

# NANoREG

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## Deliverable D 2.12

*Framework and procedures for characterization and reporting of manufactured nanomaterials for regulatory use*

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2	2017/01/24	Draft report submitted to the coordinator to document for status and coordinators comment and evaluation of scope in light of D1.11. Revisions include changes due to release of several revised ECHA documents in November and December 2016 and further elaboration of text.
3	2017/02/10	Second draft submitted to the coordinator for pre-approval by the NANoREG MC after further elaboration of text
4	2017/03/13	Final draft submitted to the coordinator after finalization with revision and final elaboration of text considering review comments received from E. Bleeker (RIVM). Additional changes were made due to a significantly revised Appendix 4 v.1 released by ECHA in January 2017.
5	2017/04/21	Project Office harmonized lay-out

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# 1 Description of task

This deliverable combines the work performed in WP2 of NANoREG in regards the establishment of a Framework and procedures for characterization and reporting of manufactured nanomaterials for regulatory use. The work is based on NANoREG D2.05, which provided a revised system and criteria for proper identification of nanoforms and their categorization in the REACH framework. Specifically this task focus on the

The ultimate aim of this work is to propose revisions to the existing REACH system for substance identification and naming that can on one hand enable improved quality in the reporting during registration of manufactured (nano)materials and more systematic and clear categorization, which on the longer term can facilitate higher transparency and evidence-based or empirical grouping rules. The system in D2.05 is re-assessed considering recent proposals in the literature and characterization methods/standard operation procedures (SOPs) available from NANoREG.

## 2 Description of work & main achievements

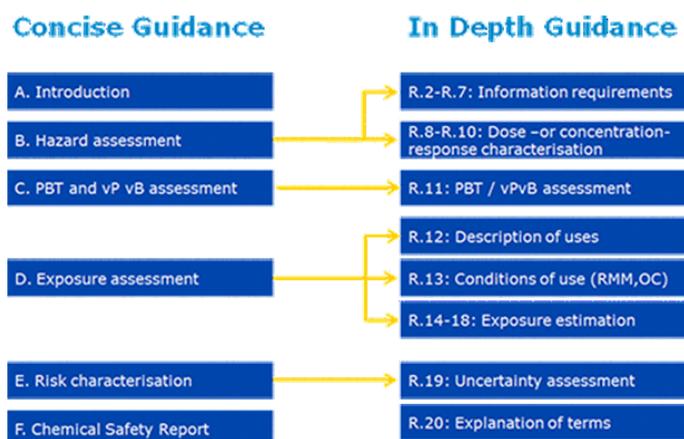
### 2.1 Summary

Previous reviews and analysis of the OECD Technical guidelines recommended to generate the physicochemical information requested in Appendix 4 (Public) Draft v. 1.0 (2007) and Appendix R.6-1 (Public) Draft v. 2.0 (2016) have previously found to be not suitable for characterization of MNM (Manufactured Nanomaterials). A sub-group of the key OECD TGs were assessed in both the OECD Working Party on Manufactured Nanomaterials (WPMN) and Work-Package 2 of the EU FP7 NANoREG project. It was found in NANoREG D2.03, that none of the analysed OECD TGs were suitable for characterization of MNM. Proposed revisions of several of the OECD TGs were proposed or proposed to be replaced with alternative or new methods and presented in NANoREG D2.09. Other methods to support the substance identification and reactivity and fate issues were developed and presented in NANoREG D2.04, D2.08, D2.10, and D2.11. It is evident that further testing and/or acceptance of existing draft methods is urgently needed in international guidelines and standard methods to support the regulatory process.

### 2.2 Background of the task

ECHA (The European Chemicals Agency) is currently developing guidance documents and appendixes to facilitate registration and risk assessment of manufactured nanomaterials (MNM) under REACH and CLP. This report consequently adhere to the recommended regulatory definition of a nanomaterial proposed by the EC (2011/696/EU) (Potocnik 2011) and adopted by ECHA for implementation in REACH. In line with the purpose of the REACH regulation, ECHA considers only manufactured nanomaterials and not incidental and natural nanomaterials, which are also covered by the EC recommendation for definition of nanomaterial.

To structure the registration of material and chemical substances, REACH provides a number of guidance documents, annexes, appendixes to guide the registrants on required end-points to be reported and recommended methods for data generation. The Guidance consists of two major parts: Concise guidance (Part A to F) and supporting reference guidance (Chapters R.2 to R.20) and are linked as illustrated below in Figure 1.



**Figure 1. Structure and interlinkage between concise guidance documents and reference guidance documents provided by ECHA to so support generation of REACH information requirements and chemical safety assessments (from: <https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment>).**

In the guidance structure illustrated in Figure 1, substance identification and naming is indirectly linked as a sub-part of Guidance B: Hazard assessment. However, the Guidance for substance identification and naming is as such introduced under ECHA > Regulations > REACH > Registration on <https://echa.europa.eu> while direct general information on nanomaterials is given under ECHA > Regulations > Nanomaterials linking back to the general Guidance structure depicted in Figure 1. The general guidance on substance identification is also found via ECHA > Support > Guidance > Guidance Documents > Guidance.

Relevant for substance identification and naming, there is currently no reference to nanosubstances in the main Concise Guidance documents A and B. However, several underlying documents in the guidance structure have been recently updated and some are still available in draft versions. An updated general Guidance for identification and naming of substances was released in December 2016 (ECHA-16-B-37-EN) together with Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a (ECHA-16-G-10-EN) covering endpoint specific guidance. Chapter R.7a refers to Appendix 4: Recommendations for nanomaterials applicable to the Guidance on Registration available as Draft (Public) of which the latest draft is v. 1.0 of January 2017.

Chapter R.7a is supported by Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7a Endpoint specific guidance from 2012 (ECHA-12-G-03-EN) and its proposed revisions in Draft (Public) v. 2.0 (2016) as well as proposed revisions to Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7a, R7b and R7c Endpoint specific guidance, which are all currently available as Drafts (Public) v. 2.0 of December 2016. These documents are also all relevant to support Appendix R.6-1: Recommendations for nanomaterials applicable to the Guidance in QSARs and Grouping of Chemicals Draft (Public) v. 1.0 of December 2016.

The purpose of this deliverable is first of all to review the strategies and schemes given in the above-mentioned documents with specific focus on substance identification and naming (Appendix 4 and the parent Guidance for identification and naming of substances under REACH and CLP (ECHA-16-B-37-EN) and the grouping and read-across scheme for MNM (Appendix R.6-1: Recommendations for nanomaterials applicable to the Guidance in QSARs and Grouping of Chemicals Draft (Public) v. 1.0 of December 2016). Based on the review results, modifications are proposed respecting the general conceptual idea in the attempt to create a sustainable solution by further harmonizing the substance identification and naming scheme considering also future material technological developments.

This deliverable is the result of an over-arching WP2 leader activity bridging especially Task 2.2: Identification of MNM according to the EC regulatory definition, Task 2.3: NM characterization SOPs for regulatory purposes and Task 2.4 Test item preparation, exposure, dose and fate for regulatory purposes and toxicology. By having more specific focus on the physicochemical aspects in the substance identification scheme and grouping/read-across schemes proposed by ECHA, the current deliverable supplements D1.11 describing NANoREG – "Definitive framework", which gives an introduction to the regulatory context and thereafter considering mainly a MNM safety assessment procedure following the hazard assessment strategy and endpoints developed in WP5.

Consequently D2.12 is intended to provide observations and recommendations for the further harmonization and improvement of the materials identification and registration schemes and guidance in REACH. As part of this task, the aim is to increase the systematics in substance identification and naming and thereby to increase the future ability in grouping, read-across and predictive risk assessment based on physicochemical endpoints and a minimum set of toxicological test results.

### **2.3 Description of the work carried out**

This deliverable is based on a review of the existing and emerging ECHA Guidance for substance identification, grouping, QSAR and read-across. The review was focused on a number of issues:

- 1) Applicability of the schemes for substance identification considering the chemical and structural complexity of manufactured nanomaterials as well as modern nanomaterials in general.
- 2) Similarities and contradictions between guidance and recommendations given for non-nanomaterials as compared to the guidance given for manufactured nanomaterials.
- 3) Applicability of the recommended methods to generate the key physicochemical information requirements.

- 4) The advancement provided from NANoREG Work-Package 2 in regards to further development and documentation of standard operational procedures for physicochemical characterization of manufactured nanomaterials in regulatory context.

Recommendations are given for revision of schemes for substance identification and methods to generate the minimum set of physicochemical end-points required for the substance identification, grouping, QSAR and read-across schemes for manufactured nanomaterials in REACH.

## 3 Results

This chapter first discusses in section 3.1 issues related to characterization and identification of MNM as substances on nanoforms as considered in the REACH guidance documents and appendices to support substance identification, which ultimately should be part of the Guidance for identification and naming substances under REACH and CLP. In section 3.2, a revised substance identification scheme is proposed. In Section 3.3, the current ECHA recommendations on characterization methods for generating data on the minimum number of end-points for substance identification, grouping, QSAR and read-across is identified followed by a brief presentation on the advancements in characterization methods achieved in the NANoREG project with an evaluation of their readiness level towards implementation.

### 3.1 Discussion on the REACH guidance on substance identification, naming and grouping/read-across and the recommendations for nanomaterials.

#### 3.1.1 Contradictions in the guidances on chemical definition and reporting of substances

First, to have a common starting point, a substance in REACH (the Registration, Evaluation, Authorisation and Restriction of Chemicals Regulation, European Parliament and Council, 2006) is defined as *"a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition"*. In REACH, the substance therefore consists not only of a single pure molecular structure or solid, but principally also includes other constituents such as impurities and additives. However, it should be noted that conventionally substances in REACH have primarily been identified according to their primary chemical composition.

As listed in the introduction, ECHA recently released several documents with guidance and recommendations to generate data to satisfy the information requirements for substance identification, naming and grouping of MNM under REACH and CLP. However, as the Guidance document (ECHA-16-B-37-EN) is the key document for substance identification, and was newly revised, it is remarkable that this document mentions "nano" in only one section saying that:

*"The current developments in nano-technology and insights in related hazard effects may cause the need for additional information on size of the substances in the future. The current state of development is not mature enough to include guidance on the identification of substances in the nanoform in this guidance document."*

This is in contrast to the specific information requirements in Appendix 4: Recommendations for nanomaterials applicable to the Guidance on Registration available as Draft (Public) v. 1.0 of May 2016 (and later update January 2017), which explicitly, even-though not as legal advice, requests that MNM are registered and reported with additional information on both size/size-range, shape and surface chemistry. It is critical to harmonize the General guidance document for substance identification documents in regards to their reference to MNM to ensure clear and transparent guidance for the registrants.

The lack of reference to "nano" in Guidance document (ECHA-16-B-37-EN) is also in contrast to Appendix R.6-1: Recommendations for nanomaterials applicable to the Guidance on QSARs and Grouping of Chemicals, Draft (Public) v. 1.0 December 2016, that requests information on 14 to 15 characterization end-points, which are further discussed below. It should, however, be mentioned that the Guidance on registration v. 4.0 (ECHA-16-G-06-EN) does refer to Appendix 4 after commenting on the "nano-issue" under the scope saying:

*"When the manufacturer or importer intends to register more than one composition or form of a substance (e.g. nanomaterial (NM)) in the same registration dossier, they would need to ensure that the relevant Annex VII-XI information takes into account all compositions or forms registered, and that this is transparently reported in the corresponding registration dossiers submitted to ECHA."*

Looking back, to consider some of the background for revision of the Guidance on substance identification, size and chemical surface treatments were already topics for discussion during the so-called REACH Implementation Project where substance identification was the topic of the RIP-oN 1 report (European Commission 2011). The RIP-oN 1 report reflects that, at this point in time, there was already high focus on the nanoscale criteria and the role of surface-chemical modification (coatings). In the RIP-oN 1 report conclusions:

- Experts could not agree on whether the size-criterion should be considered an identifier or a trigger for new identifiers in relation to substance identity. Industry judged that size is a "characterizer" whereas the substance identity is based on chemistry. The other RIP-oN 1 experts supported careful consideration of whether size should become an identifier or an identifier trigger. However, if size was

used as an identifier, this would align the concepts of substance identification logically to the proposed EC definition of a nanomaterial for regulatory use.

- Experts could not agree on whether a surface treatment should be included as an identifier of MNM. All experts agreed that surface treatments of materials may result in unpredictable changes in their hazard. Industry claimed that there was no need for to include surface treatment as an identifier as the effect of these issues would be covered by REACH FAQ 6.3.8 (Do I have to register chemically surface treated substances?). ECHA pointed out that MNM were not mentioned in FAQ 6.3.8 and therefore did not specifically cover MNM.

Due to the lack of revisions in regards to “nanofoms” in the parent Guidance on substance identification and naming (ECHA-16-B-37-EN), a precise size-criterion for MNM is currently only specified in Appendix 4. However, there are remarks in ECHA-16-B-37-EN on the fact that in regards to establishment of each substance identity profile (SIP), it may be necessary to include physicochemical parameters such as physical form and/or morphological parameters like porosity, particle size, and particle shape. I.e. when establishing SIPs, these aspects may be taken into consideration case by case as agreed and documented by the registrants. A detailed scheme for defining a SIP is given in Appendix II to the parent Guidance on identification and naming (ECHA-16-B-37-EN).

Regarding surface-chemical modifications, there are not any specific definitions or descriptions thereof in the parent Guidance on substance identification and naming (ECHA-16-B-37-EN). However, there is again reference to coatings, which can be defined for each SIP defined under the provisions mentioned above:

*“...generic ranges of these parameters covered by the SIP would need to be provided transparently (e.g. particle size ranges applicable to all registrants and list of their shape(s) and list of surface chemistries). Thus, the comprehensiveness of the hazard data jointly submitted in relation to the SIP is ensured.”*

None of the documents refer to REACH FAQ 6.3.8 (2010) or its later revision (2015), which today, however, can found under the Q/A: “Do I have to register chemically surface treated substances?” on <https://echa.europa.eu/support/qas-support/browse/-/qa/70Qx/view/scope/reach/REACH+Registration>). In this Q/A, it is said that:

*“The surface treatment of a substance is a “two dimensional” modification of macroscopic particles. A “two dimensional” modification means a chemical reaction between the functional groups only on the surface of a macroscopic particle with a substance which is called a surface treating substance. By this definition it becomes clear that this kind of modification means a reaction of only a minor part (surface) of a macroscopic particle with the surface treating substance, i.e. most of the macroscopic particle is unmodified.*

*Therefore a chemically surface treated substance cannot be regarded as a mixture nor be defined by the criteria of the Guidance for identification and naming of substances under REACH: <http://echa.europa.eu/guidance-documents/guidance-on-reach>.*

*With the same reasoning, a chemically surface treated substance could not be reported for EINECS nor be notified according to Directive 67/548/EEC because it was covered by the separate EINECS entries of both the basis substance (macroscopic particle) and the surface treating substance. Taking this decision up under REACH means a consequent continuation of former decisions. Using the same line of arguments, chemically surface treated substances should not be registered as such under REACH, but the following requirements should be fulfilled:*

1. *Registration of the basis substance (macroscopic particle)*
2. *Registration of the surface treating substance*
3. *Description of the use “surface treatment” in the registration dossier of the surface treating substance and in the registration dossier of the basis substance*
4. *Any specific hazards or risks of the surface treated substance should be appropriately covered by the classification and labelling and by the chemicals safety assessment and resulting exposure scenarios.”*

However, due to the wording in the above-mentioned Q/A: “...chemical reaction between the functional groups only on the surface of a macroscopic particle...”, it appears that surface treatments in general Guidance (ECHA-16-B-37-EN) does not include surface coatings that are just physically coatings or loosely attached by electrostatic or van der Waal forces. Yet, the parent Guidance (ECHA-16-B-37-EN) does request that the nature and concentrations is added for all additives, but it is not clear whether non-chemically bound coatings are understood as additives. Normally, this would not be the understanding of materials scientist.

The reporting scheme for size and surface treatments and their definitions is significantly improved in the Appendix 4 recommendations for registration of nanomaterials (Draft (Public) v. 1.0; January 2017). The size

defines the nanoform in the SIP according to the recommended EC definition of a MNM and differences between them can be identified due to shape and the surface chemistry (“*surface treatment with additional agents and/or modification of the surface of the particle*”). Noteworthy, Appendix 4 states that all surface chemical modifications and treatments should be reported, including e.g., acid washing, O<sub>2</sub> treatment etc.

From the analysis above, it is evident that there is not agreement between the definitions of surface treatments in Appendix 4 and parent Guidance (ECHA-16-B-37-EN). Currently, it appears that surface chemistries on materials, which are not on nanoform, may either be reported as an individual substance associated with the material or as an additive. Consequently the definition on surface modifications given in the Q/A should as a minimum be replaced with the current definitions on surface modifications in Appendix 4. It is recommended that the strategy in Appendix 4 is further elaborated to facilitate clear reporting to cover all higher generation MNM and this revision is also implemented for materials that are not on nanoform. This will increase the subsequent ability to group materials on a broader scale.

The volume specific surface area (VSSA) is proposed as a supporting criterion for identifying a MNM in the EC recommended definition of a nanomaterial. Surface area is mentioned as important information to be provided for minerals in the General guidance for substance identification (ECHA-16-B-37-EN). Porosity is also mentioned as a potential identifier for specific SIPs in (ECHA-16-B-37-EN). VSSA is not mentioned in the parent Guidance (ECHA-16-B-37-EN), but has been re-introduced in the latest version of Appendix 4 Draft (Public) v.1.0 (2017). The specific surface area is also identified as an essential information requirement for QSAR and grouping in Appendix R.6-1 Draft (Public) v. 1.0 (2016) where the surface area to report in this case should include the surface area due to porosity (see footnote 3 in Figure 2 of Appendix R.6.1). The porosity issue in surface area measurements is not mentioned in Appendix 4 and the parent Guidance (ECHA-16-B-37-EN).

Information on particle shape is requested as primary information substance identification in Appendix 4 as well as for QSAR and grouping in R.6-1. Information on shape was also requested previously as part of Appendix R7-1 Recommendation applicable to Chapter R7a v. 5.0 Endpoint specific guidance (ECHA-16-G-10-EN) and partially suggested under granulometry as part of revisions to Appendix 7-1 Recommendations for nanomaterials applicable to Chapter R7a Endpoint specific guidance (EN-12-G-03-EN), but this section is taken out in the later revision to Appendix R7-1 Draft (Public) v. 2.0 2016. This may be due to the new shape definitions introduced in Appendix 4.

**If the basic foundation of the REACH definition of a substance must be followed, it is strongly recommended to harmonize the schemes for substance identification and SIPs, irrespective of whether it is a MNM or not. It is recommended to continue along the strategy set out in Appendix 4. Currently, different strategies are at play. In regards to surface treatments and surface chemistry, the strategies and guidance in the existing, as well as the newly drafted revisions of the ECHA guidance, appears to vary considerably depending on whether the guidance is intended for materials on nanoform or not. Harmonization of the phrasing and definition of the information requirements and the reporting scheme through-out would be highly beneficial.**

**The parent Guidance (ECHA-16-B-37-EN) does not point registrants of nanoforms towards Appendix 4 and the relevant and further supporting Appendices 7-1. Appendix 4 is, however, referred to in the introductory Guidance on registration (ECHA-16-G-06-EN). It is also an issue that, the registrant is not clearly guided on how to define a surface treatment in the parent Guidance and it is unclear how the registrant should identify the definitions and requests in the Q/A on surface chemistry, as it is not referred to in the parent Guidance. Moreover, the strategy in ECHA-16-B-37-EN and for definition of SIPs differs from the recommendations for MNM in Appendix 4 Draft v. 1.0 (2017) and R.6.1. Draft v. 1.0 (2016). A specific scheme for reporting surface chemical treatments appears only to exist for registration of MNM following Appendix 4.**

In regards to size of the primary particles, the previous and current parent Guidance for substance identification (ECHA-16-B-37-EN) did not include this criterion (or rather the minimum diameter size-distribution according to the definition of a nanomaterial proposed by the EC) as such for substance identification and materials registration. However, size could be essential for establishment of a SIP as described in the parent Guidance for substance identification (ECHA-16-B-37-EN). Appendix 4 Draft v. 1.0 (2016) and Appendix R.6.1 Draft v. 1.0 (2016) includes request to report the primary particle size-distribution according to the EC proposed definition of MNM for substance identification and QSAR / Grouping purposes, respectively. In Appendix 4, this is to document the material as a MNM. Shape information, as requested in Appendix 4.0, is not used for substance identification, but to distinguish between different nanoforms within a dossier. It is worth noting that reporting on shape was already included in the previous Appendix R7-1 (EN-12-G-03-EN), but then removed in the current revision Draft (Public) v. 2.0 2016. Consequently, the reference to R7-1 in R.7a (ECHA-16-G-10-EN) may need further clarification and update by reference to Appendix 4.

**Further details on these issues will be discussed below.**

### 3.1.2 *Need for a more rigid substance identification scheme for materials*

When it comes to the scheme for substance identification, ECHA continues the original substance identification scheme and criteria in Appendix 4, which divides substances into:

- 1) Well-defined monoconstituent substances, which may contain up to 20 wt.% of another compound or impurities and up to 10 wt.% if it contains two or more additional phases and/or impurities;
- 2) Well-defined multiconstituent substances, which consists of 10 to 80 wt.% of more than one constituent;
- a) Substances with Unspecified or Variable compositions, Complex reaction products or Biological materials, called UVCB substances, which are substances that cannot be sufficiently identified by their chemical composition, because:
  - o The number of constituents is relatively large and/or
  - o The composition is, to a significant part, unknown and/or.
  - o The variability of composition is relatively large or poorly predictable.

In UVCB substances, all known constituents and all constituents present at concentrations  $\geq 10$  wt.% should be specified and all constituents, which are relevant for the classification must be reported. Importantly, for all substance categories, *“intentionally-added substances other than those added to stabilise the substance are separate substances that are not to be considered in the main mass balance.”*

According to the parent Guidance for identification and naming of substances (ECHA-16-B-37-EN), this initial identification of a substance is as a minimum based on information on:

- 1) chemical composition of the substance, considering, where appropriate, impurities and additives besides main constituent(s) and respective typical concentrations and concentration ranges;
- 2) chemical identity of the constituent(s) by means of IUPAC name plus other identifiers when available, e.g. EC number, CAS number. For UVCB substances information on the source and manufacturing process is also necessary;
- 3) molecular and structural information; this must be defined, when available and appropriate, by molecular and structural formula, information on optical activity, ratio of isomers, molecular weight or molecular weight range;
- 4) Spectral and analytical data sufficient to confirm the structure and the composition of the substance.<sup>1</sup>

As mentioned, Appendix 4 Draft v. 1.0 (2016) adds three additional minimum information requirements for registration of MNM:

- 1) The particle size (in one or more dimensions);
- 2) The particle shape; and
- 3) The surface chemistry (surface treatment with additional agents and/or modification of the surface of the particle)

Consequently the parent Guidance (ECHA-16-B-37-EN) and Appendix 4 on recommendations for registration of nanomaterials in total request information on a minimum of 12 different physicochemical endpoints summarized below in

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<sup>1</sup> It is specified in the ECHA online supporting information that ultra-violet (UV), infra-red (IR) and nuclear magnetic resonance spectroscopy (NMR) or mass spectrometry (MS) should to be conducted alongside either high performance liquid chromatography (HPLC) or gas chromatography (GC). For inorganic substances x-ray diffraction (XRD) spectrometry and atomic absorption spectroscopy (AAS) are mentioned potential alternatives.

Table 1. It should be noted, however, that size (as granulometry) and shape were included already as characterization endpoints in 2012 (Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7a Endpoint specific guidance; ECHA-12-G-03-EN), and that the entire list of physicochemical characterization end-points for > 1 ton materials contains numerous other endpoints as listed in Chapter R.7a: Endpoint specific guidance v. 5.0 (ECHA-16-G-10-EN) and revisions to R7-1 (Draft (Public) v. 2.0; 2016).

The addition in Appendix 4, requesting size, shape and surface chemistry information on the primary particulate objects, is certainly an improvement. The volume specific surface area, which is also mentioned in the EC recommendation, is not included as a mandatory information requirement for identification. However, the surface area, including the surface area due to porosity, is considered requirements for grouping. It is recognized in the parent document that surface area may be an important identifier for materials and this information is typically required for minerals (ECHA-16-B-37-EN). The specific or volume-specific surface area is also a key parameter for identifying different types of powder materials (Atluri and Jensen 2017).

To understand the applicability of the new requested information requirements in Appendix 4 and ECHA-16-B-37-EN, it is necessary to take a more specific look into what is written in regards to the specific end-points.

**Table 1: Minimum information requirements to identify a substance under REACH and CLP as specified in parent Guidance (ECHA-16-B-37-EN), Appendix R7-1 (ECHA-12-G-03-EN) and Appendix 4 v. 1.0 (2017).**

<b>Name or other identifier of each substance</b>
Name(s) in the IUPAC nomenclature or other international chemical name(s)
Other names (usual name, trade name, abbreviation)
EINECS or ELINCS number (if available and appropriate)
CAS name and CAS number (if available)
Other identity code (if available)
<b>Information related to molecular and structural formula of each substance</b>
Molecular and structural formula (including SMILES notation, if available)
Information on optical activity and typical ratio of (stereo) isomer (if applicable and appropriate)
Molecular weight or molecular weight range
<b>Composition of each substance</b>
Degree of purity (%)
Nature of impurities, including isomers and by-products
Percentage of (significant) main impurities
Nature and order of magnitude (... ppm, ... %) of any additives (e.g. stabilising agents or inhibitors)
Spectral data (ultra-violet, infra-red, nuclear magnetic resonance or mass spectrum)
High-performance liquid chromatogram, gas chromatogram
Description of the analytical methods or the appropriate bibliographical references for the identification of the substance and, where appropriate, for the identification of impurities and additives. This information shall be sufficient to allow the methods to be reproduced.
NOTE 1: If the listed information requirements are not given, the reason therefore must be clearly stated in the report.
<b><i>Specify end-points for MNM in Appendix 4 and R7-1 (Possible requirement in parent Guidance)</i></b>
Size (size-distribution of the primary objects for assessment of compliance with the proposed EC definition of a NM)
Shape (divided into four ECHA categories: spheroidal-like, high aspect ratio, two-dimensional, other)
Surface chemistry (chemical identity/ies or the chemistry they impart on the surface)

## Size

Size is the key measure by which to identify the MNM in accordance with the recommended EC-definition of a MNM. Appendix 4 says that the registrant is required to give the range in the D50 values (50% of minimum diameter dimensions by number). The registrant is required to provide more detailed size-information due to cut-off sizes where for example a nanof orm will obtain size-related “quantum-confinement effects” (e.g. catalytic activity, conductivity, optical and electronic properties, etc.). As for any substance, potential registrants will need to consider all the information available and determine the impact of size on the hazard profile(s).

**To harmonize data sets and to avoid confusion, it is considered necessary, that the primary particle size-distribution is always reported when a nanomaterial (and in in fact any particulate material) is registered. This, on one hand, ensures a clear description of the material and, on the other hand, prevents accidental non-reporting of materials where the size-ranges produced inadvertently results in higher biological activity and/or quantum phenomena, which needs to be considered during the risk assessment. Today, quantitative size-distribution measurements will usually not be a significant extra cost for a registrant as compared to generate D50 values and the value of the information.**

## Shape

Appendix 4 request reporting of shape considering four new morphology groups, which have been defined by ECHA:

- Spheroidal-like forms: particles with three similar external dimensions (i.e. approximately equi-axial forms). This includes a number of different shapes such as spheres, cubes, prisms, etc.;
- High aspect ratio forms: particles with two similar external dimensions and a significantly larger third dimension (aspect ratio of 5:1 or greater) and substantially parallel sides. This includes high aspect ratio forms with hollow structures (nanotubes), with electrically conducting or semi-conducting properties (wires), solid – non-hollow high aspect ratio forms (nanorods);
- Two-dimensional forms: particles with one external dimension significantly smaller than the other two external dimensions. The smaller external dimension is the thickness of the form (e.g. flakes or platelets);
- An “Other” category: which comprise particles with any other irregular shape and for situations where mixtures of particles with different shapes (e.g. spheres and rods) are produced and therefore none of the options reported above would be suitable.

Regarding mixtures of materials with different morphologies, the substance can belong to the same shape category as long as there is a majority of 50% or higher of one shape (assumed to be by number). If none of the nano-objects has majority of 50% or higher, a shape category must be established for each of the morphology groups under the specific substance identity profile (SIP). Similar to the situation for size, further consideration of the shape categories must be made if the shape may have impact on the properties and toxicological effects.

In contrast to the earlier version (March, 2016), it is clearly stated that the shape groups in the January 2017 version of Appendix 4 v. 1.0 are different from, but inspired by, the shapes specified in ISO/TS 80004-2. One of the reasons given is that, the new morphology groups developed by ECHA ensures that particles in the spheroidal-like forms would still be a nanomaterial even-though only one diameter was between 1 and 100 nm; and that this would not be the case if the ISO specification of a nanoparticle was followed (all dimensions 100 nm or below).

**It is the author’s opinion that the restriction to the four groups in the morphological categorization scheme by ECHA and absence of clear guidance on how to define other morphological classes likely will result in confusion. Moreover, this lack of guidance and the proposed morphological grouping also likely will make it impossible for ECHA to use the information given by the registrants directly for later grouping and read-across purposes.**

**The observation by ECHA that a material would not be a nanoparticle according to ISO specifications, if it had a near-spheroidal shape, but only had one D50 diameter between 1 and 100 nm, is a clear consequence of the lack of neutral terms in ISO specifications on shapes. In the given example, however, the material would in any case be identified as a nanomaterial according to the recommended EC definition. It would also be identified as a nano-object according to ISO/TS 27687 (2008) and ISO/TS 80004-2 (2010). This is because the EC-definition of a nanomaterial and ISO specification of nano-objects requires only one diameter in the nano-range. ECHA has rightly observed that there is a gap in the shape specifications by ISO, however, following general morphological terms, the nano-object in the given example would simply be “classified” to have a particle (or equidimensional) shape following typical shape definitions (if none of the diameters were less than 3 times larger than the shortest diameter).**

**It is unclear why already highly developed morphological classification systems are not at all applied by ECHA in general. The ISO developed already an elaborate scheme for shape type-classification in ISO/TR 11360:2010(E) and the refinements in ISO/TS 80004-1:2015 to ISO/TS 80004-4:2015 series than the basic naming specification to which Appendix 4 refers to. ISO standards were also previously produced to describe morphologies as given in ISO 9276-6(EN). Even earlier a suite of neutral descriptive morphology (habitus) terms were developed for mineralogy and petrography. From this early work, the shape definitions (equidimensional, prolate [elongated / fibre], oblate [disc / flake], and bladed [belt]) (Zingg 1935) are implemented in many areas, including powder technology, mineralogy and microscopy.**

**In the context of generating shape information, the dimensions defined by ECHA for grouping of the different shapes are of concern. Since available, the morphologies should be referred to in internationally approved terms to ensure clear and uniform communication. For example, considering the near-spheroidal group defined by ECHA, there is important information already transferred by discriminating between near-spheroidal nano-objects with 1) near-spherical, 2) euhedral or idiomorf, and 3) subhedral equidimensional morphologies as they describe that the nano-objects have a crystal shape in morphology category 2 and a partial crystalline shape in morphology category 3.**

Another issue is that, if all nanomaterials with aspect ratios greater than 5:1 are grouped together into a single group as done in Appendix 4 (Public) v. 1.0 January 2017, this group will comprise many MNM ranging from nano-objects with lengths of 5 nm and diameters of 1 nm or technically even shorter if the diameters are below 1 nm. None of the cited references in ECHA Appendix 4 defines high aspect ratio nanomaterials as nano-objects with aspect ratios greater than 5:1. Typically high aspect ratio materials are considered when they comply with the WHO fiber paradigm, where the fibre length must exceed 5 µm and the aspect ratio be greater than 3. In R7a, a fiber has an aspect ratio of 3 and a maximum length of 100 µm. ISO/TS 27687 (2008) and ISO/TS 80004-2 (2010) also adhere to the aspect ratio 3 or above to define a fibre. Nanoplates, on the other hand, are defined to have typically 2 dimensions greater than 100 nm and one dimension between 1 and 100 nm where the aspect ratios between the two lateral diameters over the plate thickness are 3 or higher. In the ECHA guidance all morphology groups are defined by aspect ratios of 5 or higher.

For registration, it is recommended to develop a scheme to report the pre-defined morphologies and define a trigger for nanomaterials with very high aspect ratios for which the lengths approach the critical length in the WHO paradigm. This is because fibre-associated hazards are so far only observed for long and relatively straight fiber materials and discussed for high aspect ratio flakes (Catalaín et al. 2016; Zumwalde et al. 2013). This systematic work still remains.

## Surface chemistry

In regards to the information requirement on surface chemistry, the ECHA Appendix 4 (Draft) v. 1.0 January 2017 states that:

*“Given the impact that surface chemistry has on particle properties, variability in surface chemistry will always need to be addressed by potential registrants in determining how to fulfil their registration obligations. Consequently, the starting point is based on chemical identity. For example, when registrants need to demonstrate how they took surface chemistry variability into account when their registration covers surface treated nanoforms, they would need to report minimum elements based on the chemical identity/ies of the surface treating agent(s) in their corresponding registration dossiers.*

*Consequently, chemical identity is the minimum element that needs to be reported for the surface chemistry of nanoforms; e.g. the chemical identities of the surface treating agents, the identifiers of the functionalities introduced by chemical treatment such as acid washing, oxygen treatment, etc.*

Surface chemistry is considered an highly important parameter for any substance and in any system it may to a high degree control, at least the initial properties and fate on a material. The text in Appendix 4 (Public) v. 1.0 January 2017, requests that the registrant report *any* surface chemical modification and highlights that the 80%-20% rule in the overarching substance categorization scheme still applies for substance identification of MNM. If any surface chemical modification will be reported and characterized, this is in line with the proposal in D2.5 and will be an important development towards establishment of grouping and read-across principles for this parameter.

It is unfortunate, however, that surface-chemistry is the only chemical modification to be considered as the minimum reporting requirements for nanoforms in ECHA Appendix 4 4 (Public) v. 1.0 January 2017. As described in NANoREG D2.05 and seen from scientific literature (Atluri and Jensen 2017; Garcia-Lopez et al. 2007; Wu et al. 2008; Sainsbury et al. 2010; Buesen et al. 2014), MNMs may be modified in many different ways in modern material design including: atomic substitution, doping, porosity-filling, physical coating and chemical functionalization. More than one of these modifications may also be applied to the MNM and are thence not only affecting the surface chemistries within the 20% one-sustance rule. Hence, reporting of all types of surface, porosity and matrix modifications should be mandatory as they all potentially change the properties, reactivity, fate and hazard of the MNM.

### 3.1.3 Consequences of the registration exemptions for natural minerals?

An additional consideration, in regards to the substance identification and registration, is that mined minerals may be exempt from registration as defined in REACH article 3(39). It is said in the ECHA guidance for REACH and CLP v. 2.0 (ECHA-16-B-37-EN):

*“Minerals which occur in nature are covered by the exemption if they are not chemically modified. This applies to naturally occurring minerals, which have undergone a chemical process or treatment, or a physical mineralogical transformation, for instance to remove impurities, provided that none of the constituents of the final isolated substance has been chemically modified. Thus, if both the above conditions are fulfilled, then the mineral is exempted from the obligation to register.”*

Examples of such exempt minerals are:

Asbestos minerals:

- Crocidolite (CAS: 12001-28-4);
- Amosite (CAS: 12172-73-5);
- Anthophyllite (CAS: 77536-67-5);
- Actinolite (CAS: 7536-66-4);
- Tremolite (CAS: 77536-68-6)
- Chrysotile (CAS: 12001-29-5 and 132207-32-0)

Dolomite (CAS number 16389-88-1);

Barite (CAS number 13462-86-7);

Limestone (CAS number 1317-65-3), which is an interesting mineral example as it is a sedimentary rock type;

Fluorapatite (CAS number 1306-05-4), the most common phosphate rock mineral.

Etc. ....

**Importantly, the exemption for minerals does not per default apply to synthetic substances having the same compositions and structures as the natural occurring minerals. This means that manufactured material substances, including MNM in principle must be registered according to the REACH tonnage and CMR requirements. However, natural minerals, which have been only physically modified or purified, may escape REACH registration due to the exemption rule: “*provided that none of the constituents of the final isolated substance has been chemically modified.*” In this case, the minerals will be administered in compliance with regular risk assessment (e.g., occupational exposure limits). However, if a mined (natural) mineral was a nanoform by the size-definition, or separated from a matrix as such, or reduced in size by physicochemical treatment, it would not be easily understood whether reporting was needed and consequently not easily identified by risk management as a potentially biologically more hazardous variant of the substance than the mineral on µm-size.**

### **3.2 Proposed revision of the substance identification scheme and material categorization**

Based on the analysis presented above, a number of improvements in the substance identification scheme are considered. Despite modifications have been made in some of the relevant Guidance documents and supporting appendixes to accommodate substance identification of MNM, it appears still that the criteria for distinguishing between substances under REACH and CLP are insufficient to achieve structured identification and naming of MNM. In fact, the established criteria for identification and naming of substances in REACH appear to be sub-optimal for materials in general and may limit the possibilities for realization of data-supported grouping and read-across between bulk and nanoforms as well as across substances.

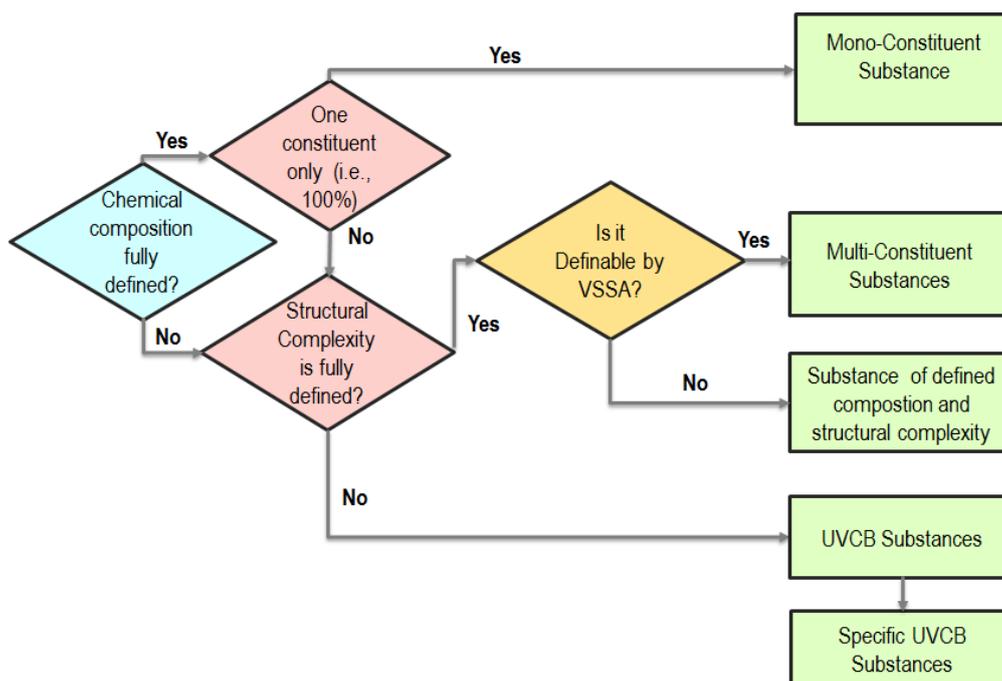
First, the reliability of the established grouping and read-across scheme depends on the specific material characteristics by which the substances and the associated types of nanoforms are defined in the individual SIPs. The criteria for qualifying for the different SIPs may vary, because the registrants are allowed to define the SIPs. In such a strategy, it is considered important to establish a highly systematic structure and criteria for identifying differences. Preferably, the hazard of a material should be fully separated from its physicochemical identification.

The general strategy for substance identification appears to have caused a major loss of opportunity. If a fully structured approach had been established, material comparisons could be made at a more detailed level and allow more facile substance reporting and administration. A fully structured approach would also give higher confidence in substance grouping and read-across, which could have been extrapolated to MNM. This view also penetrates through in several recent scientific publications by authors from both industry and academia, in which new paradigms to improve grouping and read-across have been presented (Arts et al. 2016; Arts et al. 2015; Landsiedel 2016; Oomen et al. 2015). These papers reflect a general concern about the practicality of the established regulatory system for registering MNM. The recent ECHA grouping strategy in R.6-1 Recommendations for nanomaterials applicable to the Guidance on QSARS and Grouping of Chemicals is an important step in this direction.

The most critical aspects in the existing REACH guidance on substance identification, categorization and grouping in regards to manufactured nanomaterials are that:

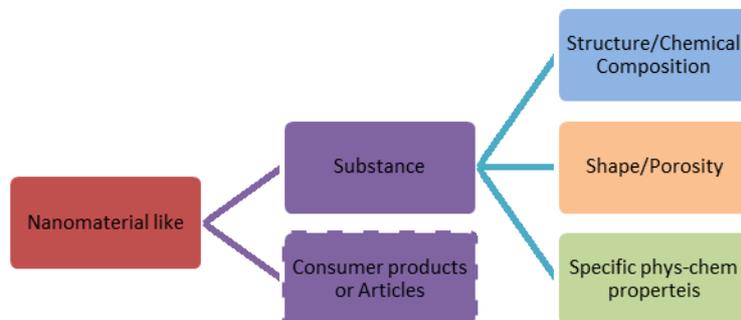
- 1) Nanomaterials are, as bulk materials, divided into substance groups following the conventional definitions for well-defined monoconstituent, multiconstituent and substances of unknown or variable composition, complex reaction products or biological materials (UVCB). The consequence is that especially already produced second and higher generation materials and nanomaterials will end up in different substance groups and each substance group may contain materials with highly different physicochemical properties and biological effects;
- 2) The scheme for defining SIPs allows registrant(s) to define their own individual compositional ranges for substances, which can result in un-intended differences in the specific SIPs and sub-division into different compositional profiles thereunder. This can reduce the ability to perform grouping and read-across;
- 3) The proposed scheme for identification of substances on nanoforms according to the recommended EC regulatory definition of nanomaterials, further division into the four new “ECHA shape groups” and different surface chemistry profiles, does not appear to take into account that (nano-)materials may be chemically and structurally tailored materials comprising also for example shells, doped, porous, pore-filled, internally chemically modified/coated/functionalized materials or combinations thereof. Stringently, such materials will be identified following the overarching substance identification rules based on percentage and presence of chemically attached surface-chemical modifications.
- 4) The criteria for when to report intentionally-added substances can result in some mistakes and also allows the producer to add stabilizers without reporting;
- 5) Mined minerals and refinements thereof, on nanoform, may not registered under REACH due to exemption rules in the parent REACH Guidance for substance identification requesting registration only when the mineral is chemically changed.
- 6) The guidelines recommended in Appendix R7-1 for generating the physicochemical data for substance identification of nanoforms in Appendix 4 and grouping in Appendix R6-1 are rarely applicable to nanomaterials. For example, update in regards to the minimum diameter size-distribution of individual particles has not been made.

With the aim to propose an optimization of the REACH substance identification and categorization, a modified categorization scheme was already proposed in NANoREG D2.05, which includes identification of impurities and chemical modifications and application of the volume-specific surface area of powder materials for the categorization (Figure 2). Unless a very strict no tolerance approach is applied, the threshold for acceptable impurity levels in or associated with a substance needs to be defined for each group and production method of MNM. It is, however, recommended that impurities and their structural and chemical association with the substance in question is always reported.



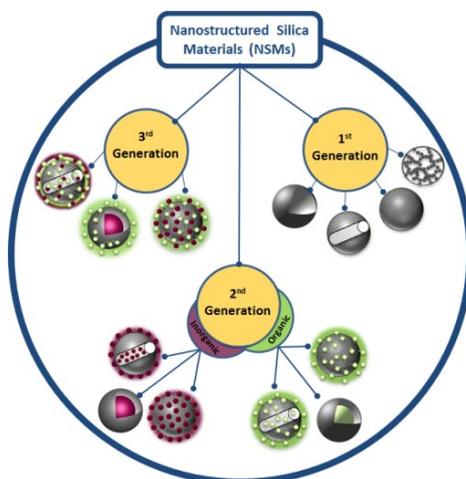
**Figure 2: Proposed REACH substance categorization scheme for (nano-)materials adopted from the scheme proposed for nano-silica in NANoREG D2.05.**

In D2.05 it was also suggested that the final identification and reporting should include characterization of physicochemical properties according to the (nano-)materials by subgrouping them into 1) Structure/Chemical composition, 2) Shape/Porosity, and 3) Specific Physicochemical properties as shown in the general overview scheme in Figure 3. The number of characterization end-points under “Specific physicochemical properties” could vary from limited to rather extensive depending on the material type and information needs.



**Figure 3. Scheme with key physicochemical characteristics used for categorisation of MNMs in NANoREG D2.05.**

The specific categorization of the materials D2.05 was suggested to be done according to the principal nature of the 1<sup>st</sup> generation nanomaterial (solid, capsule/hollow, porous) and then subsequently according to the physical location and extent of structural and chemical modifications to achieve the 2<sup>nd</sup> generation NM (modified internally or externally by either organic or inorganic compounds) or 3<sup>rd</sup> generation NM with organic and inorganic chemical modifications. The proposed classification and proposed descriptive codes enables quick identification of various NMs by their complexity, which could also be an easy way to identify requirements for new risk assessments. Figure 4 shows an example of this structure considering nanostructured silica nanomaterials.

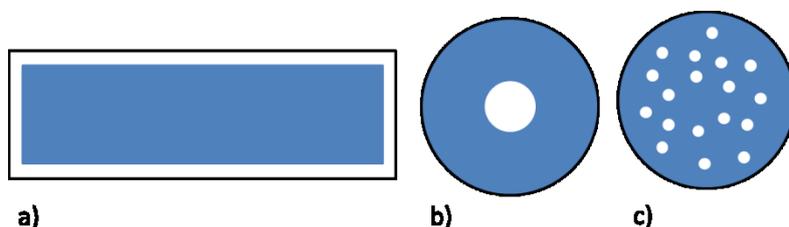


**Figure 4. Illustration of the proposed 3 generation chemical substance classification paradigm in NANoREG D2.05 given as an example for nanostructured silica materials (NSMs) with sub-groups illustrating non-porous and porous types and presence of surface-treating agents as organic and/or inorganic as their counterparts.**

In ECHA Appendix 4 (Public) Draft v. 1.0 (2017), the subgrouping of nanoforms is made under each SIP considering: 1) Size, 2) Shape, and 3) Surface chemistry. Hence, following the draft ECHA guidance, the composition is used two times; first for substance identification and then for identifying subgroups within a substance. There are likely pro’s and con’s in both approaches.

To illustrate some of the potential consequences, we consider a set of different physical combinations of nanomaterials with the same chemical composition (85 wt.% of A and 15 wt.% of B) as illustrated in Figure 5:

- Following the ECHA Appendix 4 (Draft) v. 1.0 January 2017, example a): 85 wt.% “A” physically coated with 15 wt.% of “B” will fall under the monoconstituent SIP for compound “A”. In the “A” SIP, the substance will be a nanoform due to size (possibly further grouped in a subcategory considering size-class (e.g., D50 = 60 to 80 nm)) and then according to shape in accord to the new five “ECHA shapes”; and finally according to its surface chemistry (amorphous silica or even the end-groups in the silica-coating).
- Following the scheme in Figure 2, example a) in Figure 5 would be a multiconstituent nanomaterial defined as a compound “A” coated with “B” and sub-sequently sorted into a shape group according to e.g., ISO or the five new “ECHA-shapes” and described with additional physicochemical properties as needed.



**Figure 5. Three examples a, b, and c of a nanomaterial with the exact same chemical composition (Blue: 85 wt.% of A; White: 15 wt.% of B), which are all a well-defined monoconstituent substance according to ECHA Appendix 4 (Draft) Public v. 1.0 (2017) substance identification scheme, but well-defined multiconstituent according the scheme in Figure 2. A) A is coated with B; B) B is coated with A; c) A has inclusions of B; either as pore-fillings or matrix-embedded or encapsulating coating.**

We now invert the material in example a) compositionally to obtain examples b and c in Figure 5, so “B” is coated with “A” or “B” occurs as inclusions or porosity fillings in “A” with the same 85/15 wt.% proportions, then:

- Following the draft ECHA strategy the material in example b) and c) would again fall under the SIP for “A” following, but now there would be no chemical modification, and B might be reported as impurities or cause further division depending on the accepted compositional profiles established in the SIP.
- Following the scheme in Figure 2, example b) will be a multiconstituent substance and example c) would be either a multiconstituent or UVCB substance depending on whether the level of structural complexity can be controlled. However, the specific categorization of the substance at SIP level would for example b) be substance “B” coated with “A” and for example c) substance “A” with internal porosity-filling of “B” or “A” with inclusions of “B” depending on the manufacturing process. Again further division into shape categories will depend on the shape definitions applied.

From these simple examples it is evident that the strategy in Figure 2 to Figure 4 can enable a more specific identification of 2<sup>nd</sup> and higher generation materials than in the currently proposed ECHA guidance. For full use of the substance categorization scheme in Figure 2, the format for reporting the structural complexity and extent of nanomaterials given in D2.05 or similar should be considered.

### 3.3 Methods to support data generation for substance identification, categorization and grouping

Generation of reliable and comparable data is one of the most critical aspects for establishment of reliable substance identifications and SIPs as well as for grouping. Chemicals registration is normally completed using OECD technical guidelines and several reviews have shown that many of these methods were not applicable to MNM.

Both ECHA Appendix 4 (Draft) v. 1.0 January 2017 on substance identification and Appendix R.6-1 (Public) Draft v. 2.0 December 2016 on guidance for QSAR and grouping of NM, do not advice registrants on how to generate the requested physicochemical data to fulfil the information requirements.

In this section, the need for update and revision of the Endpoint specific guidance on physicochemical characterization methods in ECHA Appendices R7-1 is briefly discussed. This is followed by a step-by-step reference to and assessment of the contributions provided by NANoREG WP2 in regards to further development of methods and standard operation procedures for NM characterization in a regulatory context.

### 3.3.1 Current ECHA recommendations for generating physicochemical characterization for substance identification, QSAR and read-across

ECHA Appendix 4 (Draft) v. 1.0 January 2017 and ECHA Appendix R.6-1 (Public) Draft v. 2.0 December 2016 request information on a minimum of 13 physicochemical characterization end-points. These 13 end-points are all summarized in Table 2 along with the current ECHA recommendations on how to generate the data.

For identification of the specific characterization methods to be used, ECHA Appendix 4 (Draft) and ECHA Appendix R.6-1 refers to the following ECHA Guidance and R-7-1 Appendixes:

Chapter R.7a: Endpoint specific guidance v. 5.0 – December 2016 (ECHA-16-G-10-EN)

Appendix R7-1 to Chapter R.7a of the Guidance on IR&CSA

Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7a Endpoint specific guidance,” Draft (Public) v. 2.0 December 2016.

Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7b Endpoint specific guidance,” Draft (Public) v. 2.0 December 2016.

Appendix R7-2 “Recommendations for nanomaterials applicable to Chapter R7c Endpoint specific guidance,” Draft (Public) v. 2.0 December 2016.

There is also (non-specific) reference to the methods described in scientific paper on DF4nanoGrouping by Arts et al. (Arts et al. 2015; Arts et al. 2016).

Both of the original Appendixes to Chapter R.7a (ECHA-12-G-03-EN) and the proposed new revisions in draft express that the recommended methods are rarely fully applicable (or validated) for characterization of MNM. Looking into the specific recommendations on how to characterize MNMs, it is evident that the nanospecific guidance in Appendix 7-1 and 7-2 is limited.

Appendix 7-1 to Chapter R.7a addresses the following end-points (recommendations and key statements in parenthesis):

- Water solubility (OECD TG-105 may not be appropriate for especially nanomaterials with low and/or slow water-solubility; Section R.7.1.7 in parent guidance; OECD No. 62 and OECD No. 29 are considered applicable for determination of dissolution rate over one-week to 28 days, respectively. However, it is noted that applicable methods for nanomaterials are currently under development in OECD and should be used when available. It is also recommended to perform tests in specifically relevant biological and environmental media)
- n-Octanol/water partition coefficient (OECD TG-107, OECD TG-117 and OECD TG-123 are in most cases not applicable to nanoparticles; the end-point may be relevant for water-soluble organic nanomaterials; care must be taken to not misinterpret dispersed for dissolved nanomaterials, which are two distinctly different phenomena)
- Adsorption/desorption for environmental fate-modelling (OECD TG-106 is not applicable to nanomaterials. For some aspect the OECD TG-312 for soil mobility and leaching testing in columns studies may be applicable. OECD TG 303A for determination of interaction with sewage sludge may be used for indirect assessment of sorption to sludge)

In Appendix to Chapter R7b it is said that there are no standard methods for any of the end-points listed below, but some recommendations are given on:

- Abiotic degradation (photocatalysis test may be performed following OECD TG 316);
- Dissolution (refers back to Chapter R7a and section 2.2.1 in Appendix R7-1);
- Adsorption/desorption (no standard method available for testing of nanomaterials; see guidance in section 2.2.4 in Appendix R7-1 to Chapter R7a; which states that if the NM is highly dissolved with a fast dissolution kinetics, the original OECD TG-106 can be used).
- Agglomeration (sections 2.2.1, 2.2.2, and 2.2.4 in Appendix R7-1 to chapter R7a where there is reference to a new OECD TG in preparation)
- Aggregation (section 2.2.1, 2.2.2, and 2.2.4 in Appendix R7-1 to chapter R7a where there is reference to a new OECD TG in preparation)

Comparing the nanospecific guidance on characterization covered in the Appendixes above and requested in Table 2, it is evident that several of the end-points are not addressed in the nanospecific recommendations provided. In fact none of them are fully or directly addressed using the same terminology.

Consequently, the guidance on methods to generate the characterization end-points requested for substance identification, QSAR, grouping and read-across for MNM, is still mainly covered by the parent Guidance: Chapter R.7a Endpoint specific guidance Draft (Public) v. 5.0 December 2016 (ECHA-16-G-10-EN) and Appendix R7-1 to Chapter R.7a of IR&CSA Guidance. For additional information and recommendation, the ECHA “Practical guide for SME managers and REACH coordinators – How to fulfil your information requirements at tonnages 1-10 and 10-1000 tonnes per year v.1.0 July 2016 (ECHA-16-B-24-EN) also often provides useful and clear information, which is not always reflected in the Parent Guidance(s) and Appendixes.

**Previous reviews and analysis that the OECD Technical guidelines recommended to generate the physicochemical information requested in Appendix 4 (Public) Draft v. 1.0 (2007) and Appendix R.6-1 (Public) Draft v. 2.0 (2016) have previously found to be not suitable for characterization of MNM. A subgroup of the key OECD TGs were assessed in both the OECD Working Party on Manufactured Nanomaterials (WPMNM) and Work-Package 2 of the EU FP7 NANoREG project. It was found in Deliverable 2.03, that none of the analysed OECD TGs were immediately suitable for characterization of MNM. Revisions of several of the OECD TGs were made or proposed or suggested to be replaced with alternative or new methods and presented in NANoREG D2.09. Other methods to support the substance identification and reactivity and fate issues were developed and presented in D2.04, D2.08, D2.10, and D2.11. It is evident that further testing and/or acceptance of existing draft methods is urgently needed in international guidelines and standard methods to support the regulatory process.**

A common European approach to the regulatory testing of nanomaterials

**Table 2. Minimum list of physicochemical information requirements to be used in the grouping scheme proposed in R6-1 v.1 (2016) and proposed characterization methods from Appendix 4 and Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R.7a End-point specific guidance (ECHA-12-G-03-EN and Draft (Public) v. 2.0 2016).**

What they are!	Remarks from R6-1 v.1	Proposed Methods in ECHA guidance
Chemical composition	<p>Detailed information on chemical composition is fundamental for determining human health and environmental effects of nanoforms, as is the case for non-nanoforms. However, size, shape and surface characteristics of a nanoform may cause the nanoform to exhibit a different behaviour compared to the non-nanoform of a material with the same composition.</p> <p><b>NOTE 1:</b> In this table, remarks on crystalline structure have been separated from chemical composition and is described below as an individual parameter renamed as atomic structure.</p>	<p>Practical guide for SME and REACH coordinators v. 1.0 (ECHA-16-B-24-EN) specifically informs that spectral or other analytical data are required to determine the chemical composition. Examples are:</p> <p>UV/Vis (OECD TG 101), IR; MS, GC, HPLC, XRF, AAS, ICP-OES, and IC.</p> <p>And any other method known to be suitable for quantifying the substance.</p> <p>No NM-specific methods were considered in Appendix 4 or any of the R7-1 Appendixes.</p>
Atomic structure	<p>Crystalline structure may for some nanoforms influence other properties of the material (e.g. reactivity, zeta potential, Hamaker constant) in a way that affects human and environmental toxicity. Decreasing size of particles may introduce crystallographic changes in the material (contraction of the crystalline lattice or deformation). Based on the present understanding of nanoparticle behaviour, differences in the crystalline structure may be relevant for metals, metal-oxides or carbon based nanomaterials.</p>	<p>Practical guide for SME and REACH coordinators v. 1.0 (ECHA-16-B-24-EN) recommends:</p> <p>XRD, IR, MS, GC, and HPLC.</p> <p>And any other method known to be suitable for quantifying the substance. There is no reference to standards.</p> <p>No NM-specific methods were considered in any of the R7-1 Appendixes.</p>

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Impurities	As for non-nanoforms, impurities can substantially contribute to the human and environmental toxicity of nanoforms.	No specific recommendation, but the end-point should be determined as a result of the chemical composition analysis above.  No NM-specific methods were considered in any of the R7-1 Appendixes.
Surface chemistry (e.g. chemical coating, surface treatment)	The term surface chemistry indicates the chemical composition at the surface of the particles as a result of chemical coating and/or surface treatment of the particle. Surface chemistry influences dissolution behavior and agglomeration behavior of nanoforms. Considering hazard endpoints, the surface chemistry of a nanoform affects its reactivity and systemic absorption. Surface modification(s) may determine which biomolecules adhere to the nanoform, its distribution and cellular uptake, and its toxic effects. In the environment surface chemistry will influence sorption to environmental or biological media and the reactivity of a nanoform.	No NM-specific methods were considered in any of the R7-1 Appendixes.
Physical size/range	The size of the nanoform affects other physicochemical parameters, such as crystallinity, zeta potential and specific surface area, and may determine exposure, and whether the nanoparticle can be internalised into an organism. Once internalised, particle size may also affect the distribution within the body, and the toxicity at both the point of entry and distally. Size distribution is not a static parameter; it may also change during the course of (environmental) toxicity testing (as well as during the life cycle of the material) due to e.g. partial dissolution, interaction with test media or preferential absorption of smaller particles.	<p>Practical guide for SME and REACH coordinators v. 1.0 (ECHA-16-B-24-EN) refers to OECD Test No. 110 (1981), but also refers to Chapter R.7a (ECHA-16-G-10-EN), which refers to Appendix R7-1 for Chapter R7a (ECHA-12-G-03-EN) on the granulometry and shapes. The recommended methods therein includes:</p> <p>TEM (ISO/TR 27628:2007; ISO/13322-1:2004; and ISO/1322-2 general guidance on size-measurements); SEM (As for TEM); SPM (ISO/TR 27628); Ultrasonic spectroscopy (ISO/20998); SAXS (ISO/TS 13762:2001); DLS (ISO/22412:2008; ISO/13321:1996; and ASTM E2490); and XRD (BS EN 13925-1, BS EN 13925-2 and BS 13925-3). <i>Continued</i></p> <p><b>NOTE 1:</b> There is no change to measurement of granulometry in revisions proposed in R7a Draft (Public) v. 2.0 (2016).</p> <p><b>NOTE 2:</b> It should be taken into consideration that granulometry in the ECHA guidance, normally is considered to be the particle size-distribution of powder dispersed in relevant</p>

		mediums (air and waters) and is used for assessment of migration.
Shape	<p>Particle shape may affect the internalisation of a nanoform (e.g. the ability of a nanoform to penetrate into a cell) and its (environmental) toxicity. In inhalation studies, particle shape may influence nanoform deposition within the lungs and may also influence its persistence in the lungs and probably in other sites. Particle shape may also influence other parameters, such as zeta potential. For advice on characterization for shape see also section 2.2.3.3 of Appendix R.7-1 to Chapter R7a of the Guidance on IR&amp;CSA.</p>	<p>Chapter R.7a (ECHA-16-G-10-EN) refers to Appendix R7-1 for Chapter R7a (ECHA-12-G-03-EN) on the granulometry and particle shapes. The recommended methods includes:</p> <p>TEM (ISO/TR 27628:2007; ISO/13322-1:2004; and ISO/1322-2 general guidance on size-measurements); SEM (As for TEM); SPM (ISO/TR 27628); and Nomenclature: ISO/9276-6:2008</p> <p>There is no reference to shape in Practical guide for SME and REACH coordinators v. 1.0 (ECHA-16-B-24-EN).</p> <p><b>NOTE 1:</b> In Appendix 4 Draft (Public) v. 1.0 January 2017), the shape groups were changed so high aspect ratio nanomaterials are any objects with an aspect ratio <math>\geq 5</math> as compared to the fibre definition with an aspect ratio <math>&gt; 3</math> and a length <math>&lt; 100 \mu\text{m}</math> in Chapter R.7a (ECHA-16-G-10-EN).</p> <p style="text-align: right;"><i>Continued</i></p>

<p>Surface area, including porosity</p>	<p>The increase of relative surface area with decreasing particle size may increase the reactivity of a nanoform relative to its mass and/or volume. Furthermore, as a consequence of the increased surface to volume ratio, porosity may affect the crystalline structure.</p>	<p>Chapter R.7a (ECHA-16-G-10-EN) refers to Appendix R7-1 for Chapter R7a (ECHA-12-G-03-EN) for methods to determine specific surface area and porosity:</p> <p>BET (ISO/9277:2010 (outer surface area and porosity); ISO/18757:2005 (total surface area including porosity).</p> <p>Determination of VSSA requires quantification of relative density: Chapter R.7a (ECHA-16-G-10-EN) defines to the conditions and possible methods and further refers to a.o. the Practical Guide Practical guide for SME managers and REACH coordinators – How to fulfil your information requirements at tonnages 1-10 and 10-100 tonnes per year (ECHA-16-B-24-EN).</p> <p>Pycnometry: OECD TG-109; ISO/3507.</p> <p>There is no reference to surface area and porosity in Practical guide for SME and REACH coordinators v. 1.0 (ECHA-16-B-24-EN). No methods are specifically recommended in Appendix 4 Public (Draft) v.1.0 January 2017; nor in Appendix R.6-1 Public (Draft) v.1.0 December 2016.</p> <p style="text-align: right;"><i>Continued</i></p>
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<b>Behaviour (Where they go )</b>		
Solubility: Rate of dissolution / Equilibrium solubility	The rate of dissolution depends on factors including, but not limited to the chemical composition, particle size, coating, surface treatment, stability, manufacturing process, and biological environment. The rate of dissolution gives information on how many ions/molecules are released from the particle over time. The ion(s)/ molecules released may also dictate the toxicity of the nanoforms, which will be an important aspect of the evaluation. 'Water solubility' is an intrinsic material property, but in most cases the system-dependent property 'dissolution rate in relevant biological media' will be more relevant as this fundamentally affects the bioavailability of substances in the (biological) environment. The relevance of the different media depends on the actual route of exposure and/or the environmental compartment under evaluation.	R7a Draft (Public) v. 2.0 (2016) and Appendix R7-1 to Chapter R7a Draft (Public) v.2.0 December 2016 suggest state that:  OECD Test No. 62 and OECD Test No. 29 may with caution be considered applicable for determination of 7-day and 28-day dissolution rate, respectively.  There is no reference to solubility testing in Practical guide for SME and REACH coordinators v. 1.0 (ECHA-16-B-24-EN).
Hydrophobicity	Hydrophobicity for nanoforms is dependent on e.g. Van der Waals energy (as represented by the Hamaker constant) and surface charge. Analytical determination of the hydrophobicity of nanoforms is still under development, e.g. sessile drop contact angle, dye adsorption. While these parameters can influence agglomeration and sorption, as well as 'dispersibility in biological media' and dustiness, currently the exact relationships between them are not clear. Hydrophobicity is influenced by surface chemistry of the particles. Thus, knowledge on the surface chemistry can give qualitative information about the hydrophobicity of the nanoforms.	No NM-specific methods were considered applicable in R.6-1 Draft (Public) v.1.0 December 2016 or any of the R7-1 Appendixes.  There is no reference to hydrophobicity testing in Practical guide for SME and REACH coordinators v. 1.0 (ECHA-16-B-24-EN).  <i>Continued</i>
Zeta-potential	Zeta potential can be used as a proxy for surface charge and may provide information in dispersion stability, degree agglomeration/de-agglomeration of particles in relevant media. Surface charge may influence systemic distribution and cellular uptake of a nanoform, and ultimately its toxicity.	No methods were recommended in R.6-1 Draft (Public) v.1.0 December 2016 or any of the R7-1 Appendixes.  There is no reference to determination of surface charge in Practical guide for SME and REACH coordinators v. 1.0 (ECHA-16-B-24-EN).

	Additionally there is evidence linking zeta potential to the inflammogenicity of nanoscale particles of metals and minerals.	
Dispersibility	This parameter can influence the degree of environmental transport and (environmental) exposure. Furthermore, this parameter may influence the degree of internal exposure (particularly by the oral route; however particle dispersibility also affects nanomaterial mobility within the lung and hence its potential for systemic uptake). For further information, see Appendix R.7-1 to Chapter R.7a.	No methods were recommended in R.6-1 Draft (Public) v.1.0 December 2016 or any of the R7-1 Appendixes.
Dustiness	This parameter is mainly relevant for exposure via air (particularly by inhalation) and transport through air.  In the environment this parameter is not relevant to aquatic/sediment exposures and only to a limited extent for soil exposures.	No NM-specific methods were considered in any of the R7-1 Appendixes.  Chapter R.7a refers to the prEN-15051-2 (rotating drum dustiness method) and prEN-15051-2 (continuous drop test method) under granulometry from which the respirable, thoracic and inhalable dustiness levels can be assessed from both methods and the mass median aerodynamic diameter (MMAD) from prEN15051-3  <i>Continued</i>

<b>Reactivity (What they do )</b>		
Biological (re)activity (or surface reactivity)	The biological (re)activity or surface reactivity of a nanoform of a substance appears to generate reactive oxygen species (ROS) which induce inflammation, and thus may elicit cellular toxicity.	No NM-specific methods were considered in any of the R7-1 Appendixes.
Photoreactivity	<p>Photoreactivity may increase with decreasing particle size. In human toxicity testing, this parameter may be particularly relevant when considering dermal exposure, but it may also play a role in other exposure routes.</p> <p>In the environment this parameter may be particularly relevant when considering the air and aquatic compartment, but it may also play a role in other compartments. If oxygen radicals are induced (i.e. reactive oxygen species or ROS), they may easily react with other molecules, which in some cases may lead to severe effects (e.g. reaction with DNA leads to genotoxicity). This parameter is relevant for nanomaterials that are photoactive.</p>	OECD TG-316 could be used to some extent to determine photocatalysis

### 3.3.2 NANOREG Procedures for characterization end-points

The key objective of WP2 was to test or develop standard operation procedures (SOPs) for key physicochemical characterization end-points to be used in a regulatory context today and in anticipated future developments. The SOP development and testing was organized in three process themes considering:

- 1) Methods and characterization protocols to enable implementation of the proposed EC-definition for identification of a MNM (D2.10 and D2.11).
- 2) Analysis of the suitability of selected existing OECD TGs for physicochemical characterization of MNM (D2.03 and D2.09)
- 3) Development of SOPs for new intrinsic end-points, which were not considered in the existing Guidance (D2.04; D2.08).
- 4) Development of SOPs for deriving data for new contextual end-points, including hydrochemical reactivity, dissolution, and biomolecule-interaction (D2.06; D2.08).

Many of the end-points selected for SOP test and development in WP2 excellently support the needs for methods as a result of the published ECHA Appendix 4 v.1.0 January 2017 and ECHA Appendix R6.1 v.1.0 (Draft) December 2016. Below, a brief description is given on the key results from the SOP developments and testing in NANoREG WP2 and considerations related to analysis of the specific end-points and future needs for development.

#### 3.3.2.1 Particle size-distributions

In D2.10, several methods were assessed for quantification of particle size-distributions according to the EC recommended definition of a NM. It was concluded that electron microscopy was the most suitable technique for sizing the minimum particle diameter size-distribution of a NM sample.

A suite of thoroughly tested SOPs were developed to enable production of comparable results. The SOPs include sample preparation methods, imaging methods, image analysis, and data-treatment. The SOPs were all applied on near-spherical and fractal-like MNM in an inter-laboratory comparison test using up to 9 different laboratories. The results showed that all materials could be sized quantitatively with in most cases with less than 10% uncertainty. It should be mentioned that the relative uncertainty is expected to vary with size and the morphological complexity of the materials.

It was found that the sample preparation was the most critical step to enable a good analysis and that a full quantification following the established SOPs can be done with reasonable work-effort using semi-automatic analysis. There are on-going developments to enable higher levels of automation, which will reduce the time for analysis even further.

WP2 did not specifically test SOPs for sizing flakes and fibrous NM. SOPs based on Scanning Electron Microscopy -imaging were applied for fibers and tubes (nanocellulose and carbon nanotubes). Whereas fibrous objects that can be dispersed can be sized for both length and diameters, it is still not possible to provide good quantitative length data for entangled materials. This challenge may be solved by improved sample preparation techniques.

The quantitative measurement of fibers, entangled fibers and odd structures should be considered in future work. Whereas, electron microscopy techniques are technologically well advanced, there are still challenges in accurate sizing around the lower end of the nanoscale. The application of Atomic Force Microscopy (AFM) for sizing flakes should also be considered in future research. A tiered approach applying also other techniques (e.g., mass spectrometry methods) for sizing very small nanoparticles around 1 nm-size may also need to be considered.

#### 3.3.2.2 Shape

The SOPs developed in D2.10 also includes analysis of shape parameters. In total 23 parameters are collected simultaneously during the image analysis. Methods for quantification of the shapes of fibers, tubes, and flakes were not tested in NANoREG WP2, but dimensions have been provided using single laboratory procedures. Further work is needed to improve sample preparation techniques for fibers and tubes. In future

work, the application of Atomic Force Microscopy (AFM) for sizing flakes should also be considered. The reliability of shape analysis of very small nano-objects around 1 nm may also need further attention.

### **3.3.2.3 Chemical composition and impurities**

In NANoREG, there was only limited effort dedicated to thoroughly testing the quality of chemical analysis of MNM. Several different methods may be applied depending on the type of materials and impurities to be analyzed (Table 2).

In D2.04, a strategy was proposed for identification and quantification of inorganic and organic coatings, which implies a bulk sample analysis of the inorganic elemental composition as well as combustion analysis to identify presence and amount of evaporable and combustible compounds.

It is recommended that the elemental analysis is made using a technique that covers as much of the periodic table as possible (or at least the range of expected elements) and does not require extraction or dissolution. In addition, the method should be applicable to analyze both high and low concentrations ranging from ppm-range to 100 wt.%. If this is not possible complementary techniques should be applied. One should be aware that when the analysis requires extraction or dissolution, the quantification depends on the efficiency of extraction and dissolution and final recovery of the elements/compounds to analyze. For elemental analysis, a SOP for screening and quantification of the elemental composition using a standard-less WD-XRF method was applied.

Presence and abundance of evaporable and combustible compounds can be assessed by either the water-loss and loss-on-ignition methods, which implies weighing of the mass-loss after heating a powder sample sufficiently to allow evaporation of adsorbed water and combustion of organic compounds associated with the powder, respectively. However, it is recommended to apply differential thermogravimetric analysis (TGA or DTA) combined with gas chromatography (GC) or Fourier Transform InfraRed spectroscopy (FTIR), which allows analysis of molecules released during combustion and temperature-resolved information on the mass-loss. Note in these analysis, the type of material should be considered as phase changes and decomposition of the main substance, such as carbonates and carbon nanotubes, should be understood.

### **3.3.2.4 Surface chemistry (D2.04)**

A technical guideline was developed in D2.04 for identification and quantification of surface chemical modifications to MNM. The initial step for bulk analysis was described above in section 3.3.2.3. After identification of the chemical composition, the stoichiometric composition should be calculated considering the structural formula of the substance. Deviations from structural formula compositions can be used to determine the presence of impurities and whether chemical modifications have been made.

After a chemical deviation has been described or decided, a specific strategy is made for specific identification and quantification of the chemical modification. This may require the use of a series of techniques, including high-resolution TEM (HR-TEM) and High Angle Annular Dark-Field Scanning TEM (HAADF-STEM), High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) coupled with mass-spectrometry (MS).

However, it should be considered that a producer normally knows what the chemical modifications to a material are and therefore easily can identify the suitable techniques for the analysis. This also includes treatments that do not result in chemical changes detectable in bulk chemical analysis, but only changes in surface chemistry due to e.g., ozone treatment.

The chemical analytical techniques are highly established technologies. Future work, should include further analytical methods and reliability testing for screening surface chemistry and interlaboratory testing of SOPs for chemical quantification to deliver a global guideline for surface-chemical analysis for regulatory use.

### **3.3.2.5 Atomic structure**

WP2 did not develop or test any SOPs for quantification of atomic structure. Crystalline phases were determined using X-ray diffraction (XRD) and Raman spectroscopy was applied to describe the overall atomic structures of carbon nanotubes and graphene. Limitations by the techniques should be considered. For example, in XRD, there is an instrument-dependent material-specific lower limit for detection of the crystalline structure due to peak-broadening. I.e. a material may be seen as amorphous, but in fact consists of small crystallites of nanomaterials. Therefore, it is recommended to include HR-TEM in the description of atomic structure of materials. Methods with higher size-resolution for describing atomic structures may also be considered.

For molecular and organic nanomaterials, the suitability of the methods must be considered. Often such materials can be described using GC or HPLC or MALDI.

Whereas the current techniques for structural characterization of materials are well established, future work should consider how to analyze and structurally describe higher generation composite (hybrid) nanomaterials consisting of several phases, which include also mixed organic and inorganic compounds.

#### **3.3.2.6 Specific surface area and porosity (and VSSA)**

WP2 developed SOP for improved specific surface area analysis and quantification of the porosity in powder materials based on a so-called t-plot analysis on data from multi-point BET gas-adsorption tests. The SOP was presented in D2.11. An interlaboratory comparison study was made on 9 different samples in 3 laboratories. The results showed that the total specific surface areas could generally be with an uncertainty of less than 10% between the laboratories when the specific surface area was higher than 20 m<sup>2</sup>/g. The comparability between laboratories strongly depends on the degassing conditions applied to the samples. For comparable degassing conditions, an acceptable reproducibility is obtained i.e. about 7%. This corresponds to the generally accepted margin of error for adsorption data. In this study, most of the uncertainty was linked to one laboratory.

The refined BET and t-plot analysis in NANoREG D2.11 enables users to improve the gas adsorption data and the ability to determine the fraction of the surface area, which is ascribed to the porosity of MNM. It also specifies that any surface-coated material should be analysed following the multi-point BET gas adsorption. In certain cases, the aggregate/agglomerate surface area may also be determined from the t-plot analysis. It should be mentioned that there were no direct comparison with the methods described in ISO/9277:2010 (outer surface area and porosity); ISO/18757:2005 (total surface area including porosity) recommended by ECHA (Table 2) or the newer ISO 15901-2 (2006) and -3 (2007) Pore size distribution and porosity of solid materials by mercury porosimetry and gas adsorption – Part 2: Analysis of mesopores and macropores by gas adsorption.

If the specific surface area is to be used for calculation of the volume-specific surface area (VSSA), it is necessary to also determine the relative (skeletal) density of the material. It was found in NANoREG D2.09 that the He-pycnometry method, which is one of the methods in TG-109 (Table 2), is in principle valid, but, the He-pycnometer required refurbishing to ensure a test volume of at least 1-5 g MNM and re-calibration to enable accurate data for MNM with typically lower density. Moreover, a longer vacuum-treatment was added to obtain repeatable results.

In future work, documentation of methods should be improved by running an interlaboratory comparison involving several more laboratories than the 3 that volunteered collaboration in the NANoREG project.

#### **3.3.2.7 Solubility: Rate of dissolution / Equilibrium solubility (D2.07 and D2.09)**

This end-point has typically been covered by OECD TG-105 that is determination through sedimentation using a flask method, which was found inadequate for testing MNM. NANoREG D2.09 investigated modifications to this method where quantification of the amount of dissolved MNM was determined by chemical analysis after 3KD<sub>a</sub> centrifuge-filtration or direct in situ measurement of released ions using Ion Selective Electrodes (ISE) or the Absence of Gradients and Nernstian Equilibrium Stripping (AGNES) technique. Alternative methods were stirred batch-reactor testing in controlled atmosphere and temperature and chemical quantification of the dissolved NM after 3KDa centrifuge-filtration or quantification using the AGNES technique.

It is clear that the optimal dissolution analyses are provided if reliable in situ time-resolved analyses are made of the dissolved matter, but this is not always feasible. It was also observed that harmonization of procedures in regards to definition and control of the atmospheric and temperature test conditions is necessary. Moreover, for risk assessment, the dissolution (or time-fixed solubility) should not be studied in water alone. It is recommended in NANoREG D2.08 that dissolution studies should be conducted in a series of relevant mediums that represent or simulated key biological and environmental compartments and conditions.

Further work, should consider harmonization of SOPs, evaluation of separation techniques and in situ analysis, and interlaboratory comparison testing. Technologically, fast response in situ measurement methods should be further developed to ensure more accurate measurements in the future.

#### **3.3.2.8 Zeta-potential**

The zeta-potential described the charge that particles have at specific conditions in a specific medium. In NANoREG D2.09, it was proposed to replace the ECHA characterization request on the dissociation constant

in water (TG 112) with a request for determination of the zeta-potential of MNM by electrophoretic mobility using electrophoretic light scattering and as function of pH in water. Such data can be used to determine the iso-electrical point (IEP; or pH with zero charge) of the MNM. The pH is varied by acid-base titration using simple salts (NaOH and HCl), which provides the negative positive and negative ions that create the electrical double-layer around the particles and their zeta-charge. Other acid-base pairs, such as HNO<sub>3</sub>, NaOH and NaNO<sub>3</sub> may also be used as applied in the SOP developed in NANOGENOTOX (Guiot and Spalla 2013). In any case, the procedure requires that the test material can be dispersed and kept in dispersion long enough to allow the analysis. In the work proposed by NANoREG, these dispersions are made by probe-sonication using harmonized dispersion energies, established after calibration using the NANoREG probe-sonicator calibration protocol (NANoREG D2.05).

It should be noted that the surface-charge of a material also varies with the specific medium composition. Therefore, the surface charge should be determined in relevant mediums, which was demonstrated and applied on both ecotoxicological test media, cell media and synthetic saliva fluid in NANoREG D2.08.

Technically, the measurement of zeta-potential is highly established. Further developments should be harmonization on acceptable SOPs and media that should be analyzed in a regulatory aspect and interlaboratory comparison.

### **3.3.2.9 Dustiness**

Methods for dustiness testing were not specifically developed as part of the NANoREG project. However, the Small Rotating Drum and Vortex shaker methods for dustiness testing of nanomaterial powders, which were applied in NANoREG, were under standardization in a working group under CEN/TC137/WG3 almost in parallel with the NANoREG project.

In total, 4 new CEN standards are currently circulated for comments in CEN (Jensen et al. 2016). The methods all report the respirable particle size-distributions from ca. 10 nm to 10 µm in addition to the number-based dustiness index, and the conventional respirable mass-based dustiness. Two of the methods are modifications to EN15051-2 (rotating drum method) and EN15051-3 (continuous drop method). The Vortex shaker and the Small Rotating Drum methods are new standard methods.

The different dustiness test methods are considered well-established. Future work could include interlaboratory testing to further demonstrate the repeatability of the methods.

### **3.3.2.10 Biological (re)activity**

There is currently no agreement on how one should determine the biological surface reactivity of a MNM. Several more or less specific methods may be applied to determine the formation of reactive oxygen species (ROS), which is the key requirement in R6-1 v. 1.0 (2016). In D2.08, SOPs were tested on methods to assess the pH and oxidative reactivity in cell media using a Sensor Dish Reader (SDR) and synthetic biological fluids using an Atmosphere-pH-Temperature-controlled Stirred Batch Reactor (ATempH-SBR).

In the SDR method, the ability of a MNM to affect the pH and O<sub>2</sub> concentrations in cell media is measured in situ during specific cell incubation conditions. In this case the difference between O<sub>2</sub> concentrations in exposed and unexposed wells can be used to indirectly calculate the change in redox potential and thereby the theoretical MNM-induced change in free electron equivalents in the media during incubation. This procedure is under development for technical specification in CEN.

In the ATempH-SBR method, the pH, temperature and atmosphere is fixed to mimic conditions in e.g., a biological compartment such as the lung-lining or the phagolysosome. Fixing these conditions enables a rather accurate measurement of the redox potential in the test medium. The difference in redox potential between the MNM-free test media and the MNM-exposed media can be used to calculate the MNM-induced change in free electron equivalents during incubation in the tested compartment.

In both cases future work should include further assessment of the data generated by using of these methods and their comparability with biological effects as well as other methods for determination of ROS. This should be followed by interlaboratory comparison studies and development of technical guidance documents.

### **3.3.2.11 Photoreactivity**

This end-point was not studied in NANoREG. ECHA considers that OECD TG-316 could be used to some extent.

Future work should include assessment of candidate methods, their validation, and demonstration to support development or revision of the OECD TG.

### 3.4 Evaluation and conclusions

This deliverable, attempts to:

- 1) Provide an analysis of the current state-of-art and development in the REACH guidance towards substance identification and grouping, QSAR and read-across of MNM and to what extent the guidance for non-nanomaterials aligns with the new requirements for MNM
- 2) Assess the suitability of the ECHA scheme for substance identification of MNM considering the modern material characteristics and propose alternative solutions
- 3) Provide an analysis of the preparedness of the ECHA guidance documents and Appendixes in regards to recommended methods suitable for characterizing the requested end-points for substance identification, QSAR and read-across of MNM
- 4) Demonstrate how the developments in NANoREG WP2 support the potential further development of the ECHA guidance and advancements in physicochemical characterization methods specifically designed for characterization and testing of MNM.

It is found that the scheme and criteria for identification of material substances on nanoform is significantly improved as compared to the scheme for non-MNM. However, the substance category criteria are considered to be sub-optimal for materials in general as they allow up to 20 wt.% of another phase (or 10 wt.% of a second phase plus a maximum of 10 wt.% of tertiary substances), before it is no longer a monoconstituent substance. A revised set of criteria for defining mono and multi-constituent substances is proposed, in which deviation from structural formula and stoichiometry of the known phase(s) is used as a criterion.

A difference was observed between the type of information requirements requested on MNM and non-MNM as well as the definition of shapes and surface chemistry in the Guidance documents. These differences are likely to cause confusion and inability to group across MNM and between non-MNM and MNM. It is strongly recommended to harmonize the schemes for substance identification and substance identification profiles, irrespective of whether it is a MNM or not.

Regarding size-information, it is considered necessary, that the primary particle size-distribution is always reported when a nanomaterial (and in fact any particulate material) is registered. This, on one hand, ensures a clear description of the material and, on the other hand, prevents accidental non-reporting of materials where the size-ranges produced inadvertently results in higher biological activity and/or quantum phenomena, which needs to be considered during the risk assessment.

Regarding shape, ECHA has developed four new groups in the morphological categorization scheme building on aspect ratios of 5 or greater, but there appears to be no clear explanation on the motivation to define these new shape classes. As the MNM shape-classes differ from the shape classes for non-MNM, application of the new shape classes will likely result in confusion. Moreover, the lack of guidance and the proposed morphological grouping will also likely make it impossible for ECHA to use the information given by the registrants directly for later grouping and read-across purposes. It is unclear why already highly developed morphological classification systems are not at all applied by ECHA in general. For example, neutral shape definitions (equidimensional, prolate [elongated / fibre], oblate [disc / flake], and bladed [belt]) by Zingg et al. (Zingg 1935) are implemented in many areas, including powder technology, mineralogy and microscopy. For MNM, the ISO developed already an elaborate scheme for shape type-classification in ISO/TR 11360:2010(E) and the refinements in ISO/TS 80004-1:2015 to ISO/TS 80004-4:2015 series. For registration, it is recommended to develop a scheme to report the pre-defined morphologies and define a trigger for nanomaterials with very high aspect ratios for which the lengths approach the critical length in the WHO paradigm.

Regarding surface chemistry, this end-point is considered a highly important parameter for any substance and in any system may to high degree control, at least the initial properties and fate on a material. The text in Appendix 4 (Public) v. 1.0 January 2017, requests that the registrant report *any* surface chemical modification and highlights that the 80%-20% rule in the overarching substance categorization scheme still applies for substance identification of MNM. If any surface chemical modification will be reported and characterized, this is in line with the proposal in NANoREG D2.05 and will be an important development towards establishment of

grouping and read-across principles for this parameter. It is unfortunate, however, that surface-chemistry is the only chemical modification to be considered as the minimum reporting requirements for nanoforms in ECHA Appendix 4 4 (Public) v. 1.0 January 2017. MNMs may be modified in many different ways in modern material design including: atomic substitution, doping, porosity-filling, physical coating and chemical functionalization. More than one of these modifications may also be applied to the MNM and are thence not only affecting the surface chemistries within the 20% one-substance rule. Hence, reporting of all types of surface, porosity and matrix modifications should be mandatory as they all potentially change the properties, reactivity, fate and hazard of the MNM.

Regarding natural minerals, the exemption for minerals does not per default apply to synthetic substances having the same compositions and structures as the natural occurring minerals. This means that manufactured material substances, including MNM in principle must be registered according to the REACH tonnage and CMR requirements. However, natural minerals, which have been only physically modified or purified, may escape REACH registration due to the exemption rule: *“provided that none of the constituents of the final isolated substance has been chemically modified.”* It is recommended that mechanically processed minerals are also reported in REACH.

Analysis of the recommended methods to generate the 13 minimum physicochemical characterization data end-points requested in Appendix 4 (Public) Draft v. 1.0 (2007) and Appendix R.6-1 (Public) Draft v. 2.0 (2016), shows a lack of methods verified to be suitable for the characterization of MNM. NANoREG WP2 developed and demonstrated SOPs for identification of MNM by sizing using electron microscopy and BET gas-adsorption and de-sorption profiles. Procedures were developed for several other end-points of regulatory relevance in NANoREG WP2, including identification and quantification of surface chemical modifications, dissolution testing, and reactivity. Revisions of several of the OECD TGs were also proposed or proposed to be replaced with alternative or new methods and presented in NANoREG D2.09. The established SOPs for sizing dispersed near-spherical particles and primary nano-objects in agglomerates and aggregates are already documented by interlaboratory testing in 9 laboratories. It is evident that further testing and/or acceptance of the other SOPs is urgently needed to establish international guidelines and standard methods to support the regulatory process. A revision to clarify the guidance and recommended characterization methods in general would be of great benefit for the registrants.

### 3.5 Data management

Not relevant. No data are generated as a result of this deliverable.

## 4 Deviations from the work plan

*If relevant explain why a delay has occurred and/or why the task as described in the DoW has been modified.*

D2.12 was delayed due to prioritization of finalization of other delayed deliverables, which among others feed into D2.12, and final data generation to fill as many data gaps as possible under the NANoREG activity. The recent serendipitous access to ECHA Appendix 4: "Recommendations for nanomaterials applicable to the Guidance on Registration vs. 1" (ECHA, 2016) has also had influence on the decisions for the D2.12 report and its contents and issues to be considered.

## 5 References / Selected sources of information (optional)

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