ECHA
EUROPEAN CHEMICALS AGENCY

GUIDANCE

Appendix for nanoforms applicable to the Guidance on Registration and substance identification

Draft (Public) Version 1.0
February 2019

This document aims to assist users in complying with their obligations under the REACH Regulation. However, users are reminded that the text of the REACH Regulation is the only authentic legal reference and that the information in this document does not constitute legal advice. Usage of the information remains under the sole responsibility of the user. The European Chemicals Agency does not accept any liability with regard to the use that may be made of the information contained in this document.

Version	Changes	Date
Version 1.0	First edition	xxx 2019

Appendix for nanoforms applicable to the Guidance on Registration and substance identification

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

If you have questions or comments in relation to this document please send them (quote the reference and issue date) using the information request form. The information request form can be accessed via the Contact ECHA page at:
http://echa.europa.eu/contact

European Chemicals Agency

Mailing address: P.O. Box 400, FI-00121 Helsinki, Finland

Visiting address: Annankatu 18, Helsinki, Finland

PREFACE

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

This appendix intends to provide advice specific to nanoforms and 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 set 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]).

Table of Contents

Table of Figures

1. Introduction

This document 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 a nanoform, and how to distinguish a nanoform from another.

Section 4 focuses on how to create and justify sets of different nanoforms.

2. General considerations

The guidance on registration outlines the steps that potential registrants need to follow, from determining their registration obligations to establishing the identity of the substance, considering joint submissions with other registrants where relevant, and collecting/generating relevant Annex VII-XI data, until 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 document provides additional advice for potential registrants to assist them in understanding what nanoforms are and how to characterize 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 of 3 December 2018 amending REACH to address nanoforms of substances makes explicit that nanoforms of substance need to be covered by the registration dossier. Annex VI defines the term 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 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 include some specific information requirements (e.g. dustiness) or modification to the existing ones in the forms of adaptations or limitations of waiving possibilities.

The tonnage trigger requirements apply as explained in the Guidance on Registration. This means that the tonnage triggers for registration apply to the total tonnage of a substance manufactured or imported by a registrant [7]. Thus, for registrants of non-nanoforms and nanoforms, the total volume determines the need for registration and the information requirements for the registered substance.

Legal entity specific information requirements are triggered by his aggregated tonnage.

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 of the substance.

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 and sets of nanoforms

The revised Annex VI of REACH introduces the concept of "nanoform" into the Regulation. It establishes the principles that all the nanoforms of a substance 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 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.

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, shape, surface treatment and functionalisation and specific surface area).

Any variation of one or several of the parameters defined in section 2.4.2-2.4.5. must result 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 fixed process parameters (e.g. starting materials, solvents, temperature, order of manufacturing steps, purification steps, etc.). Batch-to-batch variations cannot result from any modification of the manufacturing process parameters.

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.

3.1.1 Particle size distribution and number fraction of constituent particles

REACH Annex VI section 2.4.2. requires to report number based particle size distribution with indication of the number fraction of constituent particles in the size range within 1 – 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 a batch-to-batch variability. Any variability in the particle size distribution beyond batch-to-batch variability creates another nanoform.

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 the methods are suitable for all the nanoforms. For example, shape, size range as well as the chemical and physical nature of the particles needs to be taken into consideration when the method is selected [8]. The registrant is recommended to use at least one microscopy technique to measure the particle size distribution and the number fraction of constituent particles. For high-aspect ratio and two-dimensional particles, as described under the section 3.1.2.1.2 of this Guidance, the microscopy techniques can also provide essential information to report the length of the high-aspect ratio particles and three Cartesian dimensions of the primary structure of the two-dimensional particles.

3.1.1.2.1 Reporting in the dossier

The Registrant needs to provide in the dossier a graph and a table showing the particle size distribution of the smallest external dimension of the particles of the nanoform and the number fraction of constituent particles with their smallest external dimension in the size range 1 – 100 nm as a value between 50 and 100 %. In the context of reporting of the particle size distribution, a d10[1], d50[2] and d90[3] value with an indication of the measurement uncertainty 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 used method(s) and provide all the relevant biographical references in the dossier. The description of the method(s) needs to include the description of sample preparation, instrument parameters, functions and calculation applied, as appropriate, as well as the description of the method used to estimate the smallest external dimension of the particles (e.g. Feret diameter or maximum inscribed circle diameter).

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

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 characterization 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).

However, when deciding whether to distinguish between nanoforms, both crystallinity and the

[1] Size at which 10 % of the particles in number based distribution has size less than this value
[2] Median size of the particles
[3] Size at which 90 % of the particles in number based distribution has size less than this value

rest of the parameters described in 3.1.2.1 must be taken into account.

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 different shapes, such as spheres, cubes, tubes, wires, plates, etc. Each nanoform, as a result of a defined manufacturing process, can be made by particles of a same shape (e.g. cubic) or particles with different shapes can be present simultaneously (e.g. 30% spheres and 70% cubes).

Nanoforms made of particles with a different shape or with different combinations of mixed shapes, are different nanoforms.

Same shapes (e.g. nanorod) but with a different aspect ratio (length to diameter ratio), are also different nanoforms. As the aspect ratio is related to the parameter size (i.e. to the diameter in case of high aspect ratio nanoforms) and to the length, the particle size and the aspect ratio of a certain nanoform are linked together.

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

Regarding an 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 be likely captured by other parameters such as size, and the result will in any 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.

Further description on the possible types of shapes and considerations on what will be considered as a different nanoform are given on 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 for a certain nanoform, the registrant must always provide for each nanoform a representative electron microscopy image with a scale bar on the image, accompanied by an indication of the magnification used and a description of the sample preparation method, suspending medium, temperature, and a reference to the standards and reference materials used. Fundamental is the representativeness of the sample used for the measurements. The ISO standard ISO 14488:2007 provides indications on how to obtain a sample that can be considered as representative of the entire sample of a particulate material.

3.1.2.1.3. Reporting in the dossier

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

As the number of possible shapes of particles making a certain nanoform is very large, for organisation purposes, four broad categories of shapes, with indication of the specific types of shapes included in each different category, are defined and reported below:

- **Spheroidal-like particles** with three similar external dimensions in all projections (i.e.

approximately equiaxial forms with aspect ratio smaller than 5:1). This includes a number of different shapes such as spherical, pyramidal, cubic, star shaped, orthorhombic, polyhedral, etc. Nano-onions made by concentric multiple shell structure are also falling under the spheroidal-like category.

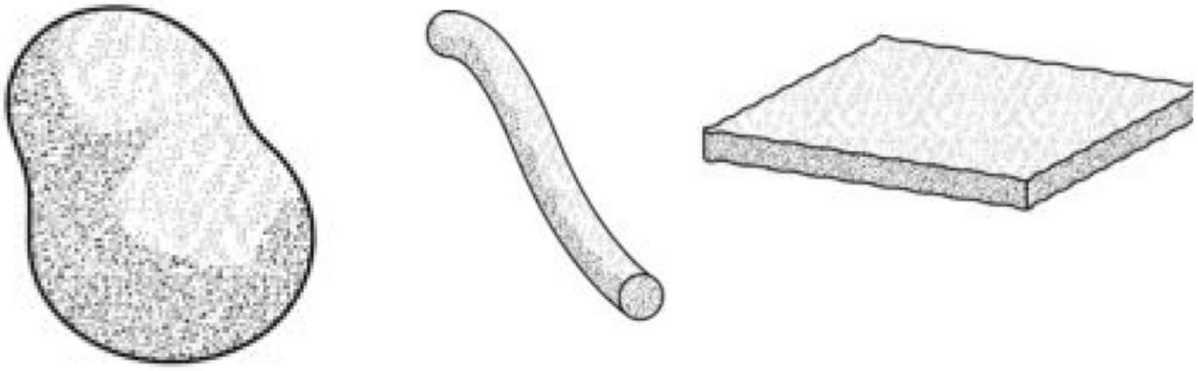
- **High aspect ratio**: particles with two similar external dimensions and a significantly larger third dimension (aspect ratio of 5:1 or greater) [9], [10], [11], [12] and substantially parallel sides [10]. This includes high aspect ratio particles with hollow structures (nanotubes), as well as solid, non-hollow high aspect ratio particles (nanorods) and electrical conducting or semi-conducting high aspect ratio particles (nanowires).

- **Two-dimensional**: particles with one external dimension significantly smaller than the other two external dimensions. The smaller external dimension is the thickness of the particle (e.g. platelets).

- **Other**: this fourth category includes nanoforms manufactured as mixtures of particles whose shapes belong to different categories (e.g. spheres and rods), with no category of shape being present in the nanoform at more than 80%, and therefore none of the categories above would be suitable.

[Note 1]. The definitions of the shape categories defined in these documents closely resemble the terms used, namely nanoparticle, nanofiber and nanoplate as defined in ISO TS 80004-2, and indeed the terms used by ISO served as a basis for the shape categories used in this document. However, there are subtle differences between the terms as defined in ISO TS 80004-2 and the terms used in this document, and therefore the terms used here are deliberately different in order to avoid confusion. More specifically, the definition of nanoform included in Annex VI of REACH regulation requires that one or more external dimensions is in the size range 1-100 nm, whereas the ISO terminology for nanoparticle requires all three dimensions to be in the nano range and the ISO terminology for nanofibers requires the presence of two dimensions in the nano range. Therefore, it is at least theoretically possible for a nanoform to meet the definition of spheroidal-like according to the terminology used in this guidance, but to not meet the definition of a nanoparticle according to ISO terminology. Registrants should be aware of this potential difference.

These four categories of shape are illustrated in Figure 1.



a) spheroidal-like b) high-aspect ratio c) two dimensional

Figure 1: Schematic representation of some shapes for the categories a) spheroidal-like, b) high-aspect ratio and c) two dimensional. Figure adapted from ISO/TS 80004-2 'Nanotechnologies —

Vocabulary — Part 2: Nano-objects: nanoparticle, nanofibre and nanoplate'.

1) 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-like, high-aspect ratio, two-dimensional, other) the specific nanoform would fall in. The shape of an individual 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 two shapes falling within a same category (e.g. spherical and cubical) are to be considered as two different nanoforms.

2) 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-like").

3) Further specific information must be reported in the situations explained below:

i. For high-aspect ratio nanoforms the aspect ratio value must be provided. The aspect ratio is a geometrical shape descriptor defined as the length to diameter ratio of a particle. It is obtained from size measurements performed on the nanoform: i.e. by measuring the length and diameter of individual particles in the nanoform (geometric mean length, geometric mean diameter and their standard deviation) [13]. Where the nanoform in question is a high aspect ratio nanoform, the registrant should report the range of the aspect ratios covered, as well as the range of the lengths (longest dimension of the particle), in addition to the particle diameter size range. This information concerns specifically high aspect ratio nanoforms.

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 ISO/TS 80004-2 [14, 15]. For this kind of more complex structures, information on the number of multiple walls/multiple shells formed will need to be provided.

iii. For high-aspect ratio nanoforms, registrants must provide information on rigidity. The rigidity parameter, together with aspect ratio, is known to influence the toxicity of all high aspect ratio nanoparticles (HARN) [16]. The rigidity is dependent on the diameter of the particles and information on the diameter will be covered by the requirement under section 2.4.2 of Annex VI of REACH. In any case, the registrant must clearly state in the dossier if the high-aspect ratio form is rigid or not and support this information by appropriate microscopy images.

Reporting of nanoforms with mixed shapes

It should be noted that some nanomaterials may contain a mixture of different shaped particles due to the manufacturing process. In such a case, as also explained above, the shape of the majority of the particles should be used to determine under which shape category the particles belong to. That is, if 80% or more of the particles belong to one shape category, then the particles should be allocated to that particular shape category. If no one particle shape is in such a majority (e.g. 30% of the particles are spheroidal-like, 30% are high-aspect ratio particles, and 40% are plate like particles), then it is recommended to report such particles under the "other" shape category. In cases where a mixture of shapes exist, the registrants must also report further details of the shape (e.g. 60% of the particles are spherical and 40% of the particles are rods).

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 in (e.g. spheroidal-like)
- The specific shape of the nanoform (e.g. cubic)
- An electron microscopy image

In addition to the above,

For a **high-aspect ratio nanoform** the registrant must provide:

- The value of the aspect ratio with an indication of the measurement uncertainty
- The value of the geometric mean length (longest dimension) of the particles and its standard deviation
- An indication of the (average) number of walls for high aspect ratio particles with hollow structures (nanotubes)
- An indication of the rigidity the registrant must clearly state in the dossier if the high aspect ratio nanoform is rigid or not

For **a two-dimensional nanoform:**

- The value of size of the other two Cartesian dimensions, other than thickness (already covered under the requirement 2.4.2), of the two dimensional nanoform.

For a **nanoform manufactured as mixture of different shapes**, the registrant must provide:

- The shape category: either the shape category represented by the particle shape present in majority or the "other" shape category
- An indicative composition in terms of specific shapes of the individual nanoform (i.e. 30% spherical particles and 70% cubic)
- Reporting of size according to the shape categories, as described above

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 be made by atoms organized in periodic arrays (crystalline nanoform) or by atoms arranged in random assemblies without any atomic/molecular periodicity (amorphous nanoform). Moreover, in case of crystalline nanoforms of a substance, different crystal structures may exist.

3.1.2.2.1 Distinguishing one nanoform from another

Each nanoform of a substance has a specific crystallinity, achieved by using defined manufacturing process parameters. **Each nanoform of a substance has a specific amorphous or crystalline structure. Any change in the structure beyond batch-to-batch variability creates another nanoform.**

It must be noted that certain nanoforms can be made by particles with different crystal structures present simultaneously. This kind of materials 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 [16]. **When a variation on the proportion of the different crystal structures occurs that goes beyond the batch-to-batch variability, a different nanoform is created.**

3.1.2.2.2 Requirements for measurements or calculation method

Information on crystallinity is obtained 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 [17].

For the characterization 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 [18]. A quantitative analysis using the Rietveld method can be performed on a 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 amorphous structures [19]. High-resolution TEM images may also be needed as support information 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. The description should be given in such detail that the method can be reproduced.

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

For **crystalline nanoform** made by particles with more than one **different crystal structure**:

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

For **partially crystalline nanoform**:

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

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

According to subsection 2.4.3.of Annex VI of the REACH Regulation, characterization 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*".

The term "surface chemistry" is used in this guidance to cover both the terms "surface functionalization or treatment of nanoforms". This terminology intends to cover any chemical modification (i.e. the result of a chemical reaction) applied on the surface of a particle and the resulting chemistry obtained on the surface of the nanoform (i.e. the functionalities introduced by such chemical modifications).

3.1.3.1 Distinguishing one nanoform from another

Core particles with nominally identical compositions may have very different surface chemistries due to the differing synthesis methods used (e.g. high temperature pyrolysis vs. wet chemical synthesis), the addition of other 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 may have very different surface chemistries (e.g. alumina, trichloromethysilane, low silanol group density, high silanol group density, etc.).

Surface chemistry is intentionally varied 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 (e.g. treatment of calcium carbonate, silver, ZnO, etc.), etc.

The modification of particle surface chemistry essentially introduces a "wild card" because the variability in surface chemistry may be as broad as the definition of substance itself as in principle any substance may be added to the surface of a particle. For example, modification of surface chemistry 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).

Any variation on the surface treating agent applied, of the reaction conditions, of the molar ratio of surface treating agent applied, that results in a change of the surface chemistry of a particle generates a different nanoform.

3.1.3.2 Requirements for measurement or calculation method

Information on surface chemistry of a nanoform can be obtained through a combination of analytical techniques. 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 the NANOREG project. A project aimed at the development of a new guidance document (GD) for identification and quantification of organic and inorganic surface chemistry of nanoscale materials is currently ongoing at OECD level. Registrant must select the most appropriate analytical method(s) that allow obtaining a full picture on the composition of the nanoform, including its surface treatment.

3.1.3.3 Reporting in the dossier

When characterizing a nanoform, registrant must provide a description of the surface chemistry of the nanoform and identification of each agent(s) used for surface functionalization/treatment, including IUPAC name and CAS or EC number.

The description of the surface functionalization/treatment must include details of the chemical treatment applied (e.g. acid washing, oxygen treatment, etc.) and of the functionalities introduced by the chemical treatment. Schematics of the particle surface chemistry can be provided to visually describe the surface chemistry of the nanoform(s).

For example, organosilianes are important coupling agents used to modify surface chemistry [23]. The organosilane itself is not attached to the surface but rather it reacts with groups on

the surface to covalently attach functional siloxanes. An illustrative example of an organosilane coupling chemistry is given in Figure 2.

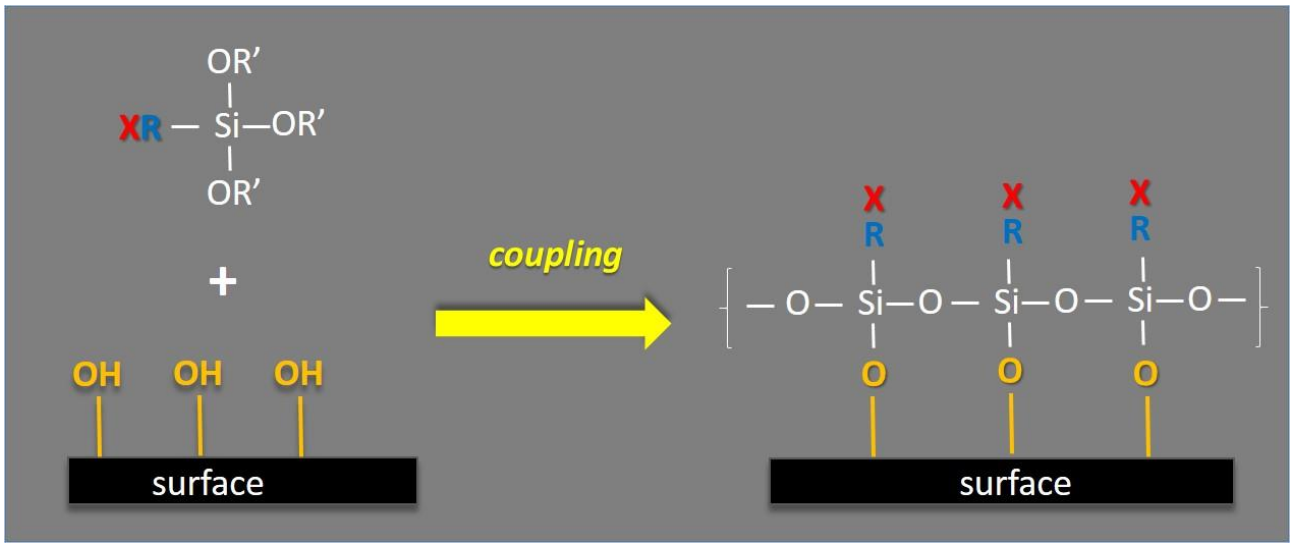


Figure 2: a schematic of an organosilane surface treating agent XR-Si-(OR')3 and the chemistry it imparts the surface post-surface treatment.

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 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.

Information on the weight-by-weight contribution of the surface treating layer and, when possible, an indication of the amount of coverage of the particles surface must be provided.

Multiple/sequential layers

When sequential surface treatments are applied to a nanoform (see Figure 3), information on surface chemistry as described above must be provided for each different surface treatment layer. The registrant must therefore provide identification of each agent used for each sequential surface functionalization/treatment, including IUPAC name and CAS or EC number.

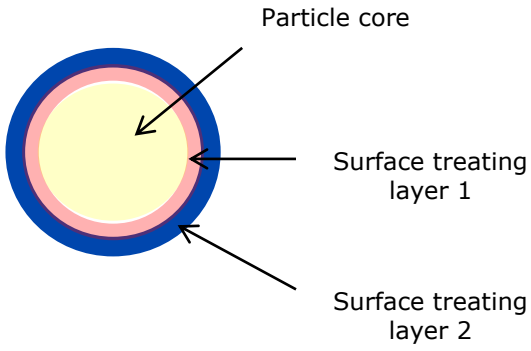


Figure 3 Idealised schematic representation of a nanoform whose surface chemistry has been modified by sequential surface treatments.

The registrant must provide the weight-by-weight contribution of each surface treating layer and, when possible, an indication of the amount of coverage of the particles surface.

The description of the surface treatment must be supported by appropriate analytical data (e.g. IR, NMR, TGA, ICP-MS, XRF, etc.). Registrant is also recommended to provide, when feasible, analytical data that would support specifically the identification of the surface chemistry of the nanoform (e.g. XPS and EDX). Registrants must always provide a description of the analytical methods used. The description of the methods must be given at a level of details that would allow the method to be reproduced.

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.

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.

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

Therefore, when defining a particular nanoform, registrants should fix the nanoform based on size (assuming the same shape, and no changes to the porosity of the particle), and characterise the specific surface area of the nanoform in question. The reverse process is also valid: registrants can choose to fix a defined surface area for the nanoform in question, and then characterise the particle size of the nanoform.

3.1.4.2 Requirement of measurement or calculation method

The surface area is a measurement of 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 measurement of the specific surface by volume requires information on the density of the substance in question. Surface area is a property of solid particles, and therefore not relevant for liquids or suspensions.

The surface of a nanoform is generally measured using gas adsorption using the Brunayer-Emmett-Teller (BET) isotherm. In this technique, an inert gas, typically nitrogen, are 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 principle of the method is to measure the amount of a monolayer of the adsorbate that is adsorbed to the surface of the material. The technique measures that amount of the adsorbed as a function of pressure, while holding the temperature constant, and this amount of gas adsorbed is plotted against the relative pressure in order to obtain an adsorption isotherm. The instruments are generally supplied with software that calculates the surface area based on the adsorption isotherm.

The calculation of a volume specific surface area 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 [20]. 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 Chapter R.7a of the Guidance on IR&CSA [20]. Nevertheless, in order to report relative density, information on true density is needed. Furthermore, density often can refer to different values, including:

- Bulk density
- Tapped density
- 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 tapped density are inappropriate for the purposes of calculating 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 standard deviation for the measured surface area
- A description of the method used to determine the surface area
- When reporting volume specific surface area, 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.

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

1) clearly defined boundaries for the parameters in 2.4.2-2.4.5 must be defined. The variations will in this case arise from merging of information on different nanoforms (i.e. parameters such as shape, size, surface treatment, surface area, have on purpose been modified).

2) A justification must be provided as to:

- Why the hazard assessment can be performed jointly. This means that 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 (e.g. gradual changes when reducing particle size, see section 3.1.1)

  The development of a set of nanoform must not replace the development of a read-across approach between nanoforms. More specifically, the justification that the hazard assessment of various nanoforms can be performed jointly in a set must be based on a generic premise applicable to all the applicable endpoints. By contrast, the registrants must rely on a read across can justify that the properties of nanoforms are the same by developing a premise specific to the applicable endpoints. In such case, they have to report the various nanoforms individually and to submit in the relevant endpoint of these nanoforms a justification in accordance with Section 1.5 of Annex XI of REACH.

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

  The exposure and risk assessment result from the analysis and evaluation of the risk associated with the hazard identified. The assessment of the hazards of nanoforms serves as a basis for the exposure and risk assessment. The developments 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 safe.

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

Where the registrant constructs a set of nanoforms, the information reported must be applicable to the entire set

4.1 Particle size distribution and number fraction of constituent particles

4.1.1 Principles on the boundaries of sets of nanoforms

In general, it is expected that if certain forms of a substance are nanoforms, the variation in size within the size range 1-100 nm would not affect the hazard profile of the different nanoforms **and they can be reported in one set of similar nanoforms**.

However, if existing scientific knowledge shows that for a certain substance there is a threshold in the particle size, which induces a specific effect for particles with size below/above that size, the registrant must create two different sets of nanoforms. The threshold size is substance dependent and the impact on some properties can be more or less significant in each specific case. Threshold may be related to quantum confinement or other properties affecting hazard (e.g. rigidity of CNTs related to diameter). **Registrant must justify why there are no threshold effects for the nanoforms included in a set.**

4.1.2 Reporting in the dossier

When a registrant is reporting a set of similar nanoforms, he needs to provide, as a minimum and in accordance with the requirements under section 3.1.1.2.1 for a single nanoform, the particle size distribution and the number fraction of constituent particles of the nanoforms included in the set with the smallest and largest d10, d50, and d90 value. The boundaries for the set of similar nanoforms are defined by smallest d10 and largest d90 value.

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

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

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 and thereby the toxicity) [21] and may affect the kinetics of deposition and absorption in the body [22]. Particle shape can also influence the deposition of nanomaterials in the lungs upon inhalation [22].

Given the impact that shape can have on the toxicological properties of nanoforms, variability in shape must always be considered when building sets of similar nanoforms. **If nanoforms of the registered substance fall under different shape categories** (as defined in section 3.1.2.1.3) **different sets of nanoforms must be created at least for each different shape category (spheroidal-like, high aspect ratio, two-dimensional or others).**

Spheroidal-like nanoforms

Nanoforms of different shape within the spheroidal-like category (i.e. spherical and pyramidal nanoforms) may or may not have a different hazard profile. Separate reporting in different sets

maybe necessary if scientific publications/toxicological tests indicate that the difference in shape leads to a difference in the toxicological profile. Therefore, if the registrant decides to report nanoforms of different shapes within the spheroidal-like category in a same set, he must justify why those changes in shape do not affect the hazard profile of the different nanoforms (e.g. by providing supporting literature, or screening testing).

Two-dimensional nanoforms

Two-dimensional nanoforms can variate in terms of shape of the primary structure (plates, discs, etc. can be formed) and size of the particles in the three Cartesian dimensions. The registrant must justify how these parameters will affect the toxicological profile of the different nanoforms and, when those different forms are reported together, justify why the variability does not affect the hazard profile.

High aspect ratio nanoforms

Nanoforms of different shape within the high aspect ratio category (nanotubes, nanowires, nanorods) are likely to have different properties and hazard profile and should not be included in the same set. Moreover, within the high aspect ratio nanoforms different parameters, often interlinked, can have an influence on the toxicity of these nanoforms. Registrants will first need to consider the variation in diameter. Diameter 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 size in point 2.4.2. of Annex VI of REACH and the registrant must justify how the variation in diameter of the different forms will affect their rigidity and consequently the toxicological profile of the different nanoforms. When there is a variability of the diameter 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.

The length of high aspect ratio nanoforms, considered as unchanged the diameter, must also be taken into account as a parameter that can affect the hazard of these forms. When there is a variability of the aspect ratio 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, registrant needs to assess if to further subdivide nanoforms in different sets based on these additional parameters and justify their choices in the registration dossier. When cut-of values in length and diameter are known (e.g. from literature or from tests) to trigger a different behaviour, i.e. are linked to carcinogenic potential typical of fibre-like materials, the registrant must split nanoforms in different sets based on these cut-off values.

Mixed shapes

In the unlikely situation of a certain nanoform made by particles falling under different shape categories (i.e. of spheres and rods), it is expected as basic rule that this nanoform is reported on its own (i.e. a different set is created). The registrant may still consider including such nanoform in a certain set with other nanoforms made uniquely by high-aspect ratio forms or uniquely by spheroidal-like forms, but the decision must be justified. For instance, it could be known already that the high aspect ratio nanoform has a higher toxicity and the new nanoforms can be allocated there (justification via worst-case scenario may be included).

4.2.1.2 Reporting in the dossier

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

- The shape category under which the nanoforms that are part of the set fall in (e.g. spheroidal-like)
- A list of the specific shapes covered under a certain set (e.g. spherical, cubic, pyramidal)
- An electron microscopy image for each different shape included within the set (i.e. one for the spherical, one for cubic) or for each combination of different shapes.

In addition to the above, for a set of **high-aspect ratio nanoforms** the registrant must provide:

- The range the aspect ratios of the different nanoforms covered under the set
- The range of the geometric mean lengths (longest dimension) of the particles
- An indication of the (average) number of walls for high aspect ratio particles with hollow structures (nanotubes)
- An indication of the rigidity

For a set including **two-dimensional nanoforms:**

- The range of values of size of the other two Cartesian dimensions, other than thickness (already covered under the requirement 2.4.2)

For **a set including nanoforms manufactured as mixtures of different shapes**:

- An indicative composition in terms of shapes of each individual nanoform within the set (i.e. nanoform 1: 30% spherical particles and 70% cubic, nanoform 2: 40% spherical 60% cubic, etc.)
- Reporting of size ranges according to the shape categories, as described above

Based on the principles on the boundaries described above, a justification must sometimes be submitted to demonstrate that the hazards of the nanoforms covered by the set can be performed jointly. When such justification must be submitted, the registrant must report the scientific information on which this justification is based or indicate if this justification is 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 toxicity of nanoforms. Amorphous and crystalline forms (e.g. amorphous versus crystalline silica) can have a different hazard profile and the same can be valid for different crystal structures of a same substance.

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

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

When it comes to nanoforms of mixed crystallinity, the following situations are possible:

1. Nanoform manufactured as mixture of amorphous particles and particles with one precise crystal structure **(30% amorphous TiO2 and 70% rutile)**

2. Nanoform manufactured as mixture of amorphous particles and particles with more than one crystal structure **(20% amorphous TiO2, 30% rutile, 50% anatase)**

3. Nanoform manufactured as mixture of particles with two or more precise crystal

structures **(70% rutile, 30% anatase)**

The number of combinations increases exponentially 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). Information on the characterization parameters size, shape, surface treatment and specific surface area is always to be combined with information on chemical composition of the different nanoforms (e.g. impurities profile) when building the sets.

4.2.2.2 Reporting in the dossier

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

For a **set including amorphous nanoforms**:

- A representative analysis 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 sets includes only amorphous nanoforms

For a **set including crystalline nanoforms**:

- The specific crystal structure covered (e.g. rutile)
- A typical diffraction pattern recorded on one of the nanoforms that is part of the set
- A description of the analytical method(s) used
- A clear indication that the set includes only a specific crystalline structure (e.g. rutile)

For a **set including crystalline nanoforms** manufactured as mixtures of particles with **different crystal structure**:

- The percentage and type of each different crystalline structure present in each nanoform that is part of the set (e.g. nanoform 1: 20 w/w% rutile, 80 w/w% anatase, nanoform 2: 30 w/w% rutile, 70 w/w% anatase, etc.).
- A typical diffraction pattern recorded on at least two of the nanoforms that are part of the set
- A description of the analytical method(s) used

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

- The percentage and type of crystalline structure(s) and the percentage of amorphous fraction (e.g. 20 w/w% rutile, 70 w/w% anatase, 10 w/w% amorphous titanium dioxide) of each nanoform that is part of the set.
- A typical diffraction pattern recorded on at least two of the nanoforms that are part of the set
- A description of the analytical method(s) used

4.3 Surface functionalization 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 ([23], [24], [25]).

Where both treated and non-surface treated nanoforms are covered by a registration, surface treated and non-surface treated nanoforms must not be included in one unique set of similar nanoforms. Registrant must rather create, as a minimum, two sets of similar 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 difference in reaction conditions can result in a different surface chemistry of the resulting nanoform. Consequently, the different resulting surface chemistries are likely to result in a nanoform with a different hazard profile.

Accordingly, as a matter of 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.

By derogation to the above principle, registrants may decide to group different surface treated nanoforms under one set of similar nanoforms, only if the following conditions are 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 on the overall composition of the particle surface.

3) No significant variability is expected on the amount of coverage of the particle surface.

4) If there is no difference in the intrinsic 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 different surface treated nanoforms that are part of the set.

Where nanoforms surface chemistry has been modified by sequential surface treatments (i.e. multiple surface treatment layers are formed), the different order of the layers must be taken into account, and not only the nature/composition of the more 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 similar nanoforms, a registrant must provide a list of each agent used for surface treatment of each nanoform covered under a set (e.g. list of IUPAC names, CAS and EC numbers), a description of the common chemistry applied and of the functionalities introduced by the chemical treatment. Schematics of the particles surface chemistry may be provided to visually describe the surface chemistry of the nanoform(s) included in the set, as already detailed in 3.1.3.1.

Registrants must provide representative analytical data in support of the identification of the

common surface chemistries of the nanoforms that are part of the set.

Based on the principles on the boundaries described above, a justification must be sometimes be submitted to demonstrate that the hazards of the nanoforms covered by the set can be performed jointly. When such justification must be submitted, the registrant must report the scientific information on which this justification is based or indicate if this justification is 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 total rates of reactivity on the surface of the nanoform[4]. This in turn impacts properties such as water solubility, as well as toxicity and (eco)toxicity.

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 provide a justification for why the surface area of the different nanoforms included within the set do not change the properties of those nanoforms. The registrant's justification must address at 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 biological availability of the set members?
  How does the surface area of the different nanoforms within the set impact the (inhalation) toxicity of the set members? Is there a direct relationship between the surface area and the (inhalation toxicity)?

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

At the same time, as pointed out earlier, the surface area of a particular nanoform is closely related to the particle size of the particular nanoform. Similarly, for a set of nanoform, the boundaries for the size of a set of nanoforms will affect the boundaries of the surface area for the particular set. Similarly, the justification provided when considering the impact of particle size of the substance on the hazardous properties of the set, is likely to be related to the justification for the surface area boundaries of the set in question.

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 toxicity/inert materials will naturally have a lower toxicity per unit surface area (e.g. higher EC(50) values), whereas reactive materials such as transition metal particles will have a higher toxicity per unit surface area (lower EC(50)) values.

[4] The reactivity can be normalised per unit surface area. In such cases, the reactivity per unit surface area will remain constant, although the total reactivity will increase as the surface area is increased

4.4.2 Reporting in the dossier

Given that a set of nanoforms may cover nanoforms with different 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 surface areas covered by the particular set, including **the minimum and maximum** surface areas covered. Where the registrant reports the volume specific surface area of the set, they should also provide information on the skeletal density of the substance under the IUCLID section on density.

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

References

[1] ECHA, "Guidance on registration," [Online]. Available: http://echa.europa.eu/guidance-documents/guidance-on-reach.

[2] ECHA, "Guidance for identification and naming of substances under REACH and CLP," [Online]. Available: http://echa.europa.eu/guidance-documents/guidance-on-reach.

[3] ECHA, "[DRAFT] Appendix R.6-1: Recommendations for nanomaterials applicable to the Guidance on QSARs and Grouping," [Online]. Available: http://echa.europa.eu/support/guidance/consultation-procedure/ongoing-reach.

[4] ECHA, "Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7a Endpoint specific guidance," [Online]. Available: http://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment.

[5] ECHA, "Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7b Endpoint specific guidance," [Online]. Available: http://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment.

[6] ECHA, "Appendix R7-2 Recommendations for nanomaterials applicable to Chapter R7c Endpoint specific guidance," [Online]. Available: http://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment.

[7] "CA/59/2008: Nanomaterial in REACH," 2008.

[8] C. Gaillard, A. Mech and R. H, "The NanoDefine Methods Manual - NanoDefine Technical Report D7.6," [Online]. Available: http://www.nanodefine.eu/publications/reports/NanoDefine_TechnicalReport_D7.6.pdf.

[9] A. G. Wylie, "Fiber length and aspect ratio of some selected asbestos samples," [Online]. Available: http://onlinelibrary.wiley.com/doi/10.1111/j.1749-6632.1979.tb18766.x/pdf.

[10] US-EPA, [Online]. Available: https://www.gpo.gov/fdsys/pkg/CFR-2012-title40-vol32/pdf/CFR-2012-title40-vol32-part763-subpartE-appA.pdf.

[11] C. Tran, S. Hankin, B. Ross, R. Aitken and A. Jones, "An outline scoping study to determine whether high aspect ratio nanoparticles (HARN) should raise the same concerns as do asbestos fibres. IOM," 2008. [Online]. Available: http://nanotech.law.asu.edu/Documents/2009/07/Michael%20Vincent%20IOM%20(2008),%20An%20outline%20scoping%20study_182_2184.pdf.

[12] "NIOSH method 7400 . NIOSH Manual of Analytical Methods (NMAM)," [Online]. Available: http://www.cdc.gov/niosh/docs/2003-154/pdfs/7400.pdf.

[13] NIOSH, "NIOSH Manual of Analytical Methods. MEASUREMENT OF FIBERS," [Online]. Available: https://www.cdc.gov/niosh/docs/2003-154/pdfs/chapter-l.pdf.

[14] ISO, "ISO/TS 80004-2 'Nanotechnologies —Vocabulary — Part 2: Nano-objects: nanoparticle, nanofibre and nanoplate'," [Online].

[15] ISO, "ISO/TS 80004-1: Nanotechnologies -- Vocabulary -- Part 1: Core terms," [Online].

[16] T. Ohno, K. Sarukawa, K. Tokieda and M. Matsumura, "Morphology of a TiO2 Photocatalyst (Degussa, P-25) Consisting of Anatase and Rutile Crystalline Phases," *Journal of Catalysis,* vol. 203, no. 1, pp. 82-86, 2001.

[17] C. Giannini, M. Ladisa, D. Altamura, D. Siliqi, T. Sibillano and L. D. Caro, "X-ray Diffraction: A Powerful Technique for the Multiple-Length-Scale Structural Analysis of Nanomaterials," *Crystals,* vol. 6, no. 8, 2016.

[18] Liane M. Moreau, "1.Defining Crystalline/Amorphous Phases of Nanoparticles through X-ray Absorption Spectroscopy and X-ray Diffraction: The Case of Nickel Phosphide," *Chemistry of Materials,* vol. 25, no. 12, pp. 2394-2403 , 2013.

[19] D. L. Bish and H. S. A., "Quantitative phase analysis using the Rietveld method," *Journal of Applied Crystallography ,* vol. 21, pp. 86-91, 1988.

[20] ECHA, "Guidance on information requirements and chemical safety assessment Chapter R.7a: Endpoint specific guidance," [Online]. Available: http://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment.

[21] K. Kettler, K. Veltman, D. v. d. Meent, A. v. Wezel and A. Hendriks, "Cellular uptake of nanoparticles as determined by particle properties, experimental conditions, and cell type," *Environmental Toxicology and Chemistry,* vol. 33, no. 3, pp. 481-492, 2014.

[22] G. Oberdörster, A. Maynard, K. Donaldson, V. Castranova, J. Fitzpatrick, K. Ausman, J. Carter, B. Karn, W. Kreyling, D. Lai, S. Olin, N. Monteiro-Riviere, D. Warheit and H. Yang, "Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy," *Particle and Fibre Toxicology,* vol. 2, no. 8, 2005.

[23] ECETOC, "Synthetic Amorphous Silica. ECETOC JACC REPORT No. 51," [Online]. Available: http://www.ecetoc.org/publication/jacc-report-51-synthetic-amorphous-silica.

[24] US-EPA, "Fact Sheet: Nanoscale Materials," [Online]. Available: https://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/fact-sheet-nanoscale-materials.

[25] ECHA, "Assessing human health and environmental hazards of nanomaterials-Best practice for REACH Registrants-Second GAARN meeting," 2013. [Online]. Available: http://echa.europa.eu/documents/10162/5399565/best_practices_human_health_environment_nano_en.pdf.

EUROPEAN CHEMICALS AGENCY
ANNANKATU 18, P.O. BOX 400,
FI-00121 HELSINKI, FINLAND
ECHA.EUROPA.EU