard of the substance, the registrant must take into account the impact of surface area when constructing any sets. The registrant must justify why the range of specific surface areas of the different nanoforms included within the set does not change the hazard assessment, exposure assessment, and risk assessment of those nanoforms. The registrant's justification must address as a minimum the following:

- How does the surface area of the different nanoforms impact the dissolution rate and solubility of the set members?
- How does the surface area of the different nanoforms within the set impact the toxicokinetic behaviour as well as fate and (bio)availability of the set members?
- How does the surface area of the different nanoforms within the set impact the (eco)toxicity of the set members? Is there a direct relationship between the surface area and the (eco)toxicity?

Where needed for the purposes of the hazard assessment, registrants should build separate sets for high surface area and low surface area nanoforms.

This guidance does not provide any specific numerical boundaries for the ranges of surface area within a particular set. This is because the guidance recognises that the boundaries will be dependent on the material in question. Low (eco)toxicity/inert materials will naturally have a lower (eco)toxicity per unit surface area (e.g. higher EC(50) values), whereas reactive materials such as many transition metal particles will have a higher (eco)toxicity per unit surface area (lower EC(50)) values.

4.4.2 Reporting in the dossier

Given that a set of nanoforms may cover nanoforms with different specific surface areas, and given that the boundaries of a particular set must be clearly specified, registrants who construct a set of nanoforms must report the range of specific surface areas covered by the particular set (**the minimum and maximum** specific surface areas covered). Where the registrant reports the volume specific surface area range of the set, derived from BET measurements, they should also provide information on the skeletal density of the substance under the section 1.2 of IUCLID. Information on the method(s) used to measure the (volume) specific surface area must also be provided.

Based on the principles on the boundaries described above, a justification must be submitted to demonstrate that the hazards of the nanoforms covered by the set can be assessed jointly. The registrant must report the scientific information on which this justification is based or indicate if this justification is in the form of a hypothesis not based on scientific information.

5. Nanoforms, sets of nanoforms and joint submission

The previous sections of the guidance focus mainly on principles on how to define nanoforms and sets and what are the reporting obligations of the individual registrants. This section provides an overview of how this process occurs within a joint registration and describes it in three steps that may in practice overlap or be iterative. Detailed information on how to carry out this reporting in IUCLID will be provided in relevant IUCLID manuals.
Figure 4 below provides an overview of the process to identify nanoforms and define sets of nanoforms.

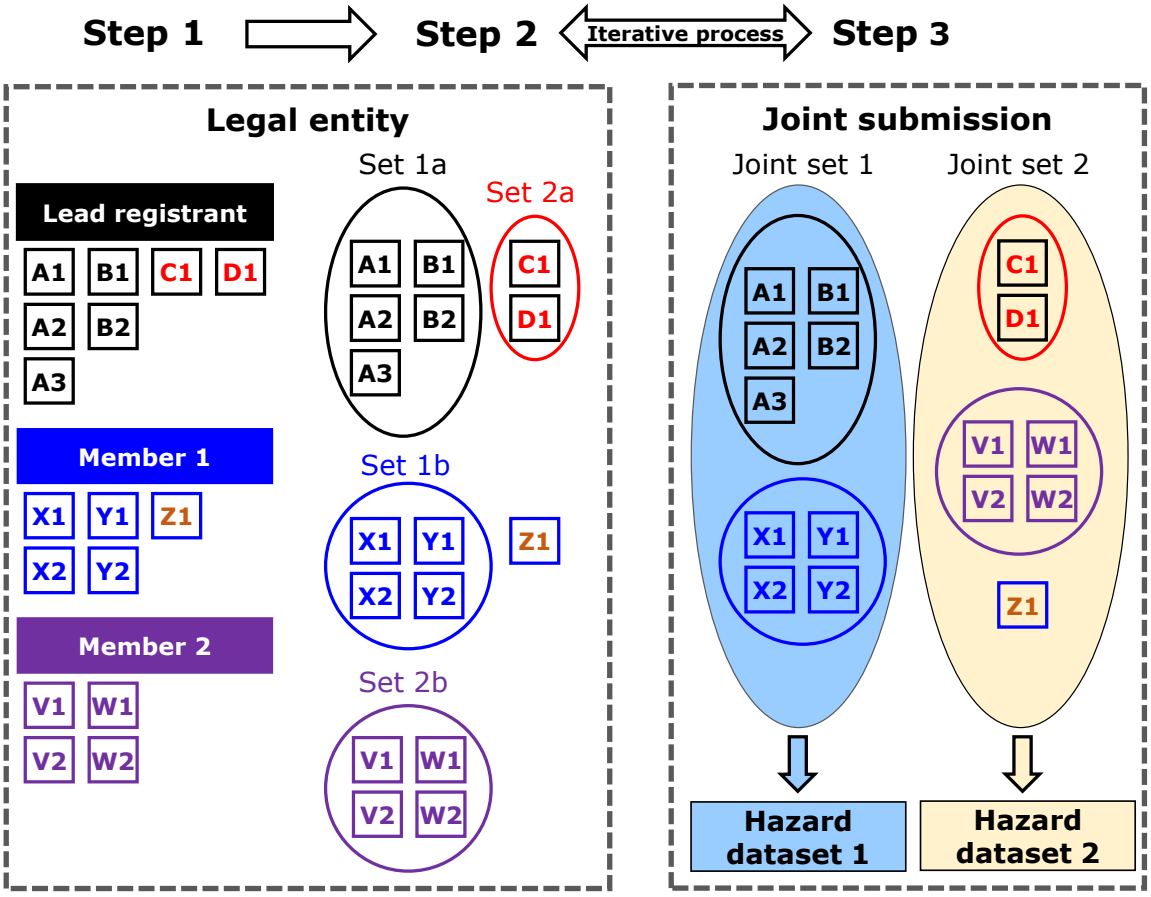

Figure 4: A schematic overview of the steps to identify nanoforms, define the sets at the level of each legal entity and at the level of the joint submission (boundary compositions) and ultimately submit the dataset(s) (REACH Annex VII-XI data).

Each box with letter-number combination represents a nanoform. The nanoforms with same letter but different number are nanoforms with same specifications. The nanoforms with black edges of the box are manufactured/imported by the lead registrant, those with blue edges by Member 1 and those with purple edges by Member 2. Black, red, blue and purple ovals/circles represent boundaries for sets of nanoforms defined by each co-registrant (Set 1a, 1b, 2a, 2b). The nanoform Z1 is different from nanoforms X1, X2, Y1 and Y2 and cannot be in the same set with them. Then needs to be reported on its' own.
Joint set 1 (oval with light blue background) represents the combined boundaries of the Set 1a and 1b at the joint submission level. These boundaries are defined for the purposes of linking full hazard dataset (Hazard dataset 1) to nanoforms A1, A2, A3, B1, B2, X1, X2, Y1 and Y2 (reported as Set 1a and 1b in the dossiers of the Lead registrant and Member 1, respectively) and for developing a justification that the hazard assessment, exposure assessment and risk

assessment of these nanoforms can be performed jointly. Same applies in analogy to Joint set 2 (oval with yellow background) and the Hazard dataset 2. The Hazard dataset 2 is applicable for nanoforms C1, D1, V1, V2, W1, W2 and Z1.

Step 1

Each registrant (Member 1 and 2 and the Lead registrant in Figure 4 must identify first the nanoforms (e.g. A1, A2, X1, V2, etc.) that he manufactures/imports. Each box in Figure 4 represents a nanoform (see section 3).

Step 2

Sets of nanoforms can be defined, based on the characterization parameters of Annex VI (particle size distribution, shape, surface treatment, and surface area), following the principles outlined in this Guidance. Each registrant may compile initial sets of nanoforms, taking into account all the available scientific information that can be used to justify inclusion of certain nanoforms within set(s). For example, in Figure 4, the Lead registrant is creating Set 1a and 2a, Member 1 Set 1b and Member 2 Set 2b.

Step 3

The co-registrants of nanoforms of the same substance will need to discuss and agree on the reporting of sets of nanoforms for the full joint submission. All the parameters listed in Annex VI must be specified for the sets agreed at joint submission level and reported in the form of a boundary composition in IUCLID (Joint set 1 and 2 in Figure 4).

Defining the sets for each legal entity and for the full joint submission can be seen as an iterative process. The sets defined at the level of joint submission may have an impact on the sets that each legal entity reports in section 1.2 of their own registration dossier and vice versa. This involves also agreeing on a justification why the hazard assessment, exposure assessment and risk assessment of the nanoforms in the set can be performed jointly.

For each set agreed at joint submission level, a full hazard dataset needs to be provided by the lead registrant (in Figure 4 Hazard dataset 1 for the Joint set 1 and Hazard dataset 2 for the Joint set 2).

Each legal entity has to report the boundaries of their own individual sets that are likely to be narrower than those of the joint set (reported as boundary composition) and must always fall within those boundaries. Each legal entity should provide the link between the nanoforms or set identified in the individual registrations and the relevant information in the joint submission and the justification why the hazard assessment, exposure assessment and risk assessment of the nanoforms in the set can be performed jointly.

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