



**NANO SUPPORT Project**

**Scientific technical support on  
assessment of nanomaterials in  
REACH registration dossiers and adequacy of  
available information**

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between DG Environment (DG ENV) and the Joint Research Centre (JRC)

**Final Report  
on analysis and assessment  
(Task I, step 3&4&5) and  
options for adapting REACH (Task II, step 1)**

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*It should be stressed that this project is not a compliance check or any other formal REACH evaluation of the dossiers/substances analysed.*

*It should be noted that the original identification/selection of dossiers for this project (i.e. Task I, step 1&2 of the project) was done prior the adoption of the EC Recommendation on the definition of nanomaterial<sup>1</sup>. However, as will be discussed in this report, the information needed to identifying nanomaterials according to the definition is generally not included in the REACH dossiers as it is not an explicit requirement. Thus, it is not expected that Task I, step 1&2 would have identified more/other dossiers had the nano-definition been available. Please refer to the Task I, step 1&2 reporting about uncertainties related to the identification and selection of dossiers.*

*It has been questioned by several stakeholders whether the REACH tonnage triggers should be lowered for nanomaterials due to the potential for increased hazards and risks expected on a weight basis as compared to larger sized materials. This project is not aimed at addressing the issue of tonnage triggers for nanomaterials. Given that the dossiers examined in this project were submitted as part of the 2010 REACH registration deadline, all except 3 of these dossiers are registered according to the highest tonnage level of > 1000 tpa.*

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<sup>1</sup> 2011/696/EU, OJ L275/38-40

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**Appendix 1: Analysis and assessment template used for assessing the individual dossiers included in project**

## List of abbreviations:

ADME	Absorption, Distribution, Metabolism, Excretion
AF	Assessment Factor
BAL(F)	Broncho-Alveolar Lavage (Fluid)
BCF	Bio Concentration Factor
BET	Brunauer, Emmett, Teller theory (used in calculating surface area based on gas adsorption/desorption)
C&L	Classification and Labelling
CAS	Chemical Abstract Service
CASG-nano	(REACH) Competent Authorities Sub-Group on Nanomaterials
CMR	Carcinogenic Mutagenic Reprotoxic
CSR	Chemical Safety Report
DMEL	Derived Minimum Effect Level
DNA	Deoxyribonucleic acid
DNEL	Derived No Effect Level
DLS	Dynamic Light Scattering
DSD	Dangerous Substances Directive
EC number	European Commission number for chemicals
EC <sub>x</sub>	Effect Concentration (causing x% effect)
ECETOC TRA	European Centre for Ecotoxicology and Toxicology of Chemicals - Targeted Risk Assessment tools
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EUSES	European Union System for the Evaluation of Substances
FE-SEM	Field Emission Scanning Electron Microscopy
FP project	Framework Programme project (EU research project)
FPR	Final Project Report
GHS	Global Harmonized System
GLP	Good Laboratory Practice
GSD	Geometric Standard Deviation
HPRT	Hypoxanthine PhosphoribosylTransferase
IARC	International Agency for Research on Cancer
ISO	International Organization for Standardization
IUCLID	International Uniform Chemical Information Database ( <a href="https://www.iuclid.eu">https:// www.iuclid.eu</a> )
JSO	Joint Submission Object
LEV	Local Exhaust Ventilation
LOEC	Lowest Observed Effect Concentration
MAK	Maximale Arbeitsplatz Konzentration
MMAD	Mass Median Aerodynamic Diameter
MSCA	Member State Competent Authority
NIOSH	National Institute for Occupational Safety and Health
NO(A)EL	No Observed (Adverse) Effect Level
NO(A)EC	No Observed (Adverse) Effect Concentration
NTA	Nanoparticle Tracking Analysis
OECD	Organisation for Economic Co-operation and Development
OECD WPMN	OECD Working Party on Manufactured Nanomaterials
OEL	Occupational Exposure Limit
PBT	Persistent, Bioaccumulative, Toxic

PNEC	Predicted No Effect Concentration
PSPs	Poorly Soluble Particles
QA	Quality Assurance
QSAR	Quantitative Structure-Activity Relationship
RA	Risk Assessment
RC	Risk Characterisation
RDT	Repeated Dose Toxicity
REL	Recommended Exposure Level
RIP-oN	REACH Implementation Project on Nanomaterials
SEM	Scanning Electron Microscopy
SID	Substance Identification
SIDS	Screening Information Data Set
SIEF	Substance Information Exchange Forum
SSA	Specific Surface Area
SU	Sector of Use
TEM	Transmission Electron Microscopy
TG	Test Guideline
TOF-SIMS	Time-of-Flight – Secondary Ion Mass Spectrometry
vPvB	Very Persistent & Very Bioaccumulative
VSSA	Volume Specific Surface Area
WAF	Water Accommodated Fraction
WSF	Water Soluble Fraction

# 1 INTRODUCTION AND OBJECTIVES

## *Scope of this report*

This report presents the results of Task I, steps 3&4 (relating to analysis and assessment of the identified REACH registration dossiers) and Task II, step 1 (options for adapting REACH) of the NANO SUPPORT project. The report should be read in light of the CASG-nano document (CASG-nano/10/2011) specifying the methodology and tasks in the project, and the Task I, step 1&2 report (CASG-nano/10/2011\_Annex II) explaining how the dossiers analysed and assessed in this project have been selected. That report identified 45 registration dossiers possibly addressing nanoforms/nanomaterials within their scope.

The current report supersedes the previous versions (CASG-nano/10/2011\_Annex III and CASG-nano/10/2011\_rev1) integrating written comments from CASG-nano (acting as stakeholder group for the project) received to those versions. The report will serve as the input for Task II, step 2 (Assessment of consequences for economy, environment, consumers and human health).

## *Structure of the report*

The methodology for the analysis and assessment addressing Task I, step 3 (assessment criteria) is presented in Chapter 2. The end of Chapter 2 and the beginning of Chapter 3 also describe an initial analysis and assessment reducing the number of dossiers for detailed analysis and assessment from 45 to 25<sup>2</sup>, and suggest a division of those in three categories. Thereafter the findings and conclusions from the detailed analysis and assessment (Task I, step 4) and options for REACH adaptation (Task II, step 1) are presented:

- Substance identification, physico-chemical properties and manufacturing and use (Chapter 3)
- Human health and environment endpoints, including PBT assessment and classification and labelling (Chapter 4)
- Exposure assessment and risk characterisation (Chapter 5).

Finally, the summary and main conclusions will be presented in Chapter 6.

Each Chapter contains a summary of main findings. In combination with dedicated sections, outlining the options for adaptation of REACH, the summaries should give a good overview. The relevant sections are:

- Section 3.2: Summary and options for adaptation of REACH: Substance Identification, characterisation and physico-chemical properties
- Section 4.1.6: Summary for human health and environment
- Section 4.2: Options for adaptation of REACH: Human health and environment
- Section 5.1.3: Summary for exposure assessment and risk characterisation
- Section 5.2: Options for adaptation of REACH: Exposure assessment and risk characterisation.

Readers interested in further details are invited to read the entire report.

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<sup>2</sup> Of these 25 dossiers, 4 dossiers are member dossiers in which the registrants have not opted-out. This means that these dossiers do not contain any information in the endpoint specific sections relating to physico-chemical, environmental fate and (eco-)toxicological properties. Please also refer to flowchart in Section 3.1.1 for an overview.

## 2 METHODOLOGY

### 2.1 Assessment criteria / Generating Template (Task I, step 3)

The criteria for analysis and assessment of selected REACH dossiers have been defined to provide a set of questions to guide the assessors in examining each dossier. A template has been designed including all assessment criteria, as well as general instructions for the assessor. The assessment template is included as Appendix 1. The template has been used as a working document when examining each dossier analysed in this project. The template includes questions addressing substance identification (SID), physico-chemical, toxicological, and eco-toxicological properties, as well as properties related to environmental fate and behaviour. These included the REACH information requirements in Annex VI(2), column 1 of Annexes VII-X, as well as the specific and general rules for adaptation of the information requirements in column 2 of the Annexes VII-X and Annex XI. In addition, the assessor has been asked to examine the classification and labelling of the substance, information on manufacturing and use, PBT assessment, as well as exposure assessment and risk characterisation, where available. In addition, questions related to a number of endpoints that are not part of the standard information requirements in the REACH annexes, but were considered of potential relevance for the assessment of nanomaterials, have been included in the template. Consideration of these additional endpoints was largely motivated by the findings in the REACH Implementation Projects on Nanomaterials (RIP-oNs)<sup>3</sup>. For each of the endpoints in the template, a number of guiding questions, deemed to be relevant for assessing nanomaterials, were included for the assessor to consider when going through a given dossier. These were linked either to a specific endpoint (following the IUCLID structure) or were of a more general nature and included in an annex to the template.

It should be stressed that the inclusion of questions on additional endpoints does not imply that this information or those endpoints were considered to be part of the standard information requirements for a REACH dossier on nanomaterials. Furthermore, the aim of the project was not to assess compliance of the dossiers or to conduct any other type of REACH evaluation, and the conclusions should not be interpreted to be an indication of the potential (in)compliance of any individual dossier. For this reason and to avoid release of sensitive information, this report does not refer to any individual dossier or substance name, and aim to identify and summarise general findings and conclusions.

The dossiers have been analysed and assessed as stand-alone documents based on the information provided by the registrants in their dossiers, and the assessment was not intended as a review of the available information on any particular nanomaterial. Thus the assessors have not been required to check original sources/literature cited by registrants in their dossiers, though the use of background knowledge was not excluded. Finally, the assessment was not limited to the issues described in the template, as the assessment was intended to include some flexibility and to allow the assessors to learn from the way nanomaterials were addressed and assessed by the registrants.

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<sup>3</sup> Available at: <http://ec.europa.eu/environment/chemicals/nanotech/index.htm#ripon>

The criteria have been developed in an iterative fashion; a draft template for the assessment was designed, which was used for the initial assessment of a sample number of dossiers. Based on the experience gained from this exercise, the template and the assessment criteria were refined, and a final version of the template including all assessment criteria was agreed and fixed. This final template has been used to examine all dossiers. All analysis and assessments were subject to a quality assessment by a second expert in order to enhance consistency among different experts as well as among different dossiers. Given the assessor's challenges in inferring the registrant's intentions in relation to addressing nanomaterials (as will be evident in Chapters 3, 4 and 5) and the related sub-division of the dossiers into categories (Section 3.1.1), further alignment was made in relation to drafting the final report.

For Substance Identification (SID), the assessment criteria were based on the REACH requirements specified in Annex VI(2). Annex VI(2) states that the information included in the registration dossiers shall be sufficient to enable the substance to be identified and specifies information that shall be included in the registration dossier to fulfil this requirement. Therefore, the key question was whether sufficient information was included in the dossier to enable the nanomaterial substance or nanoform of a substance to be identified and/or characterized. It is important to note that all the substances considered in this project are inorganic substances, and therefore a number of the specific requirements listed in Annex VI(2), such as those referring to chromatographic (Annex VI(2) point 2.3.6) and spectral (Annex VI(2) point 2.3.5) data are generally either not applicable or will not provide sufficient information to enable the substance to be identified. Therefore for such substances, it is necessary to consider the use of other analytical data than those specifically listed in Annex VI(2). This issue is not specific to substances at the nanoscale, rather it is an issue for inorganic substances in general, and is noted in the ECHA Guidance for identification and naming of substances under REACH (for example see section 4.2.1.3. and 4.2.2.3 of the Guidance). In addition to this, other questions have been formulated that are not explicitly defined by REACH requirements; these include whether any additional nano-specific identification or characterisation data was included in section 1.4 of the IUCLID dossier and whether any limitations of the methods used to obtain the data were reported. Furthermore, the assessment has examined whether or not surface chemistry was taken into account in the substance composition reported in the dossier. The questions concerning surface chemistry and analytical data were specific for nanomaterial substances and/or nanoforms of a bulk substance. These additional questions were based on the possible identifiers/characterisers for nanomaterials/nanoforms discussed during the RIP-oN1 project and outlined in the final RIP-oN1 project report<sup>3</sup>. Please refer to that report in relation to further discussion of 'identifier' (triggering separate registrations) and 'characteriser'. This project does not take a stand in relation to the identifier vs. characteriser issue.

For physico-chemical properties, the questions focused on the examination of the information requirements in Annex VII 7.1-7.14, as well as Annex IX, 7.15-7.17. Again, it is important to note that as all of the substances are inorganic solids, a number of these endpoints are not needed as per the specific rules for adaptation in column 2 of the relevant Annex (e.g. viscosity, flashpoint). Nevertheless, the assessors were asked to examine whether any justifications were given in relation to whether the waivers would apply to a nanoform and/or whether such waivers apply for the nanomaterial. In addition to the standard information requirements, assessors were asked to examine whether any information was provided on the following endpoints of interest: morphology/shape,

specific surface area, dustiness, agglomeration/aggregation, crystalline phase, crystallite size, zeta potential, surface chemistry, photocatalytic activity, pour density, porosity, redox potential, radical formation potential, and (representative) SEM/TEM pictures. These endpoints were identified to be of interest based on the work done in the RIP-oN2 project as well as the OECD WPMN.

For information on environmental fate, toxicological and ecotoxicological endpoints, the template addressed the related information requirements in REACH Annexes VII-X and followed the structure by which these have been implemented in IUCLID. Additional information requirements were not included in the template, but as for other parts of the template, questions potentially relevant for assessing nanomaterials were included.

Finally, the term 'nanomaterial' has in this report been used for dossiers addressing nanomaterials only whereas the term 'nanoform' has been used for dossiers that (seem to) also address other forms (e.g. bulk). Thus, a nanoform registered 'alone' (not along with non-nanoforms) would be a nanomaterial. In essence, the terms therefore cover the same, but a distinction was found useful for reporting the results in this project.

## **2.2 First detailed analysis and assessment**

A total of 45 dossiers were identified for inclusion in the assessment based on the results of the search in the IUCLID database (Task I, step 1&2). The selection process of the dossiers is described in the Task I, step 1&2 reporting and, therefore, will not be addressed in any detail here. As a first step of the detailed analysis and assessment, an examination of the substance identity as well as key physico-chemical information requirements, was performed before the full detailed analysis and assessment. This was necessary as the initial selection of the dossiers for the project was based on a combination of automated searches of the IUCLID database, as well as knowledge of the substances selected for assessment in the OECD WPMN (see Task I, step 1&2 reporting). As such, the selected dossiers (45 dossiers) were identified as being likely, but not definitively, to cover nanoforms/nanomaterials. Therefore it was necessary to further verify the relevance of these dossiers for the assessment, by examining the substance identity and key physico-chemical parameters.

In this first stage of the analysis and assessment, the following information has been considered:

- Substance Identity (entire IUCLID section I)
- Physico-chemical endpoints on state of the substance (REACH Annex VII, 7.1, IUCLID section 4.1), and granulometry (REACH Annex VII, IUCLID section 7.14)
- Other relevant endpoint specific information found in the dossiers (e.g. surface area, not a REACH endpoint, where included)
- A screening of other contextual information in the dossiers that could be of relevance (e.g. the 'nano' hits from the ECHA search in Task I, step 1 and a screening of 'scoping' information in the CSRs).

As a result of this assessment, 20 of the original 45 dossiers were considered to be less relevant for further detailed analysis and assessment in the project, as it was not possible, based on the information provided, to conclude whether nanoforms/nanomaterials were likely to be addressed - or it was clearly stated by the registrant that the nanoform was outside the scope of the registration. No further detailed

analysis and assessment was done for these dossiers. However, findings from this initial assessment, which were deemed relevant for identifying and describing substances/forms with a possible fraction of primary particles in the nanorange (1-100nm), are, where considered relevant, included in Chapter 3, in particular in relation to substance ID (Section 3.1.2) and Granulometry (Section 3.1.3.1). The introductory parts of Chapter 3 provide further details on this first detailed analysis and assessment. The 25 remaining dossiers were examined for all endpoints.

### **3 SUBSTANCE IDENTIFICATION, PHYSICO-CHEMICAL PROPERTIES AND MANUFACTURE AND USE**

#### **3.1 Findings and conclusions**

##### **3.1.1 Examination of the scope of the selected dossiers, categorisation, and comparison with the EC Recommendation on the definition of nanomaterial**

IUCLID version 5.2 allows registrants to explicitly declare their substance as a nanomaterial (or containing a nanoform of a bulk substance) in two locations (IUCLID Section 2.1, Classification and labelling, and IUCLID Section 4.1, Appearance and physical state). However, there is no explicit requirement for registrants to use this functionality and/or define their dossiers as (including) nanomaterials. Additionally, the absence of an agreed definition for nanomaterials at the time of registrations may have prevented registrants from using these options effectively. The absence of a requirement to explicitly identify the presence of nanomaterials in their dossier means that in many cases the assessors had to infer the registrants' intentions regarding whether the substances registered in the dossier are indeed nanomaterials and/or include nanoform(s) of a substance, or whether the dossier does not cover nanomaterials/nanoforms. This inference relied on information found in other sections of the technical dossiers as well as the Chemical Safety Reports (CSR) (see also section 2.2), and on expert judgement from the assessors. This ambiguity concerns not only the identity and forms of the registered substance, but in many cases the identity and form of the tested material in the submitted test results as well.

In order to address this ambiguity, the substance identity and other key information (see list in section 2.2) of the 45 selected dossiers were thoroughly examined in the initial detailed analysis and assessment. As a result, 20 dossiers were excluded from further detailed analysis and assessment, mainly for two (non-mutually exclusive) reasons:

1. The registrant includes statements explicitly excluding nanomaterials from the scope of the registration dossier. There are registrants' statements such as "this dossier does not consider nanoparticles of...". It was not possible to identify and exclude those dossiers in the initial search (Task I, step 1) due to the fact that registrants make such statements in various parts of the dossier, and there are currently no tickboxes in IUCLID that allow registrants to explicitly **exclude** the presence of nanomaterials/nanoforms from the scope of the registration. Therefore, these dossiers were not excluded by the largely automated searches performed in Task I, step 1, and a more detailed manual examination of the dossiers were necessary. Some of these dossiers related to the substances considered by OECD-WPMN as representative nanomaterials in its sponsorship programme and were automatically included in Task I, step 1 (See Task I, step 1&2 reporting)<sup>4</sup>. Several of the substances covered by these dossiers (e.g. metal

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<sup>4</sup> Note: Inclusion of a substance in the OECD WPMN does not automatically imply that all forms of the substance are nanomaterials.

and metal oxides) can exist in the nanoform and in other forms. It should be noted that as the registrant did not define what “excluded” nanoforms were (e.g. in terms of size distribution), these dossiers may still address nanoforms, e.g. if the registrant does not consider an aggregated/agglomerated form of primary nanoparticles to be a nanomaterial, or if a registrant applied a different nanomaterial definition. Based on the information provided in the registrations, it was not possible to explicitly determine the validity of such statements and thus the dossiers were typically excluded.

2. The information on granulometry provided in the dossier shows that there are no nanoparticles or there is no significant fraction (see below discussion in relation to Category III dossiers) with a (primary) particle size smaller than 100 nm (the so-called ‘nano-tail’ in the tested substance)<sup>5</sup>. It is necessary to note that while these dossiers were excluded from further detailed analysis and assessment, it is still possible that either the lead registrant or one of the member registrants intended to include nanoforms/nanomaterials within the scope of some of those registrations. It should also be noted that there is no single test for particle size distribution that is capable of characterising particle sizes in the entire range from 1 nm up to 1000 µm, as is already noted in the ECHA Guidance on information requirements (Guidance on information requirements and chemical safety assessment, R.7a: R.7.1.14.). For example, a sieving method that uses a smallest sieve size of 1 µm can only provide information on the proportion of particles with sizes above or below 1 µm, but not on the proportion of particles with a size below 100 nm. Thus, the assessment of the particle size information reported in a dossier should take the method used into account, and it is possible that the information on granulometry do not detect the fraction of primary particles below 100 nm, either due to the detection limit or due to the methods measuring aggregates/agglomerates rather than primary particles.

It should be noted that while these 20 dossiers (referring to 15 substances) were excluded from further detailed analysis and assessment, they do often contain hits for the search term ‘nano’ in the automated searches undertaken as part of Task I, step 1. These included for example test results on materials labelled as ‘nano’ from other substances, used in a read-across approach. However, while a registrant may include information on tests conducted using various nanomaterials, this does not imply that the dossier is intended to register nanoforms/nanomaterials. Registrants may include such information in a read-across context, or as a worst case scenario, or as additional supporting and/or weight of evidence information for the registered substance. The aim of the project was to assess information in registration dossiers covering nanoforms/nanomaterials, rather than dossiers containing information on nano-tests in another context.

After the above exclusions, a total of 25 dossiers (of which 4 were member dossiers and one related to a non-phase-in substance) remained that were deemed to be relevant for further detailed analysis and assessment as these dossiers were judged to cover

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<sup>5</sup> A significant exception to this rule has been for dossiers for substances that have been considered not to have a macroscale form and therefore not included in a registration of a macroscale substance (see Category II). These dossiers have been included for further detailed analysis and assessment even when the results of the granulometry did not indicate the presence of a significant fraction with a particle size smaller than 100 nm.

nanomaterials or nanoforms of a substance. These dossiers can be divided into three categories, with different levels of ambiguity:

1. **Category I - Dossiers that cover nanoforms (eight dossiers):** These are dossiers where the registrant explicitly marks the substance as covering a nanoform using one of the IUCLID picklists, labels a form/composition of the substance as 'nano', specifies that the primary particle size is below 100 nm, or states that all sizes are included in the scope of the registered substance and that primary particles for one form are < 100 nm. These eight dossiers refer to five substances.

Of these eight dossiers, only one contained sufficient analytical information to conclude that it does indeed meet the criteria for nanomaterials as described in the EC Recommendation on the definition of nanomaterial adopted on 18 October 2011. The information in the dossier indicated that for the nanoforms, 50% of the particles by volume are under 100 nm, which means necessarily that at least 50% of the particles in number based distribution will also be under 100 nm. A second dossier contained a statement by the registrant saying that one form of the substance is composed of primary particles under 100 nm in size, though no experimental information was provided to confirm this, and the information on granulometry showed particle size distributions in the micrometre range. The remaining six dossiers did not contain sufficient analytical information to confirm whether the substance meets the criteria set in the definition for three reasons. First, the information on particle size distributions referred to mass/volume based distributions, and no information on number based distributions was provided. Second, it was unclear if the results referred to aggregates/agglomerates or primary particles, and third the method used was unsuitable to measure primary particle size distributions below 100 nm. Despite these shortcomings, it has been concluded that these dossiers do indeed cover nanoforms. This is because the registrants described at least one form/composition of the substance as 'nano' in one or more sections of the dossier (using the IUCLID picklist, labelling a form/composition as 'nano', or in one case stating that a form is composed of primary particles in "the nano size"). This absence of information on number based particle size distributions is not altogether surprising, as there is no requirement to provide this information in a registration dossier.

2. **Category II - Dossiers for substances considered to be nanomaterials (12 dossiers):** There were 12 dossiers for 9 substances which are generally considered not to have a bulk form. 11 of these dossiers refer to 3 distinct types of substances, grouped by similarity in their synthesis and/or chemistry. The primary particles are in the nano-range and there is no corresponding macroscale counterpart (one of these dossiers used the IUCLID picklist for nanomaterials). For the 12<sup>th</sup> dossier, the information included by the registrant on substance identity indicates that the substance consists of core-shell nanoparticles<sup>6</sup>. It should be noted that some of these registrants did not consider their substances to be nanomaterials, because the primary particles (which are smaller than 100 nm) form aggregates and agglomerates that are > 100 nm in diameter.

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<sup>6</sup> A core-shell particle is a commonly used term to refer to a particle that contains a 'core' at the center of the particle with a specific chemistry and a 'shell' with a different chemistry.

Out of these 12 dossiers, 3 contained sufficient information to conclude they meet the definition for nanomaterials, although the nature of the information varied among them. For the core shell type nanoparticle, the assessors have concluded that the substance meets the definition because the registrant described it under the substance identity section as a core-shell nanomaterial consisting of particles under 100 nm, although the granulometry results showed the particles are > 100 µm. For another substance, the assessors have concluded that the substance meets the definition because one of the results under the granulometry section indicated the substance has an average primary particle size under 100 nm. For a third dossier, the assessors have concluded that the substance meets the definition because the information on volume specific surface (VSSA) showed a VSSA > 60 m<sup>2</sup>/cm<sup>3</sup> <sup>7</sup>. The remaining dossiers did not contain sufficient analytical information to determine if they meet the criteria set in the definition for the same reasons described for the Category I dossiers.

3. **Category III - Dossiers for substances with a percentage of the reported particle size under 100 nm ('nano-tail') based on granulometry (five dossiers):** These are dossiers where the results of the granulometry information showed the presence of a size fraction below 100 nm, i.e. substances which have a 'nano-tail', but do not fall under either Category I or II. These dossiers refer to five substances.

None of these five dossiers contained sufficient information to explicitly verify whether they meet the criteria set in the nanomaterial definition. Nevertheless, the information provided in the dossiers suggested that they most likely meet the criteria. The information on the particle size in these five dossiers is summarised in the table below with the caveat that what "particle" refers to has not been defined in the dossiers.

**Table 1. Information related to particle sizes for Category III dossiers.**

<b>Substance</b>	<b>Fraction of particles<sup>8</sup> under 100 nm</b>
Substance 1	10% of particles by mass
Substance 2	Approximately 2% of particles by mass
Substance 3	Less than 10% of particles by mass <sup>9</sup>
Substance 4	Approximately 5-10% of particles by mass <sup>10</sup>
Substance 5	The mean particle size by TEM is 0.2 µm

<sup>7</sup> Note: in this case, the registrant provided the specific surface area (SSA) as well as the bulk density of the substance, but not the Volume SSA (VSSA). The VSSA can be obtained by dividing these values.

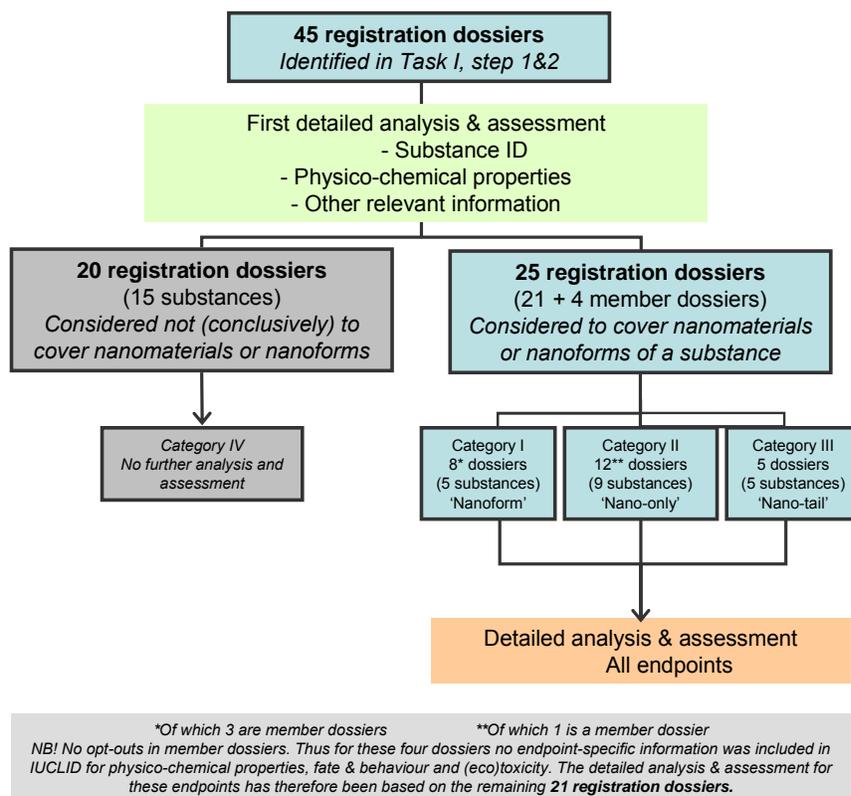
<sup>8</sup> Note: the term particle is not defined by the registrants, and may refer to primary particles and/or aggregates and agglomerates.

<sup>9</sup> Note: for this substance the assessors have estimated this value based on the size distribution information in the dossier. It was not possible to determine an exact value due to the limited information provided.

<sup>10</sup> Note: for this substance the assessors have estimated this value based on the size distribution histogram provided. It was not possible to determine an exact value due to the limited information provided.

Although a direct conversion of mass based particle size distribution to number based particle size distribution is not possible, a 2-10% of particles by mass under 100 nm may easily result in >50% of the particles numbers falling under 100 nm. Thus except perhaps for substance 5, the substances are likely to meet the 50% particle number threshold in the EC definition of nanomaterials.

The above 3 groups of dossiers will, in the remaining part of this report generally be referred to as Category I, II and III dossiers as appropriate. The deselected 20 dossiers will be referred to as Category IV dossiers to aid the readability of the report. The following flowchart gives an overview of dossiers identified, selected, excluded, analysed etc.



As can be seen from the above analysis, it has generally been difficult, if not impossible, to explicitly conclude whether nanoforms/nanomaterials are included in the scope of the registration dossier given the (analytical) information available in the dossiers. The decision to consider the above dossiers/substances as including nanoforms/nanomaterials within the scope of the registrations therefore required expert judgement. This represented one of the key challenges of this assessment and this finding in itself motivates some of the options suggested in the following sections for explicitly identifying/characterising nanoforms/nanomaterials within the scope of a registration<sup>11</sup>.

<sup>11</sup> On the other hand, it should also be realised that meeting the definition is not a criterion for a material being hazardous or not. Thus materials within the definition are not automatically very hazardous and materials not covered by a definition can still be hazardous, also because of their size. Findings based on

### 3.1.2 Substance identification (SID)

As discussed in section 3.1.1, the substance identity information in all 45 dossiers was assessed. Therefore, this section refers as appropriate to all 45 dossiers identified in Task I, step 1. The 20 deselected dossiers are referred to as Category IV. A detailed analysis and assessment of the information relevant to substance identification included in all dossiers is given in the following section.

#### 3.1.2.1 Specific requirements of Annex VI(2)

Annex VI(2) defines the information on substance identity that shall be included in registration dossiers. This information is included in Section 1 of the IUCLID dossiers. A key provision of Annex VI(2) is that the information submitted shall be sufficient to enable the substance to be identified. Annex VI(2) lists the identifiers that can be included to fulfil this when applicable and appropriate. These include numerical identifiers (EC and CAS numbers), names (e.g. IUPAC name, chemical name) and a list of spectral and chromatographic requirements that will enable the substance identity to be verified.

**Name and other identifiers (Annex VI (2.1), IUCLID section 1.1):** The majority of the 45 dossiers referred to EINECS listed substances with only four substances using list numbers assigned during the pre-registration phase of REACH as the numerical identifiers (so-called 900 numbers). The names included for the EINECS listed substances were typically those listed in EINECS. For those substances not listed on EINECS, the names were more specific for the particular form/composition being registered. Broken down by Category, only one substance from Category II and three substances in Categories IV respectively referred to non-EINECS listed substances. All Category I and III substances were EINECS listed. From the IUPAC, CAS and EC names included for the substances in all 45 dossiers, it would not have been possible without additional information to determine that the substance registered was a nanoform. Exceptions were e.g. one member dossier for a Category I substance where 'nano' was added to the name, and established nanomaterials (Category II substances).

All Category I and III substances were named based on their chemical composition. Considering the nine Category II substances, one substance was named based on chemical composition and crystal phase, two were named based on chemical composition and manufacturing process, four were named solely based on chemical composition; one was named based on mineral type while the last was named based on mineral type and further processing. 12 of the 15 Category IV substances were named based solely on chemical composition while one was named based in mineral type and two were named based on a combination of composition, processing and morphology.

**Composition (Annex VI (2.3), IUCLID section 1.2):** 10 dossiers (4 Category I and 6 Category IV) reported more than one composition. Three were explicit for nanoforms (all Category I substances). Note in these cases, the composition was reported generically simply as "nano-substance name". All Category III substances reported a single generic composition in 1.2, five of the twenty Category IV and one of the twelve Category II

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analysis and assessment of dossiers for materials which would just fail a nanodefinition may thus be very relevant for nanomaterials as well.

dossiers included more than one composition but none were specific for nanoforms. For Category II substances, different composition blocks were not reported for possible different forms (e.g. particle sizes, surface area, surface treatment, etc.). Note for those Category I dossiers that included a nano-composition in section 1.2, the analytical information included in Section 1.4 of the dossiers would not enable it to be verified.

Of the three Category I substances that included a nano-composition, the lead registrant for one included the nano-compositions in the lead registration dossier in IUCLID section 1.2 (and selected “nanomaterial” as the form of the substance in section 2.1, but not in section 4.1), although his (lead) registration was specific for the bulk form of the substance (the nanoform was for a member who reported the nanoform as the sole form of the substance in the IUCLID Section 1.2, Composition). The lead registrant of another of Category I substance specified both bulk and nanoforms as compositions of the substance in his registration in IUCLID section 1.2 (and also used the picklist in IUCLID section 2.1, but not in 4.1). Two member dossiers for the third substance included a nano-composition in section 1.2 while the lead registrant did not. The lead registrant for a Category I substance that did not include a nano composition in Section 1.2 included a report in the substance identity section of the dossier (section 1.4) that defined the scope of the registration to explicitly include all sizes and physical forms of the substance, but without providing detailed information on these forms in the IUCLID fields (the nanomaterial pick lists were not used by this registrant).

#### **Chromatographic and spectral data (Annex XI (2.3.5 and 2.3.6), IUCLID section 1.4)**

As all the substances considered in the project are inorganic, the specific requirements listed in Annex VI(2) referring to chromatographic (2.3.6) and spectral (2.3.5) data are generally either not applicable or will not provide information that would enable the substance to be identified. This issue with inorganic substances is recognised in the Guidance document for the identification and naming of substances under REACH where alternative methods that may be more suitable are suggested. Almost all of the 45 dossiers included quantitative analytical information not specifically defined in Annex VI(2) that enabled the substance to be identified based on its chemical composition (e.g. elemental analysis, X-Ray fluorescence). With the exception of two Category IV dossiers, all dossiers included some analytical data that would aid the identification of the substance being registered. For example, the dossiers for 20 substances from the list of 45 dossiers considered (16 substances/19 dossiers out of 25 Cat I-III dossiers) also included X-ray diffraction (XRD) data that would enable the substance to be identified based on its crystal phase. Note that the dossier for one Category II substance composed of an inorganic nanoparticle coated with an organic layer; the required chromatographic and spectral (IR, UV & NMR) data were included. However, these methods are only applicable to characterise the organic layer and the data submitted would not enable the substance to be identified or its composition quantified.

The dossiers for 12 substances from the list of 45 dossiers (nine of these were Category I-III dossiers) considered, included specific information relevant for nanomaterials, which are not part of the information requirements of Annex VI(2) (e.g. specific surface area, particle size distribution) in the substance identity section. However, typically the term 'particle' was not defined and details of the sample preparation were not included and/or the method used would only determine aggregate/agglomerate size and/or the measurement detection limit for the method used was above 100 nm. This ambiguity in terms of the size reported in the dossiers made it challenging for the assessors to explicitly determine based on the analytical information whether the form(s) registered

should be considered as a nanoforms/nanomaterials. The data submitted generally did not enable the nanomaterial or nanoform to be characterised or to determine if more than one nanoform were within the scope of the registration. Interestingly, however, as this information was included in IUCLID Section 1.4, the registrants for these 12 substances did consider information on size (distributions) to be of some relevance for the SID.

#### **Description of the methods (Annex VI (2.3.7), IUCLID section 1.4)**

For those dossiers that included data relevant for the characterization/identification of nanoforms/nanomaterials, descriptions of the methods used in such detail that the methods may be reproduced were not included. This has the outcome that the data submitted cannot be verified. In particular the absence of a description for what 'particle' means in the context of the methods used for example to measure particle size distribution and the lack of sample preparation description sufficient for repeating the measurement, makes the data submitted ambiguous and difficult to interpret.

#### **3.1.2.2 Information on surface treatment**

The substance identity sections of all 45 dossiers were assessed for information on surface chemistry or surface treatment.<sup>12</sup> For two Category II substances, surface treatment was explicitly included in the substance identity. For one of these substances (Category II), the surface layer was considered explicitly in the name given for the substance. For the other (Category II), substance, the surface treatment was included in the name for the substance in section 1.1 of the dossier. However, no additional details on the extent of surface modification were included in the dossier.

The lead dossier for a third substance (Category I) included a separate document in section 1.4 detailing an extensive list of surface treatments that the substance can be subjected to. However, no other details were reported and the registrant included a conclusion that surface treatment did not lead to any relevant difference in properties. Further, the registrant stated that the provisions of the FAQ question 6.3.8 on the exemption for surface treatment were applicable for all forms and sizes covered by the scope of the registration<sup>13</sup>. For a fourth substance (Category III), the surface treating agent was considered as a stabilizer and information on the identity of the agent were included in the additive fields in section 1.2 of the dossier. However the analytical data included did not include any specific information on the stabilizer. None of the remaining 41 dossiers included information on surface treatment in the substance identity sections.

Note, however that for approximately half of the 45 dossiers, it was stated in other IUCLID sections that the substance may be surface treated; e.g. in IUCLID Section 3.1, there were statements such as "the product may be surface treated to promote hydrophobicity" without additional details given in the dossier. Generally, information on

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<sup>12</sup>The aim of this section is to focus on surface functionalisation/treatment in the dossiers, whereas the term surface chemistry is a more broad term. However, for some substances, such as the core-shell type particle described here, surface chemistry may be a more appropriate term.

<sup>13</sup> It should be noted that ECHA has clarified that this FAQ refers to macro-sized particles only. If the FAQ is considered applicable for nanomaterials, it should be noted that the FAQ clearly states that any specific hazards or risks associated with a surface treated substance should be appropriately covered by the classification and labeling and by the CSR and resulting exposure scenarios.

the type or extent of such surface treatment was not included (with the exception of a few dossiers with some information under SID, see above)<sup>14</sup>.

It is interesting to note that most of the 34 substances registered in the 45 dossiers considered here are known to be extensively surface treated<sup>15</sup>. Surface treatment is used to control the extent of dispersion/aggregation/agglomeration (i.e. overall size) and/or to introduce specific functional groups on the surface of the particle and is application driven.

Surface treatment of nanomaterials was discussed in detail in the RIP-oN1 project on the substance identification of nanomaterials, and while it was agreed that it was relevant for assessing the properties of a given material and thus needs to be dealt with in a transparent way, there was no consensus on *how* it should be considered in the context of REACH, i.e. whether it should be part of substance identification or as a characteriser for specifying different forms within a dossier.

### 3.1.2.3 Adequacy of the SID information

*From the assessment of the SID information included in all 45 dossiers, it has been concluded by the assessors that it was generally considered sufficient to enable the substance to be identified based on its chemical composition, i.e. the substance is generically named based on chemical composition and analytical data, that enables the chemical composition to be verified, were included. Note that based on the RIP-oN1 report, the identification of the substance based on solely chemical composition implies that all other parameters (e.g. size, shape, crystalline form etc.) are relevant only for its characterization and not its identification. 20 substances from the list of 45 dossiers additionally included information that would enable the crystalline form of the substance to be identified while others listed forms in section 1.2 of the dossier, which they labelled as “bulk” and “powder” forms, and in three cases “nano” was specifically listed. For the case where more than one composition or form was reported in section 1.2, the corresponding analytical data that would enable each composition or form to be verified were not typically included in section 1.4. Furthermore, for the substances considered in the project, the information included in the substance identity sections of the dossiers alone did generally not enable the form of the substance to be identified or characterised as a nanomaterial substance or a nanoform of a substance. Where information on particle size was included, it was generally not specified what the term “particle” referred to (primary, aggregates, agglomerates or other). In addition, for the dossiers where size was reported, detailed information was not provided on the method used and in particular not on the sample preparation. This is required by Annex VI (2.3.7) to enable that the method/results can be reproduced/verified.*

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<sup>14</sup> In this context, it should be noted that in the detailed analysis assessment of the Category I-III dossiers, the influence surface treatment may have on the properties of the substances/forms registered were generally not addressed in the endpoint specific sections in other parts of the dossiers.

<sup>15</sup> A search of the manufacturer’s websites for example shows multiple surface treated products for these substances

### 3.1.3 Characterisation

This section describes the information found in the dossiers that is relevant for basic characterisation of nanomaterials/nanoforms in addition to surface treatment already addressed above. This includes information on particle size (granulometry) and specific surface area (SSA). As this information is vital for the characterisation of nanoforms/nanomaterials, it is presented separately from other physico-chemical properties. It is recognised that some of this information may be relevant for both substance identification and information on physico-chemical properties.

As for substance identification, findings from all 45 dossiers identified in Task I, step 1&2 will be addressed where appropriate. A summary of the available data on granulometry and surface area/specific surface area given in Table 2, divided across the four categories of substances.

Category (substances/dossiers)	Experimental data on granulometry in IUCLID section 4.5	Granulometry method assessed appropriate for nanomaterials	Size distribution of primary particles	Provided surface area
<b>Category I (5/8)</b>	5	1	1	1
<b>Category II (9/12)</b>	11	3	3	5
<b>Category III (5/5)</b>	5	1	0	3
<b>Category IV (15/20)</b>	13*	2	0	5

**Table 2: Summary of characterization data.** The numbers in brackets refer to the number of lead registrant dossiers, followed by total number of dossiers, so Category I has 5 lead registrants with 8 dossiers analysed and assessed, etc. \* 1 dossier in this category waived the test, while another submitted information from a different substance as read-across. Note that for one Category III dossier, an appropriate method for nanomaterials was used, but it is not clear if the results reported refer to primary particles or aggregates.

#### 3.1.3.1 Granulometry (Annex VII 7.14, IUCLID section 4.5)

The endpoint on granulometry is a key endpoint for nanomaterials, especially as particle size forms an essential part of any definition of nanomaterials and is believed to be an important driver for other properties of the substance. Therefore, it is necessary to have high quality information on this endpoint. The assessment criteria included several questions on granulometry in order to provide a detailed analysis of the information provided on particle size. However, in many cases it was difficult to address the questions posed by the assessment criteria due to the absence of this information in the dossiers, as there are no provisions explicitly requiring such information as part of the current REACH standard information requirements.

In principle, information on granulometry included in section 4.5 of the dossier could have been used as part of the assessment of the characterisation/identification of the nanoform/nanomaterial. However, several shortcomings were identified. First, only lead registrant dossiers contained information on granulometry in section 4.5 of the technical dossier, and none of the joint member dossiers submitted information individually by opting out. This means that the registrants considered the data on granulometry in the

lead registrant's dossier to be valid for all members of the joint submission.<sup>16</sup> However, as the particle size of each substance depends on the specific manufacturing process, it is doubtful whether data from one registrant could be used for others.

Second, registrants rarely provided experimental information on the primary particle size distribution. Out of 45 dossiers, only one Category I dossier provided experimental information on the primary particle size, whereas 3 Category II dossiers contained experimental information on primary particle size (2 dossiers contained some experimental results for primary particles under IUCLID section 4.5, and 1, the core-shell particle, provided granulometry in section 4.5 of the dossier that appears to be for the aggregate/agglomerate, while providing information on the primary particle size distribution in section 1.4 of the dossier). The Category III or IV dossiers did not contain information on the primary particle size.

While not containing experimental data on primary particle size, four dossiers contained statements describing the primary particle size of the substance in various parts of the dossiers. A Category I dossier contained a statement (under the summary for IUCLID section 4), stating that that one form of the substance is composed of primary particles under 100 nm, and the primary particles form aggregates and agglomerates greater than 100 nm. A Category II dossier contained a statement (under IUCLID section 4.1) stating that the substance forms colloids in water composed of discs 1nm thick and 25 nm wide, but also state that the material sold is not a nanof orm, rather it has a PSD in the micron range. Two other Category II dossiers (referring to one substance), contain statements (in the CSR and in the section on granulometry, respectively) indicating that the primary particle size is between 10-500 nm.

Most of the granulometry methods used are incapable of distinguishing between primary particles on one hand, and aggregates and agglomerates on the other. Some registrants did include statements saying the particles are composed of tightly bound aggregates/agglomerates and explicitly stated the granulometry results are for these particles, without providing the particle size distribution of the primary particles. This was the case for three Category II dossiers. The assessors were thus mostly forced to assume what the term 'particle' might refer to. This has made it difficult to draw explicit conclusions as to whether a nanomaterial/nanof orm was included within the scope of the registration based on the granulometry data alone. Overall, it can be concluded that it was not possible to explicitly characterise nanof orms/nanomaterials based on the information on granulometry for most dossiers. Further information would be necessary to fully characterise the particle size in these dossiers.

Furthermore, as can be seen from the analysis and assessment of environmental fate & behaviour and (eco-)toxicity in Chapter 4, it was not always evident how the test materials for such test corresponded to the test material used for granulometry.

In addition to information on the particle size distribution, it is necessary to have information on the stability of any aggregates/agglomerates forms in order to evaluate if these particles could break up to reform the primary particles during the life cycle of the

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<sup>16</sup> Note: while the exercise contains only 45 dossiers mostly related to lead dossiers and only a few member dossiers, in reality several joint submissions contain a large number of member dossiers, which have not been selected in this project as the member dossiers generally did not contain any opt outs that would lead to additional information.

substance. However, registrants generally did not provide any information on the stability of the particles in terms of possible deaggregation/deagglomeration. Qualitative statements such as the primary particles form aggregates/agglomerates which are bound together, or that the primary particles do not exist in isolation or outside the reaction chamber were provided. This was seen in one Category I dossier, and five Category II dossiers.

In four of the Category II dossiers the registrants subjected the particles to “destructive forces” in the granulometry tests to support that the particles are bound as aggregates. In these cases the use of “destructive forces” resulted in the breakdown of agglomerates/aggregates to give smaller aggregates. One Category II dossier provided a study on dustiness to illustrate that only a small fraction of the particles are respirable, although a dustiness study does not evaluate aggregate/agglomerate stability.

Two main issues affecting the interpretation of the data, in relation to whether nanoforms are addressed, were identified regarding the methods used to measure granulometry. First, registrants often used methods that are inadequate to measure particle sizes below 100 nm. In several cases, the lower cut-off was significantly above 100 nm, and the only information available was that x% of the substance (by volume/mass) falls below the cut-off value. Out of 45 Category I-IV dossiers, 6 dossiers (4 out of the 25 Category I-III dossiers), used methods appropriate for detecting primary particles below 100 nm, such as TEM. Second, registrants generally did not provide sufficient information about the sample preparation used for the analysis. Information on sample preparation was one of the questions asked in the assessment criteria, because it is known that the results of granulometry studies depend significantly on sample preparation (as also concluded in RIP-oN2). In order to obtain reproducible data, as well as to aid in the interpretation of these studies and comparison of the results, not only the type of sample preparation but also the detailed preparation parameters (e.g. pre-wetting, duration of sonication, power used for sonication etc.) must be known.

One final issue noted during the analysis and assessment relates to the joint submission of data. In order to encourage data sharing and reduce unnecessary testing, registrants are required to submit data jointly. Registrants may submit information on granulometry separately, although this requires them to ‘opt-out’, which results in a higher fee and is a criterion used to select dossiers for a targeted compliance check under dossier evaluation. It should be noted (see above) that for the substances in the 25 Category I-III dossiers analysed and assessed (and also all the substances in the Category IV dossiers), there were no opt-outs in the joint submission for this endpoint. The particle size of a substance, unlike many other physico-chemical properties, is not determined by the chemical structure or composition, and is highly dependent on the manufacturing process. Therefore, the particle size measured for one registrant may be significantly different from other members of the joint submission, and the data from one registrant may not be representative for members of the joint submission. The same may be true for other characterisation parameters, which will be elaborated further under options in section 3.2.

### **3.1.3.2 Specific surface area (SSA) (Not a REACH standard information requirement)**

Information on volume specific surface area (VSSA) can be relevant for the identification of nanomaterials, and is indeed included in the EC Recommendation on the definition of nanomaterial. While information on this endpoint is not currently part of the standard information requirements, some registrants (9 of the Category I-III dossiers, 5 of Category IV dossiers) provided information on (mass) specific surface area, either in IUCLID section 1 or 4. In such cases where information on this endpoint was available, gas adsorption/desorption measurements were performed and analysed with the BET method. However, there was limited information provided on the performance of the test or on sample preparation.

For two Category IV substances (four dossiers), the specific surface area (SSA) was high ( $> 1000 \text{ m}^2/\text{g}$ ), however the information on Particle Size Distribution (PSD) indicated that the particles were micron sized. As the substances are not considered to be nanomaterials *per se* (in this particular case the high surface area was due to the highly porous nature of the substance, rather than due to it being a nanomaterial/consisting of primary particles in the 1-100 nm range), these two substances have not been considered to be nanomaterials in the scope of this project and are thus part of the 20 'deselected' dossiers. However, the information included in the dossiers did not enable this to be definitively verified.

*In general, the available information in the dossiers for characterisation of these substances (granulometry and surface area) are not sufficient for most of these dossiers to determine if they are a nanomaterial using the criteria in the EC Recommendation on the definition of nanomaterial, as information is lacking on the number based size distributions and surface area. This is not surprising, as such information is not a REACH standard information requirement and as the methods generally used for granulometry do not enable the generation of number based particle size distribution for primary particles under 100 nm. Finally, the current practice of submitting data jointly for joint submissions is not applicable for the granulometry endpoint (and for SSA) as these endpoints are dependent on the manufacturing process, and are not derived directly from the chemical composition of the substance, and therefore information from one registrant may not be relevant for others.*

### **3.1.3.3 Scope of the registration dossier (in terms of substance/forms covered)**

From the assessment of the SID and 'characterisation' information included in the 45 dossiers, it became apparent that a key issue was the determination of the 'scope of the registered substance'; i.e. what forms/compositions of a substance are considered to be part of the substance registered. In particular for substances identified with very generic EINECS entries, e.g. metal oxides, it was not possible to determine the scope in terms of nanomaterials/nanoforms addressed based on the information included. Note that this was also problematic for Category II substances, which are assumed to be a priori nanomaterials as it could not be determined if different nanoforms were within the scope of the registered substance (e.g. different surface treatments).

Note that the scope of the registered substance is a key concept in REACH as it relates to substance sameness. According to the REACH requirements for SID, each registrant regardless of whether the registration is part of a joint submission or not, is required to submit his own information for the substance that he manufactures and/or imports. For the substances analysed and assessed, it was found that while the lead and member registrants included their own specific SID information, (analytical) information was typically not explicitly included neither in the SID nor in other parts of the registrations that would enable an independent assessor to determine if nanomaterials/nanofoms are intended to be within the scope of the registration dossier.

As outlined in the “Guidance on the identification and naming of substances under REACH”, the composition and form of a given substance can differ between manufacturers/importers and it is up to the registrants to agree on “substance sameness” for the purpose of data sharing in the joint submission for that substance. Rules of thumb to aid registrants making these decisions are given in the Guidance document. Substance sameness criteria agreed by the registrants determine the scope of the registered substance in terms of compositions/forms covered by the registration. The validity of substance sameness decisions made by registrants in a joint submission is assessed by ECHA and Member State Competent Authorities (MSCAs) in cases when that substance is chosen for evaluation. It should also be noted that there is currently no guidance available to aid either registrants or regulators to determine substance sameness for nanomaterials.<sup>17</sup>

*While it is not explicitly required in Annex VI(2) or other REACH standard information requirements to define the scope of the registered substance in terms of forms/compositions, this information (either considered as identifiers or characterisers) would be necessary and helpful for the registrant to demonstrate that risks are controlled for all forms and uses of the substance registered, as some inherent/hazard properties can differ based on the form of the substance.*

### **3.1.4 Other physico-chemical properties**

It should be noted that out of the 25 dossiers included for further detailed analysis and assessment, 4 dossiers were members of a joint submission. As these dossiers did not include any opt-outs for any of the information requirements on physico-chemical properties, the information below refers to 21 dossiers. For Category II dossiers, which do not have a bulk form, it may be possible to assume that the information provided is relevant for the nanoform. However, as will be seen below, the dossiers in Category II, with few exceptions (noted where present), did not provide specific information indicating that the test materials was indeed a nanomaterial. Where experimental data was provided, there was no information on the particle size of the tested material nor of possible difference related to surface functionalisation/treatment.

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<sup>17</sup> It should be noted that the process of inquiry as described in Article 26 of the REACH regulation does require ECHA to put registrants for the same substance in contact with each other for the purpose of data sharing. Thus ECHA does in principle assess substance sameness in a joint submission when processing Article 26 inquiries. However, although ECHA may put potential registrants and existing registrants of a given substance into contact based on substance identity information, it is for the registrants to decide whether data-sharing is meaningful such that the safe use of the substance can be demonstrated. The duty of potential registrants to make an Article 26 inquire is limited to non-phase in substances and phase in substances that were not pre-registered.

#### **3.1.4.1 State of the substance (Annex VII 7.1, IUCLID section 4.1)**

This endpoint is used to provide a general description of the state of the substance (e.g. liquid, solid), and form (e.g. powder). The information on this endpoint is usually obtained via visual inspection or based on handbook data. It is generally not expected that this information will differ between nanoforms and bulk forms, though in some instances it is possible (e.g. the colour of some substances change from the bulk to the nanoform).

The most important aspect of this endpoint for nanomaterials/nanoforms stems from the fact that IUCLID (in version 5.2, released February 2010) was updated to allow registrants to indicate “nanomaterial” as a form of the substance. Dossiers that were submitted using an older version of IUCLID did not have this option. Although all the dossiers included in the project were submitted or updated following the release of IUCLID version 5.2, only one dossier (Category II) chose this value in section 4.1 of the dossier. In another two dossiers (both Category I), a nanoform was indicated in IUCLID section 2.1 on classification and labelling. Therefore, only three dossiers in total explicitly, indicated via use of the nano picklists, that they were registering a nanomaterial or a nanoform of a substance. No dossiers used the picklist in both section 2.1 and section 4.1. For one specific dossier, the registrant states in this endpoint that the substance is not sold as a nanomaterial, though the substance is described in this IUCLID section as being composed of particles with dimensions less than 100 nm in aqueous dispersion. For the remaining dossiers (Categories I-III) there is no information in this endpoint that specifically mentions nanomaterials/nanoforms as the form of the substance.

#### **3.1.4.2 Melting/freezing point (Annex VII 7.2, IUCLID section 4.5)**

The dossiers identified as relevant for this project were inorganic materials with high melting points, significantly higher than 300 °C. The registrants generally provided data on this endpoint, but did not provide information on the particle size of the test substance. For Category I dossiers, addressing several forms, registrants did not provide any justification for why the results - which are presumed to be for a bulk/macroscale form - would or could apply to a nanoform. In one particular Category I dossier, the registrant explicitly labelled the study on melting/freezing point as “bulk”, but did not provide any information on the nanoforms. The remaining dossiers (Categories I-III) did not specifically mention nanomaterials/nanoforms, and where experimental data was available, no information was provided on the particle size of the tested substance.

High melting points can be used to waive other REACH information requirements (boiling point and vapour pressure). For some substances, melting point depression is known to occur for the nanoform<sup>18</sup>, resulting in lower melting points compared to the corresponding bulk substance. However, in many cases, the melting point reported in the dossiers was high (>1000 °C). It is expected that even if the melting point was reduced, it is likely to remain above 300 °C, which is the REACH cut-off for waiving tests on boiling point and vapour pressure.

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<sup>18</sup> For example, see Goldstein, A.N.; Echer, C.M.; Alivisatos, A.P. Melting in Semiconductor Nanocrystals, *Science*, **1992** 256, 1425-1427

### 3.1.4.3 Boiling point, and vapour pressure (Annex VII 7.3 and 7.5, IUCLID sections 4.3 and 4.6 respectively)

The tests for these endpoints were generally (18 of 21 dossiers) waived by registrants based on the high melting point of the substance (adaptations in column 2, Annex VII for both endpoints) or due to the decomposition of the substance. In cases where the dossier contained multiple forms (e.g. a bulk form and a nanoform – Category I) the justifications for waiving were not specifically marked for the nanoform, and therefore it is not clear if the registrant intended to waive the test for the bulk form, nanoform, or both. In one particular Category I dossier, the registrant explicitly labelled the study on boiling point and a waiver for vapour pressure as “bulk”, but did not provide any information on the nanoform. As the registrants generally did not have data on the melting points of a nanoform, the waiving statements may not directly apply to the nanoform. However, it is expected that the melting point will nevertheless be above 300 °C in most if not all cases, and the registrants would be able to waive these tests using the appropriate column 2 adaptation.

### 3.1.4.4 Relative density (Annex VII 7.4, IUCLID section 4.4)

All registrants provided experimental data on this endpoint, however, as for other endpoints on physico-chemical properties, no information was provided on the particle size of the tested material. For dossiers, where a bulk and nanoforms exist (Category I), registrants did not provide any justification for whether the test results apply to the nanoform. In one particular Category I dossier, the registrant explicitly labelled the study on density as “bulk”, but did not provide any information on the nanoform. Nevertheless, it is not expected that the density of substances would be significantly affected by the particle size. It has to be noted that in some cases for particulate material, it is unclear whether the *bulk density*, i.e., the density of a solid block of the material, or the *pour density* is reported. However, one has to be aware, that the maximum pour density of a material that consists of particles is always lower than the bulk density. In the case of spherical particles the maximum pour density is  $\frac{\pi}{\sqrt{18}}$  ( $\approx 0.74$ ) of the bulk density.

### 3.1.4.5 Surface tension (Annex VII 7.6, IUCLID section 4.10)

Registrants generally waived this endpoint (20 of the 21 dossiers) with either of the following justifications a) low water solubility or b) based on the structure, surface activity is not expected or desired. In cases where the dossier contained multiple forms (Category I), the justifications for waiving were not specifically marked for the nanoform, and therefore it is not clear if the registrant intended to waive the test for the bulk form, nanoform, or both. In one particular Category I dossier, the registrant explicitly labelled the study on surface tensions point as “bulk”, but did not provide any information on the nanoform. Nevertheless, these are valid Column 2 waivers in Annex VII, and excluding the effects of significant surface treatment there is no expectation that surface tension will significantly change for the nanomaterial/nanoform. For one particular case where the study was waived due to water solubility, it was noted that the surface activity of the substance caused problems when it was attempted to conduct a partition coefficient test. The lowered surface tension was most likely due to the fact that this substance is a core-

shell particle with surface active functional groups. Many of the substances included in the project can be surface treated (see Section 3.1.2.2). The possible effects of surface treatment/functionalisation were not considered by the registrants for the dossiers assessed.

#### **3.1.4.6 Water solubility (Annex VII 7.7, IUCLID section 4.8)**

All registrants provided information on this endpoint, however, as for other endpoints on physico-chemical properties, no information was provided on the particle size of the tested material. In one particular Category I dossier, the registrant provided different studies on water solubility labelled as “bulk” and “nano”, although no information on the particle size of this nanoform was provided. In a second Category I dossier, the registrant explicitly indicated that the study on water solubility is for the “bulk” form, but did not provide any information on the nanoforms. For the remaining Category I dossiers, the registrants did not provide any justification for whether the test results apply to the nanoform.

A common challenge with the assessment of the water solubility of nanomaterials is the distinction between solubility, dispersion, and ion leaching from the particles. In at least five cases (all Category II dossiers), it appears that the registrants may be measuring ion leaching from the particles, although this was not always clear, or whether the measured value was actually a dispersion of (fine) nanoparticles.

Many of the substances included in the project can be surface treated. The possible effects of surface treatment on the apparent water solubility and/or dispersion were not considered by the registrants for the dossiers analysed and assessed.

The possible effects of particle size on the water solubility was generally not discussed in the dossiers, although the (primary) particle size and/or surface area can have an impact on the solubility of a substance. Information on the particle size of the tested material was provided only in 3 cases. In two Category III dossiers the information on particle size showed the particles to be > 1 µm, whereas one Category I dossier showed the particle size to be between 200-250 nm.<sup>19</sup> Finally, in one Category II dossier the water solubility was measured for different grades of the substance with different surface areas and registrant concludes that primary particle size/SSA has “biggest influence” on water solubility.

#### **3.1.4.7 Partition coefficient n-octanol/water (Annex VII 7.8, IUCLID section 4.7)**

All registrants waived this endpoint with the justification that the substance is inorganic (in one case due to surface active properties, where the registrant provided instead a QSAR estimate of the surface active group). In a total of two cases the registrant included a QSAR estimate in addition to the waiving statement. In cases where the dossier contained multiple forms (a bulk form and a nanoform), the justifications for waiving were not specifically marked for the nanoform (with one exception), and therefore it has not been clear if the registrant intended to waive the test for the bulk

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<sup>19</sup> Average size, no information on number or mass of substances below 100 nm, or on agglomeration/aggregation state.

form, nanoform, or both. Nevertheless, as all of the dossiers examined refer to inorganic substances the waiver must be considered to apply. It is important to note that in cases a substance is surface treated with organic groups (e.g. hydrophobic or hydrophilic surface treatment), the relevance and applicability of this waiver should be questioned, and registrants should more carefully examine the partition of the substance between octanol/water.

#### **3.1.4.8 Flash-point (Annex VII 7.9, IUCLID section 4.11)**

All registrants waived this endpoint with the justification that the substance is a solid. When different forms were addressed in one dossier (Category I), the justifications for waiving were not specifically marked for the nanoform, and therefore it is not clear if the registrant intended to waive the test for the bulk form, nanoform, or both. Nevertheless, this endpoint is not relevant for solids with high melting points, as flash-point is a property of liquids, and the waiver must be considered to apply.

#### **3.1.4.9 Flammability, explosive properties, self-ignition temperature, and oxidising properties (Annex VII 7.10-7.13, IUCLID sections 4.12-4.15)**

The results of physico-chemical hazard tests are generally known to depend on the particle size. Finer particles are generally more reactive (flammable, explosive, oxidising) compared to larger particles. The registrants' approach to these endpoints varied according to substance. In approximately half the cases registrants provided data on flammability or autoflammability (3 dossiers provided information on only on flammability, 1 dossier provided information only on auto-flammability, and 8 dossiers provided some information on both), whereas in other cases the test was waived based on chemical structure (e.g. no structural alerts for explosive or oxidising properties, metal oxides with a metal at the highest possible oxidation state, and therefore incapable of reacting further with oxygen). In total, seven dossiers contained some information on explosive properties. Split across the categories, this included one Category I dossier, which contained a study for the bulk form, but not for the nanoform, five Category II dossiers (four contained data on dust explosion hazard, with one of these showing that the substance presents a dust explosion hazard, and one contained an expert statement), and one Category III dossier.

In cases where the dossier contained multiple forms (Category I) regardless of which approach was taken, the registrants did not specifically address the nanoform for 4 out of the 5 substances, and therefore it is not clear if the registrant intended to cover/waive the test for the bulk form, nanoform, or both. In one Category I dossier, the information on flammability, explosive properties, and self-ignition temperature were performed using the nanoform as a worst case scenario. In one separate Category I dossier the registrant explicitly labelled hazard studies provided as being for the "bulk", but provided no information on the nanoform, or any discussion on the applicability of the information from the bulk form to the nanoform.

#### **3.1.4.10 Stability in organic solvents (Annex IX 7.15, IUCLID section 4.17)**

All registrants waived this endpoint with the justification that the substance is inorganic (except for two, where the information was not required due to tonnage), or that the stability is not considered critical. For Category I dossiers the justifications for waiving were not specifically marked for the nanoform, and therefore it is not clear if the registrant intended to waive the test for the bulk form, nanoform, or both. Nevertheless, according to the REACH regulation this endpoint is “only required if stability of the substance is considered to be critical”, and is not required for inorganic substances. It should be noted that many of the substances in this report can be surface treated/functionalised, and in waiving this endpoint, the registrants have not considered the potential effects of any surface treatment/functionalisation.

#### **3.1.4.11 Dissociation constant (Annex IX 7.16, IUCLID section 4.21)**

All but one registrants waived this endpoint with the justification that the substance does not contain functional groups capable of dissociation/ionization at relevant pH ranges, two registrants did not include any information due to tonnage considerations, and two registrants included read-across information from another substance in addition to a waiving statement. With the exception of one Category I dossier, the justifications for waiving were not specifically marked for the nanoform, and therefore it was not clear if the registrant intended to waive the test for the bulk form, nanoform, or both. In one case the registrant explicitly indicated that the waiving statement was for the bulk form, but did not provide any information on the nanoform. Nevertheless, it is possible to make a qualitative prediction on a substance’s potential for dissociation regardless of the size of the particle. Note as discussed in previous sections that many of the substances assessed can be surface treated. Depending on the treatment, the surface of the particles may have functional groups that are relevant for this endpoint. However, this was not considered by the registrants for the dossiers assessed.

#### **3.1.4.12 Viscosity (Annex IX 7.17, IUCLID section 4.22)**

All registrants waived this endpoint with the justification that the substance is a solid, except for two registrants where this information was not required due to tonnage. For Category I dossiers that contained bulk and nanoforms, the justifications for waiving were not specifically marked for the nanoform, and therefore it is not clear if the registrant intended to waive the test for the bulk form, nanoform, or both. In one case the registrant explicitly indicated that the waiving statement was for the bulk form, but did not provide any information on the nanoform. Nevertheless, this endpoint is not relevant for solids.

#### **3.1.4.13 Additional Characterisers/Physico-chemical properties**

The examination template contained questions on a number of endpoints that are not part of the REACH information requirements (see Appendix 1). Most dossiers did not contain information on these additional endpoints, however, some exceptions are noted. Two Category III dossiers as well as one Category II dossier contained SEM/TEM pictures. In addition, three Category IV dossiers also contained SEM/TEM images.

Some dossiers contained information on crystalline phase, see discussion in Section 3.1.2 on crystal structure/XRD data. Three Category I dossiers contained information on dustiness, and two dossiers contained information on the shape of the primary particles). Finally, some dossiers contained information on aggregation/agglomeration of particles (see Section 3.1.3.1 on Granulometry).

*As a summary, several endpoints on physico-chemical properties have been waived by registrants in their dossiers. In many cases, this waiving may be applicable regardless of the particle size of the substance, such as waivers for viscosity, flashpoint, and vapour pressure. However, registrants could improve the transparency of the dossiers by indicating if the waivers and/or information provided is applicable to the nanoform (when a bulk and nanoform are present). Furthermore, for some endpoints (e.g. water solubility, physico-chemical hazard endpoints such as explosiveness and flammability) particle size can have a significant impact on the results of the tests, and therefore registrants should examine the potential effects of particle size on these properties. For some endpoints (such as the partition coefficient, surface tension, water solubility), surface treatment could have an impact on the performance and the results obtained from these tests, and surface treatment was generally not addressed by the registrants.*

### **3.1.5 Manufacture and Use**

#### **3.1.5.1 Technological process (Annex VI 3.1, IUCLID section 3.1)**

The eight Category I dossiers did not report any specific information on the technological processes used to manufacturing the nanoforms. In these dossiers a generic description of the manufacture was generally reported without distinction between the different forms covered. In one case a dossier mentioned “fractionation of the particles according to the size” as last step of the process but there was not enough information to judge whether a nanosized fraction was generated. In another case, the lead dossier addressing both bulk and nanoform was associated with a member dossier specifically addressing the nanoform: the technological process described in the member dossier can be doubtlessly considered as nano-specific since the nanoform was the only substance covered. However, the description reported was the same as in the lead dossier and this implies that based on the information provided by the registrants there are no differences in the manufacture of the bulk and the nanoform. In spite of this, all dossiers reported “SU9 Manufacture of fine chemicals” as sector of use associated to some of the uses by workers in industrial settings and by professional workers listed under the dedicated IUCLID section 3.5, Identified uses. This sector of use may include nanoforms of the substances, but there was not enough information that could support a final interpretation of this issue.

In all Category II dossiers, the technological process has been considered by the assessors to address the nanoform following the assumption that the substance exists only as a nanoform with no corresponding bulk form. However, the information provided by the registrants did generally not explicitly mention that a nanomaterial was manufactured. In one dossier a nano-specific manufacturing process was clearly reported. There was also one case of a dossier mentioning “fine powder” as output of the manufacturing process, but there was not enough information to judge the size of the fine powder. Moreover, in eight dossiers the production process of the substance is associated to the “SU9 Manufacturing of fine chemicals” sector of use, which may also

cover smaller sizes. However, there were no additional details that could support a final conclusion on this issue.

The Category III dossiers did not specifically address that the processes could result in a nano-tail. However, one dossier stated that “quenching, sizing, milling and chemical treatments to adjust particle size distribution and final chemical purity” are applied, while in another dossier, processes, aimed to change the shape and the polarization of the crystal lattice of the material in order to enable specific applications, were mentioned. The formation of a coating in order to improve properties of the substance was also specified. Moreover, four out of six dossiers reported “SU9 Manufacture of fine chemicals” as sector of use associated to the production processes, which may also cover smaller size fractions.

In relation to the above analysis, it should be stressed that further detailed background knowledge on the processes listed might have enabled the assessors to implicitly infer what processes could lead to particles in the nano-range.

In two dossiers (one Category I member dossier and one Category II dossier for an imported substance), no information on manufacture was reported.

*In conclusion, for Category I dossiers addressing different forms (including nanoforms) of the same substance, the manufacturing process is generally not clearly specified for each form (exempt for one case). If the same processes are applied regardless the form, this was not explicitly stated. Regarding Category II dossiers, manufacture has been assumed to be addressed for the nanoform as the substance does not have a bulk counterpart. However nano-specific processes were not mentioned (except for one case). Finally, if the manufacturing process could intentionally or unintentionally produce a nano-tail, this was not addressed in the Category III dossiers.*

### **3.1.5.2 Estimated quantities (Annex VI 3.3, IUCLID section 3.2)**

All dossiers reported estimated manufactured and/or imported quantities.

Almost all dossiers referred to the >1000 tonnage band and registered a phase-in substance. There were some exemptions:

- 1) two phase-in substances produced at lower quantities, one by a JSO member (in the 100-1000 t/y range) and another one by a JSO lead registering a CMR substance (in the 10-100 t/y band)
- 2) a non phase-in substance that has been voluntarily registered (imported at <1 t/y)
- 3) two cases where the estimated quantities of the lead registrant are in the 100-1000 range, but the registration was for >1000 t/y as there were some members with >1000 t/y. Thus, the registration in those two cases was done in accordance with the >1000 t/y requirements.

Among the Category I dossiers, for one substance (covered by one lead and one member dossier), the JSO lead dossier listed three compositions (one is nanoform) while the associated member dossier specifically addressed the nanoform. The reported manufactured and imported tonnages in that member dossier were thus specific for the nanoform. In another Category I dossier, which explicitly addressed both bulk and nanoform, the quantities related to the nanoform were specified in the “Remarks” box in the IUCLID section 3.2 and represented approximately 5-10% of the total tonnages in

2010. In the remaining five Category I dossiers, the total tonnage was reported without any distinction between the different forms.

For the 12 Category II dossiers, the estimated quantities have been assumed by the assessors to address the nanoform following the assumption that the substance itself is a nanoform with no corresponding bulk form. The registrants for these substances did not explicitly mention 'nano' or 'nanomaterial' under the estimated quantities.

For the four Category III dossiers, the reported tonnages did not explicitly refer to 'nano'.

*In conclusion, for Category I dossiers explicitly addressing different forms (including nanoforms), one dossier explicitly addressed tonnages for the nanoform (being a member registration specifically for the nanoform) and one dossier did so in a remark field. For the remaining Category I dossiers, no specific tonnage information for the nanoform was provided. For the Category II dossiers, tonnages must implicitly be assumed to be for the nanoform, although this was not explicitly noted by the registrants. For the Category III dossiers, the reported tonnages did not explicitly refer to 'nano'.*

### **3.1.5.3 Identified uses (Annex VI 3.5, IUCLID section 3.5) and Uses advised against (Annex VI 3.7, IUCLID section 3.6)**

Firstly, it should be noted that identification of uses in IUCLID section 3.5, Identified uses is based on the predefined use descriptors, mainly intended to give a general description of uses and not give details that would often be needed in relation to building actual exposure scenarios. Further, the use descriptor system was developed without considering specific uses of nanomaterials. See REACH guidance on Information Requirements and Chemical Safety Reports Chapter R.12 for further details on the use descriptors system. However, IUCLID allows with some free text to indicate for the uses selected what they refer to, e.g. to which form.

Almost all dossiers (with two exceptions) reported uses by workers in industrial settings and professional workers and half of the dossiers (i.e. 13) identified uses by consumers. There were no uses advised against except for one use by consumers in one of the Category II dossiers. In another Category II dossier it was stated that "the substance should be used as intended by professionals" and specific conditions of uses were reported. It should be noted that for two other dossiers the substance was classified as CMR and information advising against consumer uses was given in the exposure assessment in the CSR.

Within Category I dossiers, two dossiers explicitly addressed nano-specific uses. In the first dossier both bulk and nanoform were covered and the use names were labeled with "bulk" and/or "nano". The identified uses by workers in industrial settings were common for bulk and nanoform (i.e. they had the same name). The reported use by professional workers and the consumer use were nano-specific. In the second dossier, the nanoform was the only substance covered by a JSO member and therefore all identified uses reported in that dossier must be considered as nano-specific. However, these uses were not different from the ones identified in the corresponding JSO lead dossier covering the bulk form. The remaining Category I dossiers did not explicitly report specific nanoform uses. Based on what was reported by the registrant under dedicated fields, such as "Identified use name", "Market sector by type of chemical product", and "Sector of use" (e.g. "SU9 Manufacturing of fine chemicals" as mentioned in Section 3.1.5.1), some uses could refer to the nanoform, but there were no explicit references to the nanoform.

As far as Category II dossiers were concerned, the identified uses have been considered by the assessors to address the nanoform following the assumption that the substance itself is a nanoform with no corresponding bulk form. The registrant did not explicitly mention 'nano' or 'nanomaterial' under the specified uses. In one case, it was stated in a "Remarks" box that the physico-chemical properties of the substance enable specific uses in a variety of applications, but there was not enough information to conclude whether those properties were considered nano properties. However, based on what was reported by the registrant under dedicated fields, such as "Identified use name", "Market sector by type of chemical product", and "Sector of use" (e.g. "SU9 Manufacturing of fine chemicals" as mentioned in Section 3.1.5.1), this could be the case for some uses.

The Category III dossiers did not refer to 'nano' or 'nanomaterials' in their uses. However, based on what was reported by the registrants under dedicated fields, such as "Identified use name", "Market sector by type of chemical product", and "Sector of use" (e.g. "SU9 Manufacturing of fine chemicals" as mentioned in Section 3.1.5.1), some uses could refer to the nanoform, but there were no explicit references to the nanoform.

In the IUCLID section 3.5, Identified uses, the registrant has the opportunity to include information on exposure routes, targets and patterns according to REACH Annex VI, item 6, which generally concerns 1-10 tons registrations. This section is occasionally reflected in the dossiers. It has not been considered relevant to further report the information provided, which by nature is very general as this IUCLID section consists of generic tick boxes.

*In conclusion, for Category I dossiers addressing different forms (including nanoforms), the use of a nanoform was explicitly addressed in two cases. For one of these cases there was no difference between the nano and bulk uses, whereas some nano-specific uses as well as uses which could relate to both nano and bulk were indicated in the other dossier. For the Category II and III dossiers, there were no explicit references to 'nano', although, at least for the Category II dossiers, the listed uses must logically be assumed to refer to the nanoform.*

#### **3.1.5.4 Form in the supply chain (Annex VI 3.4, IUCLID section 3.4)**

All dossiers specified the form in the supply chain in this IUCLID section. In eight dossiers, the substance was provided as such to the supply chain, in four cases in a mixture, in nine cases both as such and in a mixture. There was also one substance provided to the supply chain in an article, another one provided both in a mixture and in an article, and one substance provided to the supply chain in all forms, i.e. as such, in mixture and in article. Even if the substance was available as such, according to what was reported by the registrant in this IUCLID section, seven out of eight dossiers specified in the IUCLID section 3.5, Identified uses that the substance can also be provided in a mixture for some uses. As far as the form of a mixture is concerned, 7 substances were provided as dispersion, four as powders, one as liquid, one as slurry, one as coating, one as thick paste, one as granulate and one as castables. There was also a dossier specifying that the form was an inorganic mixture of the registered substance and other hydroxides, but there was no indication of the physical state.

Nano-specific information was reported in two Category I dossiers. The first one represented the JSO member dossier covering the nanoform only. In this case the nanoform was explicitly addressed and it was specified that the substance is supplied in

a mixture as “nano/powder”. The second dossier has both bulk and nanoform as registered compositions. Two forms in the supply chain were indicated: as a substance and in a mixture (more specifically, as powder or coating). For the mixture, a list of trade marks referring to the nanoform were reported in a “Remarks” box and therefore it must be assumed to refer to the nanoform. The remaining Category I dossiers reported how the substance was provided to the supply chain without distinguishing by form.

In all the Category II dossiers, the form in the supply chain reported in this IUCLID section has been considered by the assessors to address the nanoform following the assumption that the substance exists only in a nanoform with no corresponding bulk form. For these dossiers, the registrants did not explicitly mention ‘nano’ or ‘nanomaterial’ in this IUCLID section.

The Category III dossiers reported how the substance was provided to the supply chain but did not contain any specifications relating to ‘nano’ or ‘nanomaterial’.

*In conclusion, a varying level of information was found in this IUCLID section within and among categories. For the Category I dossiers, nanoforms were in two cases addressed explicitly, whereas for Category II and III substances no nano-specific information was provided, although at least for the Category II dossiers, the listed information must logically be assumed to refer to the nanoform.*

#### **3.1.5.5 Exposure estimates (IUCLID section 3.8)**

Firstly, it should be noted that according to REACH the exposure assessment is not required if the substance does not meet the criteria for classification as dangerous in accordance with Directive 67/548/EEC. Nevertheless, three out of five dossiers reporting information on exposure estimates were not obliged to include an exposure assessment.

Exposure estimates related to production and use were not reported in Category I and III dossiers.

Some information was reported in five Category II dossiers and has been considered by the assessors to address the related nanoform, following the assumption that the substance exists only in a nanoform with no corresponding bulk form. However, it should be noted that the information provided was not mentioning ‘nano’ or ‘nanomaterial’.

These dossiers reported exposure estimates found in literature. No clear link was made in this section in relation to whether the reported data were representative for the manufactures and uses covered by the dossiers.

For one substance, the JSO lead dossier reported a brief but comprehensive summary of what was described in the exposure assessment in the CSR. It has to be pointed out that the exposure in working places and the exposure to consumers were evaluated as “not expected to occur” and “negligible” respectively, since the substance was used in both cases as bound into a matrix. Moreover, the specification “even if fine powdered” was added. In addition to that, an individual registrant of the same substance briefly reported the CSR conclusion (i.e. “no need for additional testing and risk management measures”) in this IUCLID section and referred to the whole document for more details.

The remaining three Category II dossiers referred to similar substances and reported similar literature information. There was one explicit case of read-across based on the assumption that the substance is a structural analogue. As far as exposure related to production and use in working environments was concerned, total inhalable/respirable

dust concentrations (mg/m<sup>3</sup>) from monitoring campaigns were reported. In some cases, the measured concentrations were compared to thresholds in order to demonstrate their safety. Indirect exposure to humans was generally considered as “negligible” or “not significant”. Regarding the environmental exposure, the main emission sources were generally identified and shortly analyzed. In two cases low bioavailability in water was assumed.

*In conclusion, no exposure estimates were found in Category I and III dossiers, although several of these include an exposure assessment in the CSR. Information was provided in five Category II dossiers. In three cases, some quantitative and/or qualitative data were reported where no exposure assessment was included in the CSR.*

### **3.1.5.6 Waste from production and use (Annex VI 3.6, IUCLID section 3.7)**

16 out of 25 dossiers reported information on waste production distributed over the three categories of substances.

Six Category I dossiers reported information on waste production. For one substance, the lead dossier (registering both bulk and nano compositions), as well as the member dossier (specifically addressing the nanoform), referred to the exposure assessment described in the CSR. In the remaining dossiers, the registrant did not refer to waste production for the nanoforms. In one dossier, waste estimates were considered as “not quantifiable” and depending on the amount of substance which is sold. For one substance, the three related dossiers (lead and two members) specified that “minimum quantities” are produced, water purification plants are used, and waste is not disposed on agricultural soils.

Information on waste production was reported in 9 out of 12 Category II dossiers, which have been considered by the assessors to address the nanoform following the assumption that the substance exist only as a nanoform with no corresponding bulk form. The information provided did not explicitly refer to ‘nano’ or ‘nanomaterial’. Among these dossiers, one reported “no waste”, for two dossiers waste production was considered as “of limited concern”, two dossiers specified the exact quantities (t and t/a respectively), 1 dossier considered it as “not quantifiable” and depending on the amount of substance which is used. Moreover, water treatments, disposal via landfill and incineration were mentioned in two dossiers while recycling was mentioned in three dossiers.

As far as the Category III dossiers were concerned, information on waste production was found in two dossiers. No reference was made to ‘nano’ or ‘nanomaterial’. In one case, it was stated that the waste was sold as end products or re-introduced in the first step of the process, while in the second case, the percentage of waste produced and sent to external waste treatment was specified.

*In conclusion, more than half of dossiers reported information on waste production. For the Category I dossiers, the registrants did not refer to waste production for the nanoforms, exempt for a member dossier only registering the nanoform. The data reported in the Category II dossiers have been considered as nano-specific but the registrants did not mention ‘nano’ or ‘nanomaterial’. A few Category III dossiers reported waste information without any references to nano.*

### 3.1.5.7 Summary – main issues

For Category I dossiers addressing different forms (including nanoforms), the information reported in IUCLID section 3, Manufacture use and exposure was in 1-2 cases (depending on IUCLID entry field) addressed to some extent. In the remaining cases nanoforms were not explicitly addressed. In Category II dossiers, the information reported has been considered as nano-specific by the assessors following the assumption that the substance exists only as a nanoform with no corresponding bulk form. However, the information provided did generally not make explicit reference to 'nano' or 'nanomaterial'. Statements such as "fine powder", reference to "manufacture of fine chemicals", and uses that are known to be related to nanomaterials were found in several dossiers. The Category III dossiers did not explicitly refer to 'nano' or 'nanomaterial'. Some steps of the manufacturing process could be related to the intentional production of a nano-tail but there was not enough information to draw a conclusion on this issue.

With the exception of manufacturing process and estimated quantities, the IUCLID section 3, Manufacture use and exposure in general provided general/statistical information that is not designed for supporting detailed assessment purposes. It is worth noting however that the dossiers reported that the substances can be used in several different stages throughout their life cycle potentially enabling various types of exposures to consumers and workers, as well as releases to the environment. This would be important in relation to assessing the hazards, exposures and risks of the different states of a given nanoform, when exposure assessment and risk characterization is triggered. See also Chapter 5.

## 3.2 Options for adapting REACH

### 3.2.1 Substance Identification/Characterisation

As described in section 3.1.1, one of the key challenges associated with the assessment of the selected dossiers was the ambiguity of the scope of the registration dossier and the registrants' intentions regarding which nanomaterials/nanoforms fall under the scope of the registration. This ambiguity was not limited to the identity/characterisation of the registered substance, but extended to the information on the exact form/particle size of the tested material for many physico-chemical and hazard endpoints (the latter to be addressed in Chapter 4). The impact of this ambiguity on the assessment of dossiers containing nanomaterials/nanomaterials cannot be overstated. Without clear knowledge of the scope of the registration, it has been difficult for the assessors to determine if the presented data on inherent properties (and the associated test material descriptions) were sufficient for risk assessment of the (possibly) different (nano)forms of the substance covered by the registration dossiers. Therefore, any options for adapting REACH must begin with adaptations aiming at resolving such ambiguities. The following recommendations are made:

#### **Option 3.1. Explicitly require registrants to describe the scope of the registration dossier.**

The scope of the registered substance in terms of forms and compositions should be explicitly described in the lead registrant dossier. The scope should state whether and which nanoforms are included. It should be noted that IUCLID already has certain picklists that allow registrants to indicate whether nanoforms/nanomaterials are within

the scope of a dossier, although at this moment there is no requirement for the registrant to use these picklists. Further, the picklists in themselves do not allow for appropriate characterisation of the nanoforms/nanomaterials (see Option 3.2 below). When different forms are covered, the dossier should include a scientifically justified discussion as to when data can be shared and when not among different forms. The need for a sound scientific justification for data sharing among different forms is an important principle, and this motivates the need for clear information on the scope of the registration dossier. It is acknowledged that practical implementation of this should be balanced against the need to protect confidential business information between different registrants. Given the importance of this starting point, it is suggested to explicitly clarify this in the enacting terms of the REACH text, or alternatively in relevant annexes.

Further, the current requirements for SID are based on Annex VI(2) and include no provisions for nanomaterials beyond the general requirement that “the information provided shall be sufficient to enable the substance to be identified”. From the discussions in RIP-oN1, there was no consensus on whether parameters like size should be considered as identifiers or characterizers for nanomaterials. Based on the analysis and assessment of the dossiers included in this project, it seems less clear to registrants that where a registration covers different forms of a substance (e.g. bulk and nanoforms, different nanoforms) each form should be reported separately in the IUCLID composition Section 1.2 and that analytical data should be included in IUCLID Section 1.4 for each form reported in IUCLID Section 1.2. This information was not included in the dossiers assessed. Without information on the characterisation of the form, it is not possible to verify any form specific information that is included to fulfil other information requirements, such as risk management measures that are form-specific (e.g. specific risk management measures for powders). Therefore an option would be:

- To reword the requirement of Annex VI(2) to state that the information included shall be sufficient to enable the substance and its forms (if there are more than one) to be identified.

The description of nanoforms within the scope of a registration should at least address the characterisers listed under ‘Option 3.2. Characterisation’. As can be seen it is suggested that those characterisation data, which are largely manufacturing specific are provided by each registrant. In relation to describing nanoforms within the scope of a lead registration, it is acknowledged that this should be done in a way that confidential business information among registrants is not revealed. At the same time however, the description of different nanoforms within the scope should be sufficiently detailed to assure that the properties of different forms are explicitly addressed throughout the dossier.

In addition, as the scope of the registered substance also relates to substance sameness and in turn the obligation to make a joint registration, it is also recommended that guidance be developed to aid both registrants and regulators to define substance sameness criteria for nanomaterials. This will aid assessment of when it can be considered meaningful to share data between registrants of substances that exist in different forms.

**Option 3.2. Explicitly require registrants to provide more detailed characterisation of nanomaterials/nanofoms.**

During the preparation of the assessment criteria, a number of physico-chemical characterisers were identified as being of interest to nanomaterials (mainly based on the results of the RIP-oN projects). However, with few exceptions (see Section 3.1.3.2 and Section 3.1.4.13), registrants did not provide any information on these endpoints. Note there is no explicit legal requirement for registrants to provide information on these endpoints (i.e. they are not part of the standard information requirements). However, it illustrates that changes to the standard information requirements in Annexes VI-XI if information on these, or other characterisers, will facilitate achieving the objectives of REACH for nanomaterials.

Registrations with nanomaterials/nanofoms within the scope of the registration should include sufficient information to enable the characterisation of the nanoform(s). In order to clarify the REACH requirements for nanomaterials, it is suggested that the following information requirements are explicitly required as a minimum:

- 3.2.a. primary particle size distribution with indication of the number fraction of primary particles smaller than 100 nm (in line with the EC recommendation for definition of a nanomaterials and guidance to be developed in support of its implementation)
- 3.2.b. other particle size distributions representing possible agglomerated/aggregated forms during uses and following (environmental) release of the substances, or other information demonstrating the stability of aggregates/agglomerates
- 3.2c. description of surface functionalisation/treatment
- 3.2d. shape (based on the recommendations of the RIP-oN2 project)
- 3.2e. surface area (Volume specific surface area and/or mass specific surface area)
- 3.2f. possible other characterisers.

**Ad 3.2a and 3.2b: Information on particle size (whether required as part of substance identification in IULCID section 1 or in 4.5 Granulometry)**

For nanomaterials<sup>20</sup>, it is suggested to include as standard information requirements:

- primary particle size distribution with indication of the number fraction of primary particles smaller than 100 nm (in line with the EC recommendation for definition of a nanomaterials)
- other particle size distributions representing possible agglomerated/aggregated forms during uses and following (environmental) release of the substances, or other information demonstrating the stability of aggregates/agglomerates.

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<sup>20</sup> In relation to implementing this, it should be considered that a manufacturer/importer of a material may not always be able to determine upfront whether a certain material is a nanomaterial or not. Therefore, it might often be needed to generate information for 'small particle' materials to determine if they are nanomaterials or not.

Given the fact that particle size is a property dependent on the manufacturing process of a substance, and not strictly based on the substance's chemical identity, the current practice of requiring registrants to submit information on granulometry jointly does not generally seem justified. Instead of the current practice, it is recommended that registrants be required to submit data individually for this endpoint as it is the current practice for information on substance identity and manufacturing specific information. However, it needs to be noted that such an individual submission of an information requirement will lead to a higher registration fee as it is considered as "opting out" from a joint submission. Note any change to this would likely involve changes to the REACH enacting terms (including article 10, 11 and 74).

In addition to the above, it is necessary to develop a set of common methods for the measurement of particle size at the nanometer scale, as it is difficult to compare the results of studies conducted using different methods. In relation to the EC Recommendation on the definition of nanomaterial, it is expected that guidance, including specification of methods, will be developed to support its implementation.

For any methods applied, registrants should be required to provide detailed information on the sample preparation involved, and to indicate what type of particle the results refer to (primary particle, aggregate, agglomerate or a combination) and which metrics are used in reporting particle size distribution. It should also be clearly mentioned whether a mean value or a median is reported. In general, the methods used should be appropriate for the particle size to be measured and the limitations of the methods, e. g. cut-off for size range, should be clearly indicated. Finally, it is recommended that, as part of the information requirement on particle size distribution(s), registrants should provide (quantitative) information on the stability of any aggregates/agglomerates under reasonably expected use conditions. This could be done for example by subjecting the aggregated/agglomerated particles to pre-defined forces or sonication and observe the effects on particle size, though a standardised method would be required for comparability.

### **Ad 3.2c: Surface functionalisation/treatment**

Based on the analysis and assessment of the dossiers, surface chemistry/surface treatment of nanoforms/nanomaterials was not or scarcely reported in the substance identity sections of the registration dossiers nor was it addressed in detail in the other dossier sections. Based on the OECD-WPMN templates, developed for the dossier development plans (DDPs), surface treatment/coating/functionalisation is considered to be one of the key parameters relevant for risk assessment of nanomaterials. Surface treatment/surface chemistry was also discussed in detail in the RIP-oN1 project and although there was no consensus in terms of whether it should be an identifier or characteriser under REACH, there was agreement that it was relevant for nanomaterials when assessing their properties. It thus seems clear that the registrant needs to consider surface treatment/functionalisation of his nanomaterial/nanoforms and in particular assess what impact this may have on the inherent property data and on the hazard/risk assessment. Therefore, clarification on this issue is needed.

Clarification of the RIP-oN1 open questions on this issue is needed. In particular there is a need to consider whether surface functionalisation/treatment trigger the need for a separate registration. This would determine in which REACH annexes to include these information requirements (e.g. Annex VI(2) on substance identity, or Annexes VII-X).

Various possible sub-options are proposed:

- surface functionalisation/treatment is considered relevant to the substance identity (and included in the name for the substance) and thus require separate registrations for each surface treated form. In this case the information included based on Annex VI(2) would be required to be sufficient to identify the surface functionalised/treated form(s)
- surface functionalised/treated forms are considered as “forms” of the substance and are reported in the composition section (1.2) with the corresponding analytical information for each form included in section 1.4. In that case the information included in section 1.4 would be required to be sufficient to characterise each surface functionalised/treated form listed in the composition section 1.2

These options would require the rewording of the requirement of Annex VI(2) to state that the information included shall be sufficient to enable the substance and **its forms** to be identified. In any case, registrants should always consider the effect that surface functionalisation has on the performance and interpretation of various tests, and ensure that control of risks of all forms of the substance can be demonstrated.

There might be other ways to implement this requirement. In any case, the bottom-line is that each registrant clearly identify/characterise surface functionalisation/treatment as part of his registration and that the scope of a lead registration in terms of surface functionalisation/treatment is clearly outlined.

#### **Ad 3.2d and 3.2e: Shape and surface area**

As specified in RIP-oN2, information on shape and surface area would also be key characterisers. These are assumed to be manufacture specific and are thus suggested to be supplied by each registrant as part of the standard information requirements. How to address this in the REACH annexes would depend on whether the information requirement is covered by the information requirement on granulometry or as separate information requirements (see RIP-oN2 report) and/or as part of the substance identity information. The inclusion of these endpoints in the standard information requirements would require further development of both the test methods and the guidance in order to aid registrants in providing this information.

#### **Ad 3.2f: Other characterisers**

Given the rapidly developing nature of this field, registrants should be encouraged to include information on any characteriser (or other endpoint) which to the knowledge of the registrant might affect the properties of his material. For example, any information on the variability of the (size distribution) of the nanof orm during the production process could be helpful. This could e.g. be specified in REACH annex VI (Step 1 to 4).

Reporting of additional physico-chemical endpoints that are of potential interest to nanomaterials (and nano-particulate materials in general), such as surface charge/zeta potential, porosity, surface energy and acidity etc. could eventually become part of the standard information requirements for nanomaterials, whenever they are/become considered important for characterization and/or assessment of hazards/risks. The decision on whether to include (or not) information on such endpoints as part of the standard information requirements on physico-chemical properties should be motivated by the potential impact of these endpoints on the assessment of a substance's

hazard/risk, and therefore may require further research on the relevance of these parameters. In addition, the inclusion of these endpoints as part of the standard information requirements would require the development of standard guidelines/methods for the determination of these properties.

It should be noted that guidance on assessment of nanomaterials have been developed or are in development in support of other EU legislation (e.g. for food and cosmetics). It is acknowledged that e.g. the EFSA guidance document<sup>21</sup> on assessment of nanomaterials in food goes further in terms of characterisation as compared to what is suggested above (the above is based on the RIP-oN projects).

Realising that REACH is considered an ‘upstream regulation’, between others with the purpose of supporting downstream legislation, it could be considered by the responsible policy makers whether and how REACH should support those legislations. In this respect, it should also be considered that a good upfront characterisation of nanomaterials used in various testing/research would facilitate the much needed development of non-testing approaches such as e.g. QSAR models.

### **3.2.2 Other options related to Physico-chemical properties and general manufacture and use information (IUCLID section 3)**

For the vast majority of dossiers, the registrants did not provide (detailed) information on the particle size of the tested material in the various physico-chemical endpoints (see also Chapter 4 in relation to other endpoints). Moreover, where studies or waivers were provided, registrants did generally not specify whether the provided data or waiver applied to the nanoform/nanomaterial, even though it is possible to do this in a IUCLID dossier using different endpoint study records (e.g. using an endpoint study record labelled as “bulk” or “nano”). It should be noted that information on particle size and other characterisers has not always been reported for all tests in the past, and this may limit the availability of this information to registrants in particular when using historical literature/data. Knowledge of the particle size and other characterisers is not necessary for the assessment of the available information on some endpoints (e.g. bulk density, vapour pressure, flashpoint – but see discussion on relative density in Section 3.1.4.4) as the particle size and other characterisers are not expected to have a significant impact on the outcome of the test or the applicability of any waivers used. In general however, the absence of this information leads to reduced transparency in the dossier, and it is difficult to judge whether the registrant considers that the supplied physico-chemical (and hazard) information is applicable to one or all forms of the registered substance. For other endpoints, especially physico-chemical hazards such as flammability and water solubility, it is generally known that particle size can have a significant impact on the outcome of the test.

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<sup>21</sup> European Food Safety Authority (EFSA). Scientific opinion. Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain. EFSA Journal 2011; 9(5): 2140.

Based on the above, the following general recommendations are made:

**Option 3.3. Require that nanoforms are explicitly addressed in the endpoint sections.**

If a dossier contains multiple forms of a substance (e.g. nano and bulk forms, or multiple nanoforms), the registrants should clearly indicate whether the results of a test or a waiver are intended to refer to a particular form(s) and where necessary justify this. This could be specified in relevant REACH annexes. This can technically be achieved using multiple endpoint study records for each form, with a clear indication as to a specific form is addressed by each endpoint study record. The different forms should also be addressed explicitly in the associated endpoint study summaries. For several endpoints, it may be scientifically possible to omit testing under the existing column 2 adaptations, or using Annex XI, scientifically unjustified (e.g. boiling point, vapour pressure, surface tension, flashpoint, viscosity, and stability in organic solvents). However, as the registrants' intentions are often unclear, registrants are recommended, as suggested above, to use explicit waiving statements for each (nano)form within the scope of a registration to improve the transparency of the dossier.

For certain information requirements, registrants are recommended to provide more detailed information:

Information requirement on physico-chemical hazards (flammability, auto-ignition temperature, oxidising properties, and explosivity): Under some circumstances, the information on these endpoints can be adapted based only on knowledge of the chemical structure of the substance (e.g. for a metal in its highest oxidation state, no further reaction with oxygen, and therefore no flammability is expected). This applies to substances regardless of their size. In general however, the particle size of a substance can have a significant impact on its reactivity and hazard, with smaller particles being more reactive. Therefore, it is recommended that in cases where a dossier contains multiple forms within its scope, the most reactive form where technically feasible (for example using the smallest particle size, or the surface functionalised form expected to lead to the highest reactivity, etc.) should be tested as a minimum, though testing of other forms may also be necessary to provide adequate information on classification and labelling of the different forms.

Water solubility and ion leaching: It has been recognised that water solubility of and ion leaching from nanomaterials may be higher than for the corresponding bulk form. It is recommended that registrants take these factors into account, and one suggestion may be to perform tests on different forms (size distributions) of the substance. As also mentioned in the RIP-oN 2 Final Report (3.5.193) dissolution kinetics may be included as an information requirement under the 'Water solubility' endpoint. It should be underlined that dispersability is a different phenomenon. The issue of dispersion vs. solution may require further development of the existing guidelines on the testing of water solubility and/or the guidance on information requirements for this endpoint.

**Option 3.4. Require detailed description of the test material/sample and sample preparation.**

The results of several tests may depend on both specific particle characteristics (e.g. particle size, surface area) and the techniques used for sample preparation. Clear and detailed information on the test material/sample (particle size, surface area, etc) and sample preparation (e.g. sonication) is necessary in order to interpret the results of some

studies. Therefore, it should be included as part of the study summary in the IUCLID dossier.

In cases where this information is not available (e.g. for already existing tests/older data/literature references), the registrant should explicitly consider the uncertainties arising from the absence of this information as in this case, it is not known what exactly has been tested. In cases where the characterisation and/or sample preparation is limited or not known, the registrant should analyze whether the results could apply to the nanoform(s) addressed by the dossier, and for each form provide a scientific justification when this is considered to be the case. If the information is not available for certain endpoints, where the results are known to depend on the characterisation (e.g. certain physico-chemical hazard endpoints such as water solubility, flammability, explosiveness), the registrant should consider additional testing using the nanomaterial/nanoform.

Particle size (distribution) of the test material is a key parameter, especially for certain endpoints. It is acknowledged that nanoparticles may agglomerate/aggregate. As substance properties may be related to both primary particles and their agglomerates/aggregates, it is important to obtain information on both. Thus a MMAD is not considered sufficient alone, and it is important to report the size distribution of the materials as tested (indicating agglomeration/aggregation state and any remaining primary particles). The primary particle size distribution may not be necessary to generate during every testing if well-characterised nanomaterials are used of the testing. It should also be considered whether the data provided for each form (including dispersion state and (de-) aggregation/agglomeration) are relevant for the different expected uses of the different (nano)forms. The same is relevant in relation to any changes that may happen to the shape and surface of the nanomaterial.

It is suggested that this requirement is explicitly mentioned in the REACH annexes, including reference to the latest OECD guidelines/recommendations for sample preparation and dosimetry. Application of this recommendation will also require an update of the guidance documents (e.g. additional specific guidance may be needed on the information needed for a robust study summary). In addition, the Agency (ECHA) has the authority to request additional tests for the nanoform in a compliance check or during the testing proposal examination if this is deemed necessary.

**Option 3.5. Require scientific justifications for grouping/read-across/QSAR and other non-testing approaches for different forms.**

When data is read-across (from one substance to another), or when data from one form (e.g. bulk) is considered by the registrant to be applicable to other forms, the registrant should always provide an adequate scientific justification of the applied read-across. The requirement for sufficient justification is not unique to nanomaterials, and is equally applicable for all read-across cases. Annex XI (1.5) already states that in all cases, adequate and reliable documentation of the applied method shall be provided. Although the read-across approach is currently applied when using data from one substance to another, this was not explicitly done on a form to form basis in dossiers containing multiple forms of the substance. Therefore, this report recommends that a justification should be explicitly required when data from one form of a substance is used to cover the properties/hazards/risks associated with another form, especially for endpoints where particle size is known to have a significant impact on the outcome of the test. It is suggested to explicitly state in Annex XI that read-across from one form to another

should follow the same requirements as from one substance to another. The same is recommended for other non-testing approaches. In line with this, QSAR models should only be used for nanomaterials if the materials are within the applicability range. In general, discussion of the uncertainties related to possible use of QSAR, grouping and read-across should be explicitly required to be included/specified in the dossier. This is in line with the recommendations in the RIP-oN projects.

**Option 3.6. Include information on dustiness.**

The inhalation route is generally very important when assessing nanomaterials hazards, exposures and risks. As suggested by a registrant (see Section 5.1), dustiness heavily influences the ability of a (nano)material to become airborne. Also recent Japanese approaches<sup>22</sup> for assessing the exposure and risks of nanomaterials use this parameter in the exposure estimation. Consequently, dustiness is also expected to become an important parameter input for models for nanomaterials exposure estimation. For these reasons, it is suggested to include Dustiness as an option for a physico-chemical parameter in the REACH standard information requirements and assess the impact of this in the next phase of the project.

### **3.2.3 Manufacture and use**

The general recommendation suggesting to explicitly address different forms throughout a registration dossier (Option 3.3) also applies here. For example, as the particle size of a substance is strongly linked to the manufacturing process, it is recommended that registrants include information on the manufacturing where this has an impact on the particle size, as this can be of benefit (e.g. when comparing the similarity of two substances or two members of a joint submission). No further specific options are currently suggested for general information on 'Manufacture, use and exposure'. See Chapter 5 in relation to options for exposure assessment.

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<sup>22</sup> See e.g. Hanai et al. 2009. Risk assessment of manufactured nanomaterials – titanium dioxide (TiO<sub>2</sub>). Interim report. NEDO project "Research and Development of nanoparticle characterization methods (P06041).



## **4 HUMAN HEALTH AND ENVIRONMENT ENDPOINTS, INCLUDING PBT AND CLASSIFICATION AND LABELLING (C&L)**

### **4.1 Findings and conclusions**

#### **4.1.1 Inherent properties – Human Health**

4 of the 25 dossiers addressed in the detailed analysis and assessment were member dossiers without any data in the human health endpoint sections. Thus the discussions in the endpoint sections below relate to **21 dossiers**.

As description of test material size (distributions) and sample preparation were hardly described in the studies under the various endpoints, these issues have been summarised/addressed under main issues in section 4.1.6 under the heading “Sample preparation and dosimetry for (eco)toxicological testing”. If information was available to individual substances or tests, it will be highlighted in the below endpoint sections.

Based on the available information, the assessors have attempted to group the dossiers in relation to the presence (or absence) and kind of data provided under each endpoint. This was not always a clear-cut assessment as several dossiers provided data in combination with read-across and/or waiving and/or a test proposal. Therefore the presented ‘grouping’/‘statistics’ are based on the view of the assessors. There might thus be different views on these statistics, but that would not affect any of the overall conclusions of the report. In addition, as will be further discussed in Section 4.1.6, it often had to be inferred by the assessors whether and how nanoforms/nano properties were addressed in the endpoint sections. Anyway, the assessors have attempted to give a description of the available information.

##### **4.1.1.1 Toxicokinetics, metabolism and distribution (Annex VIII 8.8, IUCLID section 7.1)**

At present, an ADME study is not a standard information requirement under REACH. “Only” an assessment of the toxicokinetic behaviour of the substance to the extent that it can be derived from the relevant available information shall be provided by the registrant.

###### **4.1.1.1.1 Metabolism and distribution**

The toxicokinetic section of a registration where the behaviour of a substance in the body is investigated is of very high importance as it evaluates the possibility of systemic exposure and systemic toxicity. It thus provides part of the basis for e.g. a decision on the validity of extrapolating available data from the bulk material to nanoforms. It is also key in relation to justifying whether a read across to and grouping with other related substances, e.g. based on the soluble ion or same parent compound, is appropriate.

Three Category I dossiers presented toxicokinetic studies, of which two dossiers referred in one study to a nanoform. Another Category I dossier, using the nanomaterial pick-list in IUCLID section 2.1 extrapolated the conclusions for the nanoform from results with what they called a 'microform'. Yet another Category I dossier concluded on low bioavailability (not specific for nano) based on reviews with the bulkform.

Two Category II dossiers presented experimental data. Four Category II dossiers applied read across, two of them in combination with data from the registered substance<sup>23</sup>. Two Category II dossiers concluded based on a weight of evidence or reviews. One Category II dossier did not provide any data and two dossiers provided only solubility studies to demonstrate that the substance is not soluble and therefore no oral or dermal absorption would be expected. One of these dossiers also concluded that inhalation was unlikely to happen due to the big particle size (there was no indication whether this would be the primary particle size or the size of aggregates/agglomerates).

One Category III dossier presented experimental data for the registered substance. Three dossiers drew conclusions applying grouping based on the same (metal) ion. In yet another dossier testing was waived based on the insolubility of the ion.

NB: Weight of evidence conclusions and data waiving justified by the non-solubility of particles should be considered critically. First of all, this justification would not be valid for inhalation exposure as especially the insoluble particles would accumulate in the lungs and could induce particle specific effects. Second, for oral and dermal exposure it may be as well of relevance, as insoluble nanosized material could be absorbed and distributed to target organs, different from coarser particles. Therefore insolubility alone does not seem sufficient to justify not addressing toxicokinetics in more detail. Further, as discussed elsewhere in the reporting, solubility (of metals and metal oxides) can increase with lower particle size and justification for insolubility of the bulk material might not be applicable to the nanoform.

Comparative analysis of different particle sizes would be relevant when comparing and extrapolating results from different substances and/or forms with different particle sizes. Only one dossier of a Category I substance, using the IUCLID picklist 2.1 presented a comparative study of nano and microsized material. It concluded that the bioavailability of the nanosized materials was increased compared to the microsized material following oral exposure.

For read across to other related substances based on solubility various justifications for the suitability of read across was given. In the case of metal compounds it was assumed that it would be the metal ion mainly contributing to the toxicity. In other (Category II) dossiers, similar solubility was used as additional justification for read across. This was in one dossier supported by presenting an *in vitro* dissolution study in e.g. different biological solutions (phosphate buffer, PBS; Gamble's solution, GMB; artificial sweat, ASW; artificial lysosomal fluid, ALF; artificial gastric fluid, GST). It was also proposed in the same dossiers, that higher surface area (smaller) particles have a higher solubility

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<sup>23</sup> Nanomaterials are known to differ depending on the production method and the properties of the 'registered substance' for nanomaterials will therefore be process-specific. Although the registrant reports that the tested material is the same as the registered substance they may therefore differ in physico-chemical properties—even if the EC number is the same. In this context, however, what is meant is that testing data is provided for the chemical identity given in IUCLID section 1.

than particles with a lower surface area. The same studies were also used in the dossiers of related compounds (salts) of that substance.

One comparative study of high surface and low surface forms concluded that the low surface form was more efficiently cleared from the lungs than was the high-surface form. This useful information that shows that smaller particles can resist longer in the lung than their coarser counterparts was however not followed up in the assessment in other parts of that dossier.

For eight dossiers, human data were presented in addition to experimental animal data; however these studies did not provide any information on particle size. This makes it difficult to evaluate the relevance of such information/conclusions for the nanoform of specific substances.

Many of the substances analysed and assessed contain abundant atoms or ions, which are (in low doses) not toxic, or are essential elements for the human body. In several cases, the registrants concluded that a dissolution and absorption of particles containing these atoms or ions, is not expected to represent a risk to humans (in low doses). However, nanosized particles may behave differently from bulk particles as they might have different absorption and distribution patterns and could therefore reach different target organs/cells. This is especially relevant to be considered for insoluble particles that do not dissociate sufficiently to the ions, to which the body is expected to be used.

Translocation of nanosized particles in the body was described in one case where the registrant concluded that there was no evidence of a quantitatively important translocation of 100-nm particles to the systemic circulation from the lungs in humans. For all other substances no conclusions on the transport of different particle sizes were drawn.

#### 4.1.1.1.2 Dermal absorption

Two Category I dossiers presented data for dermal absorption, two dossiers (Category II and Category III) concluded based on read across and two dossiers (Category I and Category II) waived testing. Most dossiers did not (nine dossiers) or not directly (six dossiers) address dermal absorption in the IUCLID endpoint sections.

In the latter six dossiers there was either some information on dermal absorption presented in the description of other endpoints (toxicokinetics, dermal toxicity studies) or as a general statement (without further explanation) in the CSR or as justification used for waiving (e.g. for dermal toxicity tests).

The conclusion of no or very low dermal absorption was drawn for 12 substances as a consequence of physico-chemical properties, mostly referring to the insolubility of the substance in water or organic solvents. The absorption of the ion from a soluble salt was tested for three substances. Different solubility of nanoform as compared to the bulkforms and therefore having potentially different absorption properties were not discussed.

Dossiers for two Category I substances, that are extensively used for dermal applications also in the nanoform, included several studies of either different related compounds and/or particle sizes. One of these dossiers presented several studies with

different related compounds containing the same common ion which aimed at investigating the absorption of the ion. This was partly done radiochemically and radioautographically and the nanoform of that substance was addressed in at least one study. The penetration of nanosized material was investigated in UVB damaged porcine skin. It was shown that the nanoparticles remained on the surface and upper stratum corneum layers, while in UVB unexposed skin, it remained on the surface. The ion was found to penetrate into the stratum corneum by TEM and into the epidermis by TOF-SIMS. There was no definitive evidence by these optical methods that the nanoparticles penetrated into the perfusate. Summaries of human studies with the same nanomaterial concluded that no penetration of healthy skin is anticipated. In the other Category I dossier it was concluded that the substance did not penetrate but is retained in the outmost layer of the stratum corneum of pig and human skin. The key study tested the nanoform; from the supporting studies no information on the size is available.

The presented studies suggested that both the nano and the bulkform were not absorbed via the skin. These results indicate that the size did not influence the absorption rate for these two substances; however this should not be generalised.

*In conclusion, several studies investigated the adsorption, distribution, metabolism and excretion of the substances for different application routes. In several dossiers the conclusions made were based on data from toxicity studies, which provided some information on toxicokinetics, e.g. from acute or repeated dose toxicity studies or combined studies with sub-chronic exposure or human data.*

*Some dossiers applied read across to chemically related substances. The particle nature and/or different particle sizes were not given special attention. Insolubility was used as justification to conclude on the non-availability of the particles to the body; however this may not be appropriate for nanosized particles. Firstly the solubility may change with particle size and corresponding bigger surface area and secondly in particular insoluble nanoparticles may behave differently from their coarser counterparts in the body and may be absorbed because of their small size.*

*The registrants considered different exposure routes in relation to concluding on systemic exposure. Internal exposure was usually considered to be very low (but this conclusion was not referring explicitly to nanoforms) or it would be to ions or compounds which are abundant,(except the few toxic ones).*

*Dermal absorption was for most substances either not addressed or it was excluded based on physico-chemical properties. For two substances, where dermal exposure is significant, tests of the penetration of nanoforms were provided, which did not suggest that these nanoparticles were absorbed. Specifically addressing dermal absorption of the nanoform(s) as indicated in these two dossiers seems relevant when dermal exposure occurs. However the results with these two substances should not be used for a general conclusion on nanoparticles and might merit closer investigation.*

*Few dossiers indicated that smaller particles have a higher solubility in biological solutions and slower lung clearance in lungs. However in general the description of test material, sample preparation and dosimetry was very poor.*

*In conclusion, the fact that size and other characterisers may alter the toxicokinetics of nanomaterials/nanoforms was generally not addressed. Such information would be extremely important in relation to e.g. read-across between nanoforms and from bulk to nano.*

#### **4.1.1.2 Acute toxicity (Annex VII 8.5, Annex VIII 8.5, IUCLID section 7.2)**

Almost all dossiers presented experimental data for oral (20 dossiers) and inhalation (17 dossiers) exposure, either with the registered substance (12 and 11, respectively) or by read across (8 and 5, respectively) from related substances. For dermal exposure such data was presented in seven dossiers and read across in four dossiers.

All dossiers did not specifically address particle sizes and sample preparation was not described in detail. For two Category II substances and their related compounds mostly trade names were reported as test substance and limited test material description was available. Read-across was made for two substances to related particulate compounds, however based on the same leading ion and no discussion on the particle size was provided, which was sometimes indicated to be in the nanorange and sometimes in the micro-range.

Also in two Category III dossiers the hazard assessment was based on read across to substances containing the same ion. In one of these dossiers the other component than the leading ion of the substance (i.e. oxide) was considered of minor role for the toxic effect, while in the other case the toxicity of both ions were considered of comparable concern. The particle nature of the substance was in these two cases not taken into consideration.

##### **4.1.1.2.1 Acute toxicity: oral**

In 12 dossiers at least one oral toxicity study has been presented with the registered substance, while 8 dossiers were reading across. Application mode/media ranged from oral feed and gavage to drinking water. Only one dossier waived this endpoint as it was not regarded a relevant route of exposure. However, two other dossiers for the same substance presented several available data, including OECD reviews.

Four dossiers (three Category I, one Category III) presented oral studies with a nano and a non-nanoform. In all but one case both forms showed similarly low acute oral toxicity and an LD50 >> 2000 mg/kg bw/d or a NOAEL of > 1000 mg/kg bw/d. For one substance (in Category I) results suggested that the 120 nm form was more toxic than the 20 nm form, however the registrant concluded, "that the relevance of this study is unclear as it could not be explained how these effects could have been attributed to size differences". One Category III dossier presented a study testing two nanoforms of 30 and 40 nm together with what they called bulk form (50-200 nm, thus also containing particles in the nanorange). However the conclusions and LD50 were only reported for the bulk form. The results were thus not used to make a direct comparison between the effects of different particle sizes and from the presented data it could not be inferred, whether there were differences in the results.

For substances considered to exist only in the nanoform (Category II) several studies with different types of different particle sizes were tested, however the registrant did not make an attempt to draw size specific conclusions on the effects.

#### 4.1.1.2.2 Acute toxicity: Inhalation

The inhalation route was in terms of data provided the second important route of exposure. Studies were provided in 11 dossiers, read across was made in 5 cases and a weight of evidence approach applied in one case. In four cases this endpoint was waived. Justifications for waiving given were:

- existence of several sub-chronic and chronic studies (but not always according to test guidelines),
- the substance acutely behaves as an inert particle (a function of particle size and number), and is even used as a negative control in many acute inhalation studies.
- The substance is a genotoxic carcinogen. Appropriate measures should already be taken to prevent exposure.
- No justification for a dossier for a low tonnage substance

Animals were usually exposed in inhalation chambers either by whole body or head or nose only inhalation. Studies using other application routes (e.g. intratracheal instillation or insufflation) were also presented, but usually not considered as key studies and not used for drawing conclusions.

Information on the primary particle size was limited/not provided in the test material description (see also Section 4.1.6). One Category I dossier provided particle size and surface area for both the nanoform and the fine (bulk) form.

For most reported studies, especially the ones performed according to test guideline, it was reported that the concentration was controlled and, but not consistently how it was controlled.

For two Category I substances, the key study was performed with a 'nanoform'. However this study was given equal importance as studies of bulk forms and there was no comparison of the results to draw a conclusion on different effects due to different particle sizes or surface coating.

In a Category II dossier, tests on different particle sizes were reported within one study and different potencies were observed, e.g. results in a low reliability study suggested that ultrafine (20 nm) was more effective than 200 nm in inducing oxidative stress and inflammation in the lung. These differences in effects were noted without any further discussion/conclusion of the consequences of these findings in the dossier. Possible agglomeration/aggregation of the test material was not discussed.

For one Category I dossier, 'nano' and 'fine material' were tested in one study applying intratracheal instillation. Both forms produced substantial lung inflammatory responses which were reversible after 1-3 months. Different potencies of the different forms were not reported.

#### 4.1.1.2.3 Acute toxicity: Dermal

Compared to the other routes of exposure, the dermal route was generally the one for which least information was provided in the dossiers analysed and assessed. Nine waived the endpoint, three dossiers presented experimental data for the registered substances, eight were reading across and one dossier addressed the endpoint with weight of evidence.

In one Category I dossier, the registrant reported a peer reviewed study with a nanoform, which was not further characterised. "The nano form was tested because the form was anticipated to represent the worst case as the smaller size particles are more likely to be transported through the skin". The other dossiers, that provided data for this endpoint, did not address the particle size and possible different behavior due to smaller particle size.

In nine dossiers the acute dermal toxicity study was waived for the following reasons:

- the available physico-chemical properties (limited water solubility or insolubility) and/or toxicological properties (low dermal toxicity) suggest no potential for a significant rate of absorption through the skin
- lack of acute oral toxicity (in that case in combination with read across).
- an acute inhalation toxicity study is available, which is considered as the major route of exposure.
- data from a comparable substance (however with a larger particle size) are available for a weight of evidence approach
- the substance is a genotoxic carcinogen. Appropriate measures are already taken to prevent exposure.

The justifications provided were not specific for the nanosize and did not take into consideration the particle nature. As size can impact physico-chemical properties and dissolution, some of the justification might not be applicable for the nanoform of the same substance.

#### 4.1.1.2.4 Acute toxicity: Other routes

In this IUCLID section any kind of data can be inserted, which would not be covered by the exposure routes above. It is not a requirement, but gives the possibility to provide additional data, which could be used for a weight of evidence approach or to support results from key studies for relevant exposure routes (e.g. comparable studies with different forms of the substance).

17 dossiers did not report any information under this endpoint. Experimental data for the registered substances was provided in two dossiers and two other dossiers addressed this endpoint indirectly in other parts of the dossier. For the two dossiers providing data, studies using administration via intravenous or intraperitoneal injection or intratracheal instillation were reported. In one Category II dossier, particle sizes of the test materials were reported. In that case it was shown that smaller sizes were more potent in inducing pre-inflammatory effects in the lungs following intratracheal instillation. This result could be relevant for deriving conclusions and evaluating other tests, but this was not done in the dossier.

*Most acute toxicity studies were performed via the oral or inhalation route. For these exposure routes, mainly bulk forms were tested or no specific test material information was available. In several cases only the trade name was reported and read across was mostly based on the leading ion, not taking into consideration the particle nature of the material. Only few dossiers presented data with nanosized materials, however in these cases the description of the test material was limited, in relation to e.g. primary particle vs. agglomerate/aggregate size, and sample preparation was not described in detail.*

*Acute dermal toxicity testing was in many cases waived, mostly referring to the insolubility of the substance (an argument which may be challenged for nanosized materials) and/or lack of oral toxicity or dermal toxicity or absorption. Few dossiers presented studies with different particle sizes and only one study tested them within one test. In general from the available data it is difficult, if not impossible to draw any conclusion on equal or different toxicity between forms. Also the registrants did not make an attempt to draw any conclusion in relation to the influence of size.*

*All data provided for substances in all categories showed a low acute toxicity via all exposure routes and no classification for acute toxicity was suggested.*

#### **4.1.1.3 Irritation/corrosion (Annex VII, 8.1/8.2, Annex VIII 8.1/8.2, IUCLID section 7.3)**

##### 4.1.1.3.1 Skin irritation

Experimental data (13) or read across (6) were presented in all dossiers, except two. One of the latter registrants justified waiving of testing based on a weight of evidence (review data available), and the other with the justification that an acute study by the dermal route did not indicate skin irritation up to the limit dose level of 2,000 mg/kg body weight.

Of the two Category I dossiers using the IUCLID picklist (nano) in section 2.1, one dossier provided data only with the bulk form. In the other dossier data on the nanomaterial was provided from an *in vitro* EpiDerm test.

Different particle sizes were tested for two substances (one in Category I and one in Category II) in different studies and showed no obvious difference (all not irritating), however no direct comparisons within one test were presented.

An *in vitro* skin irritation test with a Human skin model test was presented for one Category I substance, however with no indication of particle size.

For six substances (Category II and III) the conclusion was drawn, based on studies with the registered substance in combination with read across.

'Human experience' was also used to draw conclusions. One Category II dossier reported signs of irritation to the unprotected skin of workers. Case reports for the working environment were available, describing dryness or eczema of the skin in workers following prolonged and repeated exposure due to the desiccative and defatting property of the substance. The registrant concluded that these reactions do not warrant

classification and can be avoided by skin care. In another Category II dossier it was stated that direct contact may cause mild irritation as with any particulate matter.

#### 4.1.1.3.2 Eye irritation

Experimental data (13 dossiers) and/or read across (7 dossiers) were presented in all dossiers, except one that waived testing with the same justification as for skin irritation (no dermal toxicity).

Two Category I dossiers using the IUCLID 2.1 picklist (nano) provided *in vivo* data with the bulk form without justification in relation to the nanoform. One Category II dossier provided *in vitro* data for eye irritation without information on the particle size.

Different particle sizes were tested for two substances (one Category I and one Category II) in different studies and showed no obvious difference – they were all non-irritating; however no direct comparison within one test was presented.

Unspecific effects due to particles were described in some cases for substances in Category II, which were however reversible and did not warrant classification. The observed reactions were described as slightly irritating mechanically produced due to superficial foreign bodies.

#### 4.1.1.3.3 Respiratory irritation

There is no dedicated test guideline available for testing this endpoint and information can only be retrieved from other studies like e.g. inhalation studies or from human experience. Ten dossiers did not present any data, while other ten dossiers concluded on this endpoint in the CSR, mainly based on results from repeated inhalation exposure studies, human experience or without any reference.

Only one Category II dossier provided a non-validated study and described in a direct comparison that only smaller particles (<30nm) and not larger particles (> 200 nm) induced airway inflammation. This information was however not taken into consideration in the overall conclusions in that dossier. Four Category II dossiers described in the CSR (one substance and its related salts) dryness of mucous membranes that may result in mechanical irritation of the respiratory tract following dust inhalation. The registrant concluded that irritation to the respiratory system is related to deposition of particles, but is no particular inherent property of the substance.

*All substances were concluded to be non-irritating, to skin, eye and respiratory tract. Particle sizes were however generally not reported and a possible influence of different particle sizes was not discussed in relation to drawing conclusions. As for other toxicity studies, the description of the test materials and of sample preparation was limited. Results from specific nanomaterials (other than Category II, implicitly covering nanomaterials) was reported only from one in vitro skin and eye irritation test. Some dossiers reported unspecific effects, either to the eye or the respiratory tract, which could be the consequence of exposure to and deposition of any particulate matter, but concluded that these would not be a particular inherent property of the substance and could not be regarded as irritation requiring classification.*

#### 4.1.1.4 Sensitisation (Annex VII 8.3, IUCLID section 7.4)

##### 4.1.1.4.1 Skin sensitisation

Nine dossiers provided experimental data and four dossiers applied read across for skin sensitisation, all with limited information on particle sizes. Two dossiers concluded based on a weight of evidence and six dossiers waived testing.

For two Category I dossiers (one of them using the IUCLID 2.1 picklist (nano)) a nanoform of a substance was tested in reliable studies, showing no sensitising effect. The same substance was also tested in the bulkform in other studies and showed the same lack of effects, suggesting that at least for this substance there were no differences related to the particle size.

Waiving of testing was justified based on the structure, physico-chemical properties and abundance of the substance or components (ions) of it in the environment, which suggested that the substance would not cause skin sensitisation. Also the experience of several decades from exposed workers or use in care products, which gave no evidence of skin sensitisation/allergic contact dermatitis, was used as justification to further confirm the conclusion. One Category II dossier also made reference to the absence of skin-sensitising properties of its main impurities, which have been shown to be dissolved in artificial biological fluids, (iron, magnesium, lead, aluminium and zinc). One substance is a genotoxic carcinogen and therefore waiving was based on the argument that appropriate measures are already taken to prevent exposure.

Only one Category II substance was sensitising in a Local Lymph Node Assay (OECD 429), but the test material was not well-defined and seemed to be a mixture of substances.

##### 4.1.1.4.2 Respiratory sensitisation

This endpoint is not a standard information requirement under REACH and there is no dedicated test guideline available. Nine dossiers did not address this endpoint and nine dossiers concluded on that endpoint in the CSR and/or waived testing without presenting specific data. Two dossiers (one Category II and one III) concluded based on a weight of evidence, either based on human data or by presenting a study testing allergic inflammation after intratracheal instillation of 1- 5 µm particles.

Only one Category II dossier presented a key study which was however not a guideline study, investigating one relevant endpoint: allergic airway inflammation of ultrafine (< 30 nm) and fine (> 200 nm) particles. From this study it was concluded that only the smaller sized particles could induce this effect. This conclusion was however not followed throughout the dossier.

The nine dossiers that addressed the endpoint in the CSR and concluded on the endpoint without testing, justified that the substance structure, physico-chemical properties and/or abundance the substance is not expected to cause respiratory sensitisation. Also the absence of evidence of respiratory sensitisation after decades of experience from exposed workers was used as justification for waiving, however no information on the 'test material' was given in relation to the referred studies.

*Many dossiers did not provide data for sensitisation, especially respiratory sensitisation was hardly addressed. Several dossiers concluded on non-sensitising effects based on physical-chemical properties or on the absence of effects in humans over long exposure periods. Only for one substance classification for skin sensitisation was suggested. Particle sizes were not reported for the tested materials and the possible influence of different particle sizes on these conclusions or justifications for waiving were not discussed.*

#### **4.1.1.5 Repeated dose toxicity (Annex VIII 8.6, Annex IX 8.6, IUCLID section 7.5)**

##### 4.1.1.5.1 Repeated dose toxicity: oral

For nine dossiers at least one oral toxicity study was presented. 11 dossiers addressed this endpoint by read across (8) and/or waiving (3) and for one substance a test proposal was provided.

In the toxicity tests, the substance was applied either by oral feed, gavage or drinking water. For one Category I dossier using the IUCLID 2.1 picklist (nano), 3 studies were presented, of which 2 were supporting studies where a nanoform had been tested. In the third study the bulk form was tested and there were no obvious differences in the results of these studies observed. Another Category I dossier using the 2.1 picklist (nano) provided only a study with the bulk form.

The three dossiers that waived testing provided the justifications that the oral route is not expected to be a significant exposure route or that, based on physico-chemical properties, no absorption is expected and/or the substance has shown low toxicity in acute studies.

For one Category I substance a test proposal for an OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents) was provided, however not for the substance registered, but for a related substance. No justification for the selection of the test material was provided, but as studies for other endpoints were presented with the same substance, it can be assumed that the evaluation should be based on the common soluble ion. This also means that particle nature of the substance was not taken into account in this test proposal.

##### 4.1.1.5.2 Repeated dose toxicity: Inhalation

The inhalation route was the second important route of exposure in relation to test data provided. Eight dossiers provided repeated dose inhalation studies and four dossiers addressed this endpoint by read across from related substances. Two dossiers concluded based on a weight of evidence, with one of them using also data and read across. Five dossiers, waived the repeated dose inhalation study (for justifications see below). One Category I dossier included a test proposal for an OECD Guideline 413 (Subchronic inhalation Toxicity: 90-Day) and one low tonnage Category II dossier did not address this endpoint.

The two Category I dossiers using the IUCLID 2.1 picklist (nano) provided a study with the bulk form and a test proposal for a 90 day inhalation study, respectively. The test

proposal specified the test material as the registered substance, but it was not indicated which of the compositions (bulk or nano) listed in 'IUCLID Section 1.2, Composition' would be tested. Species to be tested was not indicated. In five dossiers (two Category I, two Category II and one Category III), tests with substances of different sizes were performed.

For one Category I substance, a nanoform was specifically addressed in one study, but differences in adverse effects due to different forms applied were not discussed. One study of a Category II substance showed more potent effects of smaller particles. However, the available information was not taken into consideration to draw any conclusion on the effect/influence of different particle sizes.

The other Category I dossier was tested in a nanoform and a non-nanoform in a subchronic inhalation study in three different species. The results indicated that the nanoform was more potent in inducing adverse pulmonary effects. These studies with reliability score 2 were indicated as supporting studies. There was no discussion of the effects of different particle sizes in the endpoint study summary and the "No effect level" from the nano study was not used for deriving the DNEL. The DNEL was instead derived from a key study in which no nanoform was indicated to have been tested. This is interesting, as the DNEL for this substance was stated to be valid also for nanoform without clear justification about why the results from the nanostudy were not taken into account. For the same dossier, a chronic inhalation study in two species and for different forms indicated induction of cancer in rats (but not in mice) for the nanoform. This was also not addressed in the endpoint study summary, but from text in the CSR, it could be implicitly inferred that the registrant did not consider these studies relevant.

This Category I dossier and a Category II dossier (+1 additional partly) discussed that the effects seen in rats following inhalation exposure to insoluble particles were not considered relevant for human exposure arguing that the rat would be specifically sensitive to particle lung overload. Based on this, the Category II dossiers registrants concluded that the results of such studies could not be taken into account for deriving a DNEL. They found their arguments further supported by epidemiological studies of exposed workers which showed no causal link between exposure and the risk of non-malignant respiratory disease in humans. Further discussion on this can be found below and in Section 4.1.6.

Reporting of the histology of the respiratory tract and BAL analysis were usually included in the studies reported, especially for the guideline studies. If this information was not included in IUCLID, it has been difficult to find out whether it has not been investigated, or whether the information is in principle available, but has not been considered relevant enough to be reported in the registration dossier.

Three dossiers (two in Category II and one in Category III) waived the repeated dose inhalation study with the justification that inhalation is not a relevant route of exposure and/or that acute toxicity studies, intratracheal instillation studies or studies with related compounds are available that showed low toxicity via inhalation. One of these dossiers (Category III) also waived the oral and dermal route for repeated dose toxicity.

One of the Category II dossiers argued that the material was not soluble and did not hydrolyse at pH's consistent with lung alveolar fluids; thus any material entering the lung would not be absorbed into the blood stream. It was also stated that as the residual

matter in the lung was not of fibrous nature or similar to crystalline substances it would not produce conditions that might result in lung disorders. Another Category II dossier described the substance as insoluble and to contain big particles (probably aggregates), which were not inhalable. One Category I dossier included results from an intracheal instillation study (13 weeks) and applied route to route extrapolation from results of a subchronic oral study. One Category III dossier provided no justification, but the justification for not performing an acute study (it is a genotoxic carcinogen) would be valid also for repeated exposure.

#### 4.1.1.5.3 Repeated dose toxicity: Dermal

The dermal route was usually the route with least information in the dossiers analysed and assessed, with only three dossiers providing data and one dossier addressing the endpoint by a weight of evidence approach, based on a study of low reliability and reading across from a related substance. In 15 dossiers the endpoint was waived and two dossiers did not address this endpoint.

The three Category II dossiers that provided data, did not report the particle size of the test material applied. One of these dossiers was reading across to a substance which is not in particulate form.

The 15 dossiers, that waived the information requirement for this endpoint, included also two substances that are used in their nanoform for dermal applications. The following justifications either alone or in combination were used for waiving of the study:

- minor/no relevance of this exposure route,
- lack of effect seen in acute dermal or oral toxicity testing
- physico-chemical properties (e.g. inert inorganic nature, anticipated lack of dissolution) of the substance do not suggest a significant rate of absorption through the skin
- lack of percutaneous absorption in dermal absorption studies
- no systemic effects or other evidence of absorption were seen in the skin or eye irritation studies
- one substance is classified as genotoxic carcinogen -> efficient protection already in place
- no specific justification provided in 1 dossier

These waiving justifications are not specific for nanomaterials and some of them do not take into consideration the particle nature.

#### 4.1.1.5.4 Repeated dose toxicity: Other routes

14 dossiers did not report data in this IUCLID section. One Category II dossier reported data which were of reliability 4. Four dossiers addressed this endpoint indirectly by either presenting studies with applications via intravenous or intraperitoneal injection or intratracheal instillations in another section (e.g. 7.9.3 Specific investigations other studies) or by reading across. In two dossiers conclusions were based on human data that were presented in the CSR, but not in IUCLID. The results of the studies relevant for this section were, if at all, taken into consideration in a weight of evidence approach, but not for deriving endpoint specific conclusions.

*Most repeated dose toxicity data were presented for the oral or the inhalation route. In the presented inhalation studies some effects of different particle sizes have been observed, with the smaller particle size being more potent in inducing toxic effects. However these differences were not considered to derive size specific conclusions for different forms addressed by the registrant, sometimes with the accompanying argument that observed adverse effects of small particles were triggered by rat lung overload not relevant for humans. In general however, the information on particle sizes was not elaborated and used for deriving nano- or size specific conclusion.*

*Most dossiers have waived the repeated dose dermal toxicity study and the provided justifications are not specific for nanomaterials and particle size was not addressed in the waiving justification. It can be assumed that several of the justifications could also be considered to be equally relevant for the nanoforms, if the nanoforms would be well characterised and the lack of dermal absorption proven also for those nanoform.*

*Several conclusions were based on a weight of evidence, using substance specific data in combination with read across or with human experience. All but 2 substances were not classified for repeated exposure.*

#### **4.1.1.6 Genetic toxicity (Annex VII 8.4, Annex VIII 8.4, IUCLID section 7.6)**

##### 4.1.1.6.1 In vitro genotoxicity

All dossiers addressed this endpoint, either by presenting data (11 dossiers) with the registered substance, or reading across (7 dossiers) from related substances, or concluding based a weight of evidence approach (3 dossiers).

Usually at least 2 different in vitro genotoxicity tests were provided, where bacterial mutagenicity tests were provided in all but one dossier. That dossier provided only non bacterial test results. Except for one study in one dossier, all test results were negative. Only one Category III dossier discussed the possible limited relevance or significance of these negative results due to the absence of confirmation that the substance was in fact taken up by bacteria.

The only positive result in an Ames test (a guideline study) for a Category II substance was found among the non-key studies of one registrant. The applicant argued that genotoxic impurities of the substance might have influenced the results and thus did not give weight to this study when concluding 'non-genotoxic'.

In addition to the Ames test there were also other *in vitro* tests in mammalian cells presented. For example: comet assay, *in vitro* mammalian gene mutation, chromosome aberration test, micronucleus test, mammalian cell transformation assay, DNA Damage and Repair. The dossiers did not contain a discussion on the appropriateness of these tests for particles or for an overall conclusion.

Two Category I dossiers presented studies (mostly Ames tests, but also one comet assay in mammalian cells) for both a nano and non-nano form (one with a surface coated nanoform), but no difference in the results/conclusions were seen. The possible influence of different particle sizes was not discussed.

One Category I dossier using the IUCLID 2.1 picklist (nano) presented only studies performed with nanoforms (OECD 471 (Bacterial reverse mutation test), 473 (In vitro mammalian chromosome aberration test) and 476 (In vitro mammalian cell gene mutation test)). All test results were negative. The registrant justified that the nanoform was tested because this form was anticipated to represent the worst case as it is likely to be more soluble than the bulk form due to the smaller particle size and hence greater surface area. Furthermore, the registrant concluded that the smaller particle size would be more likely to penetrate the cells and thus the results would be valid and worst case for both the bulk and the nanoform.

The other Category I dossier using the IUCLID 2.1 picklist (nano) provided only an Ames for the nanoform and all other tests were performed with the bulk form. The Category II dossier using the IUCLID 4.1 picklist (nano) did not report primary particle size of the testmaterial.

Secondary/indirect genotoxic effects were discussed for two substances (Category II and III), however for one of them this was not done in relation to nanoparticles but to the leading ion.

#### 4.1.1.6.2 In vivo genotoxicity

Data was provided in six dossiers, four addressed the endpoint by read across and two concluded based on a weight of evidence (one by presenting IARC reviews). For the remaining nine dossiers, the results of the *in vitro* tests were considered sufficient to prove the absence of genotoxic effects and the *in vivo* tests were waived.

The data provided for *in vivo* genotoxicity included *ex-vivo/in-vitro* HPRT assays, dominant lethal assay, chromosome aberration assay and micronucleus test, transgenic animal mutagenicity assay and host mediated assays (Salmonella typhimurium reverse mutation assay (target organism) or Saccharomyces cerevisiae D-3). Particle sizes of the test material were reported only for few studies (see below).

For two dossiers (Category I and III), *in vivo* OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test), and OECD Guideline 475 (Mammalian Bone Marrow Chromosome Aberration Test) tests with a nanoform were provided. TEM images of the test material were attached to the Category I dossier. While this substance was negative for chromosomal aberration, the other substance (Category III) seemed to have particle size dependent effects. Nanoparticles (30, 40 nm) induced chromosome aberration and micronuclei in rats following oral gavage, while the results for coarser particles of the same substance (50-200 nm) were not significantly different from those of the vehicle control. In that test particle size of 100 particles was measured and the shape reported (roughly spherical). It was also reported how the particles were dispersed (in 1% Tween80) and subjected to ultrasonication. For the first dossier it was not reported whether the systemic availability was checked in that test, while for the second dossier it was reported that tissue levels of the ion were controlled, however the bone marrow was not mentioned.

One category II dossier showed ambiguous results in several different *in vivo* tests (e.g HPRT mutations in alveolar epithelial cells; formation of 8-oxo-dG in lung). The registrant concluded that the mutagenicity was secondary to inflammation due to lung overload

and would only be elicited at high doses (threshold for lung inflammation 1 mg /m<sup>3</sup> respirable particles). The influence of particle size was not discussed.

For one Category III substance, the conclusion on mutagenicity was mainly based on the toxic ion and the particle nature of the substance was therefore not addressed.

*A few tests have tested explicitly the nanoform in vitro for mutagenicity – one considering it as worst case. Most dossiers have however presented data with the bulk-form. The dossiers did not discuss the suitability of bacterial mutagenicity tests, for which data were presented in all but one dossier. There was in general limited justification, why certain tests would be appropriate to test genotoxicity of nanomaterials.*

*Only few substances have been tested for their genotoxic potential in the nanoform in vivo and results with the bulkform are generally used to draw conclusions. Some of the effects were discussed to be secondary to lung inflammation. Effects of particle sizes were (generally) not discussed.*

*For the tests described there was limited description of the test material (e.g. particle sizes and agglomeration/aggregation state, known to affect the likely uptake in cells) and except one case no information on how the material was dispersed. Only for one (Category I) substance in one test, the material was well described and TEM pictures were provided.*

#### **4.1.1.7 Carcinogenicity (Annex X 8.9.1, IUCLID section 7.7)**

Three dossiers provided data for carcinogenicity, all of them included inhalation studies as well as oral feeding studies. No dermal studies were found in the dossiers analysed and assessed. Five dossiers addressed this endpoint by read-across and four dossiers concluded based on weight of evidence. Six dossiers waived the endpoint and three did not address the endpoint.

In three dossiers (one Category I and two Category II), guideline carcinogenicity studies were provided. For one Category I substance there was a carcinogenicity study listed under Repeated Dose Toxicity (RDT) but not under carcinogenicity. This study tested the nanoform and showed tumours in rats. The dossier referred to the conclusion of IARC for “inadequate evidence in humans” based on epidemiological data in the CSR, however did not mention that IARC has concluded that the nanoform of the substance is possibly carcinogenic to humans (Group 2B) based on “sufficient evidence in experimental animals”.

The weight of evidence approaches were either based on test data and read across (five dossiers), or by using animal and human data (two dossiers) to draw a conclusion. One Category III substance was classified for carcinogenicity. A Category II substance was classified for carcinogenicity in one dossier, but not in two dossiers addressing the same substance. These dossiers were not specifically referring to nanosized particles.

Eight dossiers waived the endpoint. The justifications for waiving of a carcinogenicity, or a chronic inhalation study were, alone or in combination:

- due to inherent physical-chemical properties the substance would not be able to induce hyperplasia and/or preneoplastic lesions.

- chronic oral or subchronic inhalation studies toxicity study available and there is no evidence that the substance is able to induce hyperplasia or pre-neoplastic lesions.
- the substance is not classified as a mutagen/has not shown mutagenic effects
- The substance is already classified as genotoxic carcinogen (e.g. based on ions)
- weight of evidence of available data does not support an association between inhalation exposure and cancer
- other effects (neurotoxic) at low dose levels, risk management would sufficiently protect from carcinogenicity
- no widespread dispersive use and there is no evidence of frequent or long-term exposure of the general population or workers (epidemiological data – for discussion see also below)

The relevance of observed lung tumours in rats were for two dossiers (Category I and Category II) considered to result from impaired lung clearance (“overload” in rats). Also there is evidence that inflammation and cell proliferation may have contributed to them and that they would therefore not be relevant for humans (see also discussion in sections 4.1.6 and 4.1.1.5.2).

*In conclusion there is limited carcinogenicity data available and only few dossiers reported particle sizes. Carcinogenicity of particles observed in rats for some nanomaterials was considered to result from rat lung overload which was considered not relevant for humans. Several dossiers waived testing and concluded applying a weight of evidence approach based on data from repeated dose toxicity studies, with related compounds, mutagenicity studies and/or epidemiological data.*

#### **4.1.1.8 Toxicity to reproduction (Annex VIII, IX, X 8.7, IUCLID section 7.8)**

For toxicity to reproduction, data was provided in the three dossiers using the IUCLID picklist (nano). Two dossiers addressed the endpoint by read across and two by a weight of evidence approach. 13 dossiers waived the two-generation reproductive toxicity test (for justification see below) and one dossier (low tonnage) did not address the endpoint.

For developmental toxicity six dossiers provided experimental data, while five were waiving the testing. Five dossiers addressed the endpoint by read across, two applied a weight of evidence approach and two dossiers (Category I and III) provided a test proposal for an OECD 414 test which however were not specific for the nanoform. One dossier (low tonnage) did not address the endpoint.

For one Category I dossier using the IUCLID 2.1 picklist (nano) an OECD 422 (Combined repeated dose toxicity study with the reproduction / developmental toxicity screening test) was performed with the nanoform in rat via oral gavage. The registrant described that the nanoform was tested because this form was anticipated to represent the worst case as it is likely to be more soluble than the bulk form due to the smaller particle size and hence greater surface area for systemic absorption. The test materials was not further described or characterised. For the same dossier a developmental toxicity/teratogenicity study with the bulk material from 1993 was provided, but it did not include any information on the size of test material. A two-generation reproductive toxicity study was waived for that substance.

The other Category I dossier using the IUCLID 2.1 picklist (nano) an OECD 422 study was performed with the microform and the two generation study was waived.

The category II dossier using the IUCLID 4.1 picklist (nano) provided a study with a precursor substance which is not in particulate form. For the other supportive study no information on the test material was provided.

In one Category I dossier toxicity to reproduction and developmental toxicity/teratogenicity was assessed with several read-across studies and the two studies for developmental toxicity/teratogenicity with the registered substance were from the 1960's with no information on particle size. In the read across studies, which based the conclusions on the soluble ion, the particle nature was not taken into consideration.

The justifications for waiving the pre-natal developmental toxicity and/or the two-generation reproductive toxicity were (alone or in combination):

- no systemic toxicity anticipated due to low solubility (essentially insoluble in water, low solubility in artificial gastric fluid and/or simulated alveolar fluid).
- no evidence that the substance is absorbed through ingestion, dermal contact or inhalation.
- absence of impairment of fertility/reproductive performance for the ions or structural analogs
- long-term toxicity/carcinogenicity studies, reproduction/ developmental screening test or other studies in animals and the relevant information on the toxicokinetic behaviour (low systemic availability/toxicity) do not indicate a reproductive toxicity hazard.
- natural abundance and high tolerance of the elements of the substance in the human body.
- availability of human data with the leading ion (in that case the presence of effects, and classification)
- a precursor material containing a much higher concentration of toxic impurities was negative at producing reproductive effects in males or females (however this precursor is not of particle nature)

*Little information is available on reproductive and developmental effects. Only one Category I dossier provided a study with a 'nanoform'. Several of the waiving arguments could be applicable to the nanoform, provided there would be sufficient characterisation of the nanoforms tested and sufficient knowledge on the toxicokinetics to decide on the systemic availability of those nanoforms.*

#### **4.1.1.9 Specific Investigations (IUCLID section 7.9.1)**

Six dossiers provided data or were reading across (one), whereas 14 dossiers did not address this endpoint.

For dossiers providing data, this section included all kind of different information which would not fit into the other IUCLID sections, e.g. *in vitro* assays, various mechanistic studies, additional published literature, unusual exposure routes like intratracheal instillation, intraperitoneal or subcutaneous injection. Usually it was not clear how the information from this section was used for the overall weight of evidence in the

registration dossiers. Some of the reported studies in the section were of reliability 2, but many were of low reliability (3, 4) or no reliability was assigned.

For example, for one Category I substance 132 mechanistic studies including *in vitro*, mathematical models, human mechanistic studies, intraperitoneal injection, etc. were summarised in a table, covering endpoints like inflammation, cytotoxicity, genotoxicity, oxidative response, biological reactivity and lung function. Test material was normally only indicated as the chemicals name, whereas the titles of the studies indicated 5 studies on nanomaterials and 21 on ultrafine test materials. For the same substance there was one comparative study using intratracheal instillation of different particle sizes. This study showed that more cytokines related to lung inflammation were released after exposure to the nanoform(s) than to the non-nanoform. These results suggest that nanoparticles have a relatively greater toxicity on a mass/lung basis. The results of this study were not reflected in other parts of the dossier of the same substance.

For another dossier, one *in vitro* study with lung epithelial cells showed that ultrafine particles induced a greater oxidative stress than fine particles. However the specific result of this study was not taken into consideration in the overall conclusion.

#### **4.1.1.10 Neurotoxicity/Immunotoxicity**

Immunotoxicity was not addressed in any of the IUCLID endpoint sections in the dossiers analysed, whereas neurotoxicity was explicitly addressed in one dossier. In that Category II dossier the conclusion was based on read across to the leading ion in neuro-developmental studies. It is interesting to note that these data have not been provided for the other related substances based on the same ion.

In all other dossiers, effects on neurotoxicity or immunotoxicity, if addressed/observed, were reported in the Repeated Dose Toxicity (RDT) section or under human observations. Some substances concluded, however without any data presenting, that “No adverse effects concerning neurotoxicity had been identified”. For one substance, which contains a neurotoxic ion, data was presented under IUCLID section 7.10.5 ‘Exposure related observations in humans: other data’. Most dossiers did however not address these endpoints in the registrations.

*Little information is reported in the registration dossiers on immunotoxicity and neurotoxicity and the few conclusions were drawn mostly based on a weight of evidence.*

#### **4.1.1.11 Secondary effects**

There is no specific test for secondary effects and no dedicated IUCLID section where such effects can be inserted, however information on secondary effects could be reported in relation to toxicity studies.

This endpoint has been explicitly addressed in two dossiers (Category I and Category II) and in the Category I dossiers specifically in relation to nanosize. These dossiers discuss the development of rat lung tumours after chronic inhalation of inert particles exposure as a secondary inflammatory/proliferative mechanism. According to the discussion, the lung tumour response to inhaled inert particles is not due to direct

genotoxicity. “Particle overload” would be the key factor leading to the development of tumours as it leads to sustained inflammation, release of various biological mediators, and oxidative stress. Accordingly, the susceptibility of the rat may reside in the fact that rat lungs show a far greater induction of several key pro-inflammatory processes and less induction of anti-inflammatory processes than other species. This is in line with the discussion presented under Carcinogenicity in a number of dossiers. Also observed positive *in vivo* results for genotoxicity were explained by secondary effects following inflammation.

*Secondary effects were discussed in few dossiers, mainly referring to induction of lung tumours as a consequence of inflammation due to lung overload of particles. Influence of nanosize was specifically discussed in one dossier.*

#### **4.1.1.12 Exposure related observations in humans**

Observations in exposed humans can be reported in several IUCLID sections, e.g.: Health surveillance data, Epidemiologic data, Direct observations: ‘Clinical cases’, ‘Poisoning’, ‘Incidents and other’, ‘Sensitisation data (humans)’, ‘Exposure related observations in humans: other data and Additional toxicological data’. ‘Epidemiologic data’ was addressed by all but 5 dossiers. The remaining sections were addressed by between 2 and 9 dossiers.

Some of the dossiers analysed and assessed cover substances on the market for many years or decades. Some substances exist in different particle sizes, not necessarily as nanoform (Category I), while for some others, the primary particle size is considered only to be in the nanorange (Category II). For such substances there is a lot of experience with human exposure: occupational exposure, epidemiology, health surveillance etc. Studies presented include for example: x-ray of workers, tests of respiratory function, medical supervision, cohort mortality studies; cohort and case-control studies, clinical trials, human volunteer studies, observations (e.g. referring to sensitisation, which is used as justification for waiving an animal study). Sometimes up to 90 studies were presented. Some dossiers presented opinions, surveys and/or reviews by e.g. OECD and/or EFSA or certificates of their company doctors to prove the absence of effects in humans. For one Category II substance all human data referred to the leading ion and its possible effects.

The assigned reliability of the presented studies varied. In some cases no reliability was assigned or the data were of low reliability.

While observations in humans provide useful information on the possible effects on humans under realistic exposure conditions to the specific materials used in those studies, it is difficult to translate these data to possible exposure to nanoforms/nanomaterials, as no particle sizes are generally reported and it is difficult to determine the particle size retrospectively. Further, the exposure could have been to a mixture of different particle sizes, some of which have probably been nanosized particles but in unknown concentrations. This is an important issue as the absence of effects in humans was used in several cases as waiving argument for not performing a test. Therefore registrants should thoroughly evaluate whether the ‘human experience’ is appropriate in the evaluation of nanomaterials.

In that context it is also worth noting that it is in general difficult based on epidemiological studies to confirm the absence of effects in humans.

*Several dossiers have presented a huge dataset on observations in humans for the substances and have used them also in a weight of evidence approach. It is difficult to judge on the relevance of this data for the assessment of nanoforms/nanomaterials, as particle sizes are usually not known.*

#### **4.1.1.13 DNELs/DMELs**

Only one dossier (Category II) has derived DNELs for all exposure situations of workers and the general public. In the other dossiers a DNEL was mostly provided for long term inhalation of workers (18 dossiers; either only systemic and or only local inhalation exposure). Two dossiers did not present any DNEL (one of them justified with exposure based waiving). In 11 dossiers, in addition to the DNEL for workers a long term oral DNEL for the general population (systemic) was provided.

The justifications for not deriving DNELs for various routes (mostly for the acute DNEL) given by the registrant were (alone and in combination):

- no acute toxic or no local (e.g. irritating) effects observed
- no systemic (oral or dermal) effects anticipated, (due to inherent substance properties and experimental evidence) or as not absorbed
- no exposure likely either for the route of exposure (dermal/oral) or of the general public or because of size that are hardly respirable (aggregates/agglomerates)
- Long term (inhalation) DNEL is considered sufficient to cover the short term effects
- substance not classified
- negligible exposure of the general public anticipated or consumers must not be exposed because of classification as mutagenic

In some cases no justifications were given, especially for not deriving an acute DNEL.

DNELs for the general public were often not derived, e.g. with the justification of negligible exposure or no application in consumer products. However the negligibility of exposure could in some cases be discussed due to a widespread use of products containing such substances.

For deriving a DNEL different methods were used in the 19 dossiers:

- 1) Use of animal data and application of assessment factors (AF) for inter-/intraspecies differences, or duration using REACH default values for assessment factors (AFs)
- 2) Applying AFs different from REACH default values, as they were considered too conservative, e.g. in comparison to existing limit values (OEL, MAK values) for similar compounds. E.g. an AF of 3 for workers and AF of 5 for the general public.
- 3) Use of the general dust limit or OEL for inhalable ( $10 \text{ mg/m}^3$ , or  $4 \text{ mg/m}^3$ ) and respirable dust ( $3 \text{ mg/m}^3$ ) fraction or the nuisance dust level
- 4) Use of NIOSH Recommended Exposure Levels (REL) (which was divided by 2 for the general public)

- 5) Use of human data (large multi-centre studies; NOEL = 3/5 of concentration with prevalences of symptoms (chronic bronchitis) without any further AF (DNEL = NOAEL in humans).
- 6) Internal values from human data without further consideration of additional AFs.
- 7) Route to route extrapolation from oral data to inhalation exposure, e.g. using a specific method or by using a specific absorption rate for the different exposure routes (= evaluation based on toxic ion and not on particle nature)
- 8) DNEL was derived from structural analogues
- 9) Oral intake recommendations for the specific ion
- 10) For the general public the same DNEL was used as for workers (based on general dust level: for inhalable airborne fraction ( $10 \text{ mg/m}^3$ ) and for the respirable airborne fraction ( $3 \text{ mg/m}^3$ ) without further AF or justification

None of the Category I dossiers that used the IUCLID 2.1 or 4.1 picklist (nano) derived a nanospecific DNEL from the available hazard data. Either the OEL of  $10 \text{ mg/m}^3$  for nuisance dust particulates, or an unspecific OEL value for respirable dust were used. The Category II using the picklist used the NIOSH recommended exposure level (REL,  $3.5 \text{ mg/m}^3$ ).

For one Category I substance, the registrant claimed that the DNEL that was derived from a study not indicating to having tested the nanoform, was also valid for the nanoform without any clear justification why the available data for the nanoform had not been taken into account. These studies with the nanoform (supporting studies, reliability 2) had a lower NOAEL than the selected key studies for deriving the DNEL. In this case, the suggested DNEL (applicable also for the nanoform) was identical with the general dust limit ( $10 \text{ mg/m}^3$ ).

Different AFs for intraspecies variability were applied. Examples of AF for intraspecies differences among workers were 1 and 3 (REACH default 5). Often an intraspecies factor of 5 was applied for the long-term oral DNEL for the general public (REACH default 10). If the dust level for workers was used as DNEL, either the same level was used for the general public, or it was divided by 2.

Usually no assessment factor for extrapolation of time (e.g. from subchronic to chronic) was applied. One dossier indicated as justification that the NOAEC would not be dependent on time, due to constantly operating clearance mechanism (while the severity of effects may increase with increased exposure duration). One dossier, which was based on read-across used a factor of 3 for long term inhalation of workers and a factor of 2 for long term oral exposure of the general public for adequacy of the database.

Some dossiers did not apply interspecies AFs but considered the correction factors for different inhalation scenarios sufficient. These were e.g.:

- 1) A correction for the different respiratory volume between standard conditions ( $6.7 \text{ m}^3$ ) and light activity ( $10 \text{ m}^3$ )
- 2) a "correction" factor of 7 for the difference in particle-size dependent fractional deposition in the respiratory tract between rats and workers.

It was also argued in some dossiers, that as the rat is more sensitive than humans no interspecies AF would be required.

Several dossiers discussed why NOAECs from animal studies would not be appropriate to be used for deriving a DNEL. The following arguments were provided:

- exposure patterns and particle characteristics in experimental animal studies do not mimic conditions in the occupational environment
- prolonged exposure does not give the animals the normal recovery period for lung clearance. This is explainable by the fact that rat studies are only hazard studies and not risk based studies.
- phenomenon caused by rat lung overload. Justification given: The development of lung tumours occurs only in rats under lung overload conditions. Neither other rodents, such as mice and hamsters, nor humans develop lung tumours under similar conditions of lung overload from PSPs (poorly soluble particles). The development of lung tumours at lung overload exposures is triggered by the inability of rats to effectively clear the particles from their lungs and an exaggerated inflammatory process.
- under occupational use conditions the substance forms aggregates/agglomerates with particle sizes of up to 100 µm that are hardly respirable. In commercial products the fraction of air-borne particles that is potentially able to reach the thoracic and alveolar site would be <1 vol %.

It should be noted, that especially the arguments presented in that section are a list of justifications or methods provided by the registrants. Several of them could be discussed for their appropriateness in the respective dossiers; however this is outside the scope of the assessment in that project.

*Different methods for deriving the DNELs were applied. Several DNELs were based on available OALs or dust limits, which are however not specific for nanomaterials. Only few DNELs using REACH default factors were derived. In general lower assessment factors were applied with different types/levels of justification. When different forms were addressed in the dossiers (Category I), specific DNELs for nanosized materials were not derived from available (nanospecific) hazard data. In general, there seems to be a big need for establishing consensus on how to derive DNELs for nanomaterials and particles in general, including whether general dust levels are applicable and what can and what cannot be done in relation to selection of assessment factors. Some of the issues related to e.g. deviation from default AFs are general and should be addressed in a broader context.*

#### **4.1.2 Inherent properties – Fate**

The following is a summary of how environmental fate endpoints were addressed in the 25 registration dossiers. For four member dossiers none of the environmental fate endpoints were addressed in the dedicated IUCLID fields as information on environmental fate was included in the lead registration. *The summaries below for specific endpoints therefore cover the remaining 21 dossiers.*

#### 4.1.2.1 Stability

##### 4.1.2.1.1 Phototransformation/photolysis in air, water and soil (IUCLID Sections 5.1.1, 5.1.3 and 5.1.4)

The endpoints for phototransformation were not addressed in 11 out of the 21 dossiers assessed (for one of the dossiers, this endpoint was not an information requirement due to its low tonnage band). In ten dossiers, the endpoints were waived based on Annex XI Section 1 (test scientifically unjustified) or Annex XI Section 2 (test technically not feasible). When the waiving justification was based on Annex XI Section 1, the argument highlighted the inorganic nature and the high stability of the registered substance and lack of functional groups capable of adsorbing solar photons that could lead to direct or indirect photolysis. When the waiving justification was based on Annex XI Section 2, the feasibility of the test was stated to be compromised due to the naturally high abundance of the elemental composition of the substance in the environment.

*In conclusion, no data was reported for this endpoint in any of the dossiers. In all dossiers neither the nanoform nor the applicability of the correspondent test guidelines to nanoforms was addressed. There was no mentioning of the phototransformation capabilities of potential capping agents.*

##### 4.1.2.1.2 Hydrolysis (Annex VIII 9.2.2, IUCLID Section 5.1.2)

Knowledge on the abiotic hydrolytic transformation helps to identify the rate of hydrolysis of a chemical substance as a function of pH; not only identifying the formation and decline of hydrolysis products, but also the nature of the compounds to which organisms may be exposed. Thus, the information obtained under this endpoint is not only important for determining the rate of transformation of the substance in the natural environment, but it is also crucial information to establish an adequate experimental design for hazard assessment

In none of the 21 dossiers was hydrolysis test data provided for the registered substances.

Hydrolysis was waived in ten dossiers based on column 2 Annex VIII, due to the low solubility resulting from the inorganic nature of the substance, whereas in another ten dossiers the study was waived based on Annex XI Section 1 or 2 (study scientifically unjustified due to the low water solubility, or technically not feasible, respectively).

Although there was no specific mention to nanomaterials under this endpoint, in four of the Category II dossiers, the waiving justification was based on the low water solubility of the particles, measured by centrifugation, dialysis or filtration techniques (recommended techniques in scientific literature to measure leaching of ions (i.e. 'solubility') from nanomaterials) and reported in IUCLID Section 4.8. Therefore the importance of the solubility of the (nano)substance and potential formation of bioavailable chemical species was considered. However, there was no further identification of the chemical species formed, but only nominal elemental concentrations that were reported. In one of these dossiers, physical and chemical information on surface area and particle size distribution were also provided under this endpoint. In two other Category II dossiers this endpoint was addressed by read-across to results on dissolution to a substance of

similar chemical composition. For the latter two substances, the registrant's justification to the read across was related to the inability to quantify analytically the degree of hydrolysis of the registered substance due to the formation of a gel-layer surrounding the particle, and also the limited role hydrolysis would have in the dissolution process.

In another three Category II dossiers, the solubility of the substance (ions leached by the registered substance) was reported under this endpoint entry. For one dossier this was calculated using the model WSKOWWIN, version 1.41, © 2000, US Environmental Protection Agency, but data on hydrolysis was missing. The suitability of the computer model and its analytical limitations to nanoparticles was not discussed. For the other two dossiers, the solubility was reported as the resulting mass concentration of ions leached from the nanoform. However, no information on the methodology was reported.

None of the dossiers addressed the hydrolytic or phototransformation potential of potential capping agents. For only one of the substances assessed, the information in the dossier indicated the presence of an outer shell that differs from the chemical composition of the core material. On the other hand, some of the impurities reported in the dossiers could potentially modify the hydrophilic nature of nanomaterials, if these would be used as surface functionalizing agents, influencing the fate in environmental compartments and specifically the aggregation/agglomeration and precipitation rates.

*In conclusion, information for this endpoint is required for chemical substances with sufficient solubility in water and indeed, it is difficult to conduct with poorly soluble substances. However, in several dossiers the water solubility reported under IUCLID 4.8 water solubility reflected the degree of ions leached by a substance of similar chemical composition, from which physical and chemical properties were not reported (e.g., most often an oxide or a salt of the same chemical composition as the substance being registered, and not on the registered substance itself). Therefore, and considering that in general smaller particles have higher ion release kinetics compared to larger sized particles, waivers based on low water solubility may not apply to the nano-form. Nevertheless, it is important to mention that the degree of chemical ions leached and reported in IUCLID Section 4.8, water solubility ranged from mg/l, to µg/l, and in the former, hydrolysis waiving based on water solubility can be questioned.*

#### **4.1.2.2 Biodegradation in water, soil and sediment (Annex VII 9.2, Annex VIII 9.2, Annex IX 9.2, Annex IX 9.2, IUCLID Section 5.2)**

Biodegradation defines the process of biologically mediated transformation of chemicals. In 20 out of 21 dossiers, the endpoint of biodegradation in water (screening) was waived mainly under Annex VII, column 2.

In one Category II dossier, a key study for readily biodegradability was reported, although registrants did not explicitly mention the test was conducted on a nanoform. The study followed the test guideline outlined in the OECD 301B: CO<sub>2</sub> Evolution test, was given a reliability 1, and was GLP. The CO<sub>2</sub> evolution test results indicated a substance degradation of 58% in 28 days, and the substance was hence not categorized as readily biodegradable. Though not explicitly stated in the registration dossier, this substance seems to be a classic core-shell type nanoparticle consisting of an inorganic core coated by a ligand shell. It should therefore be taken into consideration that the observed CO<sub>2</sub> production could stem from degradation of the particle coating. This is an

issue that has to be considered for coated / core-shell nanoparticles in general as it could potentially lead to erroneous conclusions on the biodegradation potential of the nanoparticles as a whole.

For the other two biodegradation endpoints (water/sediment and soil compartments, IUCLID Sections 5.2.2. and 5.2.3, respectively) these were mostly not addressed or waived. The waiving justification was based on the study being scientifically unjustified based on the inorganic nature of the substances, as outlined in column 2 Annex VII. Other waiving justifications were based on the stable and inert properties of the substances.

For nanomaterials with chemical compositions of inert and highly abundant inorganic/essential elements, the waiving of biodegradation studies was based on the principle that their elemental composition cannot be further degraded.

*In conclusion, only one dossier from the Category II substances test data was reported. Nevertheless, no information on the physico-chemical parameters was reported. Although it is justified to waive biodegradation studies based on the inorganic nature of the substance, recent research highlights the potential for carbonaceous nanomaterials to be biodegraded, but to which extent and to what type of nanoforms this applies is yet to be investigated. Also, and as already stated in the above Section 4.1.3.2. 'Stability', the biodegradability of potential capping agents was not address in any of the dossiers.*

#### **4.1.2.3 Bioaccumulation (Annex IX 9.3, IUCLID Section 5.3)**

Investigations of bioaccumulation potential of a chemical substance is triggered when  $\log K_{ow} > 3$  (as reported in IUCLID Section 4.7), since this indicate high lipophilicity and therefore potential for the substance to bioaccumulate. However, the applicability of  $K_{ow}$  based approached to nanomaterials are not validated, as also stated in the RIP-oN 2 Final Project Report (3.7.189), due to the particle nature of the materials. Therefore justifications based on  $\log K_{ow}$  considerations may not be appropriate for waiving of bioaccumulation endpoints for nanoparticles. Also, size, surface coatings and reactivity of nanoparticles may affect and facilitate other pathways of cellular uptake.

##### **4.1.2.3.1 Bioaccumulation: aquatic/sediment (Annex IX 9.3, IUCLID Section 5.3.1)**

In one of the assessed dossiers the endpoint was not addressed, and in this case the endpoint was not required due to the information requirements of its tonnage band. The endpoint was waived for 15 dossies, based on read-across for 3 dossiers and in two Category I dossiers, experimental data on the bioaccumulation potential of the nanoform of the registered<sup>24</sup> substances was provided.

In one of these Category I dossiers, literature data on the bioaccumulation of nanoform was provided and flagged as a key study on the bioaccumulation potential of the registered substance. The study did not follow an approved guideline and it was not

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<sup>24</sup> Nanomaterials physico-chemical properties are known to differ depending on the production method. Thus, the properties of the 'registered substance' for nanomaterials will therefore be process-specific. Although the registrant reports that the tested material is the same as the registered substance they may therefore differ in physico-chemical properties—even if the EC number is the same. In this context, however, what is meant is that testing data is provided for the chemical identity given in IUCLID section 1.

according to GLP. However, a reliability 2 score was proposed as the test was well described, including sample preparation and dosimetry. Based on the results, the registrant concluded that there was no biological uptake of the nanoform. However, the results showed uptake of the nanoform in the liver of exposed fish. In the second dossier, the results from a scientific publication on exposure to a nanoform for 14 days were reported. The study was regarded as a key study with reliability 2 and no GLP. There was limited information on the physical properties of the material used and sample preparation; however the trade name of the nanoform was reported. The results show that the BCF of the nanoform on different fish organs/tissues ranges from 30 to 200 L/Kg, but a dose response relationship was not determined and therefore the registrant's conclusion was that the nanoform was not considered bioaccumulative.

In 15 dossiers the bioaccumulation endpoint was waived. In almost all dossiers were waivings based on column 2, Annex IX, and thus the waiving statement was either based on exposure considerations, based on the a low potential to bioaccumulate (from partition coefficient results,  $\log K_{ow} < 3$ ) and /or a low potential to cross biological membranes.

In one Category II dossier, the waiving statement took into account the nature of the particles and aggregation potential in aqueous suspensions to argued that diffusion through gills and uptake via the membrane of organisms in soil and sediment compartment was not expected.

In four dossiers (three of them for Category II dossiers), the waiving was justified on the substance being insoluble. It should be noted that, as will be discussed further in subsequent chapters, solubility is not considered an adequate justification for waiving this endpoint. For another two dossiers the waiving justification was based on the published scientific literature, indicating that specific metal species tends to biodilute instead of bioaccumulate in higher trophic levels.

In the remaining 3 dossiers assessed, the data presented under this endpoint was based on literature studies on metal salts and metal oxides. For all three substances (2 of them from the nanotail Category III, and one from the nanoform Category I), the data read across to a soluble counterpart, but information on sample preparation and substance properties was lacking. Hence the particle nature of the substances was not addressed.

*In conclusion this endpoint was waived for the majority of dossiers. Waiving justifications did not take into account the (nano)particulate nature of the substances. In relation to this the comment made in the RIP-oN 2 final report (3.7.189) is relevant to consider, specifying that for nanomaterials estimations of bioaccumulation cannot be based on  $K_{ow}$  or solubility. The two dossiers, reporting experimental data for this endpoint, both reported tests of a nanoform of the substances. For both studies it was concluded that the nanoform was not considered bioaccumulative.*

#### 4.1.2.3.2 Bioaccumulation: terrestrial

Terrestrial bioaccumulation was not addressed in any of the dossiers for which aquatic/sediment bioaccumulation studies were already waived or not addressed. In 3 dossiers was the endpoint addressed by read-across. In two of these dossier (Category I and II), studies, presented as part of a weight of evidence/read across approach, had low reliability, and the reported data were for metals (the metal from which the registered substance was manufactured) in the tissue of plants on contaminated sites. In a third dossier (Category III) a similar approach was presented, but the study was regarded as a key study with a reliability score of 2. In this study, the transfer of a metal hydroxide (same element as the registered substance) was measured in tissue of plants after seven days of exposure.

*In conclusion, for both aquatic and terrestrial bioaccumulation, the uptake of certain metals is heavily regulated by organisms and BCF factors may not be adequate measurements for the bioaccumulation potential of a number of metal species. On the other hand, the bioaccumulation potential of the nanoform compared to the bulk or ionized form should be evaluated with caution and estimations of bioaccumulation cannot be based on  $K_{ow}$  or solubility. For instance, surface functionalized nanomaterials could potentially enter the cell bypassing the route for uptake of bioavailable ionized metal species, leading to a 'Trojan horse' type mechanism (i.e., the nanoform acts as a delivery vehicle for bioavailable metal ions). Although, none of the substances were registered as surface treated material, some of the impurities reported in the dossiers could potentially modify the biological availability of the nanomaterials if used as a capping agent. Hence, the coating agents used for surface functionalized or coated nanoparticles should be considered when investigating the bioaccumulation potential of the nanoform.*

#### 4.1.2.4 Transport and distribution

##### 4.1.2.4.1 Adsorption/desorption (Annex VIII 9.3.1, Annex IX 9.3.3, IUCLID Section 5.4.1)

This property indicates the capacity for a substance to bind to solid surfaces, and thus it is crucial for understanding environmental fate, and determining the most suitable approach for hazard assessment of the sediment and soil compartments.

This endpoint was waived in 12 dossiers. The waiving justification was either based on Annex VIII column 2 (based on physico-chemical properties) or Section 2 of Annex XI (testing technically not possible). For those substances composed of chemical elements highly abundant in the environment, the waiving justification based on Section 2 Annex XI was on the grounds that analytical discrimination between the natural and manufactured substance is currently not feasible.

For nine dossiers (belonging to all three categories of substances) test data was reported under this endpoint. For most dossiers, the data was on a metal species (often the most representative metal species in the registered substance was the only one reported). The information under this endpoint could be considered relevant for the soluble fraction of the nanoform. For one Category II data derived from a specialized chemical estimation model (PCKOWIN, 1.66 © 2000 US) was reported instead of

laboratory data. In relation to this it should be noted that of the applicability of this estimation model for nanoparticles is not discussed and the applied method may not be appropriate. The registrant also reported the inability to use the EU method C19 to determine the adsorption coefficient for surface active materials or organic acids, thus justifying his choice of method to use.

*In conclusion, nanomaterials adsorption/desorption is a crucial parameter as the ability of nanoforms to bind to surfaces will determine their fate in the environment. Therefore, several physico-chemical parameters, such as charge, surface area, porosity and others are parameters that should be considered when characterising nanoforms, since they can help predict the potential of the substances to adsorb or desorb to solid surfaces.*

#### **4.1.2.5 Environmental data**

##### **4.1.2.5.1 Monitoring data**

Information under this endpoint was not addressed for 10 dossiers out of 21, and was waived in three dossiers. Information was present in eight dossiers belonging to Category II) and Category I..

Only in one Category I dossier was data from a scientific publication on the nanoform of the substance reported. The study did not follow an approved OECD/EU guideline and has a reliability score of 2. The data reported on the electrophoretic mobility and rate of sedimentation of the nanoform of the substance in different waters, including lagoon, river and groundwater. However, information on the physical and chemical properties of the substance tested was not reported.

For the other seven dossiers, the information reported was based on scientific literature on the fate and behaviour of the metal species. The data reported under these endpoints could be considered relevant for the soluble fraction of the nanoform but does not address the particulate nature of the registered substance and the nanoform

*In conclusion, data on behaviour of a nanomaterial in an aquatic media was reported in one dossier. For this study, information on the electrophoretic mobility of the potential nanoform in aqueous suspension would provide information on the environmental compartments most suitable for hazard assessment. For all other dossiers, containing information on environmental monitoring data, this data concentrated on measured background concentrations of the elements, constituting the registered substance, and did not contain data the registered substance itself.*

#### **4.1.2.6 Other endpoints/observations /Other information**

No additional endpoints were addressed, except for Henry's law constant (IUCLID Section, 5.4.2). This endpoint was addressed by waiving in two nanoform only dossiers (Category II), based on Annex XI Section 1, since the vapour pressure of the substances was negligible.

It should be noted, that this, and other optional endpoints, are not part of the REACH standard information requirements.

### 4.1.3 Inherent properties – Ecotoxicity

The following is a summary of how environmental hazard endpoints were addressed in the 25 registration dossiers, which are divided into three categories: ‘nanoform covered’ (Category I), ‘nano-only’ (Category II) and ‘nano-tail’ (Category III) (see Section 3.1.1). For four member dossiers none of the environmental hazard endpoints were addressed in the dedicated IUCLID fields as information on environmental hazards were included in the lead registration. *The summaries below for specific endpoints therefore cover the remaining 21 dossiers.*

#### 4.1.3.1 Aquatic toxicity

##### 4.1.3.1.1 Acute toxicity to fish (Annex VIII 9.1.3, IUCLID Section 6.1.1)

This endpoint was addressed for all 21 dossiers: for four dossiers solely by read-across, for two dossiers by waiving and for one dossier by QSAR. Testing data on the registered substance was provided for the majority of dossiers<sup>25</sup> (14 out of 21).

Out of the 14 dossiers, which contained data under this endpoint, tests on nanoforms were reported in ten dossiers out of which four are Category I dossiers and six are considered only to exist in a nanoform (Category II). For the latter category, though not explicitly stated, it is therefore considered implicit that, when the registered substance was tested, this implies testing of a nanomaterial<sup>26</sup>. In two of the six Category II dossiers only data from testing of a filtrate/supernatant (e.g. a so-called ‘water accommodated fraction’ (WAF)) was reported. For one of these tests it is stated that “In view of the difficulties associated with the evaluation of aquatic toxicity of poorly water soluble test materials, a modification of the standard method for the preparation of aqueous media was performed. An approach endorsed by several important regulatory authorities in the EU and elsewhere (ECETOC 1996 and OECD 2000), is to expose organisms to a saturated solution of the test material in cases where the test material is of high purity and is poorly soluble in water and in the permitted auxiliary solvents and surfactants.” The concentrations of the test material in the test solution was verified analytically, showing that at 100% saturation the actual concentration of the substance in the solution (after filtration through a 0.2 µm filter) was below the limit of quantification. The LC<sub>50</sub> value was expressed as “> 100% v/v saturated solution”. For the other Category II dossier, where a single study, testing a WAF, was provided for this endpoint, no information on particle size or surface area etc. was reported. No measurements of actual concentration were reported (NB! The solubility of the substance is reported to be 48 mg/L and hence the reason for testing of a WAF is unclear). This study represents one out of two studies on aquatic toxicity endpoints in the dossier: the other study is for toxicity to aquatic algae, also testing a WAF. Remaining endpoints have been waived or addressed by read-across. Nonetheless it is reported that an aquatic PNEC value is non-quantifiable due to high tolerance in acute testing. It should be noted that the use of only nominal loading rates may cause an underestimation of the actual toxicity. It is

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<sup>25</sup> Testing data for the registered substance was in some cases provided together with read-across data and endpoint conclusions based on these data.

<sup>26</sup> It should be noted, however, that the choice of sample preparation method may not in all cases be suitable for testing nanoparticles. For example preparation of water accommodated fractions (WAFs) may exclude nanoparticles from the test suspensions/solutions whereby particle effects will not be detected. This issue is discussed further in subsequent sections specifically section 4.2.3.3.

therefore suggested that the presence of particles and actual concentrations of the test material in the WAF should be quantified in order for PNEC values to be correctly estimated.

For another Category II dossier data from testing of filtrates was provided together with data from testing particle suspensions. For this dossier it was specified in the overall discussion of 'aquatic toxicity' that, as the substance is insoluble "modifications are required to the standard ecotoxicity test procedures". Two different methods for sample preparation were described and discussed in this dossier: 1) suspensions are maintained through stirring or aeration during the test. The test may be influenced by the physical presence of the particles. 2) The substance is equilibrated with water, suspensions are subsequently filtered, and filtrates are used for testing. Discussion of filtrate versus suspension toxicity was also mentioned in the endpoint summary. It was reported that for one test material total mortality was observed for 10,000 mg/L suspensions but attributed to physical effects, whereas no mortality was observed in the filtrate from the 10,000 mg/L suspension. For yet another dossier suspensions were tested in two key studies and a supporting study (read-across) tested a WAF. For the last two Category II dossiers only suspensions were tested. The appropriateness of testing of filtrates/supernatants/WAFs is discussed further in subsequent sections, specifically Section 4.2.3.3. For none of the Category II dossiers was any information on particle sizes reported for this endpoint.

For the remaining 4 (Category I) dossiers, test data for a nanoform, and often also other forms, were presented.

In one dossier the nanoform was assumed to represent a 'worst case scenario', which is justified by the registrant due to the fact that the nanoform is "anticipated to represent the worst case as it is likely to be more soluble than the bulk form due to the smaller particle size and hence greater surface area. However, the results are directly applicable to the bulk form". Though this registrant clearly addressed the nanoform and considered it a 'worst case scenario' no information on the particle size was provided for this study. For this dossier, a filtrate (WAF) was tested and the preparation of the test 'solution' was well described (48 hours stirring followed by filtration). With a 0.2µm filter size single nanoparticles and smaller aggregates/agglomerates may pass through the filter. However, the concentration of the test material was only given as a nominal value based on the initial material mass prior to filtration (100% v/v saturated solution) and was not further quantified in the actual test suspension (due to the natural high background levels of the substance (chemical element) in the medium). For future considerations, investigations of the presence of nanoparticles (quantitative, qualitative, stability, aggregation/agglomeration state) in the test suspension would be crucial for the interpretation of the test results. Again, regarding the appropriateness of filtrates/supernatants/WAF testing refer to Section 4.2.3.3.

In another Category I dossier, for which nanoforms and bulk forms were indicated in IUCLID Section 1.2. 'Composition', both forms were addressed separately for this endpoint (2 studies were reported for the bulk form (one key study, one supporting study) and one (key) study was reported for the nanoform (3 nanoparticle sizes tested)). Information on particle sizes was reported for both the bulk studies and the nano study. For the nano study, which is a literature study, the particle sizes (14, 20 and 29 nm) seem to refer to primary particles, though this was not clearly specified. Particle size

distributions were investigated 24 hours after preparation of the test suspensions by Nanoparticle Tracking Analysis (NTA). Particle aggregation to diameters ranging from 441 to 543 nm was reported. Also information on specific surface area was reported for the nanoform study. For the bulk studies the preparation of the test suspensions was quite well described. For the nanoform study the preparation of the stock suspension was not specified. Based on both bulk and nano studies the substance was considered as not harmful to fish. This registrant clearly attempted to make a visible distinction between nanoform and bulk form throughout the registration dossier. Still no discussion on the influence of particle size was reported in the endpoint study summary.

In another Category I dossier 35 studies were included and distinction between bulk and nano forms was sometimes made in the test material description. However, except for two studies (from same literature source) with information provided on surface area, no information on size was reported in the IUCLID entries. For one study additional information of particle sizes (140 and 380 nm, not specified whether this refers to primary particles) was reported in the CSR. In some cases it can be extrapolated from the test material composition that the bulk and nanoform of the tested substance was a coated test material. Sometimes coatings were mentioned explicitly in the test material description and sometimes dispersion agents were also mentioned. However, coatings were not considered in the discussion of the test results. In one case, observations were reported considering aggregation at the beginning of the test (visual observation) but no analytical measurements of aggregate/agglomerate sizes were reported for any studies. The tested material was always considered the same as the registered one. The endpoint conclusion was described to be based on reliable results of tests with both nano and bulk forms of the registered substance taken together in a weight of evidence approach. The assignment of reliability to the individual studies was based on use of guidelines and documentation of test procedure. Though some supporting studies (reliability 3) reported EC<sub>10</sub> and LOEC values of 10 and 50 mg/L (tests of nanoforms), respectively, an EC<sub>50</sub> values of >1000 mg/L (from studies with reliability 1 (and one with reliability 2), weight of evidence studies, tests of bulk form, nanoform and material with size not specified) was reported in the endpoint study summary. It was specified that reliable results cover a range of particle sizes and all data is taken together in a weight of evidence approach. Nonetheless, specific considerations for particle sizes and differences in toxicity effects between different forms/sizes were not explicitly discussed.

In another metal oxide Category I dossier six studies were reported. One study (supporting) gives experimental testing data for the nanoform. The remaining five studies (key studies) were tests of soluble forms of the metal, as the dissolved metal was considered to be the driving mechanism behind the toxic effects of the substance. The study on a nanoform of the registered compound reports a (broad) particle size range (50-360 nm) but it was not specified whether this was for primary particles or aggregates/agglomerates and no further details or information on size was reported. For the endpoint conclusion only the five studies on the soluble metal ions have been considered as key studies and the endpoint study summary did not address particle effects or particle size issues.

In summary, for all four Category I dossiers, where testing data was provided for the substance, studies on a nanoform were provided. In one case, only the nanoform was tested. In the other three cases data on the nanoform was provided alongside data on

the bulk form or soluble metal compounds. However, the effect of form/size on the toxicity was not discussed.

For the four Category III dossiers testing data was provided but the nanosized fraction of the material was not (explicitly) addressed. For one dossier, one study contains information about particle size (1µm). However, no further details or specifications were reported and hence it is not know how (preparation, technique...) this size measurement has been obtained. For the remaining 3 Category III dossiers no information on size was reported.

In four dossiers (one Category I, two Category II and one Category III) the endpoint was addressed by read-across. In two dossiers (one Category II and one Category III) read-across was based exclusively on the soluble metal ions. Hence the particle nature of the substances was not addressed. In another dossier (Category II) read-across was done from data on a particulate substance with a chemical composition similar to that of the registered substance. However, the degree of similarity between the tested and the registered substance is unclear – also due to the fact that the physico-chemical properties of the test material were not reported in the dossier. Finally, in one metal oxide dossier (Category I), read-across was done from a metal salt. The tested metal salt was reported to be soluble (up to 900 mg/L). Still, a WAF was tested and the measured concentrations of metal in the filtrate were below the detection limit of 0.003 mg/L.

For one dossier of a Category II substance, this endpoint was addressed by QSAR (ECOSAR). It can be noted, that the results from the QSARs were much lower than the results from experimental values from the acute toxicity tests included in another dossier for the same substance. However, no information about the method and assumptions applied in this QSAR approach was reported. This makes it difficult to assess the applicability of the obtained EC values to the registered substance<sup>27</sup>.

For two nano-only (Category II) dossiers the endpoint was waived due to the insoluble nature of the substances. In relation to this it should be noted that, according to REACH Annex VIII, 9.1.3., “long-term aquatic toxicity study on fish (Annex IX, Section 9.1.6.) shall be considered if the substance is poorly water soluble”.

*In conclusion, for dossiers containing testing data, limited information was generally provided on the tested substance/form (particle sizes, surface area etc) and on particle behaviour in test media (aggregation, agglomeration, sedimentation etc.). Differences in toxicity effects between different forms/sizes were generally not discussed even if data for the different forms were available. One dossier, considering the nanoform as a ‘worst-case’ due to small size and increased solubility, was an exception but still the particle size of the test material was not specified and a WAF was tested. Hence the particle nature of the substance is not addressed. Another exception was one dossier making a distinction between nanoform and bulk form. However, in this case it was concluded, from both bulk and nano studies, that the substance is not harmful to fish and thus no further discussion on the influence of particle size was reported.*

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<sup>27</sup> In general, the applicability of QSAR modelling to nanomaterials can be considered still to be an area of great uncertainty and such approaches should therefore be used with caution and accompanied by scientific justifications.

#### 4.1.3.1.2 Long-term toxicity to fish (Annex IX 9.1.6, IUCLID Section 6.1.2)

In 2 of the 21 assessed dossiers, the endpoint was not addressed due to lower tonnage (1-10 and 10-100 tonnes/annum, respectively) and consequently reduced data requirements. This endpoint was addressed for 19 dossiers: in 4 dossiers solely by read-across and in 14 by waiving. No testing data on the registered substances was reported in any of the dossiers for this endpoint.

In four dossiers the endpoint was addressed by read-across from soluble metal compounds, where the metal was part of the compositions of the registered substances. Hence, soluble metal ions were assumed to be responsible for toxic effects and the particle nature of the registered substance was not addressed.

In 14 dossiers the endpoint was waived. Waiving was most often based on the following reasons:

- Insoluble nature of the substances (and hence non-bioavailable)
- Lack of acute toxic effects
- Natural abundance the substance in nature
- Low environmental exposure is expected
- Technical problems related to testing: difficult to maintain sufficiently high and constant concentration of the substance in the water.
- Formation of complexes with (in)organic molecules in water (and hence become non-bioavailable)

In relation to these waiving justifications it should be noted that the lack of short term toxicity (waiving argument for ten substances) does not justify omitting long term testing. Furthermore, as mentioned previously, “long-term aquatic toxicity study on fish (Annex IX, Section 9.1.6.) shall be considered if the substance is poorly water soluble” (REACH Annex VIII, 9.1.3). In the case of nanoparticles, the insoluble nature may not necessarily correspond to non-bioavailability. Insoluble nanosized particles may be bioavailable through different mechanisms, compared to water soluble chemicals, including e.g. phagocytosis. As described in the RIP-oN 2 Final project report “It is important to recognise that solubility and dispersability are two distinct phenomena” (RIP-oN2 Final Project Report, 4.1.10) and that “A dispersion of an insoluble material may elicit a different response from that anticipated from the classical molecular or elemental toxicity expected from the chemical composition” (RIP-oN2 Final Project Report, 4.1.11).

For one individual registration for a Category II substance, this endpoint was addressed by QSAR (ECOSAR) (See comments to *Acute toxicity to fish*, which would also apply here).

*In conclusion no testing data were provided for the registered substances for this endpoint. The endpoint was typically waived, but we assess that some of the waiving statements might be questioned for nanomaterials/nanoforms. In some cases, read-across to tests on specific metal main components provided information on the toxicity attributable to toxic metal ions, but did not address the possible effects of the substance in nanoparticulate form. For future considerations it may be appropriate to emphasise the importance of chronic tests especially for nanomaterials, which in many cases are insoluble by nature.*

#### 4.1.3.1.3 Short-term toxicity to aquatic invertebrates (Annex VII 9.1.1, IUCLID Section 6.1.3)

This endpoint was addressed for all 21 dossiers: In three dossiers solely by read-across, for one dossier by waiving and for one dossier by QSAR. Testing data on the registered substance was provided for 16 dossiers.

Tests of nanoforms were reported in 12 dossiers out of which 8 are considered to exist only in a nanoform (Category II). For these Category II dossiers, even not explicitly stated, it is therefore implicit that, when testing data was provided for the registered substance, the nanoform was tested<sup>28</sup>. However, no information on particles sizes, shapes, surface area etc. was not reported for any of the studies of the registered substances in these eight dossiers. For 5 of the Category II dossiers a WAF was tested and in some cases a WAF test was the only study included for this endpoint. In one dossier it was stated that “as a preliminary range-finding study showed that the *Daphnia magna* were possibly physically hampered by particles, and as the presence of particulate matter is not recommended in the OECD TG 202 for *Daphnia* tests, the final test was carried out on a water accommodated filtrate”. This issue will be discussed further in Section 4.2.3.3. For one substance a preliminary stability test was carried out and reported in IUCLID under ‘details on analytical methods’. Losses due to adsorption and/or insolubility were measured after a period of 48 hours in light or dark conditions at ambient temperature, and test samples were found to be stable. However, it is unclear whether THF (tetrahydrofuran) was used in the sample preparation and thus if these results can be applied to suspensions of the material used in the toxicity test or was related only to chemical analysis.

For the remaining four (Category I) dossiers, a nanoform of the substance was tested.

In one case the nanoform was assumed to represent a ‘worst case scenario’ due to the fact that it was expected to be more soluble as a result of smaller particle size and greater surface area compared to the bulk form. However, no information on particle size was provided.

In another dossier, where distinction was made between the bulk form and the nanoform in IUCLID Section 1.2., the two forms were addressed separately for this endpoint (5 studies were reported for the bulk form and four studies were reported for the nanoform). Information on particle sizes (or size limits e.g. <5 µm or a d50 value) was reported for part of the bulk studies and all nano studies. At least for nanoparticle studies the sizes probably refer to primary particle sizes though not explicitly stated. For some studies of a nanoform also information on specific surface area was reported. Influence of particle sizes was not discussed in the endpoint summary.

In a another dossier, no data on size was reported in the IUCLID sections on ‘Test materials’ but particle sizes were reported in a few (3 out of 46) endpoint entries in ‘Results and discussions’ in relation to the test results, when several sizes of NPs were tested within one study. No information on sizes of bulk forms tested was reported. The endpoint conclusion (EC50 >1000 / >10000 mg/L for freshwater/marine) was based on a few of these studies (with reliability 1 or 2) in a weight of evidence approach. However, similar EC values (>10 ->10000) were generally reported for supporting and disregarded

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<sup>28</sup> See footnotes in section 4.1.3.1.1

studies with a few exceptions possibly related to sample preparation method (use of tetrahydrofuran as vehicle) and illumination. A general issue with this dossier was that, for endpoints where several studies were included, the justification for the choice of reliability for the individual studies was not consistent. In general the assignment of reliability of different studies was justified based on use of guidelines and GLP compliance. However, there are examples of lack of consistency and transparency. For example one supporting read-across study, testing according to OECD 201 and ISO 6341, was assigned reliability 3 with one argument being that 'Effects based on total nominal concentrations, however undissolved particles removed by filtration prior to exposure'. On the other hand one study was assigned reliability 1 even though a WAF was tested and ECs were expressed as nominal concentrations. This was the only study which was given reliability 1. The basis for choice of key study is hence not completely transparent.

In another Category I dossier, with testing data reported for this endpoint, three studies on a metal oxide nanoform were included. No information on size was reported. The remaining 32 studies were on soluble forms of the metal and endpoint conclusion is based on effect values for soluble forms.

For the four Category III dossiers, data was provided for the registered substance but the possible nanosized fraction of the test material was not (explicitly) addressed.

For one dossier one out of 13 reported endpoint studies has tested the registered substance, but no information on size was reported. The remaining 12 studies were on the corresponding metal and metal salts.

For the remaining three nano-tail dossiers only one endpoint study was included in each dossier. In all three cases a water-accommodated fraction (WAF) of the registered substance was obtained (either by filtration or centrifugation of a stock dispersion) and tested. Results were then expressed as a loading rate rather than an actual concentration of the substance in the WAF. It should be pointed out that testing of a WAF is based on the assumption that it is the water soluble fraction, which is responsible for the toxic effects which may not be the case for some nanoparticle types. Also, the separation methods might allow for nanoparticles to be present in the WAF (depending on aggregation/agglomeration, filter sizes, centrifugation speed and time etc.) but investigations of the presence and size (distribution) of particles in the WAF were not reported in the dossiers. This will be addressed further in Section 4.2.3.3.

In three dossiers this endpoint was addressed by read-across. In one case read-across was done from a test of a WAF of structure-analogous substance. As this substance is also a particulate material the particle nature was therefore addressed - though not for the registered substance - even if the appropriateness of the preparation of the test solution can be questioned. In one metal oxide dossier (Category I), read-across was done from tests of the corresponding metal and a metal salts despite the fact that a study of the registered substance was reported in the dossier (disregarded study, reliability 3 due to insufficient documentation of test conditions). Finally in another case, read-across was done based on one study on a metal salt, where the metal corresponds to the metal in the registered substance. Hence the particle nature of the registered substance was in this case not addressed.

Again, for one dossier, this endpoint was addressed by QSAR (ECOSAR) (See comments to *Acute toxicity to fish*, which would also apply here).

*In conclusion, (similar to 'acute toxicity to fish') the testing data provided for this endpoint was generally supported by limited information on test materials (particle sizes, surface area etc) and on particle behaviour in test media (aggregation, agglomeration, sedimentation etc.). Differences between forms/sizes in relation to toxic effects were generally not discussed even if data for the different forms were available. Also, different methods for the preparation of the test solutions/suspensions were applied including direct addition and test of suspensions as well as preparation of WAFs by stirring and subsequent filtration. The appropriateness of these different methods and its implications for effects were not discussed. In weight of evidence approaches no distinction was made based on sample preparation.*

#### 4.1.3.1.4 Long-term toxicity to aquatic invertebrates (Annex IX 9.1.5, IUCLID Section 6.1.4)

In 2 of the 21 assessed dossiers, the endpoint was not addressed due to lower tonnage (1-10 and 10-100 tonnes/annum, respectively) and consequently reduced data requirements. This endpoint was addressed in 19 dossiers: in 4 dossiers solely by read-across, in 12 dossiers by waiving, in one dossier by QSAR and in two dossiers by experimental data.

In the two dossiers containing testing data (one Category I and one Category II) an experimental study was reported for the registered substance for this endpoint. However in one of the cases, for a nano-only dossier (Category II), the data was not yet available at the time of the registration and it was instead stated that 'the results and conclusions will be provided at a later stage'. For the other study, which is in a Category I dossier, the nanoform of the substance was tested. This dossier makes some distinction between nano and bulk form throughout, also in the endpoint discussion. The dossier includes one study with the nanoform (key study, reliability 2, data on particles sizes and surface area reported) and two studies with the bulk form (weight of evidence<sup>29</sup>, reliability 1 and purpose not specified, reliability 4). No information on size characterisation reported. However, for the derivation of PNEC values, the value from the nanoform study was not considered with the following explanation provided by the registrant: "due to the complexation of nanoparticles with nutritive algal cells, it cannot be excluded these effects were linked to food deprivation rather than impacts of" the substance. Hence the nanoform NOEC value was not considered to be reliable for the derivation of an aquatic PNEC. A NOEC of  $\geq 100$  mg/L (supernatant tested) from the bulk form key study was put forward in the endpoint conclusion in the dossier, disregarding lower NOEC values for the nanoform, for which the lowest NOEC (reproduction) was reported to be  $<18$  mg/L. The NOEC value for the bulk form was based on nominal loading rates and actual concentrations in the supernatant not determined analytically, which is likely to cause an overestimation of effect concentrations (and hence an underestimation of hazards). No PNEC value was calculated for the micron-sized form due to absence of effects. It was not discussed if this conclusion also applies to the nanoform.

This endpoint was addressed by read-across in four dossiers (covering all three Categories). In all cases the reported studies have tested mainly metal salts and in no

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<sup>29</sup> However, the tested substance has the same CAS no. as the registered substance

case was data on the registered substances provided. In one dossier 72 entries were reported for this endpoint but all were tests of metal salts. In another two dossiers, for two different substances containing the same metal in their chemical composition, 12 endpoint entries were included in the dossier of which 11 report tests on metal salts and one was a test of a metal powder. No information on characteristics (incl. size characterisation) of the metal powder was included in the study description. For another dossier, addressing this endpoint by read-across, two studies were reported. These studies were for two different metals, which were both part of the composition of the registered substance. In the endpoint conclusion it was stated that, due to the low solubility of the substance, “adverse chronic effects of the substance to aquatic organisms are not expected. However, a minor part of the substance may dissolve...()...(and the dissolved fraction)... may have chronic effects on aquatic invertebrates”. Hence, for all four dossiers the particle nature of the registered substance was not addressed and effects were considered to be related to metal ion toxicity.

This endpoint was waived in 12 dossiers. Again, waiving was most often based on the following reasons:

- Insoluble nature / low water solubility of the substance (and hence not bioavailable)
- Lack of acute toxic effects
- Natural occurrence of the substance in nature
- Low environmental exposure is expected
- Technical problems related to testing: difficult to maintain sufficiently high and constant concentration of the substance in the water.
- Formation of (non-bioavailable) complexes

Justifications based on lack of short term toxicity may not be appropriate as “long-term aquatic toxicity study on Daphnia (Annex IX, Section 9.1.5.) shall be considered if the substance is poorly water soluble” (REACH Annex VII, 9.1.1). (See comments to 4.1.3.1.2 ‘Long-term toxicity to fish’, which would also apply here).

In one dossier (Category I) the preparation of a WAF from a metal oxide substance resulted in a solution with a concentration of the corresponding metal below the detection limit. It was hence concluded that the study was not technically feasible and was therefore waived. Testing a particle suspension was hence not considered and the particle nature of the registered substance was not addressed.

For one dossier. a nano-only substance, this endpoint was addressed by QSAR (ECOSAR) (See comments to *Acute toxicity to fish*, which would also apply here).

*In conclusion this endpoint was waived for a large part of the dossiers. Only for two dossiers experimental data was provided, and the endpoint was addressed by read-across in four dossiers. Read-across was mainly done to tests on metal salts, assuming that the toxicity is related to metal ions and not addressing the possible effects of the substance in (nano)particulate form. For future considerations it may be appropriate to emphasise the importance of chronic tests especially for nanomaterials, which in many cases are insoluble by nature.*

#### 4.1.3.1.5 Toxicity to aquatic algae and cyanobacteria (Annex VII 9.1.2, IUCLID Section 6.1.5)

This endpoint was addressed for all 21 assessed dossiers: for four dossiers solely by read-across, for three dossiers by waiving, for one dossier by QSAR and test data on the registered substance was provided for 13 dossiers.

Tests on nanoforms were reported in 11 dossiers out of which 7 are considered only to exist in a nanoform (Category II). For these, even not explicitly stated, it is therefore considered implicit that the nanoform was tested. However, no information on particles sizes, shapes, surface areas etc. was reported for any of the studies of the registered substances. Furthermore, in five out of the seven Category II dossiers only one study was reported for each dossier and these were all testing a filtrate/supernatant/WAF. Issues of filtrate/supernatant/WAF testing and its' appropriateness to nanoparticles has been discussed briefly previously e.g. in Section 4.1.3.1.3 on 'Short-term toxicity to aquatic invertebrates' and will be addressed in more detail in Section 4.2.3.3 This is potentially an issue, which needs to be addressed for future registrations of nanoparticles.

For four Category I, dossiers, a nanoform of the substance was tested and the nanoform has hence been addressed or partly<sup>30</sup> addressed. In one case the nanoform was assumed to represent a 'worst case scenario' due to the fact that it is expected to be more soluble as a result of smaller particle size and greater surface area compared to the bulk form. However, no information on size characterisation was provided for this study. In another case, where distinction was made between the bulk form and the nanoform in IUCLID Section 1.2., the two forms were addressed separately for this endpoint, also in the endpoint discussion (3 supporting studies were reported for the bulk form and one key study was reported for the nanoform). Information on particle sizes (probably primary particle sizes, though not explicitly stated) was reported for one bulk study ( $d_{50} = 7.8 \mu\text{m}$ ) and the nano study (14, 20 and 29 nm). Specific surface areas ( $\text{m}^2/\text{g}$ ) for the nanoforms were also reported. No further characterisation information was reported. In the endpoint conclusion, despite an  $\text{EC}_{50}$  value of 10.2 mg/L for the nanoform, an  $\text{EC}_{50}$  value of >100 mg/L for the bulk form was put forward. In the derivation of PNEC values the nanoform study was disregarded as indirect effects (clustering between algal cells and nanoparticles) was considered a possible cause of observed toxicity. In another dossier (Category I) 17 endpoint entries were included and a distinction between nano and non-nanoforms was sometimes made in the test material descriptions. However, except for three endpoint entries (from two different literature sources) with information on particle sizes in the results section (where different particle sizes were tested in one study), no information on particle sizes, shapes, surface areas etc. was reported. In another Category I dossier most studies were for soluble forms and this endpoint was largely addressed by read-across. One study on the nanoform (30nm nominal particle size reported) was included but here the dissolved metal concentration was measured and effects were attributed to the dissolved fraction. The other endpoint entries were on soluble metal ions.

The endpoint was addressed by read-across in four dossiers. For one dossier (Category III), read-across was based on soluble forms of the corresponding metal. For another

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<sup>30</sup> Partly addressed is defined as a dossier endpoint for which some data is provided for the nanoform but where this data is not used as the basis of the endpoint conclusion

dossier (Category II) read-across was done based on a similar substance in particulate form. However, no information was reported that allows for comparison between the specific characteristics of the two tested substances with the respective registered substance.

The endpoint was waived for three dossiers. Waiving was based on

- insoluble nature of substance (and hence not bioavailable / likely to cause effects)
- Natural abundance of substance in nature
- Low toxicity of structure-analogous substance
- Low environmental exposure is expected

In conclusion experimental data on the registered substances was reported for slightly more than half of the dossiers. In general information on particles sizes, shapes, surface areas etc was lacking. For the Category II dossiers no such information was provided. For the Category I dossiers some information on particle sizes and specific surface area was provided for some studies. However, the extent of information varies and particle sizes were sometimes reported in the results section rather than in the material description. Except for one study, specifically considering the nanoform as a 'worst case', no endpoint conclusions were based on tests with nanoforms. In general a discussion on the influence of particulate nature on algae toxicity was limited and discussion of the influence of particle sizes was lacking.

#### 4.1.3.1.6 Toxicity to aquatic plants other than algae

For this endpoint no test data on the registered substance was provided in any dossier. The endpoint was addressed by read-across for three dossiers (one from each Category) based on data for soluble forms of the corresponding metals, which are part of the composition of the registered substance. The endpoint was waived for one dossier with the reason that "a complete endpoint study record is provided in 6.1.5<sup>31</sup>. Study not required under REACH". For 21 dossiers this endpoint was not addressed.

#### 4.1.3.1.7 Toxicity to microorganisms (Annex VIII 9.1.4, IUCLID Section 6.1.7)

This endpoint was addressed for all 21 assessed dossiers: for three dossiers solely by read-across, for nine dossiers by waiving and test data on the registered substance was provided for nine dossiers.

Tests on nanoforms were reported in eight dossiers out of which four were considered only to exist in a nanoform (Category II). For these, even not explicitly stated, it was therefore considered implicit that the nanoform was tested. However, no information on size characterisation etc. was reported for any of the test materials in these four dossiers. The descriptions of sample preparation varied from good (detailed description) to reasonable or limited. For all four Category II dossiers suspensions (unfiltered) were tested. For the remaining four Category I dossiers, a nanoform of the substance was tested. In one of these Category I dossiers 29 studies were included and a distinction between bulk and nano forms was sometimes made in the IUCLID endpoint entry, though little specific information on particle sizes etc. was reported (particle sizes

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<sup>31</sup> Toxicity to aquatic algae and cyanobacteria.

specified for one study (in results section, not in material description section) out of the 29 studies. Specific surface area was specified for seven studies in the test material description section). The endpoint conclusion was based only on one key study (reliability 1) for which no information on particle sizes was reported. Hence we conclude that the nanoform was not explicitly addressed. Other studies were given lower reliability often due to "Single species tests with... (name of microorganism)... are of low relevance for STP". Some of these other studies (where the registered substance was tested) report EC<sub>50</sub> values in the same range (>1000 mg/L). However, some (testing nanoforms) report values <500 mg/L with the lowest value of 100 mg/L (in sunlight).

In another Category I dossier, the nanoform was assumed to represent a 'worst case scenario' due to smaller particle size and hence higher solubility'. One endpoint study was reported for a material containing 'nano' in its name but no information on size was provided for this study. In a third dossier, where distinction was made between the bulk form and nanoform, two studies were reported for the bulk form and one study was reported for the nanoform. Some information on particle sizes was reported for one bulk study (< 5µm) and the nano study (9 nm). All three studies concluded that the substance is not harmful to microorganisms and the nanoform was explicitly discussed in the endpoint summary. For another Category I dossier, with data reported for this endpoint, the endpoint was addressed partly by read-across from soluble metal compounds. Out of a total of five endpoint entries two (both for a nanoform, on *B. subtilis* and *E. coli* respectively, same literature source) reported tests of the registered substance. As key study an activated sludge respiration test on a metal salt was however chosen and in the endpoint discussion it was stated that, out of the available studies, this study gives the lowest reliable EC<sub>50</sub> value. However, for the nanoform study (on *B. subtilis* and *E. coli*) a near-complete inhibition of *B. subtilis* growth was observed at the lowest tested concentration of 10 ppm (=10 mg/L) under both dark and illuminated conditions whereby a lower EC<sub>50</sub> for the nanoform cannot be excluded. It was further stated that toxicity seems not to be related to particle size although only data for one particle size was reported in the IUCLID dossier. It was specified that the test material was 'nanoparticles' but sizes were reported to be 420-640 nm (mean 480 nm). The registrant did not report whether this size characterisation was a measurement of primary particles or e.g. by light scattering techniques of suspension (aggregates/agglomerates). For the latter three mentioned dossiers it is concluded that the nanoform was either addressed or partly addressed.

For one Category III dossier two studies were included, for which the registered substance was tested. However, the purpose flag for both was reported as 'weight of evidence'. The descriptions of the studies were very limited and no information on particle sizes or sample preparation was reported.

The endpoint was addressed by read-across for three dossiers (Category I and III). One dossier (Category I) includes a study of the registered substance but was disregarded due to low reliability and *E. coli* as test organisms, which was considered not relevant. For this dossier the key study was a test of a substance containing the same metal as the registered substance and EC<sub>10</sub> expressed as dissolved metal concentration (measured). For the remaining two Category III dossiers the endpoint was addressed solely based on tests of metal ion toxicity.

The endpoint was waived for nine dossiers. Waiving was primarily based on:

- Substance will be removed in the primary settling tank before reaching the microorganisms / activated sludge (limitation of exposure)
- Will not be bioavailable due to complexation
- Will not be bioavailable due to insoluble nature
- Natural abundance of the substance (or main constituents of the substance e.g. metal)
- Low toxicity to other aquatic organisms
- Test method not technically suitable (for poorly soluble, particulate material)

With regards to the waiver based on limited exposure it should be noted that there is not yet sufficient information available to conclude that nanoparticles will be removed in primary setting tank from STP.

*In conclusion little information on particle characteristics, including particle sizes, was reported for the endpoint tests. In one case it remains unclear whether size measurement refers to primary particles or aggregates/agglomerates e.g. by light scattering techniques of suspension. This emphasizes the need of the registrant to specify if a given size refers to primary particle sizes or to the test material in the test suspension. The implications of particle sizes or nanoparticle nature of the test material was very seldom mentioned.*

#### 4.1.3.1.8 Toxicity to other aquatic organisms

This endpoint was waived for one dossier (as “no reliable data were found on other aquatic organisms”) and not addressed in the remaining dossiers.

#### 4.1.3.2 Sediment toxicity (Annex X 9.5.1, IUCLID Section 6.2)

In two dossiers the endpoint was not addressed due to low tonnage (1-10 and 10-100 tonnes/annum, respectively) and therefore reduced information requirements. The endpoint was addressed for 19 dossiers: in 2 dossiers solely by read-across, in 15 dossiers by waiving and test data on the registered substance was provided for 2 dossiers.

For one of these dossiers (Category I) the endpoint summary including key EC values reported for the substance were based on a key study (for freshwater) and a supporting study (for marine). The key study was a read across from a material containing >80% of the registered substance in its composition. No information on particle sizes etc. was reported. The supporting study was on the registered substance but it is not evident from the material description whether it was on a nanoform. For the second dossier, which is a Category II dossier, the registered substance was tested. As described for previous endpoints it is hence considered implicit that a nanoform was tested. Only one study was reported for this endpoint and no discussion of size dependent effects was reported. The dossier did not contain any detailed information regarding the size characteristics of the test material. This issue also applies to the endpoints on acute toxicity to fish, aquatic invertebrates and algae for the same dossier.

The endpoint was addressed by read-across for two dossiers. In both cases the particle nature of the substance was not taken into account as read-across was done from

soluble metal compounds. Results are hence relevant for the soluble fractions of the substances but particle specific effects were not addressed or discussed.

The endpoint was waived for 15 dossiers. Main reasons for waiving are:

- Insoluble, inert nature of the substance and hence low bioavailability
- Natural abundance of the substance
- Lack of ecotoxic effects to pelagic organisms
- Lack of ecotoxic effects to soil organisms or birds
- The substance is unlikely to end up in sediment
- Similarity with inorganic soil/sediment matter
- Low water solubility and hence not present in pore water.

In one dossier, where a distinction was generally made between nano and bulk forms, it was specified that the waiver was for the bulk form. No waiver was included for the nanoform. The justification for the waivers did not include a specific discussion of the nanoforms in any of the dossiers.

Regarding the waiving based on exposure considerations ("the substance is unlikely to end up in sediment") it should be considered that soils and sediments are expected to be sinks for (nano)materials. This was also mentioned in the CSR of one dossier, stating that "The deposition in soil or sediments is therefore the most relevant compartment of fate of... (the registered substance)... in the environment." Still, for this dossier all tests on sediment and soil organisms have been waived due to the high tolerance by aquatic organisms and natural abundance of the substance. The exposure of sediment organisms may be significantly (quantitatively and qualitatively) different from exposure to organisms in the water column. This makes toxicity studies on soil and sediment organisms relevant. Regarding waiving based on low water solubility it should firstly be noted that, as a result of smaller particle sizes, metal and metal compound nanoparticles (including metal oxide nanoparticles) may in general have different ion release kinetics compared to larger sized particles. Waivers based on low water solubility may therefore not apply to the nano-form. Secondly, with a view to current discussions in the scientific literature, the contribution of particle effects to nanoparticle toxicity is still not well understood. It is true that ions, dissolved from metal and metal containing nanomaterials (such as metal oxide nanoparticles), seem to have a key role in the toxicity observed in aqueous organisms. Though attributing effects only to ion release may hold true for bulk materials, attributing effects solely to ion release is not scientifically justified in case of nanoparticles at present.

*In conclusion only few experimental results were reported for this endpoint and without information or discussion of the influence of particle sizes. In other dossiers read-across was done to soluble metal compounds based on the assumption that toxicity can be attributed to metal ions. The fact that sediments are expected to be sinks for (nano)materials should be considered in relation to information requirements for nanomaterials.*

#### 4.1.3.3 Terrestrial toxicity

##### 4.1.3.3.1 Toxicity to soil macro-organisms except arthropods (Annex IX 9.4, Annex X 9.4, IUCLID Section 6.3.1)

In two dossiers the endpoint was not addressed as this was not an information requirement at its tonnage band (1-10 and 10-100 tonnes/annum, respectively). The endpoint was addressed for 19 of the 21 assessed dossiers: in 2 dossiers solely by read-across and in 14 dossiers by waiving. For this endpoint test data on the registered substance was provided for 3 dossiers.

In all three cases, data for an acute test (14 days) on earthworms was included. No long-term test data was reported. For two of these dossiers long-term testing was waived with the argumentation that such a study was scientifically unjustified due to the natural occurrence and low toxicity of the substance. In one dossier (Category I), specifically for a nanoform of the substance, effects on the gene expression, growth, fertility and survival of the soil nematode (*Caenorhabditis elegans*) was investigated after a 24 hour exposure period. In this dossier, where distinction was made between the bulk form and the nanoform in IUCLID Section 1.2., the two forms were addressed separately for this endpoint. Two studies (one key study with reliability 1, one with purpose and reliability not specified) were reported for the bulk form and one study (reliability 3, purpose not specified) was reported for the nanoform. However, in the endpoint discussion it was stated, for the nanoform study, that "while the publication contains some experimental details, the tested species and the applied methodology are not sufficiently standardized to reliably conclude on the toxicity of nanoparticulate...(registered substance)... on soil macro-invertebrates" and the endpoint conclusion was therefore based on data for the bulk form (NOEC > 1000 mg/kg dry soil). For the studies with nanoform 20% effect on survival was seen at 1 mg/L. However different species and only one concentration was tested, making a direct comparison difficult. Information on particle sizes (15 and 454 nm) and specific surface areas (in m<sup>2</sup>/g) was reported for the nanoform study. No further characterisation information was reported for the nanoform and no information on sizes etc., was reported for the bulk form.

For another Category I dossier, the nanoform was assumed to represent a 'worst-case' and data on the nanoform was therefore reported through a key study with reliability 1. Though the study has tested the nanoform of the substance, 'no' was selected in the IUCLID tick box regarding if "identity of test material same as for substance defined in Section 1 (is not read-across)". The reason for this was not discussed/mentioned but it is possible that the tested material did not correspond to the specific nano reference test material specified in IUCLID Section 1.2. The study contains little information about test material (e.g. no info on particles sizes, surface areas etc.). The description of the testing method and exposure conditions for the study was good.

In a third (Category III) dossier the test substrate preparation was well described but (besides appearance, purity and density) no information on particle characteristics was reported.

In general, characterisation of the nanoparticles in the test system was missing for studies in all three dossiers. However, for verification and characterisation of nanoparticles in soil, development of appropriate analytical methods is needed. In

general the applicability of soil tests for nanomaterials has to be investigated further (RIP-oN 2 Final project report). Furthermore, long term testing may have been more appropriate based on the physico-chemical properties of the substance. As mentioned in REACH (Annex IX, 9.4, Column 2) for 'Effects on terrestrial organisms' that "In particular for substances that have a high potential to adsorb to soil or that are very persistent, the registrant shall consider long-term toxicity testing instead of short-term"<sup>32</sup>.

The endpoint was addressed by read-across for two dossiers. Read-across was based on tests on metal ions and the particle nature of the registered substance was hence not addressed.

The endpoint was waived for 14 dossiers (all Category II) dossiers have either been waived or not addressed). Waiving was based on:

- Read-across data available for toxicity to terrestrial arthropods
- Natural abundance of substance (or substance component(s)) in the soil
- Considered to be essentially a mineral fraction of soil already
- The substance is not expected to enter into the terrestrial environment to any great degree.
- Inert, insoluble nature of substance
- The substance is not acutely toxic (e.g. no effects on terrestrial plants or birds).
- Not classified and hence no exposure assessment is necessary. Therefore no reason that triggers such a study.

In one case it was specified that the waiver justification (based on natural occurrence) only applies to a high-purity form of the substance and that heavy metal and organic impurities "must be dealt with separately and evaluated on a case-by-case basis, according to presented information regarding specific products/batches". However, no specific considerations related to potential nano-specific properties or nanoforms of the substances were discussed in any of the dossiers.

For one Category II) dossier, it was at one hand stated as a reason for waiving that the "majority of the applications for which this substance is used will not result in the material being released to the soil" However, at the same time it was noted that a commercial product of the substance exist, has agricultural uses. Based on this information the waiving of the endpoint seems inconsistent.

*In conclusion only few experimental results were reported for this endpoint and with little information on particle sizes etc. In other dossiers read-across was done to soluble metal compounds based on the assumption that toxicity can be attributed to metal ions. The fact that soils are expected to be sinks for (nano)materials should be considered in relation to information requirements for nanomaterials, and thus waiving may not be appropriate for this endpoint.*

#### 4.1.3.3.2 Toxicity to terrestrial arthropods (Annex IX 9.4, Annex X 9.4, IUCLID Section 6.3.2)

In two dossiers the endpoint was not addressed due to lower tonnage (1-10 and 10-100 tonnes/annum, respectively) and therefore reduced data requirements. For another one

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<sup>32</sup> Regarding 'persistence' in relation to nanomaterials see section 4.1.5.

dossier this endpoint was not addressed but the reason is unknown. Other terrestrial toxicity endpoints in the same dossier have been waived based on exposure consideration (substance not expected to end up in the soil). The endpoint was addressed in 18 dossiers: in 2 solely by read-across, in 15 by waiving and test data on the registered substance was provided for 1 dossier.

For this (Category I) dossier four entries were included for this endpoint, with data from two different sources (one study report and one scientific publication). One study was for a substance with a >80% content of the registered substance (low solubility) and was thus used for read-across (key study, reliability 2). The other study was for a nanoform (supporting, reliability 3 due to exposure through diet, not soil, and not a guideline study). It is difficult to compare test results due to differences in exposure. Both IUCLID entries lack detailed information about how the study was conducted. For example no size or size distribution was reported. Endpoint conclusions were based on read-across and the study of the nanoform (supporting study) was not discussed. Only the read-across study (key study) forms the basis of the endpoint conclusion and the reported NOEC value. The nanoform was hence only partly addressed as some data was provided for the nanoform but not used in the endpoint conclusions.

This endpoint was addressed exclusively by read-across data for two dossiers. The read-across was based on tests of the toxic metal ions and the particle nature of the registered substance was hence not addressed.

The endpoint was waived for 15 dossiers. Waiving was based on:

- No ecotoxic effects to soil macro-organisms / terrestrial plants / aquatic organisms / birds.
- Inert, insoluble nature of the substance (and hence not bioavailable)
- Natural abundance of substance (or substance component(s))
- Considered to be a mineral fraction of soil.
- Non-classified substance (no risk assessment required, and thus, the PNEC does not need to be refined)
- No release to soil expected (exposure considerations)

As for the endpoint 'Toxicity to soil macro-organisms' for one Category II dossier, exposure considerations one hand were used as a reason for waiving. However, at the same time it was noted that the substance has applications in agriculture and horticulture. Based on this information the waiving of the endpoint seems inconsistent. Similar, for one Category I dossier it was stated that the substance is used as inorganic fertiliser. In this case waiving was based on the natural abundance of the substance. However, the (nano)particulate nature of the substance was not taken into consideration.

*In conclusion only test data from one experimental study on the registered substance was reported for this endpoint and without information on particle sizes etc. In other dossiers read-across was done to soluble metal compounds based on the assumption that toxicity can be attributed to metal ions. The fact that soils are expected to be sinks for (nano)materials should be considered in relation to information requirements for nanomaterials and may not be appropriate as a waiving justification for toxicity to terrestrial arthropods.*

#### 4.1.3.3.3 Toxicity to terrestrial plants (Annex IX 9.4.3, Annex X 9.4.6, IUCLID Section 6.3.3)

In two dossiers the endpoint was not addressed due to lower tonnage (1-10 and 10-100 tonnes/annum, respectively) and therefore reduced data requirements. The endpoint was addressed in 19 dossiers: in 3 solely by read-across and in 13 by waiving. For this endpoint test data on the registered substance was provided in three dossiers.

These three are all Category I dossiers. For two of these dossiers long-term testing was waived and for the last, no long term data was provided. Regarding acute toxicity, for one dossier only a nanoform was tested (assumed to represent a 'worst case'). Few data about the test material were reported but the description of the testing method and exposure conditions was good. For the other two dossiers a nanoform was tested (reliability 2) in combination with a bulk form (reliability 1) or soluble metal compounds, respectively. For the dossier containing a study on a bulk form and a nanoform, separate discussions were reported in the endpoint summary. For the bulk form no toxic effects were observed. For the nanoform a reduction in root elongation of lettuce was observed but no effects were found for six other plants. However, in the 'short description of key findings' in the endpoint summary, only bulk data were mentioned and no discussion on the influence of particle sizes was included. For the dossier containing data on soluble metal compounds (10 studies) and nanoforms (2 studies) all studies have been assigned reliability 2. In the endpoint summary the tests of the nanoform was not mentioned. NOEC and EC10 values for the metal ion were reported and selected for PNEC derivation. No discussion of dissolved metal versus particulate material was reported. Hence, though two studies on a nanoform were included in the dossier the particle nature on the substance was not addressed in the endpoint summary.

The endpoint was addressed by read-across for three dossiers. In two cases metal ions have been tested and hence the particle nature of the substance was not addressed. For the third dossier one study was included for read-across for a substance with a >80% content of the registered substance (low solubility) (key study, reliability 1). An additional study on a metal salt (supporting study, reliability 3) was included. For both studies there was a general lack of detailed information. For the key study no information was included regarding particles sizes etc. No consideration or discussion of a potential nanoform of this substance was reported in the dossier.

The endpoint was waived for 13 dossiers. Waiving was based on:

- Natural abundance of the substance (or substance component(s))
- Exposure to soil compartment is expected to be insignificant
- Inert / insoluble nature of the substance (Not bioavailable / Insoluble in water hence uptake by plants through the root system can be excluded).
- Not acutely toxic to animals via oral exposure route
- Considered to be a mineral fraction of soil.
- No hazard classification and hence no exposure assessment and risk characterisation is required.

Regarding the lack of acute toxicity to animals via oral exposure it is unclear why this waiving is relevant for toxicity to terrestrial plants.

*In conclusion only test data from one experimental study on the registered substance was reported for this endpoint and without information on particle sizes etc. Read-across*

was done to soluble metal compounds based on the assumption that toxicity can be attributed to metal ions. The (nano)particle nature on the substances has generally not been addressed in the endpoint summaries. The fact that soils are expected to be sinks for (nano)materials should be considered in relation to information requirements for nanomaterials and may not be appropriate as a waiving justification for toxicity to terrestrial plants.

#### 4.1.3.3.4 Toxicity to soil microorganisms (Annex IX 9.4.2, IUCLID Section 6.3.4)

In two dossiers the endpoint was not addressed due to lower tonnage (1-10 and 10-100 tonnes/annum, respectively) and therefore reduced data requirements. The endpoint was addressed in 19 dossiers: in 3 solely by read-across and in 14 by waiving. For this endpoint test data on the registered substance was provided in two dossiers.

In one dossier only a nanoform was tested (assumed to represent a 'worst case'). The test procedure was well described but details on the test material (including particle size) was lacking. In another dossier, which for other endpoints generally makes a distinction between bulk form and nanoform, only test data for the bulk form was reported. The study procedure and sample preparation was generally well described but no information on particle sizes etc. was reported.

The endpoint was addressed by read-across for three dossiers. In two cases metal ions have been tested and hence the particle nature of the substance was not addressed. For the third dossier one study was included for read-across for a substance with a >80% content of the registered substance (low solubility) (key study, reliability 1). An additional three studies on a metal salt (supporting study, reliability 3) were included. Also one study of the registered substance was included (reliability 3). The reason for the assignment of low reliability to this study was that only one concentration was tested and the organisms were not exposed in soil. No consideration or discussion of potential effects of the nanoform of this substance was reported in the dossier.

The endpoint was waived for 14 dossiers. Waiving was based on:

- Natural abundance of the substance (or substance component(s))
- Exposure to soil compartment is expected to be insignificant
- Inert / insoluble nature of the substance (Not bioavailable).
- Not acutely toxic to animals via oral exposure route
- Considered to be a mineral fraction of soil.
- No hazard classification and hence no exposure assessment and risk characterisation are required. Therefore no reason that triggers the performance of a soil microorganisms study.
- No ecotoxic effects to other microorganisms.

In relation to the waiving in two dossiers (containing the same metal in their composition) one short-term and one long-term study with *Eisenia Andrei* (tiger worm) using soluble metal salts were briefly described. It was stated that the studies are "presented for completeness, but are not considered relevant for assessing" the specific metal compounds "being assessed in the dossier". Firstly, as the waiving, as well as the majority of endpoint test data reported in the dossier, was based on the metal and metal salts, this seems like a contradicting statement. Secondly, the relevance of studies on worms in relation to soil microorganisms is unclear. This information would in any case have been more relevant in relation to soil macro-organisms.

For six dossiers this endpoint was not addressed. In two dossiers the endpoint was not addressed due to lower tonnage (1-10 and 10-100 tonnes/annum, respectively) and therefore reduced data requirements.

*In conclusion only few experimental results were reported for this endpoint and with no information on particle sizes etc. In other dossiers read-across was done to soluble metal compounds based on the assumption that toxicity can be attributed to metal ions. In another case read-across was done to a substance with >80% content of the registered substance but with insufficient information (and no information on particle sizes) to allow for an assessment of the likeness of the two substances (registered and tested). The fact that soils are expected to be sinks for (nano)materials should be considered in relation to information requirements for nanomaterials and may not be appropriate as a waiving justification for soil microorganisms.*

#### 4.1.3.3.5 Toxicity to birds (Annex X 9.6.1, IUCLID Section 6.3.5)

In two dossiers the endpoint was not addressed due to lower tonnage (1-10 and 10-100 tonnes/annum, respectively) and therefore reduced data requirements. This endpoint was addressed for 19 of the 21 assessed dossiers: in 1 dossier by read-across and in 18 dossiers by waiving. For this endpoint no dossiers provide test data on the registered substance.

For one dossier the endpoint was addressed by read-across from a study of the toxicity of the registered substance to hens. For this study no information on the test material was given and reliability 4 was assigned.

The endpoint was waived for 18 dossiers. Waiving was based on:

- No indications for bioaccumulation / low bioaccumulation potential
- Large mammalian dataset
- Natural abundance of the substance (or substance component(s))
- Essential element
- No evidence of toxicity in mammalian toxicity tests (repeated dose or reproduction test)
- Exposure to the soil compartment is expected to be negligible
- Insoluble nature of the substance (not bioavailable / not expected to bioaccumulate)
- The potential for the substance as particulate material to be consumed by birds is negligible.
- The substance is not classified (no reason that triggers the performance of a toxicity study in birds)

For six dossiers this endpoint was not addressed. In two dossiers the endpoint was not addressed due to lower tonnage (1-10 and 10-100 tonnes/annum, respectively) and therefore reduced data requirements.

In conclusion no dossiers provide test data on the registered substances. It should be noted that this is not an information requirement unless a risk is identified in the CSR (particularly, for potential PBT substances).

#### 4.1.3.4 PNECs

Four dossiers reports derived PNEC values for all exposure situations (oral, aquatic, STP, soil & sediment). In other dossiers PNEC values were reported for some exposure routes. Aquatic compartment (11 dossiers) and STP (10 dossiers) were the ones where a PNEC value was most often reported, whereas soil, sediment and oral PNECs were more often not derived.

For one dossier (Category I), for which PNEC values were reported for all exposure routes, a large number of studies were included for most endpoints, both for nanoforms and bulk forms. It was stated that reliable results, covering a range of different particle sizes, were taken into account in the derivation of PNEC values. The assignment of reliability to the individual studies was based on the use of guidelines and documentation of test procedure. However, the assignment of reliability is not completely transparent and the use (or disregard) of reliability 2 studies in the read-across was not discussed. Some supporting studies (reliability 3, '*In vitro studies, insufficient information reported on test method*') report EC<sub>10</sub> and LOEC values of 10 and 50 mg/L (tests of nanoforms), respectively. However an EC<sub>50</sub> value of >1000 mg/L (based on studies with reliability 1 (and one with reliability 2), weight of evidence studies, tests of bulk form, nanoform and material with size not specified) was reported in the endpoint study summary and was used for the derivation of a PNEC value.

In some cases PNEC values were based on read-across data for toxicity of soluble metals. For example, for one metal oxide Category I dossier, except for PNEC oral, all PNEC values have been derived. However, most studies used in the derivation have been performed with soluble metal compounds such as metal salts and hence not with the registered substance and not considering any possible particle effect. For this dossier, a few metal oxide nanoform studies were reported from literature sources (algae and daphnia). For example, for acute toxicity to aquatic invertebrates there was in total 27 studies of which 13 were done with metal salts, 11 were done with the metal (assumed also to be soluble metal compounds) and 3 done with metal oxide NPs. The studies using nanoparticles have not been considered for the determination of PNEC values (or for determination of a separate nano PNEC) but were described in an annex of the CSR. The PNEC values have instead been calculated mainly from results with metal salts (only one study on bulk form of the registered substance included). By taking into account all the studies reported in IUCLID, the registrant supports that lowering the AF from 1000 to 1 was justifiable. If a separate PNEC value for the nanoform would have been determined, the AF would be higher due to the fewer available data.

For one dossier, where nano and bulk forms were addressed separately in the dossier, the IUCLID fields under 'Ecotoxicological information' (PNEC values) were addressed for the bulk form. No PNEC values were derived for the bulk form and a justification was provided. A discussion of PNEC values in relation to the nanoform was included in the discussion box but no PNEC value was reported. . For the nanoform it was argued that it is not environmentally hazardous despite a study reported in the dossier concluding that the nanoform was found "harmful to *Pseudokirchneriella subcapitata* under the tested conditions". The justification for not deriving a PNEC value was the lack of short-term effects on fish and daphnids, NOEC values for *P. subcapitata* > 1 mg/l, a NOEC for chronic toxicity to daphnids > 1 mg/L, and hence substance not classified regarding its

environmental impacts. This contradicts the conclusion that the substance was found to be harmful to algae.

In some cases derivation of PNEC values are based on tests where a filtrate/supernatant/WAF was tested. As a way of testing the toxic effects of poorly soluble substances it is suggested in the 'OECD Series on Testing and Assessment' No. 23 that a WAF is prepared. For example for the endpoint 'Short term toxicity to fish' 9 out of 14 dossiers, providing data on the registered substances, included test data based on filtrate/supernatant/WAF testing. In some cases this study was the only data provided for the endpoint. In other cases data from filtrate/supernatant/WAF testing was provided in combination with other studies testing an unfiltered suspension of the substance.

It should be noted that this method may not be appropriate for testing of nanoparticles. This is discussed further in Section 4.2.3.3. It should be noted that PNEC values should be based on actual (dissolved and particle) concentrations and not nominal concentrations or WAF loading rates. Otherwise this leads to higher PNEC values and hence an underestimation of risks when RCRs are calculated. Also solubility of the test substance should be taken into account.

The argumentation for not deriving the PNEC values for the different compartments include that the substance is:

- Not (or unlikely to be) acutely toxic
- Not bioaccumulative
- An essential element for organisms
- No or insufficient data available
- No exposure of sediment expected
- No data on secondary poisoning available.

With regards to waiving of PNECs for soils and sediments it should be considered that soils and sediments are expected to be sinks for (nano)materials with low solubility. The exposure of sediment organisms may therefore be significantly (quantitatively and qualitatively) different from exposure to organisms in the water column. This potentially makes toxicity studies on soil and sediment organisms highly relevant for the hazard identification for nanomaterials and could be proposed as a testing requirement, which would also enable the estimation of PNEC values for these compartments.

#### **4.1.4 PBT assessment (Annex XIII)**

For six dossiers the PBT assessment was not addressed. Out of those four are member dossiers, where the issue was addressed in the lead registration. For one of the remaining two (Category II) dossiers, the production volume is <1 tonne/annum and for the second dossier no specific reason for not addressing PBT properties is mentioned. For the remaining 19 dossiers (all considered inorganic substances) PBT assessment has either been waived and/or the substances were reported not to be considered a PBT or vPvB. The general waiving argument was that the substance is inorganic with reference to Annex XIII: "this annex shall not apply to inorganic substances". REACH Annex XIII is specifically mentioned in nine dossiers while another six dossiers use the same argumentation (the substance is inorganic) as a reason for waiving PBT assessment, but without specific mentioning of the Annex XIII. For four dossiers other argumentation was reported such as:

- The substance is a natural element / abundant in nature

- PBT assessment is not relevant for metals
- Although it would be considered persistent, it has no bioaccumulative potential
- The substance will dissociate and lose its identity.

Other arguments mentioned in relation to waiving of the PBT assessment – or for concluding that the substance is not PBT or vPvB – are discussed below.

For one substance the argument was that it is not considered to be bioaccumulative and was not classified as CMR and that there was no other evidence of chronic toxicity. The substance in this dossier was not considered a vPvB or PBT substance, but this was based on the lack of data. The substance is very likely a vP substance (which was also mentioned in two individual submissions for the same substance), and due to its size the potential to cross membranes and to bioaccumulate has to be investigated. A more detailed assessment on the toxicity would be needed since the data presented in the dossier did not investigate a potential nano-effect since most often organisms were exposed to the WAF of the suspension.

In one dossier it was stated in the CSR that the criterion ‘persistence’ “is not relevant for the metal and its inorganic compounds in a way as it is applied to organic substances”. This is based on the text in Annex XIII of REACH on criteria for the identification of PBT and vPvB substances, in which it is specified that it does not apply to inorganic substances also reflecting the OECD view on persistency as non-biodegradability. Though this is the commonly agreed interpretation of ‘persistency’ it has been expressed, e.g. by Skeaff et al. (2002)<sup>33</sup>, that when a substance (in this case metal and metalloid) is considered “persistent in the periodic table sense, but without a linkage to bioavailability, this persistence has no relationship to environmental hazard”. Hence if a nanomaterial is found to be bioavailable and the bioavailability is unchanged over time (e.g. the nanomaterial is not transformed into a non-bioavailable form) it could be seen as persistent. Though particularly relevant for nanoparticles, due to different bioavailability (ways of crossing biological membranes), this is part of a broader discussion which needs to be clarified in relation to the P criterion and its applicability to inorganic substances in general – not only nanomaterials.

With regards to bioaccumulation, it should be noted that the potential bioaccumulation of nanomaterials is unknown, particularly if these are coated. Due to their small size and potential to cross membranes, the bioaccumulation potential is an important parameter to investigate. Regarding toxicity, the reported testing data was often not based on an investigation of potential particle-related effects. This was especially the case for dossiers for which endpoints are addressed by read-across from soluble metal compounds or where testing was done by exposing organisms to a WAF of the test substance suspension. More detailed assessment on bioaccumulation and toxicity of particulate forms seems therefore justified.

Hence, depending on the view on the P criteria, potential PBT properties of the nanoform of the registered substances cannot be excluded, although the registrants use the ‘inorganic clause’ in Annex XIII.

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<sup>33</sup> Skeaff, J.M., Dubreuil, A.A., Brigham, S.I. (2002) The concept of persistence as applied to metals for aquatic hazard identification. *Env Tox & Chem* 21 (12) 2581-2590

#### **4.1.5 Classification and labelling (C&L) (Human Health and Environment) (Section 4 of Annex VI, IUCLID Section 2)**

In three of the assessed dossiers with C&L information, a classification according to Annex I of Directive 67/548/EEC (DSD) was reported. For the remaining dossiers, self classification by the registrant according to GHS criteria was applied. It should be noted, that self-classification proposals have in general not been discussed and agreed by expert groups, which in some cases may come to different conclusions (e.g. whether tumours seen in animals are based on a mechanism, which is considered relevant for humans).

For five of the analysed substances (covering seven dossiers), a classification as dangerous for human health (five substances) and/or the environment (four substances) was suggested. The other dossiers proposed “no classification” due to either: 1) Conclusive but not sufficient for classification or 2) data lacking. As a consequence few exposure assessments and risk characterisations were provided in the dossiers analysed and assessed, although some provided information on a voluntary basis (see Chapter 5 for further details).

The two Category I dossiers which used the nano-picklist in section 2.1, included a C&L proposal for the nanoform of the substance. For both, no classification as dangerous was proposed.. For one, the justification for the nanoform (most endpoints “data lacking”) differed from the bulk form (most endpoints “conclusive but not sufficient for classification”).

With regard to classification and labelling, as it is stated in the RIP-oN 2 Final Report, it is recognized that a change in physico-chemical properties may impact intrinsic properties. Hence information, forming the basis for classification, should relate to the “form or physical states in which the substance is placed on the market and in which it can reasonably be expected to be used”. If information is only available for a different form, e.g. a form with larger particle sizes, it “should be assessed whether this information is also applicable to nanomaterials” (RIP-oN2 Final Report, 3.3.25-26).

##### **4.1.5.1 Human Health**

Five of the assessed substances (seven dossiers) were classified with regard to human health.

One Category I substance had different classification for standard and lower grades of the substance. While the standard grade had no classification, the lower grade was classified (due to impurities > 0.3 %) for acute oral and inhalation toxicity, as well as for chronic toxicity and reproductive toxicity. However no considerations of different particle sizes were addressed.

In one Category II dossier, classification for carcinogenicity in Cat. 2 (GHS) was proposed. For the same substance there were two more dossiers (one using the picklist (nano), the other one not specific) available, which on the contrary discuss that classification for carcinogenicity is not warranted due to rat lung overload phenomena.

Another Category II dossier for a low tonnage non-phase-in nanomaterial proposed classification as “H317: May cause an allergic skin reaction”.

In two Category III dossiers human health CMR classification was proposed based on grouping and toxic ions.

The conclusions for no classification were for most endpoints and for most substances: “conclusive but not sufficient for classification” and only in few cases “data was lacking”. Respiratory sensitisation was one endpoint where data was lacking for several substances. For individual substances data was lacking for endpoints such as: ‘Toxicity to reproduction - breastfed babies’ or: ‘Acute toxicity – dermal, Aspiration hazard’ and ‘Effects on or via lactation’

#### **4.1.5.2 Environment**

With regard to classification and labelling for environmental hazards, four substances (six dossiers) were classified for environmental hazard. Four substances were classified according to the GHS in relation to ‘hazards to the aquatic environment (long-term)’: three as ‘Aquatic Chronic 1’ and one as ‘Aquatic Chronic 4’. Correspondingly, these same four substances were classified as ‘N, R50/53’ (Substances or preparations that are dangerous for the environment, very toxic to aquatic organisms/may cause long term adverse effects in the aquatic environment) (three substances) and ‘R53’ (may cause long term adverse effects in the aquatic environment) (one substance) according to the DSD.

In one Category II dossier, the registrant divides the substance in low and high grades in the CSR (but not in IUCLID). The high grade is not classified for the environment. However, the hazard data for the low grade substance leads to ‘inconclusive’ data for C&L purposes. This issue was further justified in the CSR: the low grade of the registered substance contains impurities that will dissolve and leach toxic metal ions (DSD classified element as aquatic chronic 4) to aquatic organisms. Based on this it is proposed that the lower grade of the registered substance should be classified as Aquatic Acute 3/Aquatic Chronic 2 or 3 Category. For further clarification on this matter a long term study on *Daphnia magna* was ongoing at the time of the registration of the dossier.

In the 21 dossiers assessed, no substances were classified as ‘hazardous for the ozone layer’ due to ‘data lacking for this endpoint’ (10 dossiers) or ‘conclusive but not sufficient for classification’ (11 dossiers).

#### **4.1.6 Summary - Main Issues – Human Health and Environment endpoints**

In this section, main findings identified through the detailed analysis and assessment of the 21 dossiers containing information for these endpoints, are presented. As evidenced from the discussion, it has not always been possible for the assessors to explicitly verify whether and how the registrants intended to address nanoforms/nanomaterials covered by the registrations. Consequently, some of the assessors conclusions might deviate from the registrant intentions. On the other hand, this supports the options presented in

Section 3.2 and 4.2 relating to a distinct description and assessment of nanoforms/nanomaterials addressed by a given registration dossier.

### **Testing proposals**

No testing proposals have been identified for environmental fate or ecotoxicology. Four testing proposals for human health have been identified in the 21 dossiers analysed and assessed. Three of them were identified in Category I dossiers and addressed:

- OECD Guideline 413 (Subchronic Inhalation Toxicity: 90-Day)
- OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)
- OECD Guideline 414 (Prenatal Developmental Toxicity Study) for two substances

The first test proposal (for a Category I substance) specified the test material as the registered substance, but it was not indicated which of the compositions (bulk or nano) listed in 'IUCLID Section 1.2, Composition' would be tested.

The latter two test proposals, which were for one different Category I substance, intend to make the studies not with the registered substance but with a related substance (a metal salt). No specific justification for testing this substance was provided, but read across to the same related metal salt was performed for several endpoints with the justification that both substances dissociate in water to the same leading cation, responsible for the potential effects. For these two test proposals for a related substance it has been assessed that possible (nano)-particle specific effects were not addressed.

The fourth test proposal for a Prenatal Developmental Toxicity Study (OECD Guideline 414) has been identified for a Category III substance, but without any indication of the particle size of the test material to be investigated.

In none of the test proposals the test species were specified.

For information, also one testing proposal was identified in one of the 20 dossiers, deselected after the first detailed analysis and assessment (Category IV). This was not subjected to further detailed analysis and assessment.

One Category I dossier, that used the IUCLID picklist (nano), referred to the current test programme of nanomaterials under OECD and that additional tests would not be warranted at this time. The dossier would be updated as soon as the new data becomes available.

In this context it should be noted, that no evaluation/compliance check has been carried out by ECHA yet. Therefore it cannot be judged based on this information how many additional testing proposals would actually be required according to the legal requirements. For any test proposal it would be relevant that the registrants provide characterisation of the test material up front to allow the regulator to evaluate whether the proposed test, the proposed test material and metrics are the most suitable to address data gaps in a registration. This is especially important, if the registration covers multiple forms of a substance.

### **Metrics**

For human and environmental inherent properties no other metrics beside mass were identified to be used for deriving conclusions on the hazard of the substance. No

discussion in relation to whether mass is the most relevant metric has been found in the dossiers. In line with this, the traditional mass based metric has been used for drawing conclusions and deriving DNELs and PNECs. Two dossiers presented/discussed other metrics in the exposure assessment, see Chapter 5.

### **Relation between forms/substance registered and 'what is tested' in the endpoint specific sections**

The following is closely linked to the findings, conclusions and options identified in relation to substance identification and physico-chemical properties (in particular granulometry) concerning the need for a clear discussion of scope of the registration, in particular in relation to the forms included and addressed in a registration. See Chapter 3.

#### **Characterisation of test material:**

*Identification of, and information on, forms tested:* For (eco)toxicity and environmental fate studies detailed information, on different forms tested, were often *not* specifically given within a registration dossier. Some studies were performed with the manufactured substance(s), for which trade names were often reported without any further details on characteristics. For some of these trade names more information on particle sizes or specific surface area could be found in the CSR (but not in the IUCLID endpoint fields) or on the webpages of the manufacturers. In most dossiers literature data were used to address many (eco)toxicity and fate endpoints, with varying level of substance/particle characterization (e.g. details on primary particle size, agglomeration/aggregation state, surface area and surface treatment/coating) reported. The test material description was usually restricted to the name and CAS number. For human health endpoint data, sometimes purity, surface area and very rarely primary particle size or particle size distribution was provided. For environmental hazard endpoints, surface area and particle sizes were in some cases reported but generally without specification of how the sizes were measured and whether the reported sizes referred to primary particles or aggregates/agglomerates in the test media. From the test material description it was therefore often difficult to identify which (nano)form of a substance had been tested.

Studies of nanoforms could in some cases be identified from the title of the original study and in some cases from the test materials description: In some dossiers alternative nomenclature was used for 'nano' test material description, such as 'subpigmentary', 'ultrafine' or 'fine crystalline' powder. In other cases testing of nanoparticles could be retrieved/assumed based on the particle size description, which however was often not reported in detail. Furthermore, for both human health and environmental hazards, nanospecific studies were in some dossiers identifiable by the addition of the word 'nano' in front of the author in the study title. However, for other studies, it was not always obvious or easy to recognise if nanomaterials had been tested. For Category II substances, implicitly considered to exist only as nanomaterials (Category II), agglomeration/aggregation of the tested materials were generally not specified in detail.

*Addressing differences between forms:* One Category I dossier, that had ticked the nano-box in IUCLID Section 2.1 as form of the substance, provided few data on the human health hazards of the nanoform, except for two endpoints covering three studies. For the nano-studies, the primary particle size was given. In addition several literature studies with the bulk form were presented with relatively good test materials description and measures to control it, but sometimes the bulk form was not well described (e.g.

particle sizes not given). Characterisation details or images were not presented. The nanoform studies (three *in vitro* key studies for skin irritation and mutagenicity) were used to discuss/conclude that the nanoform is not expected to behave differently from the bulk form and thus not taken further into account in the overall conclusions. The DNEL for this substance was derived for the bulk form and it was not specifically claimed that it would also be relevant for the nanoform, although this could be implicitly concluded from the fact that the substance was registered to also address the nanoform.

For environmental hazard endpoints, in the same dossier, studies of a nanoform were included for seven endpoints. Hence these endpoints, for which experimental data on the substance was provided, included data on both the nano and the bulk form. In the endpoint summary discussions, the nanoform and the bulkform were addressed separately. Except for four endpoints ('Toxicity to aquatic algae', 'Long-term toxicity to aquatic invertebrates', 'Toxicity to Soil Macroorganisms' and 'Toxicity to Soil Microorganisms'), a combination of nano and bulk form studies were used to reach one common conclusion ('not harmful'). For one endpoint ('Long-term toxicity to aquatic invertebrates') effects of the nanoform were attributed to food deprivation and endpoint conclusion ('no adverse chronic effect') based on the bulk form. For 'toxicity to aquatic algae', the nanoform was found to be harmful whereas the bulk form was not. As the effects of the nanoparticles were considered by the registrant to be linked to clustering between algal cells and nanoparticles, the EC<sub>50</sub> value from this study was not used to derive a PNEC value. In fact no PNEC values were derived for any compartments for this substance – neither in bulk form or nanoform. For 'Toxicity to Soil Macro-organisms' and 'Toxicity to Soil Micro-organisms' endpoint conclusions were based on insufficient or non-existing information on nanoparticle effects, respectively. Endpoint waivings (e.g. for long-term toxicity to fish and sediment toxicity) were justified for the bulk form without addressing the nanoform.

In general, for both human health and environmental hazard endpoints, the relevance of different particle sizes for differences in study results were discussed for some individual test results in the Category I dossiers (addressing nano and bulk forms), but not considered in the overall conclusions. This applies also in relation to different particle sizes in dossiers/substances considered to exist in the nanoform only (Category II). Such information would be useful to evaluate, i.e. whether different sizes are responsible for different effects or different severity of effects.

*Additional characterisation:* For one Category I substance the preparation of the nanosized material and characterisation by DLS (Dynamic light scattering), FE-SEM (Field Emission Scanning Electron Microscopy) and TEM (Transmission electron microscopy) was provided for an 'Acute toxicity: oral' study, but not for any other endpoints in that dossier. In another dossier TEM images were found to be attached to the IUCLID endpoint entry 'Mutagenicity'. The TEM images are not described any further nor addressed in the dossier. This would have been a useful part of the test material description.

The level of detail on test material description could be a result of the quality of the literature source and/or the data selected for the summary in IUCLID. Whatever the reason, scientific justification, in relation to whether the test material, and hence the result of that literature study, is relevant for the registered substance (and forms), was usually not provided in the IUCLID dossiers. This conclusion relates to most of the endpoint sections in most dossiers. In general, the level of detail in test material

description and characterisation is improving in more recently published studies on nanomaterials, possibly supported by scientific journals increasing the demands for characterisation.

A few dossiers reported primary particle size as part of test material description in IUCLID Section 7.5.2 on 'Repeated dose toxicity: inhalation'. In some cases the description was not very specific: e.g. in two cases the description was < 1µm and particle size distributions were not provided. One Category I dossier provided particle size and surface area for both the nanoform and the fine (bulk) form.

*Use of standard test guidelines and reliable review summaries:* Tests, for which it is clearly indicated that the test material is a nanoform of a substance (the case for some endpoints in some Category I dossiers), usually literature data, and very rarely standard test guidelines, were used for testing of human toxicity.

For Category II substances standard test guidelines were often used for environmental hazard endpoints in the studies reported in these dossiers. For literature data the test substance was sometimes well characterized in IUCLID, but no further explanation was provided on the relevance of that particular particle/study choice in relation to the substance/forms registered.

For human health hazards many of the test results provided for bulk materials and for substances without a bulk form (Category II), were either from guideline studies or similar/equivalent to guideline studies. The latter because they were performed at a time when guidelines or GLP did not yet exist. These studies were sometimes quite old (40 – 60 years) and were not conducted according to modern standards, e.g. the characterisation of the test material in those studies is relatively poor. The reliability/Klimisch codes for these studies were stated to be mostly 2, but also 1 in rare cases.

For human health hazard endpoints some dossiers were largely referring to reliable reviewed summaries of their substance, or read-across substance (e.g. OECD SIDS, IARC, EU risk assessment reports, United Nations Environment Programme (UNEP) report), which normally do not include size specific information and consequently size was not addressed in the IUCLID entries. For the same dossiers, summaries were used to a lesser extend for addressing environmental hazard endpoints, although reference to size when drawing conclusion was also largely not available.

#### **Sample preparation and dosimetry for (eco)toxicological testing**

For human toxicity tests, information on sample preparation, dispersion of the substance and agglomeration state of the material before administration was generally not reported for oral or dermal application as well as for genotoxicity tests.

For inhalation studies usually the MMAD (mass median aerodynamic diameter) and GSD (geometric standard deviation) were reported as well as the control of concentration of test material in the air (but it was rarely reported, how this was measured). These are parts of the standard protocols in guideline studies.

For acute and repeated dose inhalation studies, information on the actual exposure concentration and/or on the particle size was partly provided in other IUCLID fields, e.g.: "Details on inhalation exposure", "(Details on) Analytical verification of test atmosphere

concentrations” and “doses/concentration”. This information, when reported, covered actual exposure concentration, MMAD and GSD.

Where MMAD was reported it seemed in general (for the same reasons as described under granulometry, see Chapter 3) to be for the agglomerated/aggregated forms. The MMAD values reported were usually in the low  $\mu\text{m}$  range ( $>1 - 7 \mu\text{m}$ ) and were similar and did not seem to relate to the primary particle size. Thus it is not possible from the MMAD to retrieve any information on the primary particle size. For example it was reported in one dossier for one substance that smaller high surface nanoparticles (d: 17 nm) had a higher MMAD than bigger (low surface) particles.

As the dossiers contained little discussion of primary particle and/or agglomeration/aggregation state it has not been possible to identify for Category I & III dossiers whether (a fraction of) the primary particles were in the nanorange. Also the possibility of de-agglomeration was not discussed. Only two dossiers/substances provided information on the aggregation/agglomeration status.

For carcinogenicity studies, it was observed that the descriptions of the inhalation exposure were less detailed than for RDT toxicity studies. For one dossier, the same study listed under both, RDT and carcinogenicity, did mention MMAD in the RTD section but not in the carcinogenicity section, where it would be of equal importance. It should be noted that IUCLID does not contain this field for MMAD/GSD in the carcinogenicity section, as it is not specific for the application route (e.g. inhalation), but only in the RDT section for inhalation exposure. However the information could have to be reported under “Details of exposure”.

For ecotoxicological testing, the description of sample preparation varied greatly. In some cases sample preparation was well described in a way that would allow reproducibility. Visual observations regarding agglomeration/aggregation were in a few cases reported. In other cases no information on sample preparation was provided making it impossible to evaluate the appropriateness of the preparation method in relation to testing of nanoparticles (or a nanoform/nanotail). For studies, where a filtrate/supernatant/WAF (water accommodated fraction) was tested, a quite detailed description of the preparation method was generally included e.g. filter pore sizes. The applicability of this specific sample preparation method for nanomaterials is discussed in section 4.2.3.3. For these tests nominal loading rates were most often reported (and often used to derive PNECs) whereas actual test concentrations were not known/measured/reported.

The general lack of detailed information on sample preparation and dosimetry has made it challenging for the assessors to assess the reliability and relevance of the reported values in relation to the nanoforms/nanomaterials registered.

#### **Distinction between forms**

- Generally there is no distinction between forms in endpoint sections:
  - o For dossiers addressing several forms, ‘nano’ or ‘bulk’ was added to the study name in endpoint studies in a few dossiers. In general, however no distinction was made between forms in the endpoint study summaries
- In those cases where different forms were clearly covered by a dossier:
  - o There are examples where data for nanoforms (for some endpoints) was considered worst case

- But there are also examples where data for bulk, or unspecified forms, was given preference over nano-specific data (without a scientific justification being included in the endpoint study summary)
- When no nano-specific data exist, data from 'bulk' form studies were used as no distinction between forms based on size was made in these cases.

### **Key vs. supporting studies**

- Generally there was a lack of transparency about why certain studies were considered as 'key studies' and others as 'supporting studies'. It seemed also that the 'determination' of the 'purpose' and the 'reliability' of the studies were subjective and dependent on the registrant.
- It has also been observed that the same studies, reported for the same substance in different dossiers (i.e. by different registrants), were in one case a 'key study' and in the other, a 'supporting study'.
- There was also big variation (1-3)<sup>34</sup> of the reliability of the same studies, depending on registrant. Most variation has been observed between reliability 1 and 2. For example some registrants considered reviewed data by IARC or OECD of reliability 1 while others assigned reliability 2 to the same kind of data. Also, especially older guideline studies were by some registrants assigned reliability 1, while the same study assigned reliability 2 in a different dossier for the same substance.
- Most variation (reliability 2-4, rarely 1, or very often no reliability assigned) was observed among studies reported under epidemiology, health surveillance, exposure related observations in humans etc.
- There was an example of a 'nano-study' with reliability 2 which was indicated as 'supporting' and therefore disregarded in e.g. DNEL derivation, without any clear justification.

### **Who provides the information and how?**

In a Joint submission, the lead registrant submits all the relevant endpoint information for the joint registration. The lead registrant may not cover nanoforms in his own registration dossier while member registrant dossiers do (thus with the current implementation, the nanoform endpoint data are in the lead dossier, although the lead registrant is not himself registering nano). The lead registrant is only required to provide substance identity information for the specific composition manufactured/imported by his own legal entity. An indication that nanoforms are covered within the scope of the joint submission may not be seen from the IUCLID file but only be apparent from the CSR or in the attached SIEF substance sameness documentation, if at all.

### **Read across**

Read across to structural analogues for human health endpoints was applied for seven different substances according to the analogue approach for many endpoints, almost addressing the whole dossier. Three other dossiers applied read across to structural analogues only for some endpoints.

One Category I dossier used read-across from the bulk to the nanoform. One other Category I dossier used read-across from nano to bulk for some endpoints.

<sup>34</sup> Reliability codes: R1: reliable without restriction, R2: reliable with restrictions, R3: not reliable, R4: not assignable

For five Category II substances read-across was primarily based on similar chemical composition (the same (metal) ion or parent compound) and physico-chemical properties with the justification that the pathways leading to toxic outcomes are dominated by the chemistry and biochemistry of the common parent compound or the common metal ion, or ion complex including a hydrated metal ion. In some of these dossiers, little or no attention was given to the possible influence of different particle sizes. This parallels other parts of the dossiers, where particle size was not always given much attention (see e.g. above discussion about sample preparation and dosimetry). However for three Category II substances the registrant considered that the particle size and morphology, rather than the particle composition are the determinants of tissue responses in the lung. The registrants also assumed that under comparable testing conditions, comparable results with the substances would be expected. The dossiers presented a comparison of particle size profiles under experimental inhalation conditions (low and high shear force) which includes MMAD in the higher and lower  $\mu\text{m}$  range (please note that MMAD in the  $\mu\text{m}$  range may consist of agglomerated nanoparticles).

For three Category III substances read-across was based mainly on the common ion.

Differences in crystallinity were also taken into account in four Category II dossiers, however all read across from the same parent compound. In these cases the crystalline form was not considered to be appropriate to be used for reading across to the amorphous form.

Read across from ecotoxicity tests on metal ions to the registered substances was done for some endpoints for several dossiers, hence not addressing the particle nature of the registered substance. It is important to point out that the substances registered in these dossiers are sparingly soluble substances and often containing one or more metals in their composition. Therefore the review of the aquatic toxicity literature, where soluble metal ions (e.g. metal salts) were used for assessing the toxicity of the metal ion or hydroxide, has limited direct application to the registered substances. For one metal oxide dossier, for example, all ecotoxicity endpoints - for which testing data were provided - were addressed by read-across from metal salts with only very few tests on the registered substance reported. The registrants of this substance stated themselves that the data on soluble metals are of little relevance to assess the toxicity of poorly soluble substances, such as the one registered. No studies with the nanoform were reported for the substance, and the toxicity was addressed under the assumption that toxic effects could be attributed to soluble metals, disregarding the effects related to particulate material.

*In conclusion in almost half of the dossiers read across was applied for several endpoints for human health or environment based on the common parent compound or metal ion. The particulate nature of the substance or particle sizes were only considered in one group of dossiers covering similar substances.*

## 4.2 Options for adapting REACH

### 4.2.1 General options (including C&L and PBT)

As a prerequisite for the following options, it is assumed that the options addressing scope and characterisation of the nanoform(s) (option 3.1 and 3.2), as suggested in section 3.2, are implemented. Thereby, the registrant would have explicitly identified/characterised nanoforms/nanomaterials within the scope of a registration and outlined nanoforms differing in properties.

#### **Option 4.1. Require that nanoforms are explicitly addressed in the endpoint sections.**

For the endpoint sections relating to environmental fate & behaviour and (eco)toxicity, it is suggested that in cases where a dossier contains multiple forms of a substance (e.g. nano and bulk forms, or multiple nanoforms), the registrants should clearly indicate to which form(s), and to which extend, the results of a test or a waiver are intended to apply and why. Different forms should also be clearly addressed in the endpoint study summaries. This option parallels option 3.3 for physico-chemical properties, which sets out further details on how this could be done in IUCLID. This option could be specified in relevant REACH annexes.

#### **Option 4.2. Require detailed description of the test material/sample and sample preparation.**

The need for a detailed description of the test material and sample preparation when presenting results from testing for environmental fate & behaviour and (eco)toxicological hazards should be explicitly mentioned in the REACH annexes. This relates to any (also historical) data used in a registration dossier.

Regarding test guidance (relevant for REACH Article 13(3) and 13(4) on test methods & GLP and related to option 3.4 of this report), it is suggested that the REACH annexes and/or the test method regulation make reference to the 'latest OECD guideline(s)' on test material description, sample preparation and dosimetry. The current ECHA guidance document refers to the use of OECD Test Guidelines, which have been found by the OECD WPMN<sup>35</sup> not to provide sufficient guidance on preparation, delivery, measurements and metrology for nanomaterials. This presents a limitation for assessment of, and appropriate testing for, (eco)toxicological data (see also RIP-oN2 final report paragraph 3.5.292). It is assumed that the OECD guidelines will be regularly updated to address the scientific development in this area.

See also option 3.4 in relation to further discussion and implementation considerations.

Further, in relation to human health and the environment, the different forms should be explicitly addressed in the chemical safety assessment and especially in relation to DNEL/DMEL/PNEC derivation.

Test proposals need to include a detailed test material characterisation. Besides the registrant need to provide a justification why the selected test material is the most

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<sup>35</sup> OECD Environment, Health and Safety Publications Series on the Safety of Manufactured Nanomaterials, No. 15, Preliminary Review of the OECD Test Guidelines for their Applicability to Manufactured Nanomaterials. ENV/JM/MONO (2009)21

appropriate form for the test and whether the expected results could be used for multiple forms (see also option 4.3).

**Option 4.3 Require scientific justification for grouping/read-across/QSAR and other non-testing approaches for different forms.**

In relation to read-across/grouping under the environmental fate & behaviour and (eco)toxicological endpoints, the same principle as set out in Option 3.5 for physico-chemical properties should apply. It is suggested to specify in Annex XI.1.5 that the general principles requiring scientific justification for 'substance to substance' considerations should also apply to 'form to form' considerations. This includes extrapolation from structurally/chemically related forms/compounds as well as from 'bulk to nano' forms or vice versa. It is thus suggested to clarify in the REACH annexes (in any case in guidance) that when applying read-across and grouping beside the chemical(ion) specific effects also particle (and thus size) specific effects should be considered. In case the chemical(ion) toxicity is given more importance than the particle specific effect, this should be explicitly specified and justified by the registrant. See also RIP-oN2 Final Report, e.g. paragraph 4.3.60.

In general, the applicability of QSAR modelling to nanomaterials can be considered still to be an area of great uncertainty, see e.g. RIP-oN2 Final report, paragraph 3.3.21. (Q)SAR approaches should therefore only be used with appropriate scientific justifications. It is suggested to explicitly specify in Annex XI.1.3 that QSAR models should only be applied if the (nano)form being assessed is within the applicability domain of the model.

In particular in relation to human health and environmental endpoints, non-testing approaches should be encouraged, as should intelligent testing strategies (e.g. for ranking different forms) to avoid full testing of all forms. Testing proposals should be considered only if an endpoint cannot be addressed via other means.

**Option 4.4 Require considerations of most appropriate/relevant metric with preferable presentation in several metrics.**

See option 5.2 in section 5.2, where options for addressing the metric issue covering hazard, exposure and risk characterisation will be presented.

**Other related issues (likely to be further addressed in (IUCLID) guidance/tools, see also RIP-oN2):**

- In general, discussion of the uncertainties related to the above options should be explicitly required to be included/specified in the dossier.
- It should be explicitly required that indication as 'key' or 'supporting' study should be justified - as should the reliability indicator.
- Standard test methods and associated guidance for the determination of particle size (distributions) need to be developed to address nano-size ranges. When presenting results, it is essential to describe what the term 'particle' and the associated size distribution(s) refer to in relation to agglomerates, aggregates and primary particles.
- Further guidance should be provided with regard to limitations, applicability and relevance of current Test Methods/Test Guidelines and testing strategies when applied

to nanomaterials (e.g. methods for acute toxicity, repeated dose toxicity, mutagenicity, reproduction and developmental toxicity).

- IUCLID should be updated to enable registrants to better include nano-specific information in their dossiers, e.g. endpoints as suggested in the OECD WPMN sponsorship programme and the RIP-oN2 report.

- The 'carcinogenicity' section could include a field to report particle size information to encourage registrants to deliver more information on the inhalation conditions.

In relation to classification and labelling, the previously identified options would ensure that registration dossiers are:

1. Clearly and transparently informing whether nanoforms are covered within the scope of a registration, and
2. Where relevant (if inherent properties of a nanoform differ from bulk or other nanoforms), clearly and transparently addressing inherent properties for the nanoforms throughout the dossier.

This would, where relevant, enable a classification and labelling of the nanoform and avoid the situation where a nanoform is implicitly assumed to be covered by the 'bulk' classification, while addressing as well the situation where a nanoform is 'not classified' due to lack of data.

Provided that data on inherent properties are generated for a specific nanoform, it should be considered whether/to which extent the classification and labelling criteria and/or associated guidance should be adapted to reflect, e.g.:

- that mass may not be the most relevant metric for expressing the toxicity of nanomaterials
- toxicity seen after inhalation of nanomaterials. This relates e.g. to the discussion about human relevance of rat lung overload data, including cases where carcinogenicity is found for nanomaterials in rat studies
- issues related to mutagenicity/genotoxicity.

It is considered outside the scope of this project to make specific suggestions for changes of the CLP regulation, also taking into account that classification and labelling affects many other (downstream) legislations.

Nevertheless, it should be kept in mind that C&L is extremely important in relation to whether an exposure assessment and risk characterisation is triggered under REACH. Please refer to chapter 5 in relation to the link between C&L and exposure assessment / risk characterisation for the dossiers addressed.

In relation to PBT assessment, in the text of Annex XIII of REACH on criteria for identification of PBT and vPvB substances, it is specified that the criteria do not apply to inorganic substances. However, as will be discussed in the endpoint sections below addressing biodegradability (including persistence) and bioaccumulation, this is a topic for discussion - also in relation to nanomaterials. No specific options for REACH adaptations will be outlined for PBT assessment in this report as the issue of reconsidering the exemption of inorganics from Annex XIII should be seen in a wider context and as part of a broader discussion.

## 4.2.2 Endpoint specific options – Human Health

### *Introductory remark*

22 of the 25 dossiers (including 4 member dossiers), analysed and assessed in detail as part of this project, addressed substances manufactured or imported above 1000 tons/year. For these substances Annex X of the REACH Regulation applies and the highest amount of information is requested via the standard information requirements. For substances of lower tonnages much less information is requested via the standard information requirements, e.g. on long-term inhalation.

Depending on which properties of nanomaterials would be considered as identifiers or characterisers (see discussion in RIP-oN1), the need for a separate registration of certain ‘nanomaterials’ could be triggered. It can be expected that many of these ‘nanomaterials’ are produced or imported in low tonnages and consequently the lower standard information requirements of Annexes VII – IX apply. Further, for some of these (<10 tons/year), no CSR will be provided.

It should be noted that specific rules for adaptation (waiving, triggering and replacement) of the standard information requirements are outlined in REACH Annex VII-X, column 2 and in Annex XI.

### 4.2.2.1 Toxicokinetics (Annex VIII 8.8, IUCLID section 7.1)

At present, an ADME study is not a standard information requirement under REACH. “Only” an assessment of the toxicokinetic behaviour of the substance to the extent that it can be derived from the relevant available information shall be provided by the registrant. Therefore, it appears relevant to discuss possible changes of the legal text on this endpoint in a broader context. Thus, we will not present an option here, but like to point to a number of issues to consider for nanomaterials.

In line with the general options suggested in this report, the nanoform(s) addressed by a dossier should be specifically tackled in the endpoint sections. As part of a nanotoxicity testing strategy, it is of key relevance to compare available ADME data for a nanoform with ADME information for other nano and non-nanoforms (compare also with recent EFSA guidance<sup>36</sup>). ADME data are also relevant to decide whether or not specific tests required at specific tonnage levels need to be performed or not (e.g. carcinogenicity study in Annex X). As discussed in section 4.1.1.1 and 4.1.6, ADME data are also very relevant when considering read-across – not the least in relation to particle size.

Investigating particle translocation by using barrier transfer models (see RIP-oN2, 3.6) could deliver relevant information on the behaviour of nanomaterials, also in comparison to bulkforms, and could provide important information for *in vivo* testing design and interpretation. Guidance on appropriate methods for detecting and characterising nanoparticles within the body needs to be provided.

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<sup>36</sup> European Food Safety Authority (EFSA). Scientific opinion. Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain. EFSA Journal 2011; 9(5): 2140.

Solubility studies may be used to investigate whether the substance is dissolved. If so, its subsequent behaviour would not depend on particle size for reading across of toxicokinetic data from the bulk form (especially for the oral and dermal routes). The tendency of ion leaching from nanomaterials and differences compared to bulk materials should be taken into consideration. These issues could become part of a decision tree, but this would be rather a guidance issue.

In conclusion, the information requirement may differ from case to case. In some cases the available information or a dissolution study may be sufficient, in other cases the registrant may consider to make a separate ADME study. As ADME data may trigger additional (or reduce) testing it seems not logic to make its requirement dependent on 'available' information. It may therefore be relevant to change the text in Annex VII 8.8 and delete 'available information' from column 1. It could become one of the options in column 2. It seems more relevant that the registrant shall provide sufficient data to conclude on the toxicokinetics behaviour of the registered substance. He may also include a justification why this is the most appropriate way to do so. However, as noted above, it is found relevant to discuss possible changes to the toxicokinetic information requirements in a wider context as the endpoint is also extremely relevant for other forms and chemicals in general.

#### **4.2.2.2 Acute toxicity (Annex VII 8.5, VIII 8.5, IUCLID section 7.2)**

##### **Option 4.5. Require acute toxicity data for the most relevant route of exposure**

Annex VII, 8.5 (above 1 tonne/a) requires only one acute oral toxicity test. As inhalation is generally the most relevant route of human exposure for nanomaterials, it is suggested to clarify in the testing annexes that an inhalation study (either instead or in addition to the oral route) should be conducted if indeed this is known to be the most relevant/likely route of exposure. It could e.g. be added there and in Annex VIII: Especially for nanomaterials it should be justified when inhalation is not considered a relevant route of exposure. Further, either the test methods or the guidance should refer to extended pathology/histology determinations and examination of relevant parameters in BAL fluid.

#### **4.2.2.3 Repeated dose toxicity studies, subchronic toxicity studies (Annex VIII 8.6.1, IX 8.6.2, IUCLID section 7.5.2)**

##### **Option 4.6. Change 'particles' to '(nano)particles' for repeated dose toxicity studies (inhalation).**

For repeated dose toxicity, the text could be amended in column 2 of Annex VIII, 8.6.1 and Annex IX, 8.6.2 (and a comparable change in Annex VIII, 8.5.2 referring to acute inhalation study):

Change 'particles' to '(nano)particles', and add: Especially for (nano)particles it should be justified when inhalation is not considered a relevant route of exposure. Either the test methods (for sub-acute, sub-chronic or combined repeated dose toxicity/reproductive screening study) or the guidance should refer to extended pathology/histology determinations and examination of relevant parameters in BAL fluid.

The following endpoints should be specifically addressed: Cardiovascular toxicity, immunotoxicity and respiratory sensitisation.

Several issues are important in relation to repeated dose inhalation studies, including duration, sample preparation issues, exposure conditions, rat lung overload phenomenon, etc. Different aspects of this might be considered in different scenarios to consider in the next phase of the project, but in any case most of these issues should be reflected in detail in guidance and test methods.

#### **4.2.2.4 Mutagenicity (Annex VII 8.4, VIII 8.4, IUCLID section 7.6)**

##### **Option 4.7. Require non-bacterial in vitro gene mutation study.**

Annex VII, 8.4 requires an *in vitro* gene mutation study in bacteria and further mutagenicity studies shall be considered in case of a positive result. As it has been shown that this kind of test can give false negative results when (nano-)particles are not taken up through the bacterial wall (see RIP-oN2), it is suggested to require in the relevant REACH annexes instead a non-bacterial *in vitro* test for nanomaterials. This should be further elaborated in the guidance.

It is currently difficult to come up with an alternative test proposal for an *in vitro* test in mammalian cells, which would be suitable to all nanomaterials. It has been shown in several tests that results are influenced by the cell lines (e.g. repair capacity), appropriateness of dose ranges, test serum (e.g. to prevent cellular uptake) and by the test material and its characteristics. Some tests might be better suitable for more soluble nanomaterials and others not. This has therefore to be decided case by case, taking into consideration the scientific progress.

The RIP-oN2 gap analysis (3.6) proposes the following *in vitro* tests which could be included into guidance, however it is premature to include them into the legal text: DNA REPAIR reporter assays: e.g. GreenScreen assay, Oxidative adducts of DNA (e.g. 8-OH-dG), Lipid adducts (e.g. N-1,N2 malondialdehyde-2'-deoxyguanosine M1dG). In addition, it is proposed (as a part of nanotoxicity testing approach) to compare genotoxicity data of the nanoform with genotoxicity data of the non-nanoform (see EFSA guidance on RA of NM, 2011<sup>37</sup>).

#### **4.2.2.5 Toxicity to reproduction (Annex VIII, IX, X, 8.7, IUCLID section 7.8)**

Annex VIII, 8.7 states that studies do not need to be conducted, if it can be proven from toxicokinetic data that no systemic absorption occurs via relevant routes of exposure. At least in the guidance, it should be clarified how to address the problem posed by the difficulties with detecting and characterising nanoparticles in the body following exposure, without jeopardizing the appropriate examination of the endpoint.

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<sup>37</sup> European Food Safety Authority (EFSA). Scientific opinion. Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain. EFSA Journal 2011; 9(5): 2140.

**Nano-focused *in vitro* assays** could be useful to investigate the potential of nanomaterials to affect the reproductive system or the development. Possible tests as identified by RIP-oN2 gap analysis (3.6) would be: *In vitro* embryonic stem cell test for embryotoxicity, *in vitro* micromass embryotoxicity assay and *ex-vivo* whole rate embryotoxicity assay. Given the current state of validation of these tests for nanomaterials, it is suggested to consider these as supporting evidence where available.

#### **4.2.2.6 Further considerations**

A number of *in vitro* tests can be used to investigate the potential toxicity of nanomaterials. Although the results of such tests have to be seen in the context of relevance for physiological conditions of human exposure, they could at least be used in a tiered approach as starting point to decide on the necessity and on the focus of further (*in vivo*) testing. In addition they could deliver useful information on the sameness or differences between nano- and bulk forms and between different nanoforms - by this assisting in building the basis for read-across and grouping. The following endpoints are of specific interest: Inflammation/immunotoxicity (with relevance to acute and repeated dose), cytotoxicity, oxidative stress and fibrosis. In addition also cell uptake and cell viability are important to consider (see RIP-oN2 report for detail).

These tests should be seen in the context of the current REACH Annex XI (and therefore above option 4.3) and their use could be elaborated in guidance/testing strategies. Thus no specific option for adapting the REACH information requirements are suggested for the time being.

As many of the test guidelines were not designed to test nanomaterials and as mentioned in the RIP-oN2 report (e.g. executive summary), focus should be set on measuring, dosing, delivery and tracking of nanomaterials in the test system.

### **4.2.3 Endpoint specific options – Environment**

In relation to requirements for different tonnage bands, see introductory remarks to human health (Section 4.2.2), which also apply here.

#### **4.2.3.1 Environmental Fate**

Most of the dossiers either waived the endpoints on Environmental Fate and Behaviour or the data provided were not in line with the methodologies outlined in approved test guidelines (e.g. OECD, ISO) for these endpoints. The endpoints were often waived based on the physico-chemical properties of the substance but did not take into account the possible different characteristics of nanomaterials compared to bulk materials; thus this highlights a need to incorporate nano-specific considerations for these endpoints to predict the fate of nanoforms/nanomaterials in the environment.

##### **4.2.3.1.1 Stability: Hydrolysis and phototransformation (Annex VIII 9.2.2, IUCLID Section 5.1.1, 5.1.2, 5.1.3 and 5.1.4)**

Hydrolysis and phototransformation potential may not be the only relevant parameters to determine whether nanoforms are stable in the environment. Indeed, as hydrolysis will

depend on the water solubility, it may be negligible for most nanomaterials in the timelines outlined in recognized test guidelines. Dissolution (kinetics) of ions or organic coatings may be considered more relevant processes in relation to substance stability. Regarding options for solubility see Section 3.2.2. Possible additional/modified information requirements on dissolution kinetics would then most logically be covered under the 'Solubility' Information Requirement in REACH (Annex VII, 7.7).

On the other hand, for nanoforms, stability can also be considered as the potential for the substance to remain suspended in the water column or, instead, to agglomerate and precipitate. This, in turn, will determine the targeted compartments for which assessment of environmental hazards needs to be carried out. Thus, it is crucial that a detailed and in-depth physiochemical characterization of the nanoform under relevant environmental conditions is conducted to e.g. predict its mobility and its fate in the environment. This would follow from the general options (in particular Option 4.2) presented in Section 4.2.1. For hydrolysis and phototransformation no endpoint specific options are suggested here.

#### 4.2.3.1.2 Biodegradation and persistency (Annex VII 9.2, Annex VIII 9.2, Annex IX 9.2, Annex IX 9.2, IUCLID Section 5.2)

Conventionally, degradation is considered to be the biotic (or abiotic) process of chemical transformation of a chemical substance in the environment. Considering that the reason of concern for nanomaterials derives from their size and shape, degradation could also be viewed – in addition to chemical transformation - as morphological transformation (resulting from biotic as well as abiotic processes). This includes e.g. dissolution of the nanoform or irreversible changes in size and/or shape (physical persistence). Hence solubility and aggregation (irreversible process) could be crucial properties to determine in relation to the persistence of nanomaterials in the environment. Knowledge on solubility would assist in understanding to which extent read-across approaches from e.g. metal salts to metal oxides can be considered as an alternative to testing. It is also important to keep in mind that, if nanoforms are surface treated/functionalized or capped with (in)organic compounds (e.g. one or more (in)organic layers to prevent ion leaching and control dispersability), the properties and behaviour of the nanoparticle would be highly dependent on the properties (including biocompatibility etc.) of the surface treatment material. See general options in Chapter 3 and in Section 4.2.1, in relation to the need for a taking this into account in the characterisation and throughout the dossier. Biodegradation of substances used to cap or surface functionalize a nanoform would need to be investigated due to their crucial role on environmental fate (e.g. aggregation, dispersability, charge, etc) of the capped/functionalised nanoform.

Regarding persistency, a general argumentation for the substances in the assessed dossiers not to be considered PBT substances, is that the PBT criteria do not apply to inorganic substances (according to REACH Annex XIII). This also reflects the OECD view on persistency as non-biodegradability, which is the commonly agreed interpretation of 'persistency'. In line with the previous discussion on morphological transformation, it should be noted that for nanoparticles persistence may also be considered as persistence of size and/or shape (physical persistence) rather than only molecular persistence. In contrast to persistence of the (possibly bioavailable) nanoform is then aggregation (possibly changing bioavailability) and dissolution (with a different uptake mechanism for the metal ions). Though this issue is particularly relevant for

nanoparticles, due to their different bioavailability (ways of crossing biological membranes), this discussion has to be seen in a broader context in relation to the P criterion and its applicability to inorganic substances in general – not only for nanomaterials. Thus no specific options are suggested here.

#### 4.2.3.1.3 Bioaccumulation (Annex IX 9.3, IUCLID Section 5.3)

Regarding bioaccumulation, this endpoint is often waived based on the inorganic nature of the substances or waived based on  $K_{ow}$  considerations. Investigating the bioaccumulation potential of a chemical substance is triggered when the octanol-water partition coefficient ( $\log K_{ow}$ ) is found to be  $> 3$  (IUCLID section 4.7). A  $\log K_{ow} > 3$  indicates a high lipophilicity and therefore a high potential for the substance to bioaccumulate. However, the applicability of BCF estimations based on  $K_{ow}$  for nanomaterials is not possible, as also stated in the RIP-oN 2 Final Project Report (4.1.385) since nanoparticles are dispersed and not in solution. Therefore  $\log K_{ow} < 3$ , or justifications based on  $\log K_{ow}$  in general, may not be appropriate to waive bioaccumulation endpoints for nanoforms of chemical substances. An additional limitation, that should be taken into account, is that size, surface coatings and reactivity of nanoparticles may affect and facilitate other pathways of cellular uptake, and thus the potential to cross biological membranes may not be driven by cell membrane permeability (lipophilic behaviour), but passive diffusion and other endocytosis processes may also become relevant and should be evaluated with caution. Indeed, surface functionalized nanomaterials could potentially enter the cell bypassing the route for uptake of e.g. bioavailable ionized metal species, leading to a 'Trojan horse' type mechanism (i.e., the nanoform acts as a delivery vehicle for bioavailable metal ions).

#### **Option 4.8. Require that bioaccumulation is addressed specifically for the nanoform.**

In relation to Annex IX 9.3.2., column 2 it is suggested to specify that the bioaccumulation potential of the nanoform (as particles) could be different compared to the soluble form and bulk form and hence has to be considered specifically for nanomaterials. With reference to Option 4.2 in Section 4.2.1 it should be emphasised that the registrant needs to consider surface treatment / functionalisation of the nanomaterials/nanoforms and in particular assess what impact this may have on the inherent property data under scrutiny and on the hazard/risk assessment. It should be noted that the potential bioaccumulation of nanomaterials is unknown, particularly if these are coated, and cannot be estimated based on  $K_{ow}$  values. In cases where surface treated materials are (potentially) covered by a registration, specific justification for waiving bioaccumulation studies should be provided for the surface treated materials.

#### 4.2.3.1.4 Transport and distribution: Adsorption/desorption (Annex VIII 9.3.1, Annex IX 9.3.3, IUCLID Section 5.4.1)

Understanding and being able to quantify the potential of a nanoform for adsorption/desorption from solid surfaces is crucial to predict its environmental fate. Therefore, in-depth physico-chemical characterizations of the nanoforms suspended in environmentally relevant media are necessary to help predict the potential for the substance to partition in different environmental compartments. As also addressed in RIP-oN2 Final Project Report 3.7.187 & 4.1.22, it should be noted that

adsorption/desorption behaviour ( $K_d$ ) for nanomaterials cannot be estimated from  $K_{oc}$  and  $K_{ow}$ .

**Option 4.9. Specify that adsorption/desorption behaviour of nanomaterials should not be assessed based on  $K_d$  values derived from  $K_{oc}$  and  $K_{ow}$ .**

In relation to REACH Annexes VIII, 9.3.1 and IX, 9.3.3, it is suggested to specify that adsorption/desorption behavior of nanomaterials cannot be assessed from estimated  $K_d$  values derived from  $K_{oc}$  and  $K_{ow}$  as these distribution coefficients are not applicable to nanomaterials. Instead  $K_d$  values should be determined experimentally.

#### **4.2.3.2 Environmental hazards**

##### 4.2.3.2.1 Water solubility in relation to test waiving

**Option 4.10. Consider water solubility in relation to test waiving.**

It could be specified in the REACH Annexes that waiving tests of nanoparticles based on low solubility may not be appropriate since, due to other possible uptake mechanisms, the insoluble nature of some nanoparticles may not necessarily imply that they are not bioavailable. Moreover, and as described for 'Option 3.4', water solubility, it should be noted as well that, as a result of smaller particle sizes, metal and metal compound nanoparticles (including metal oxide nanoparticles) may in general have different ion release kinetics compared to larger sized particles.

##### 4.2.3.2.2 Long term testing (Annex IX 9.1.5 & 9.1.6, IUCLID Sections 6.1.2 and 6.1.4)

**Option 4.11. Specify that long term testing should not be waived based on lack of short term toxicity.**

In relation to long term testing<sup>38</sup> it is suggested to specify that lack of short term toxicity does not justify waiving of long term testing for nanomaterials. This may be especially relevant due to the often insoluble nature of these substances in correspondence with REACH Annex VII, 9.1.1 and 9.1.3 (short term testing) specifying that for poorly soluble compounds it shall be considered to conduct long-term tests. By performing long term tests with lower exposure concentrations it may also be possible to obtain more stable suspensions, hence overcoming some of the testing difficulties related to testing of nanoparticles in aqueous media.

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<sup>38</sup> In general (Annex IX 9.1., column 2) and in relation to the specific phases "The long-term aquatic toxicity study on *Daphnia* (Annex IX, section 9.1.5.) shall be considered if the substance is poorly soluble" (Annex VII 9.1.1., column 2), "The long-term aquatic toxicity study on fish (Annex IX, section 9.1.6.) shall be considered if the substance is poorly soluble" (Annex VIII 9.1.3., column 2) and Annex X 9.4 (terrestrial organisms).

#### 4.2.3.2.3 Algae testing (Annex VII 9.1.2, IUCLID Section 6.1.5)

##### **Option 4.12. Specify that algae testing should not be waived based on insolubility.**

Specific advice is suggested in relation to Annex VII 9.1.2., column 2 regarding nanomaterials as the insoluble nature of these substances does not necessarily justify waiving of algae testing.

#### 4.2.3.2.4 Testing on soil and sediment organisms (Annex IX 9.4, Annex X 9.4, IUCLID Section 6.3)

##### **Option 4.13. Require that testing on soil and sediment organisms is prioritised.**

In relation to REACH Annex IX, 9.4 and Annex X, 9.4, the importance of addressing soil and sediment endpoints for nanomaterials could be highlighted and it should be noted that fate of nanomaterials cannot be assessed based on equilibrium partitioning. It should be considered that soils (e.g. through application of sewage sludge) and sediments are expected to be sinks for (nano)materials with low water solubility. The exposure of sediment organisms may therefore be significantly (quantitatively and qualitatively) different from exposure to organisms in the water column. This potentially makes toxicity studies of nanoparticles to soil and sediment organisms highly relevant.

#### 4.2.3.3 Other related issues: Tests of filtrates/supernatants/WAFs

##### ***The below issue is likely to be addressed in guidance.***

In general sample preparation involving filtration / centrifugation techniques for removal of particles/aggregates/agglomerates (or certain size fractions) are not recommended for nanoparticle testing. Especially preparation and testing of supernatant (water accommodated/soluble fraction obtained by settling or centrifugation) or filtrates (obtained by filtration) (also termed WAFs or WSFs) does not seem an appropriate approach. As mentioned for specific endpoints in Section 4.1.3 , this type of sample preparation may not take into account possible effects related to the presence of particles as physical entities as filtration and sedimentation procedures leads to some degree of particle removal.

Such sample preparation is normally applied when aiming to separate the WAFs from undissolved material and thus testing the fraction that is conventionally considered bioavailable to organisms and responsible for the toxic effects (which is not the case for at least some types of nanomaterials). However, testing only one fraction (e.g. soluble and particles/aggregates/agglomerates below a certain size) may result in overlooking the effects of other fractions (e.g. larger sizes agglomerates/aggregates). In some cases nanoparticle aggregates have been found to keep the toxic characteristics of the primary particles (Rabolli et al., 2011<sup>39</sup>) and omitting this fraction may therefore lead to an underestimation of effects.

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<sup>39</sup> Rabolli, V., Thomassen, L.C.J., Uwambayinema, F., Martens, J.A., Lison, D. (2011) The cytotoxic activity of amorphous silica nanoparticles is mainly influenced by surface area and not by aggregation. Toxicology Letters 206 (2) 197-203

When the stock suspensions are filtrated, through a filter with a typical pore size of 0.22-0.45 µm, single nanoparticles and smaller aggregates/agglomerates may pass through the filter. However, concentrations of the test material (in the registration dossiers including WAF tests) are generally only given as nominal values and concentrations and characteristics of the test substance in the actual test 'solution' (WAF) are not quantified.

For future considerations – if sample preparation methods involving filtration or centrifugation are found to be appropriate for testing of nanomaterials – at least investigations of the presence of nanoparticles (quantitative, qualitative, stability, aggregation/agglomeration state) in the test suspension will be crucial for proper interpretation of the test results. Also, expressing the effect as actual measured (particle) concentrations (or other appropriate metrics), as opposed to nominal loading rates, will give a more realistic picture of the toxicity of the substance, and hence enable estimation of realistic PNEC values.

***Regarding other general considerations related to test guidance see also RIP-oN2 Final Project Report.***

## **5 EXPOSURE ASSESSMENT AND RISK CHARACTERISATION**

### **5.1 Findings and conclusions**

#### **5.1.1 What is found in the dossiers? – link to PBT assessment and C&L**

The following gives a brief overview of exposure assessments and risk characterisations found in the dossiers addressed.

REACH formally requires an exposure assessment and a risk characterisation for substances:

- registered in volumes above 10 tonne/year per manufacturer/importer *and*
- fulfilling the PBT/vPvB criteria and/or classification as dangerous criteria.

No substances were assessed as fulfilling the PBT/vPvB criteria (see section 4.1.4). PBT assessment was waived and/or a qualitative discussion was given concluding that the substances are not considered to fulfil the PBT or vPvB criteria. The general waiving argument was that the substance is inorganic with reference to the Annex XIII exemption for inorganics.

Of the eight Category I dossiers, three dossiers (one lead and two member dossiers) addressed a (single) classified substance (classified both for human health and environment). An annex to the CSR of the lead registrant termed “Potential Risks associated with nano-sized xxx” presented a semi-quantitative risk assessment, but the registrants noted that it was not according to the REACH guidance as the guidance does not address how to assess nanomaterials. There were no CSRs, and thus no exposure and risk characterisations, in the two member dossiers. For a lead and a member dossier of one other Category I non-classified substance, exposure and risk characterisations were provided.

Of the 12 Category II dossiers, 2 substances were classified as dangerous. One was classified for both human health and environment but registered in a volume below 10 tonnes/year (and thus no CSR required), whereas the other was above 1000 tonne/year and classified only for human health. For two of the 10 Category II dossiers where the registrant had not classified the substances, the registrants had included information on exposure assessment and risk characterisation.

Of the five Category III dossiers, two substances were classified for human health and environment and exposure assessment and risk characterisation were provided. The remaining four Category III dossiers/substances were not classified and no exposure assessment and/or risk characterisations were provided.

In some dossiers where exposure assessment was not included in the CSR, some exposure information was found in IUCLID section 3, Manufacture use and exposure.

This related to three Category II dossiers where some exposure estimates were found in the IUCLID section 3.8, Exposure estimates (see also Section 3.1.5.5).

## 5.1.2 Human Health and Environment

In the following, the information reported in the registration dossiers regarding exposure assessment and risk characterisation will be summarised.

### Category I classified substance with an annex to the CSR of the lead registrant termed "Potential risks associated with nano-sized xxx".

As no nano-specific REACH guidance was available, a semi-quantitative exposure assessment and risk characterisation was provided taking into consideration the approach that has been proposed by the EU's Scientific Committee on New Emerging Health Risks (SCENIHR) in 2007 and 2009<sup>40</sup>. It was noted that physico-chemical characterisers should be given attention in relation to the risk assessment of nanomaterials and that mass may not be the most appropriate metric. It was stated that alternative metrics should be considered.

The annex qualitatively described uses/processes where workers and consumers could potentially be exposed to nanomaterials. For inhalation exposure during manufacturing of the nanomaterials, reference was made to particle number concentration values (particles/cm<sup>3</sup>) measured in an EU 7<sup>th</sup> Framework Program (FP7) project. The registrant noted that it was not possible to distinguish from background during those measurements, but that the values were only marginally higher than outdoor particle number concentrations. Further, the registrant noted that enclosed processes are used during manufacturing and formulation and that in general a high degree of risk management measures and good hygiene practices are in place. No quantitative data for possible worker inhalation exposure beyond manufacturing was provided. In relation to dermal exposure, which is likely for this substance, it was concluded by the registrant that dermal penetration of the nanoform is not likely and thus no systemic exposure is expected.

With reference to the main CSR chapter addressing toxicity of the substance and with reference to recent reviews of the nanoform ("complementing the information in the CSR"), a brief discussion of toxicity of the nanoform vs. the bulk form was given. It was concluded that based on the available evidence there is no reason to assume that the toxicity of the nanoform is different from that of the bulk. In relation to this conclusion, no discussion of repeated inhalation toxicity of the nanoform vs. the non-nanoform was given.

A qualitative risk characterisation was given. Based on the use, exposure and toxicity discussions summarised above, it was qualitatively concluded that exposure was

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<sup>40</sup> SCENIHR (Scientific Committee on Emerging and Newly-Identified Health Risks). 2007. Opinion on the appropriateness of the risk assessment methodology in accordance with the technical guidance documents for new and existing substances for assessing the risk of nanomaterials.

SCENIHR (Scientific Committee on Emerging and Newly-Identified Health Risks). 2009. Opinion on the risk assessment of products of nanotechnologies.

negligible, the nanoform not likely to differ in toxicity from the bulk form and thus, use of the nanoform was considered safe for both workers and consumers.

For environment, a discussion was provided concluding that based on available evidence, toxicity is driven by an active ion of the substance and test data do not seem to indicate any difference between bulk and nano. Thus reference was made to the exposure assessment and risk characterisation for the bulk form in the CSR.

Category I not classified substance (lead and member dossier).

The lead and the member provided exposure assessment and risk characterisation in their own respective 'partial CSRs'. For this substance, the lead registrant outlined bulk and nanoforms in IUCLID section 1.2, Composition, but only registered the bulk form himself. Consequently the lead did not make any reference to 'nano' in the exposure assessment and risk characterisation in his own partial CSR. The member dossier on the other hand was specific for the nanoform and thus the exposure assessment and risk characterisation in his partial CSR has been assumed by the assessors to be specific to the nanoform, although this was not explicitly stated.

In that partial CSR, an array of uses was generically described based on the REACH use and process categories.

For human health, the registrant noted that dustiness heavily influence inhalation exposure concentration and thus low, medium and high dustiness grades were considered. Inhalation and dermal occupational exposure estimates were given for all the identified uses and for three dustiness grades. The inhalation estimates were also given with and without local exhaust ventilation (LEV) generally assumed to reduce exposure by 90%. Exposure estimation was done using the ECETOC TRA model and thus presented in the mass metric. There was no discussion of the applicability of this model for estimating exposure to nanomaterials and no comparison with exposure measurements. Some consumer exposure estimates were provided also using modelling considerations. Human exposure via the environment was considered qualitatively. Considering the abundant nature of the substance, no significant increase in exposure was considered to occur given the use of the substance.

The risk characterisation for worker inhalation compared the exposure estimates with the DNEL for repeated dose inhalation. In the absence of repeated inhalation toxicity studies (a testing proposal was made by the registrant), the registrant used the general nuisance dust level of 10 mg/m<sup>3</sup> (with no distinguishing between nano and bulk). Without LEV, this DNEL was often exceeded – with LEV, this DNEL was not exceeded and it was concluded that risks are controlled. Although dermal exposure estimates were given, this was not addressed in the risk characterisation, but elsewhere in the dossier it was concluded that there is no topical and systemic toxicity expected following dermal exposure.

No consumer exposure estimates exceeded the DNELs and human exposure via the environment was addressed qualitatively in line with the above description.

For the environment it was described qualitatively that no risks are expected given the abundance of this substance in the environment and that there are no hints that the substance may cause adverse ecotoxicological effects under natural conditions.

Two Category II dossiers addressing the same substance provided exposure assessments and risk characterisations, although only one of the registrants had classified the substance.

In the dossier where this Category II substance was considered not classifiable, a description of occupational and consumer uses, which may lead to inhalation was given. Dermal exposure was not estimated as the substance was assessed not to cause any toxicity via this route.

The substance has been manufactured and used for decades and data from extensive measuring campaigns within the production industry was provided. Repeated inhalation exposure estimates were given in the mass metric distinguishing between inhalable and respirable fractions and more detailed discussions on geometric and arithmetic means found in various studies were summarised. With reference to an IARC publication, a general statement was made that occupational downstream user exposure was likely to be lower. Actual data were not provided/summarised to support this. Peak exposures were qualitatively discussed. The registrant noted that peak exposures may likely happen, but are “unpredictable” and difficult to measure with the general measurement equipment applied. No exposure estimates were provided, but it was noted that safety data sheets instruct workers to wear respiratory protection when work conditions may result in elevated exposure.

The substance is used in an array of consumer uses. These used are generally in solid matrices and consumer exposure related to these was assumed to be negligible. One other use, which might lead to higher exposure, was described. An exposure estimate for inhalable dust (mass metric) was provided for this use.

A brief human health risk characterisation was provided concluding:

- Occupational: All repeated inhalation exposure estimates are below the repeated inhalation DNEL (being the NOAEC from a human data study assuming chronic bronchitis being the lead effect).
- Consumer: No quantitative risk characterisation provided as exposure considered negligible.
- Man via the environment: No quantitative risk characterisation provided as exposure considered negligible. Note: The exposure section did not discuss this exposure pathway.

In relation to the environment, it was stated that no adverse environmental effects were identified and thus no exposure assessment and risk characterisation were provided.

In the dossier where this Category II substances was classified, a huge number of occupational and consumer uses were outlined. These were generally described using the REACH use and process categories.

For occupational inhalation exposure, a general reference was made to measuring campaigns and a maximum exposure estimate covering respirable and inhalable fraction was given. No further details were given. With reference to an IARC publication, a general statement was made that occupational downstream user exposure was likely to

be lower. Data were not provided to support this. Likelihood and level of possible peak inhalation exposures were not provided.

It was noted that occupational dermal and oral exposure cannot be excluded but are unlikely to occur. No further details were provided.

Consumer exposure was generally assumed not to occur or being negligible as substance is used in or on a matrix. No exposure estimates were provided.

Exposure to man via the environment was discussed. Reference was made to a publication showing no elevated ambient air concentration close to production facilities and it was discussed that the substance was not likely to persist in air and water. It was concluded that exposure of man via the environment is negligible.

Quantitative human health risk characterisation was done for repeated occupational inhalation by comparing the single maximum exposure estimate with the two DNELs derived (one for 'inhalable' fraction and a lower DNEL for 'inhalable and respirable'). The lower DNEL was equal to the maximum exposure estimate.

For consumer and man via the environment, no quantitative risk characterisation was performed as no exposure was likely or anticipated.

For the environment, no exposure assessment was performed. Qualitative considerations were provided in the risk characterisation chapter stating that either no PNEC could be derived or where a PNEC was identified (for aquatic environment) that the substance was not expected to persist in water.

Another dossier for a non classified Category II substance included an exposure assessment and a risk characterisation chapter in the CSR.

The exposure assessment considered general exposure scenarios for occupational manufacturing and formulation, as well as for consumer use. For occupational exposure, it was stated that risk management measures are in place to reduce exposure to levels below the dust nuisance level of 10 mg/m<sup>3</sup>. No data were given to back up this statement. A dermal exposure was estimated assuming that part of the body was exposed and using default values in the REACH guidance document Chapter R.14. It was noted that due to the mild irritating character of the substance, excessive exposure of eye and skin should be avoided by using risk management measures.

Regarding consumer exposure, the substance is used in e.g. detergents, which may/will lead to dermal contact. No exposure was estimated, but it was noted that only a few grams are used in these preparations.

No quantitative human health risk characterisation was performed with the argument that there is no hazard threshold(s). This seems to be in contrast with the fact that DNELs were estimated in other parts of the dossiers for several exposure routes.

For the environment, it was qualitatively concluded that there will be no environmental risks associated with the use of this substance due to the substance being abundant in the environment and due to the expected releases of the substance.

For two classified Category III substances, exposure assessment and risk characterisation were provided.

For one of these dossiers only the inhalation route for workers was considered as this is the main route of exposure. It was not further specified why the dermal exposure was not considered. Considering the hazard profile of this substance, this route may have been relevant to consider further. Given the classification of the substance, consumer use is not supported and thus no consumer exposure assessment was provided.

Nine exposure scenarios were described in detail using the exposure scenario template from the REACH guidance. Inhalation exposure estimation was addressed by a mixture of measurement data and various exposure models. Applicability range of the models was not discussed. Human exposure via the environment was addressed using ECETOC TRA modelling. Applicability range of the model was not discussed. All exposure estimates were presented in the mass metric.

In the risk characterisation, the inhalation exposure estimates were compared with a DMEL for systemic toxicity (derived from a route-to-route extrapolation from oral data).

For the environment, exposure estimates were provided for the nine exposure scenarios using the ECETOC TRA model. Applicability range of the model was not discussed. All risk characterisation ratios were below 1, although in several cases close to 1.

Although it might be negligible for this substance, possible particle effects were not discussed in the exposure assessment and risk characterisation chapters.

For the other classified Category III substance, there were relatively detailed descriptions of the exposure scenarios (including operational conditions and risk management measures). However, it has not been easy for the assessors to understand the exposure routes and estimates considered. The registrant stated that consumer use is not considered given the CMR classification of the substance.

It should be noted that all hazard data and thus DNELs/PNECs derived in this dossier were based on read-across to the toxic ion.

There is an annex to the CSR with some model calculations of dermal and inhalation workers exposure estimates. Applicability range of the models was not discussed. Most of these estimates were given in the mass metric, but some of the inhalation estimates were given in a fibre metric. The latter however, does not seem to be addressed in the assessment and it was not clear to the assessors whether the substance could be supplied as fibre. It was not clear to the assessors how the exposure estimates in the annex were used to draw conclusions in the human health risk characterisation. Most emphasis was on a risk characterisation using biomonitoring data for the leading ion and comparing these to an internal DNEL for this ion.

For the environment, exposure levels for the addressed scenarios were estimated using EUSES. Applicability range of the model was not discussed. Also measurement values (for the leading ion) were provided. All risk characterisation ratios were below 1.

Although it might be negligible for this substance, possible particle effects were not discussed in the exposure and risk characterisation chapters.

### 5.1.3 Summary - General issues

The following aims at summarising the findings from the analysis and assessment of the exposure assessments and risk characterisations, where available in the dossiers addressed in this project. It is realised that one should be careful with generalising from the limited number of exposure assessments and risk characterisations provided.

#### **Nanoform (explicitly) considered?**

One Category I dossier explicitly addressed exposure assessment and risk characterisation of the nanoform in a qualitatively/semi-quantitative annex to the CSR. For another Category I member dossier, it could be inferred from the scope of that dossier that the exposure assessment and risk characterisation were for the nanoform of that substance, although without explicitly stating that a nanoform/nanomaterial was assessed in relation to the tools and models used (see below). The remaining dossiers were either for Category II or Category III substances. The assessments in the latter were largely based on the assumptions that toxicity was driven by a toxic ion, which might be correct, but no attention was given to the particle nature of the substances.

#### **Metrics**

One dossier (Category III) estimated exposure (using a model) in another metric than the mass-metric (i.e. in number of fibres). It was not clear from the dossier whether this substance was actually supplied as fibre, and the alternative metric was not used in the risk characterisation. Another dossier (Category I) noted that it is important to consider metrics alternative to mass and did list some exposure estimates in the particle number metric. As the values were considered negligible, no quantitative risk characterisation was carried out, but it also could not be possible as the DNELs estimated were in the mass-metric. For the remaining dossiers, alternative metrics to mass were not considered, neither any discussions of the relevance or uncertainty associated with using the mass-metric were given. Some dossiers distinguished between inhalable and respirable fractions of the agglomerated/aggregated particles.

Thus, no risks characterisations using metrics alternative to mass were identified. For the dossiers/substances where a quantitative risk characterisation was performed, this was usually done by comparing the DNEL to an exposure value and expressing the risk in a RCR (risk characterisation ratio). In one case there were also MOS (Margin of Safety) values presented and discussed (following the approach of the previous Technical Guidance Document for Risk assessment).

#### **Description of uses and exposure scenarios**

The approach to description of uses and exposure scenarios (including operational conditions and risk management measures), largely differed between dossiers. This ranged from very generic descriptions based on e.g. the use and process categories (i.e. essentially repeated information from IUCLID Section 3 picklists) to rather detailed descriptions using the exposure scenario format in the REACH guidance. The latter was explicitly applied in one Category III dossier.

#### **Use of exposure estimation models**

Several of the dossiers applied exposure estimation models for estimating human and environmental exposure. No discussions were found in relation to the applicability of those models for nanoforms/nanomaterials. According to the RIP-oN3 findings, these models have not been validated for nanomaterials.

## 5.2 Options for adapting REACH – exposure assessment and risk characterisation

### **Option 5.1. Require identification of uses and exposure assessment of the nanoform.**

It is suggested to specify in REACH Annex I that uses of the nanoform should be identified and that a separate hazard assessment, exposure assessment and risk characterisation should be conducted when data indicate different exposure and/or hazards/risks as compared to other forms addressed by that dossier. This follows as a logical consequence of the option 3.1-3.3 (see Section 3.2) and option 4.1 (in Section 4.2) of this report. The (de-) agglomerated/aggregated state (also indicated by registrants as being important), as well as other modifications of properties (e.g. dissolution, embedded in matrices) should be reflected in the various uses and life cycle steps of the nanoform. The same applies to any foreseen changes in the surface of the nanomaterials. This option is in line with the principle in the recently adopted EFSA guidance on nanomaterials<sup>41</sup>.

### **Option 5.2. Require considerations of most appropriate/relevant metric with preferable presentation in several metrics.**

Given the importance of metrics (see e.g. RIP-oN2 and 3 reports), it is appropriate to clarify e.g. in REACH Annex I that the exposure and hazard data should be presented in available and *relevant* metrics (as also suggested in the RIP-oN3 project guidance update proposals). Linked to this, it is suggested to specify that a justification for the choice of the most relevant metric(s) should be provided and that the influence and uncertainty, related to the choice of metrics, should be discussed in relation to exposure estimates, test results and risk assessment/characterisation. Thus, as it is realised that currently most information available is in the mass-metric, the uncertainty associated with this situation should be addressed by the registrant. In line with RIP-oN2 and 3, it is suggested to clarify that for the time being results should preferably be presented in several metrics, always including the mass metric.

Related issues, which should likely be addressed in guidance, including test guidelines:

Specifically, in relation to (animal) testing, it seems relevant to require in test guidelines that characterisation of the test material is done in a way that the results can be expressed in all metrics (also to avoid unnecessary future testing, see also the RIP-oN reports). This could in turn become legally binding via legal references to these test guidelines.

It is realised that specifically for exposure assessment there is the need for further development of appropriate equipment and tools. This includes validation of tools for nanomaterial exposure estimation (see RIP-oN3 report). The RIP-oN3 recommendation in relation to justifying use of models for nanomaterials seems highly appropriate.

It might be emphasised in guidance that when limited (hazard and/or exposure) information is available to assess the risk(s) of a given nanoform, the general principle of

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<sup>41</sup> European Food Safety Authority (EFSA). Scientific opinion. Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain. EFSA Journal 2011; 9(5): 2140.

implementing risk management measures in the interim (REACH Annex I, item 0.5) should be followed, thus focusing on exposure minimisation.

## 6 SUMMARY AND CONCLUSIONS

The current report presents:

- The results of an analysis and assessment of REACH registration dossiers (Task I, step 3&4) identified in the first phases of the project (Task I, step 1&2) as likely to address nanoforms/nanomaterials, and
- The options suggested for adapting the REACH regulation to reflect the properties of nanoforms/nanomaterials in relation to risk assessment, including identification and assessment of uses, exposures, hazards and risks (Task II, step 1).

The report will feed into Task II, step 2 of the project: the assessment of consequences for economy, environment, consumers and human health. In relation to this, and to the suggested options, it should be clarified that the project had the specific aim to investigate how the REACH regulation could be adapted in relation to nanomaterials. It was hence not within the scope of the project to consider other options for assessment of nanomaterials, such as e.g. nano-specific legislation, voluntary agreements, etc.

The detailed specifications of the project, as well as the results of the identification and selection of the REACH registration dossiers (Task I, step 1&2), can be found in CASG-nano/10/2011 (Annex I and II).

*As already noted, the current report is not a compliance check of the addressed registration dossiers. The report is rather an attempt to identify general issues, which could be used in the process of developing options for adapting the REACH regulation in relation to registration and assessment of nanomaterials.*

Related to this, it is important to mention that the current REACH regulation, including information requirements, does not contain any specific provisions related to nanomaterials. Additionally, the current REACH guidance is not tuned to address the properties of nanomaterials. In this respect, it should be noted that a REACH Competent Authority document (CA/59/2008 rev1) has clarified that the REACH provisions apply to nanomaterials and that registrants should attempt to apply the existing guidance in their registrations. An additional complicating factor for registrants was the fact that there was no adopted EC Recommendation on the definition of nanomaterial at the time of the first registration dead-line (December 2010). Moreover the REACH Implementation Projects (RIP-oNs), addressing how the REACH guidance could be updated, were not finalised by the time.

Altogether, considering the available information and the general REACH requirements, it was therefore up to each registrant to decide e.g.:

1. Whether the material registered should be considered/described as a nanomaterial
2. Whether such materials should be registered on their own or as a nanoform together with other forms of a substance
3. What nano-specific information to provide on the nanoform/nanomaterials
4. What nano-specific issues to address in the registration dossier, how to assess this information and what nano-specific conclusions to draw in the assessments in various parts of the dossier.

This naturally led to a varying level of information provided in the registration dossiers addressed. It also triggered some of the main challenges in the project related to:

- a) identification of dossiers addressing nanoforms/nanomaterials and
- b) analysing and assessing the information reported on 'nano' in those dossiers.

The first challenge was the identification of registration dossiers addressing nanomaterials. The uncertainties related to the semi-automatic searches among all registration dossiers in REACH-IT and the identification of the initial set of 45 dossiers possibly addressing nanoforms/nanomaterials are set out in the Task I, step 1&2 reporting. As outlined in Chapter 2 and 3 of this report, the initially identified 45 dossiers were reduced to 25 dossiers following a more detailed analysis and assessment of the information in relevant parts of those dossiers. It should be noted that these activities were conducted prior to adoption of the EC Recommendation on the definition of nanomaterial.

After adoption of the EC Recommendation on the definition of nanomaterial (October 2011), the information found in the registration dossiers has been compared to the criteria set out in the definition. This led to the conclusion that registrants generally did not provide the constituent/primary particle size distribution needed to explicitly verify whether a nanomaterial and/or nanoform(s) is/are addressed by a given dossier. This is not unexpected as constituent/primary particle size distribution is not a REACH standard information requirement. On the other hand, given other information found in various places of the 25 dossiers and expert judgment, it is believed that the 25 dossiers address (or with very high probability address) nanomaterials and/or nanoforms. It is far more likely that a number of dossiers, where the registrant intention was to cover nanomaterials/nanoforms, were excluded through the searches, conducted in step 1 of the project, and the 'deselection' of 20 dossiers (from 45 to 25) than mistakenly including non-nanoform/material dossiers. It can be argued that having not identified further dossiers in step 1 and having excluded/deselected 20 of the identified dossiers would weaken the current analysis and assessment. However, in relation to the latter, the exclusion of these dossiers was done to ensure that conclusions made in the project relate to nanomaterials/nanoforms. In relation to dossiers possibly not identified in step 1, such dossiers did not emerge as 'nanodossiers' (given the search strategies described). It therefore seems unlikely that they would address/discuss nano issues/properties in greater detail than the 25 dossiers, which have been subject to a detailed analysis and assessment in this project. Chapter 3 provides more discussion and detail on this issue.

Linked to the challenge of identifying dossiers addressing nanomaterials was the challenge of analysing and assessing the information on 'nano' in those dossiers.

The information on identification/characterisation of the substances addressed by a given dossier were of varying level of detail. Some of the Category I dossiers explicitly mentioned that a nanoform was covered by the registration. In those dossiers a nanoform was described in a generic way.

Regarding information on other parameters relevant for identifying or characterising nanoforms/nanomaterials, it was found that about half of the dossiers reported information (in various places of the dossiers) indicating that the registered substance

could be surface treated, but specific information (including analytical data) on the type and extent of such treatments was only indicated in one dossier.

Information on particle size (distribution) was given under the 'granulometry' endpoint and in some cases in the Substance Identity section (IUCLID section 1). The quality of the information on this endpoint varied among the different dossiers, but a number of issues with significant impacts on the assessment of nanomaterials were identified. First, the methods used were in several cases not appropriate for the measurement of particle size distributions of nanomaterials (e.g. the method does not detect particles in the 1-100 nm range). Second, the results from several methods do not distinguish between primary particles, aggregates, and agglomerates, and registrants did not clearly and consistently make a distinction between these. Thirdly, members of a joint submission did not provide their own granulometry data. For joint submissions, normally one set of particle size data were given in the lead dossier. As particle size (distribution) depends on the manufacturing process, and is logically not amenable to read-across, the project suggests that members of a joint submission should submit their own individual granulometry data. It should be noted that under the current REACH regulation, submission of granulometry data individually by members of a joint submission would require an 'opt out' for this endpoint, which has some consequences in relation to fees and the possibility for prioritisation of the dossier for compliance check.

About half of the dossiers provided some additional information on other possible characterisers such as density and surface area; for example two, three and five Category II, III and IV dossiers, respectively, included data related to surface area. However, typically the description of the method used to obtain the reported data was not included.

The ambiguity in relation to the scope and identification/characterisation of nanoforms addressed by the registrations generally cascaded through the dossiers. A few dossiers did distinguish between 'bulk' and 'nano'. Though the purpose was to explicitly address different forms, this was done in varying level of detail between dossiers, as well for information (CSRs and endpoints) within those dossiers and did not go to a level beyond considering 'nano' as one form, i.e. differences in characteristics between nanoforms of the same substance were not addressed.

Further, it was found that test data provided for physico-chemical, human health and environmental endpoints generally did not describe the test material in great detail. Further, description of sample preparation, which is an important aspect known to influence the outcome of a given study, was varying and sometimes lacking. On a positive note it seems that this situation is improving for recent (eco-)toxicological studies of nanomaterials, probably supported by the fact that scientific journals continuously raise their requirements in this respect.

It is the outcome of this assessment that, in order to address the above mentioned ambiguities, it is essential to outline in a transparent manner what is registered in terms of nanoforms and how these are addressed in terms of information requirements and assessment. This is reflected in the general options presented in Sections 3.2, 4.2.1 and 5.2 relating to:

- Description of the scope of a registration dossier in terms of nanoform(s) addressed

- Identification/characterization for each nanoform for each registrant (being a lead or member registrant)
- Addressing transparently throughout the dossiers specific nanoforms differing in uses and properties (including endpoints, manufacturing process, Classification and Labelling, uses, as well as possible exposure assessment and risk characterisation). This includes:
  - o Improved test material and sample preparation description
  - o That non-testing data, e.g. read-across, should be scientifically justified on a form-to-form basis.

These options should be logically linked to the recently adopted EC Recommendation on the definition of nanomaterial.

The information that needs to be generated should be focussed on demonstrating safety of the different forms that are manufactured, imported and used on the EU market. To facilitate generation of specific information there is a strong need for developing non-testing methods and for creating stakeholder consensus on the use of non-testing data. An important prerequisite for this is a clear understanding of the characteristics of the nanoforms within the relevant registration dossiers. In any case, it is important that a transparent scientific discussion is made by the registrant when using such methods for nanoforms/nanomaterials. See further discussion in Section 4.2.1.

The general options for REACH adaptation, suggested in this report, can be implemented either in relation to substance identification or as characterisation requirements. This should be considered in the scenario development in the next phase of this project.

It is also suggested as an option to clarify in the REACH text that the registrant should address and discuss the choice of metric as the mass metric alone may not always be the most relevant metric (see Section 5.2).

The remaining options set out in this report are largely of a more endpoint specific character (mainly outlined in Section 4.2.2 and 4.2.3). Implementation of those options would make little sense unless the above general options, explicitly setting out what is registered in terms of nanoforms, are implemented.

The following table summarises the options for adapting the REACH regulation suggested in this project, with reference to the sections where a more detailed discussion can be found.

**Table 3. Overview of options for adaptation of REACH.** Details in Sections indicated in the right column. Options 3.1-3.5, 4.1.-4.4 and 5.1-5.2. are considered fundamental.

<b>Substance identification and physico-chemical properties</b>	
<b>Option 3.1.</b> Explicitly require registrants to describe the scope of the registration dossier.	3.2.1
<b>Option 3.2.</b> Explicitly require registrants to provide more detailed characterisation of nanomaterials/nanoforms.	3.2.1

<b>Option 3.3.</b> Require that nanoforms are explicitly addressed in the endpoint sections.	3.2.2
<b>Option 3.4.</b> Require detailed description of the test material/sample and sample preparation.	3.2.2
<b>Option 3.5.</b> Require scientific justifications for grouping/read-across/QSAR and other non-testing approaches for different forms.	3.2.2
<b>Option 3.6.</b> Include information on dustiness.	3.2.2
<b>General options for human health hazards, environmental fate, environmental hazards</b>	
<b>Option 4.1.</b> Require that nanoforms are explicitly addressed in the endpoint sections.	4.2.1
<b>Option 4.2.</b> Require detailed description of the test material/sample and sample preparation.	4.2.1
<b>Option 4.3.</b> Require scientific justification for grouping/read-across/QSAR and other non-testing approaches for different forms	4.2.1
<b>Option 4.4.</b> Require considerations of most appropriate/relevant metric with preferable presentation in several metrics	4.2.1
<b>Human health hazards</b>	
<b>Option 4.5.</b> Require acute toxicity data for the most relevant route of exposure	4.2.2
<b>Option 4.6.</b> Change 'particles' to '(nano)particles' for repeated dose toxicity studies (inhalation)	4.2.2
<b>Option 4.7.</b> Require non-bacterial in vitro gene mutation study	4.2.2
<b>Environmental fate &amp; hazards</b>	
<b>Option 4.8.</b> Require that bioaccumulation is addressed specifically for the nanoform	4.2.3
<b>Option 4.9.</b> Specify that adsorption/desorption behaviour of nanomaterials should not be assessed based on $K_d$ values derived from $K_{oc}$ and $K_{ow}$	4.2.3
<b>Option 4.10.</b> Consider water solubility in relation to test waiving	4.2.3
<b>Option 4.11.</b> Specify that long term testing should not be waived based on lack of short term toxicity	4.2.3
<b>Option 4.12.</b> Specify that algae testing should not be waived based on insolubility	4.2.3
<b>Option 4.13.</b> Require that testing on soil and sediment organisms is prioritised	4.2.3

<b>Exposure assessment and risk characterization</b>	
<b>Option 5.1.</b> Require identification of uses and exposure assessment of the nanoform	5.2
<b>Option 5.2.</b> Require considerations of most appropriate/relevant metric with preferable presentation in several metrics	5.2



**Dossier** \_\_\_\_\_ **(add name of substance)**

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## **Nano Support AA**

### **Analysis and Assessment Criteria**

**(NB! The content of this file shall only be used for the purpose of the Nano Support AA and does not in any way represent a legal evaluation of the dossier addressed)**

The document will be updated after experiences with analysis and assessment of the first dossiers.

Summary information (to be filled in form each dossier analysed and assessed)

Field	Value in dossier	Assessor's Comment	Instructions
Substance			<i>Name of the substance</i>
Registration number			<i>To keep track of the analysis</i>
EC Number			<i>To keep track of the analysis</i>
CAS number			<i>To keep track of the analysis</i>
Registered tonnage band	<input type="checkbox"/> 1-10 ton per annum <input type="checkbox"/> 10-100 ton per annum <input type="checkbox"/> 100-1000 ton per annum <input type="checkbox"/> >1000 ton per annum		<i>If more forms are covered, please further specify tonnage per form</i>
IUCLID version 5.2.3	<input type="checkbox"/> Yes <input type="checkbox"/> No  Section 2.1 (C&L) <input type="checkbox"/> Yes <input type="checkbox"/> No  Section 4.1: <input type="checkbox"/> Yes <input type="checkbox"/> No		<i>Was a version of IUCLID applied, which allowed choosing the value "nano" in the pick lists?</i>  <i>If yes, in which section (2.1 or/and 4.1) was the "nano" value chosen in the pick list?</i>
Nano substance	<input type="checkbox"/> Yes <input type="checkbox"/> No		<i>Is/are the nanoform(s) registered as a substance in its own right?</i> <i>How is that verified?</i>  <i>If there are any ambiguity in relation to whether nanoforms are covered or not (e.g. on 'grey list' substances), please specify the ambiguity.</i>  <i>If the following information is on the substance in the granulometry section, please indicate it here:</i> - average diameter - particle size distribution -particle refers to aggregate/agglomerate/primary particle/not specified
Bulk + Nano form(s)	<input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not explicit (describe ambiguity in next column)		<i>Are nanoform(s) registered along with the bulk? How is that verified? Is the Nanoform explicitly reported (e.g. in composition, section 1.2)?</i>  <i>If there are any ambiguity in relation to whether nanoforms are covered or not (e.g. on 'grey list' substances), please specify the</i>

			<i>ambiguity.</i>
Several nanoforms (with or without bulk)	<input type="checkbox"/> No <input type="checkbox"/> Yes  <input type="checkbox"/> Not explicit (describe ambiguity in next column)  <input type="checkbox"/> Yes, in C&L section <ul style="list-style-type: none"> <li><input type="checkbox"/> Different C&amp;L entries for nanoform(s) and Bulk</li> </ul> <input type="checkbox"/> Yes, in form of substance (IUCOLID section 4.1)		<i>Does the dossier address or specify several nanoforms?          And if so:          - Do the nanoforms refer to different sizes/shapes of the same nanomaterials or to the presence of nanomaterials of different compositions (e.g. nanoforms with different purity profiles (e.g. 80 % SiO<sub>2</sub>, 90 % SiO<sub>2</sub>), different surface treatments or surface chemistry (e.g. SiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub> coated SiO<sub>2</sub>, ZnO/Al<sub>2</sub>O<sub>3</sub> coated SiO<sub>2</sub>), silane coated SiO<sub>2</sub>)?</i>
			<i>- In which parts of the dossier?</i>
			<i>If there are a bulk and nano-forms, do they have different C&amp;L entries?</i>
			<i>If there are any ambiguity in relation to whether nanoforms are covered or not (e.g. on 'grey list' substances), please specify the ambiguity.</i>
Other dossiers addressing the same or similar nanoform(s) (either as forms or as substances in their own right)	<input type="checkbox"/> Yes <input type="checkbox"/> No		<i>Specify links (explicit links made by registrant(s) or the basis on which assess the link (which parameters, values...)).</i>
Nano-specific test/information in endpoints	<input type="checkbox"/> Yes <ul style="list-style-type: none"> <li><input type="checkbox"/> Phys-chem</li> <li><input type="checkbox"/> Environmental fate</li> <li><input type="checkbox"/> Ecotoxicology</li> <li><input type="checkbox"/> Toxicology</li> <li> </li> <li><input type="checkbox"/> Testing information</li> <li><input type="checkbox"/> Adaptation</li> <li><input type="checkbox"/> other</li> </ul> <input type="checkbox"/> No		<i>Is there any nano specific information/tests? If yes, where? What type of information?</i>
CSR	<input type="checkbox"/> Yes, nanoform(s) considered <input type="checkbox"/> No, nanoform(s) not considered		<i>Does the assessment specifically address the nanoform(s)?</i>

	<input type="checkbox"/> Yes, surface treatment of nanoform(s) considered <input type="checkbox"/> No, surface treatment of nanoform(s) not considered		<i>Does the assessment specifically address surface treatment of the nanoform(s)?</i>
			<i>If there are any ambiguity in relation to whether nanoforms are covered or not (e.g. on 'grey list' substances), please specify the ambiguity</i>
<b>Summary of analysis and assessment</b>			<i>Brief summary</i>  <i>Highlight main issues, including:</i> - Substance ID solution - (Additional) endpoints considered? - How has metric been addressed? - AF application - How has IR been addressed (read-across, waiving, test proposal, exposure based waiving..) - Any other creative/pragmatic approaches .....

**1. General information (Please make repeatable blocks when more nanoforms are addressed for the same endpoint)**

*To the assessor: the questions below are meant to guide your assessment. Please note that information addressing each of these questions may or may not be available in the dossier.*

(IUCLID) Field	Value in dossier	Assessor's Comment	Instructions
General remarks			<i>Are the nanoform(s) covered by the dossier sufficiently described/distinguished?</i>
1.1. Identification	- Reference substance: - Type of substance: - Name of substance/form:		
1.2. Composition (s)			<i>Composition(s) of the substance</i>
			<i>Is surface chemistry considered?</i>
			<i>Is the nanoform reported as a separate composition?</i>
1.4. Analytical information			<i>Is the standard analytical information (Annex VI, 1.2) included for the nanoform?</i>  <i>For each composition in 1.2?</i>
			<i>Are there any additional analytical data that specifically addresses nanomaterials?</i>
			<i>Are methodology(ies) appropriate for the measurement of the parameter?</i>
			<i>Are the requirements of Annex VI 2.3.7 fulfilled?</i>
			<i>Is the information sufficient to enable the substance or form of a substance to be identified?</i>
			<i>Are any measurement uncertainties /limitations in terms of what is measured documented and taken into account?</i>

			<i>Does the analytical data enable the nanoform to be identified/characterised?</i>
4. Physical and chemical properties			<i>General remarks</i>
4.1 Appearance/ physical state of the substance			<i>Is the value 'nanomaterial' selected in the pick list? Are there other physical states reported (e.g. powder, dispersion)?</i>  <i>Please also cross-check with the manufacturing and uses sections, which may contain information on this.</i>
4.2 Melting/freezing point			
4.3 Boiling point			
4.4 Density			<i>Is it provided for the nanoform? (If so, in SSA or VSSA?)</i>
4.5 Particle size distribution (Granulometry) Dry and in relevant media			<i>Please record (if available):</i> <ul style="list-style-type: none"> <li>- average diameter (as reported)</li> <li>- particle size distribution (as reported)</li> <li>- particle size range (as reported)</li> <li>-particle refers to aggregate/agglomerate/primary particle/not specified</li> <li>- average size of manufactured primary particles</li> </ul> <i>Which metrics are used?</i>  <i>Is the following information reported?</i> <ol style="list-style-type: none"> <li>1. Sample preparation methods and analysis methods used</li> <li>2. Lot number, sample number</li> <li>3. Suspending medium, temperature, pH</li> <li>4. Concentration (relevant to particles or fibres)</li> <li>5. Representative image(s) from microscopy</li> <li>6. Particle size distribution histogram of Stoke's (effective hydrodynamic) radius <math>R_s</math></li> <li>7. Average particle size(s) for resolvable peaks in the distribution</li> <li>8. Expected % change of reported values in the future (e.g.</li> </ol>

			<p>variations between production batches)</p> <p>9. Reference all Standards (see e.g. ISO reported previously) and reference materials used.</p> <p>If there are multiple sources of information on granulometry (e.g. multiple tests with different results, please record these here)</p> <p>Are specific surface area and shape acknowledged in this section? Or are they treated in additional phys-chem properties?</p> <p>(Note: When assessing a given tox or ecotox endpoint study, it is important to judge whether the test material in that study is 'the same' as that in the granulometry section, e.g:</p> <ol style="list-style-type: none"> <li>1. The trade name is the same (the caveat is that a particular trade name's granulometry can drift over time)</li> <li>2) The lot number is the same</li> <li>3) There is granulometry in the tox/ecotox study and the average size in that study falls within the limits of the results of the granulometry study.</li> </ol> <p>If this is not the case, it would seem reasonable that a justification is made by the registrant in relation to how the results from a given study would fit the material being registered).</p>
4.6 Vapour pressure			
4.7 Partition coefficient, n-octanol/water			<p>OECD concluded that test guidelines 107, 117 and 123 might be applicable under some circumstances or to some classes of manufactured nanomaterials. Is the methodology used appropriate for nanomaterials? Are limits of the methodology taken into account? Other indications?</p> <p>If the registrant has waived the test because the substance is inorganic, is there a reason to believe the waiver is not applicable to the nano-form? Why?</p>

4.8 Water solubility			<i>How is it considered? Was visual/instrumental assessment carried out to check the dispersion or solubilisation state? (Due to the size of NPs, a visual assessment is questionable). Is ion leaching reported for nano substance/form(s) (or for bulk-form?)? Is dispersibility considered in this endpoint or as additional endpoint? As dispersion and solubility are used interchangeably for nanomaterials, is it clear which of these endpoints the registrant covers?</i>
4.10 Surface tension			
4.11 Flash-point			<i>Is this addressed for the nanoform(s)? If so how? (NB: Not relevant for solids)</i>
4.12 Auto flammability			<i>Is this addressed for the nanoform(s)? If so how? Is self ignition temperature reported?</i>
4.13 Flammability			<i>Is this addressed for the nanoform(s)? If so how?</i>
4.14 Explosiveness			<i>Is this addressed for the nanoform(s)? If so how?</i>
4.15 Oxidising properties			<i>Is this addressed for the nanoform(s)? If so how?</i>
4.17 Stability in organic solvents			<i>Is this addressed for the nanoform(s)? If so how? (Relevant for storage or specialized uses)</i>
4.21 Dissociation constant			
4.22 Viscosity			<i>(NB: Not relevant for solids)</i>
<b>Other endpoints/parameters</b>			<i>General questions to each endpoint (not repeated below): - Has the endpoint parameter been addressed in the dossier? - If yes, where (IUCLID section and/or CSR) and how? - Does the registrant make any conclusions regarding the importance of this information?</i>
Morphology/Shape?			<i>Shape: is the following information reported? NB! This information may likely be reported in the granulometry section.  1. Sample preparation methods and analysis methods used</i>

			<ol style="list-style-type: none"> <li>2. Lot number, sample number</li> <li>3. Suspending medium, temperature, pH</li> <li>4. Representative image(s) from microscopy</li> <li>5. Particle shape descriptor(s)</li> <li>6. Reference all Standards (e.g. ISO) used and reference materials used</li> </ol>
Surface Area and specific surface area?			<p>Surface Area measured via BET: is the following information reported?</p> <ol style="list-style-type: none"> <li>1. Sample preparation methods and analysis methods used</li> <li>2. Lot number, sample number</li> <li>3. Pre-treatment and degassing conditions, e.g. degassing in a vacuum or in inert gas flow, temperature and duration of degassing;</li> <li>4. Mass of degassed sample;</li> <li>5. Density;</li> <li>6. Adsorptive (chemical nature, purity);</li> <li>7. Adsorption isotherm (na, plotted against relative pressure, <math>p/p_0</math>), measurement temperature;</li> <li>8. Evaluation parameters: multipoint or single-point determination, BET plot or range of linearity, monolayer amount, BET parameter C, molecular cross-sectional area used;</li> <li>9. Specific surface area;</li> </ol> <p>References for all Standards (e.g. ISO) and reference materials used.</p> <p>Note to the assessor: please record whether the value in the dossier is SA, SSA, or VSSA (or possible multiple values).</p> <p>If a different method has been used (other than BET), please indicate so. If results are available from BET and other methods, how do these results compare?</p>
Dustiness			<p>NB! This information may likely be reported in the granulometry section.</p>

			<i>Check also CSR, especially exposure scenarios</i>
Agglomeration/Aggregation			<i>In alternate media: solid phase, aqueous and non-aqueous media and test media (to interpret aquatic tox. studies)</i>
Crystalline phase			<i>NB! This information may likely be reported in the:</i> 1) <i>Substance ID section (including remarks)</i> 2) <i>Granulometry section</i>
Crystallite size			
Zeta potential			<i>In test media and DI water, preferably to interpret aquatic tox results.</i>
Surface chemistry			
Photocatalytic activity			
Pour density			
Porosity			
Redox potential			
Radical formation potential			
Representative TEM picture			<i>NB! This information may likely be reported in the granulometry section.</i>
Other relevant information			
<b>Other Information</b>			<i>Please report if the analysis of the dossier revealed other relevant issues, which do not fit in one of the above fields.</i>

## 2. Manufacture and uses

<b>(IUCLID) Field</b>	<b>Value in dossier</b>	<b>Assessor's Comment</b>	<b>Instructions</b>
General remarks			
3.1 Manufacture			<i>Is there any information on the manufacture of the nano form of the substance?</i>
3.2 Estimated quantities			
3.5 Identified uses (and exposure)			<i>Are uses specified for the nanoform(s) addressed? Are different uses than for</i>

<b>(IUCLID) Field</b>	<b>Value in dossier</b>	<b>Assessor's Comment</b>	<b>Instructions</b>
			<i>bulkform specified for the nano-form of the substance? Are there any consumer uses indicated, and if so are they addressed specifically?</i>
3.6 Uses advised against			<i>Is information specified for the nanoform(s) addressed?</i>
3.8 Exposure estimates			
<b>Other Information</b>			<i>Please report if the analysis of the dossier revealed other relevant issues, which do not fit in one of the above fields.</i>

### 3. Classification and labelling

(CSR section 3, IUCLID section 2)

(IUCLID) field	Value in dossier (Hazard cat and class)	Assessor's Comment	Instructions
General remarks			
Phys-chem.			<i>Is C&amp;L indicated for the nanoform(s)?</i>
Human health			<i>Is C&amp;L indicated for the nanoform(s)?</i>
Environment			<i>Is C&amp;L indicated for the nanoform(s)?</i>
<b>Other Information</b>			<i>Please report if the analysis of the dossier revealed other relevant issues, which do not fit in one of the above fields.</i>

### 4. Environmental fate properties

(IUCLID) Field	Value in dossier	Assessor's Comment	Instructions
General remarks			
5.1.1 Phototransformation / photolysis, in air			
5.1.2 Hydrolysis			
5.1.3 Phototransformation in water			
5.1.4 Phototransformation in soil			
5.2.1 Biodegradation in water (screening)			<i>If dealing with metal and metal oxide surface treated materials, is the surface treating agent degradation addressed? If present in the dossier, is carbon-based NM degradation addressed? Are methodologies issues addressed?</i>
5.2.2 Biodegradation in (water and) sediment			<i>For metal and metal oxide surface treated materials, is the surface treating agent degradation addressed? If present in the dossier, is carbon-based NM degradation addressed? Are methodologies issues addressed?</i>
5.2.3 Biodegradation in soil			<i>For metal and metal oxide surface treated materials, is the surface treating agent degradation addressed? If present in the dossier, is carbon-based NM</i>

			<i>degradation addressed? Are methodologies issues addressed?</i>
5.2.4 Mode of degradation in actual use			
5.3.1 Bioaccumulation, aquatic/sediment			<i>What method(s) was/were used to measure bioaccumulation? Was it estimated from other properties? Are issues related to nanomaterials addressed? Is sample preparation well described?</i>
5.3.2 Bioaccumulation/terrestrial			<i>What method(s) was/were used to measure bioaccumulation? Was it estimated from other properties? Are issues related to nanomaterials addressed? Is sample preparation well described?</i>
5.4.1 Adsorption/desorption			
5.4.2 Henry's law constant			
5.4.3 Distribution modelling			
5.4.4 Other distribution data			
5.5.1 Monitoring data			
5.5.2 Field studies			
5.6 Additional information on environmental fate and behaviour			
<b>Other endpoints/parameters</b>			<i>General questions to each of these 'other endpoints' (not repeated below): - Has the endpoint parameter been addressed in the dossier? - If yes, where and how?</i>
Dispersion stability			
Identification of degradation products			
<b>Other Information</b>			<i>Please report if the analysis of the dossier revealed other relevant issues, which do not fit in one of the above fields.</i>

## 5. Human Health Hazard Assessment (Toxicological Information)

(IUCLID) Field	Value in dossier	Assessor's Comment	Instructions
General remarks			<i>For this section: see general comments/instructions</i>
7.1.1 Basic toxicokinetics			<i>In general: Is kinetics sufficiently understood for the forms addressed by the registration in relation to how data for individual endpoints are being interpreted. Has (radio-) labelling been applied? Have barrier transfer models been applied?</i>
7.1.2 Dermal absorption			
7.2.1 Acute toxicity oral			
7.2.2 Acute toxicity inhalation			<i>Has the actual concentration in breathing zones been determined (+characterisation)? BAL?</i>
7.2.3 Acute toxicity dermal			
7.2.4 Acute toxicity other routes			<i>If Injection studies used/reported: justified?</i>
7.3.1 Skin irritation/corrosion			<i>In vitro skin corrosion test – suitable?</i>
7.3.2 Eye irritation			
Respiratory irritation			
7.4.1 Skin sensitisation			
7.4.2 Respiratory sensitisation			
7.5.1 Repeated dose toxicity: oral			
7.5.2 Repeated dose toxicity: inhalation			<i>Has the actual concentration in breathing zones been determined (+characterisation)? Histology of respiratory tract; BAL, respiratory cell proliferation</i>
7.5.3 Repeated dose toxicity: dermal			
7.5.4 Repeated dose toxicity: other routes			<i>If Injection studies used/reported: justified?</i>
7.6.1 Genetic toxicity <i>in vitro</i>			
7.6.1 Genetic toxicity <i>in vivo</i>			<i>Have there been positive results for genotoxicity in <i>in vitro</i>?</i>
7.7 Carcinogenicity			
7.8.1 Toxicity to reproduction			
7.8.2 Developmental toxicity/teratogenicity			
7.8.3 Toxicity to reproduction: other studies			

7.9.1 Neurotoxicity			
7.9.2 Immunotoxicity			<i>Please describe whether and how these for NMs important endpoints were studied: Inflammation (Pro-inflammatory effects, pro-fibrogenic effects), oxidative stress.</i>  <i>Or make reference to sections where this has been described.</i>
7.9.3 Specific investigations: other studies			<i>e.g. Cardiovascular toxicity; phototoxicity,</i>
7.10.1 Health surveillance data			
7.10.2 Epidemiologic data			
7.10.3 Direct observations: clinical cases, poisoning incidents and other			
7.10.4 Sensitisation data (humans)			
7.10.5 Exposure related observations in humans: other data			
7.12 Additional toxicological data			
<b>Other endpoints/parameters</b>			<i>General questions to each endpoint (not repeated below):</i> <i>- Has the endpoint parameter been addressed in the dossier?</i> <i>- If yes, where and how?</i>
"Secondary effects"			<i>Have secondary effects been addressed? If so, where and how?</i>  <i>Or make reference to sections where this has been described.</i>
<b>Other Information</b>			<i>Please report if the analysis of the dossier revealed other relevant issues, which do not fit in one of the above fields.</i>
<b>DN(M)EL workers:</b>			
General remarks			<i>Beside DNEL give assessment factor (AF) and its composition: Interspecies (IS) or allometric scaling (AS), other interspecies differences (oIS), Intraspecies (IaS), Duration (D), dose-response (DR), severity of effects (SV), database quality (DQ)</i>
DN(M)EL dermal,			

acute (systemic)			
DN(M)EL inhalation, acute (systemic)			
DN(M)EL dermal, acute (local)			
DN(M)EL inhalation, acute (local)			
(DN(M)EL dermal, long term (systemic)			
DN(M)EL inhalation, long term (systemic)			
(DN(M)EL dermal, long term (local)			
DN(M)EL inhalation, long term (local)			
<b>DN(M)EL general population:</b>			
DN(M)EL dermal, acute (systemic)			
DN(M)EL inhalation, acute (systemic)			
(DN(M)EL oral acute (systemic)			
DN(M)EL dermal, acute (local)			
DN(M)EL inhalation, acute (local)			
(DN(M)EL dermal, long term (systemic)			
DN(M)EL inhalation long term (systemic)			
DN(M)EL oral long term (systemic)			
(DN(M)EL dermal, long term (local)			
DN(M)EL inhalation long term (local)			

## 7. Environmental hazard assessment

(IUCLID) Field	Value	Assessor's Comment	Instructions
General remarks			
6.1.1 Short-term toxicity to fish			<i>For this section: see general comments/instructions</i>
6.1.2 Long-term toxicity to fish			
6.1.3 Short-term toxicity to aquatic invertebrates			
6.1.4 Long-term toxicity to aquatic invertebrates			
6.1.5 Toxicity to			

algae and cyanobacteria			
6.1.6 Toxicity to aquatic plants other than algae			
6.1.7 Toxicity to aquatic micro-organisms			
6.1.8 Toxicity to other aquatic organisms			
6.2 Sediment toxicity			
Terrestrial compartment			
6.3.1 Toxicity to soil macro organisms			
6.3.2 Toxicity to terrestrial arthropods			
6.3.3 Toxicity to terrestrial plants			
6.3.4 Toxicity to soil micro-organisms			
Toxicity to other terrestrial organisms			
6.3.5 Toxicity to birds -secondary poisoning			
6.3.6 Toxicity to other above-ground organisms			
6.4 Biological effects monitoring			
6.5 Biotransformation and kinetics			
<b>Other endpoints/parameters</b>			<i>General questions to each endpoint (not repeated below): - Has the endpoint parameter been addressed in the dossier? - If yes, where and how?</i>
<b>Other Information</b>			<i>Please report if the analysis of the dossier revealed other relevant issues, which do not fit in one of the above fields.</i>
PNECoral (secondary poisoning)			
PNEC water			
PNEC sediment			
PNEC for sewage treatment plant			
PNEC soil			

## 8. PBT and VPVB assessment

Value	Assessor's Comment

## 9. Exposure assessment

Field	Value	Assessor's Comment	Instructions
General remarks			
Exposure scenarios			<p><i>Is it clear which exposure scenarios relate to the nanoform(s)?</i></p> <p><i>Do the ESs provide sufficient details (OC, technical RMMs, PPE)?</i></p> <p><i>Is it considered/justified that the proposed risk management measures (RMM) are appropriate for the nanoform(s)?</i></p> <p><i>Life cycle:</i></p> <ul style="list-style-type: none"> <li>- <i>Are there any ES for professional uses? (Is it considered whether suggested RMMs can be implemented for these uses?)</i></li> <li>- <i>Are there any consumer ESs?</i></li> <li>- <i>Are end-of-life issues, including recycling addressed?</i></li> <li>- <i>In general, is the life cycle appropriately considered?</i></li> </ul> <p><i>Please consider further issues raised in RIP-oN3 reporting?</i></p>
Human health exposure estimation			<p><i>In general, how is the exposure estimated (analytical techniques, models)?</i></p> <p><i>If measures, carefully consider how sampling and monitoring have been done and please specify how background has been considered.</i></p> <p><i>Are choice of exposure assessment</i></p>

			<p><i>method/s justified? How?</i></p> <p><i>Are relevant exposure routes and populations addressed (link to ES questions above)?</i></p> <p><i>Is choice of metric(s) considered/justified?</i></p> <p><i>Are uncertainties considered? How?</i></p> <p><i>Please consider further issues raised in RIP-oN3 reporting</i></p>
Environmental exposure estimation			<p><i>In general, how is the exposure estimated (analytical techniques, models)?</i></p> <p><i>If measures/analytical methods are used, carefully consider how sampling and monitoring have been done and please specify how background has been considered.</i></p> <p><i>Are choice of exposure assessment method(s) justified? How?</i></p> <p><i>Are relevant compartments considered/justified?</i></p> <p><i>Is choice of metric(s) considered/justified?</i></p> <p><i>Are uncertainties considered? How?</i></p> <p><i>Please consider further issues raised in RIP-oN3 reporting?</i></p>

## 10. Risk characterisation

Field	Value	Assessor's Comment	Instructions
General remark			
Human health			<p><i>In general, how is the risk characterised?</i></p> <p><i>Are method(s) justified?</i></p> <p><i>Are uncertainties considered, incl. choice of assessment factors and metrics?</i></p>

			<p><i>Are DN(M)ELs derived for the nanoform(s)? If yes, how?</i></p>
Environment			<p><i>In general, how is the risk characterised?</i></p> <p><i>Are method(s) justified?</i></p> <p><i>Are uncertainties considered, incl. choice of assessment factors and metrics?</i></p> <p><i>Are PNECs derived for the nanoform(s)? If yes, how?</i></p>

### 11. Other remarks

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### 12. Summary of the examination (including findings/issues that have to be taken into account in the non-confidential document summarising Task I, step 4)

*Considering the above, is it shown that risks are controlled? If not, why not and what are the limitations? What are the uncertainties?*

*Please also list any ideas that pop-up in relation to Task II, i.e. suggestions for options for changing the REACH annexes (or the general parts of REACH) in order to better address the hazards/risks of nanomaterials.*

## Annex I

For each of the phys-chem, human health, fate & behaviour and ecotox endpoint:

### **General information for each nanoform.**

Note in your assessment whether the endpoint for a given nanoform was addressed as:

- Ignored/not addressed
- Not relevant
- Not measurable
- Were (animal) testing data provided? Or a Testing proposal?
- Not technically possible (Annex XI.2)
- Waived (Column 2 or Annex XI.3)
- Adaptation (Annex XI.1.2 - WoE, XI.1.3 -(Q)SAR, XI.1.4 - In vitro, XI.1.5 – Grouping/read-across) or other
- In general, for all endpoints, make an assessment of the quality of the provided information. Is the information of sufficient quality?

And related considerations/questions:

- Does data specifically apply to the nanoform?
- Are there tests for other forms of the substance (e.g. bulk, other nanoform of the same substance, nanoform of other substance but same size,...)?

**In general answer the question: Has scientific justification been provided for the approach chosen (see also below)? Is that adequate taken into consideration REACH, OECD or scientific literature?**

**And based on this, please assess/specify whether:**

- **The information requirement can be considered covered for the nanoform**
- **The information requirement seems to be covered for the nanoform, but more details would be needed to make a final judgement (e.g. justification for adaptation is insufficient, missing information)**
- **No, the information requirement cannot be considered covered for the nanoform**

For testing of nanomaterials (*in vitro/in vivo*) consider:

- whether the test was adapted. If yes, specify how: Sample preparation, Sample characterisation, Adaptations to ensure stability, Dosimetry, Route of exposure, Other (please specify). NB! See also further endpoint specific detailed considerations below.
- If there were difficulties, how were these addressed?

**Specific for C&L:**

If GHS and DSD-DPD classification are listed, only the GHS classification should be reported. If the substance was classified under DSD-DPD, but has been “self-classified” under GHS, both should be reported.

**Proposed sequence, when reporting from a HH or ENV specific study, that has used nanomaterial**

Author, year

Purpose flag (key study, supporting study, weight of evidence)

Reliability

Test, test guideline

Test material description (e.g. particle size)

Test animal, route, duration

Details on exposure (e.g. nose only, determination of actual concentration) (probably not needed for ENV)

Results/conclusion

**Specific for environmental hazard:**

Are sample preparation and exposure conditions before, and during the test well described and suitable for nanomaterials?

Which endpoints are reported?

Which metrics are used?

Are NPs present in the test suspension at the beginning of the test? (Stock suspensions can be analysed for particle size distribution, number of particles, etc...).

Are additional endpoints considered (e.g. fish ventilation rate, fish mucus secretion, behaviour, oxidative stress)?

For all PNECs: Are PNECs provided for all forms? If so, how are PNECs generated for nanoforms?

If same PNEC for several forms, has it been scientifically justified?

**Specific for human health hazard:**

Are dose, sample preparation, and exposure conditions (dispersion, stability, agglomeration/aggregation, dustiness) before, and during the test well described and suitable for nanomaterials?

Have appropriate methodologies been selected?

Are dispersion or cell culture media suitable?

Have interactions of nanoparticles with biological molecules been investigated and how?

Is a relevant route of delivery tested?

Is the exposure duration appropriate (justification given)?

Which metrics (mass, surface area, number, other?) for the dose are reported?

Which endpoints (target organs) are reported?

Are additional endpoints considered (e.g. Inflammation, cytotoxicity, oxidative stress, cardiovascular toxicity, immunotoxicity, secondary effects)?

For genotoxicity: Any considerations on possibly limited applicability when bacteria strains are used? (Background: The validity of Bacterial reverse mutation test for testing NMs has been questioned, since not all NMs are considered to penetrate the bacterial wall (including strain specific considerations if relevant)).

For all DNELs: Are DNELs provided for all forms? If so, how are DNELs generated for nanoforms?

If same DNEL for several different forms, has it been scientifically justified?