



The EU FP7 NanoDefine Project

Development of an integrated approach based on validated and standardized methods to support the implementation of the EC recommendation for a definition of nanomaterial

Recommendations on a Revision of the EC Definition of Nanomaterial Based on Analytical Possibilities

NanoDefine Technical Report D7.10

H. Rauscher, A. Mech, C.Gaillard, M. Stintz, W. Wohlleben, S. Weigel, A. Ghanem, D. Hodoroaba, F. Babick, J. Mielke

The NanoDefine Consortium 2015

NanoDefine in a nutshell:

The EU FP7 NanoDefine project was launched in November 2013 and will run until October 2017. The project is dedicated to support the implementation of the EU Recommendation on the Definition of Nanomaterial by the provision of the required analytical tools and respective guidance. Main goal is to develop a novel tiered approach consisting of (i) rapid and cost-efficient screening methods and (ii) confirmatory measurement methods. The "NanoDefiner" eTool will guide potential end-users, such as concerned industries and regulatory bodies as well as enforcement and contract laboratories, to reliably classify if a material is nano, or not. To achieve this objective, a comprehensive inter-laboratory evaluation of the performance of current characterisation techniques, instruments and software is performed. Instruments, software and methods are further developed. Their capacity to reliably measure the size of particulates in the size range 1-100 nm and above (according to the EU definition) is validated. Technical reports on project results are published to reach out to relevant stakeholders, such as policy makers, regulators, industries and the wider scientific community, to present and discuss our goals and results, to ensure a continuous exchange of views, needs and experiences obtained from different fields of expertise and application, and to finally integrate the resulting feedback into our ongoing work on the size-related classification of nanomaterials.

Bibliographic data:

NanoDefine Technical Report D7.10

Report title: Recommendations on a Revision of the EC Definition of Nanomaterial Based on

Analytical Possibilities

Author(s): H. Rauscher, A. Mech, C. Gaillard, M. Stintz, W. Wohlleben, S. Weigel, A. Ghanem, D. Hodoroaba,

F. Babick, J. Mielke.¹

Affiliation(s): RIKILT, 6708 WB Wageningen, The Netherlands

Publication date: 30/10/2015

Publisher: The NanoDefine Consortium

© Copyright 2015: The NanoDefine Consortium

Place of publication: Wageningen, The Netherlands

Citation: H. Rauscher, A. Mech, C. Gaillard, M. Stintz, W. Wohlleben, S. Weigel, A. Ghanem, D. Hodoroaba, F. Babick, J. Mielke: Recommendations on a Revision of the EC Definition of Nanomaterial Based on Analytical Possibilities, NanoDefine Technical Report D7.10, NanoDefine Consortium, Wageningen, 2015

URL: http://www.nanodefine.eu/index.php/downloads/public-deliverables

Contact: coordinator@nanodefine.eu, www.nanodefine.eu

¹ Corresponding authors: <u>agnieszka.mech@ec.europa.eu</u>, <u>hubert.rauscher@ec.europa.eu</u>

Table of Contents

Exe	cu	tive summary	6
1.	In	troduction and scope of the document	8
	1.	1. Outline of the document	9
	1.	2. The EC recommendation on the definition of nanomaterial	10
	1.	3. Legal status of nanomaterials in the EU (REACH, CLP and product specific legislation)	11
	1.	4. REACH and CLP definition of substance, mixture and article	12
	1.	5. ECHA guidance: approach to different substances	12
2.	A	nalytical challenges: well defined substances	14
2.1		External dimensions of individual particles	15
2.2		Number based particle size distribution	22
2.2	1.	Type of quantity conversion	22
2.2	2.	Polydispersity and upper size limit	24
2.3		Aggregates and agglomerates	27
2.3	1.	Review of the possibility of physical separation of constituent particles	29
2.3	2.	Determination of the VSSA	31
2.4		Colloids	37
2.5		Proving that a material is not a nanomaterial	38
3.	A	dditional analytical challenges: Mixtures	39
4.	A	nalytical challenges: nano-ingredients and components of articles and consumer products	41
4.1		Nanomaterials in articles and consumer products	42
4.2		Specific challenges related to analysis of NM in products	42
4.3		Technical feasibility of the analysis of NM in products	44
5.	S	ummary of the Recommendations	45
5.1		The term "external dimension"	45
5.2		Particle size distribution based on particle numbers	46
5.3		Polydispersity	47
5.4		Aggregates and agglomerates	47
5.5		Colloids	48
5.6		Specific Surface Area	49
5.7		Proving that a material is not a nanomaterial	51
6.	С	onclusions	52
AN	NE	XES	53
А	nn	ex 1: Non exhaustive list of the nano-specific provisions and guidelines in EC regulatory frameworks	53
A	nn	ex 2: Definitions of nanomaterials present in specific product legislations	54

Annex 3: Overview of current and expected presence of potential nanomaterials in products	57
Annex 4: Availability of standardized, validated analytical methods and reference materials	58
Bibliography	67

List of Acronyms

AC	analytical disc- and cuvette centrifugation
AFM	atomic force microscopy
ALS	angular light scattering
AUC	analytical ultra-centrifugation
BET	Brunauer–Emmett–Teller Method
CLS	centrifugal liquid sedimentation
СМ	characterisation method
CPC	condensation particle counter
CRM	certified reference material
DLS	dynamic light scattering
DEMA	differential electrical mobility analysis
EC	European Commission
EM	electron microscopy
ESZ	electrical sensing zone
FFF	field flow fractionation
LD	laser diffraction
NP	nanoparticle(s)
PTA	particle tracking analysis
PSD	particle size distribution
SAXS	small-angle X-ray scattering
SEM	scanning electron microscopy
SOP	standard operating procedure
sp ICP-MS	single particle inductively coupled plasma mass spectrometry
ТЕМ	transmission electron microscopy
TRPS	tuneable resistive pulse sensing
TSEM	scanning electron microscopy in transmission mode
USSP	ultrasonic spectroscopy
VSSA	volume-specific surface area
WAXD	wide angle x-ray diffraction

Executive summary

In October 2011 the European Commission (EC) published a "Recommendation on the definition of nanomaterial" (2011/696/EU), to promote consistency in the interpretation of the term "nanomaterial" for legislative and policy purposes in the EU. The *EC NM Definition* includes a commitment to its review in the light of experience and of scientific and technological developments. This review is ongoing in 2015 and as a contribution to the review the Joint Research Centre of the European Commission (JRC) has already developed a series of three scientific-technical reports with the title: "Towards a review of the EC Recommendation for a definition of the term nanomaterial" which provides to the EC policy services science-based options on how the definition could be revised or supported with additional guidance.

The overarching nature and wide scope of the *EC NM Definition,* as it does not exclude a priori any particulate material regardless the state, form and size, creates many analytical challenges in its implementation for all stakeholders, including enterprises and regulators.

The NanoDefine project has as core objective to support the implementation of the *EC NM Definition*. In this report key aspects of the *EC NM Definition* are addressed, with the goal to improve the implementability of the *EC NM Definition*. These aspects are presented and discussed based on the results of two years of research performed within the framework of the project. As a result this report assesses how well the requirements of the *EC NM Definition* can be fulfilled with currently available analytical possibilities. It presents recommendations and options on a revision of the *EC NM Definition* to improve the implementability of the definition based on currently available analytical possibilities, according to the state of the art of mid-2015.

Of the technical issues considered in this report, the following seem to deserve the most attention in terms of clarification of the definition and/or provision of additional implementation guidance:

• The term 'external dimension'.

A clear definition of 'External dimension' should be included in the text of the *EC NM definition* and more precise guidance on what is considered as an external dimension and how to properly characterise it should be provided.

• The 'number based particle size distribution'.

The *EC NM Definition* uses a threshold related to the number based size distribution of particles. Yet most of the easily available techniques provide a mass-, volume- or scattered light intensity-based size distribution which needs to be converted into a number based distribution to be used for regulatory purposes. A specific guidance on the conditions under which these methods can be used to identify a nanomaterial by employing appropriate quantity or metrics conversion should be provided.

The 'polydispersity' and 'upper size limit'

Polydispersity is a challenge for the measurement of particle size distribution for the *EC NM definition*, specifically for materials with high polydispersity index and broad size distribution especially when the volume or mass of the fraction containing particles below 100 nm is very small. Therefore a dedicated guidance should be provided that allows applying an upper size limit in measurements and particle statistics.

• The term 'particle'.

This term should be precisely defined for the purpose of the definition or detailed guidance should be issued on the meaning of that term. Particular attention should be paid to the identification of (constituent) particles within **agglomerates and aggregates** and a clear guidance on analytical possibilities should be also provided.

• The 'means to prove that a material is not a nanomaterial' and 'the role of the volume specific surface area (VSSA) '.

The *EC NM definition* makes it very difficult to prove that a material is not a nanomaterial. This implementation challenge should be resolved by adding an additional criterion to identify both nano and nonnanomaterial. As there is enough scientific evidence it might be based on a VSSA threshold. Specific changes in the recommendation text and dedicated guidance with clear screening strategy should be provided.

• Some "particular materials"

Some materials like e.g. colloids pose a challenge in the process of identification as nano or nonnanomaterials. Appropriate guidance which addresses these issues should be provided.

All the above listed issues could in principle be clarified by developing a specific guidance going beyond modification of the definition itself. Accordingly this report provides a number of suggestions on technical guidance documents and screening strategies that could help in facilitating the practical implementation of the definition. To assure the harmonised implementation of *EC NM Definition* also possibilities to introduce more clarity in the definition itself are considered and discussed in the report.

1. Introduction and scope of the document

In October 2011 the European Commission (EC) published a "Recommendation on the definition of nanomaterial" (here subsequently referred to as the *EC NM Definition*), to promote consistency in the interpretation of the term "nanomaterial" for legislative and policy purposes in the EU. The *EC NM Definition* uses size (i.e., size range 1 - 100 nm) as the only defining property of the material. The size refers to the external dimensions of the constituent particles of a material which can be unbound but also may be in a form of agglomerates and/or aggregates. The *EC NM Definition* applies to all particulate materials regardless of their origin, i.e. natural, incidental or manufactured. A material is a nanomaterial if 50% or more of its constituent particles, regardless whether they are unbound or part of agglomerates or aggregates, in the number-based particle size distribution have one or more external dimensions between 1 nm and 100 nm. In specific cases that threshold of 50% can be lowered to values between 1-50%.

The current *EC NM Definition* was a result of a discussion among EC services and EU agencies. This process also involved a thorough discussion of all elements of such a definition, including the defining properties and the types of material that should be covered by the *EC NM Definition*.

The *EC NM Definition* includes a commitment to its review by December 2014 in the light of experience and of scientific and technological developments. This review is ongoing in 2015 and the Joint Research Centre of the European Commission (JRC) has developed a series of three scientifictechnical reports with the title: "Towards a review of the EC Recommendation for a definition of the term nanomaterial". Two of these reports address specific points of the Recommendation and are publicly available^{1,2}. The third report³ was released in July 2015 and provides to the EC policy services sciencebased options on how the definition could be revised or supported with additional guidance.

Still, implementability and enforceability of the *EC NM Definition* remain among the most important aspects; while they depend on the one hand on individual elements of the definition, they also include more generic aspects of further development (e.g. matrices, automation, standardisation) and support (guidance, access to instrumentation, economic considerations etc.) that often need to be tailored to specific needs.

The *EC NM Definition* does not exclude *a priori* any particulate material so that the quantitative cut-off criteria need to be measured for all particulate materials including e.g. substances with macroscopic diameters, heterogeneous size and shapes, hard and soft materials or materials with complex internal structure have to be analysed. This overarching nature of the *EC NM Definition* creates many analytical challenges in its implementation for all stakeholders, including enterprises and regulators.

In this context a consortium of European top RTD performers, metrology institutes and nanomaterials and instrument manufacturers has been established to mobilize the critical mass of expertise required to support the implementation of the definition. Within the framework of the NanoDefine project, based on a comprehensive evaluation of existing methodologies and intra-lab and inter-lab comparisons, validated measurement methods and instruments for supporting implementation of the *EC NM Definition* are being developed that are robust, readily implementable, cost-effective and capable to

reliably measure the size of particles in the range between 1 nm and 100 nm and above, with different shapes, coatings and for the widest possible range of materials, in various complex media and products.

One of the major outcomes of this project is a document which provides recommendations on a revision of the EC Recommendation for a Definition of Nanomaterial, and which assesses how well the requirements of *EC NM Definition* can be fulfilled with currently available analytical possibilities. It also presents options on how and if the *EC NM Definition* could be revised so that the definition can be implemented based on currently available analytical possibilities.

This report considers and provides options on possible ways to change/ clarify each specific element of the *EC NM Definition* which poses an analytical challenge in the appropriate implementation of *EC NM Definition*, based on the state of the art of mid-2015.

While all the presented recommendations are relevant, some are mutually incompatible. The present report recommends elements, aspects and specifications for a revision of the *EC NM Definition* but it does not constitute a revised definition. It should be also underlined that the present report should not be considered as guidance.

1.1. Outline of the document

This report considers and provides options on possible ways to change/ clarify each specific element of the *EC NM Definition* that poses an analytical challenge in its appropriate implementation. Chapter 1 briefly presents the current legal status of nanomaterials in the European Union. It also includes an overview of nanomaterial definitions, to identify where the *EC NM Definition* diverges from other definitions. An important aspect for this report is a good understanding of REACH and CLP terminology which applies to nanomaterials, and this is therefore also discussed in this section.

Chapter 2 describes the analytical challenges for the characterisation of nanomaterials considered welldefined substances under REACH, and provides appropriate examples and proposals for further development of the applicable method. This section is complemented with the discussion on additional analytical challenges encountered while characterising nanomaterials defined in mixtures (Chapter 3).

Chapter 4 describes analytical challenges when characterizing nanomaterials in articles and consumer products, where specifically the difficulties caused by the embedding of nanomaterials in a matrix and the possible co-presence of various types of natural, incidental and engineered particles are addressed.

Finally, chapter 5 presents summary of recommendations for options of introducing changes/clarifications in the *EC NM Definition* supported by the dedicated guidelines considering currently available analytical possibilities.

1.2. The EC recommendation on the definition of nanomaterial

In October 2011 the European Commission (EC) published a Recommendation on the definition of nanomaterial (2011/696/EU)⁴. The purpose of this definition is to allow determination when a material should be considered a nanomaterial (NM) for regulatory purposes in the European Union. The definition covers natural, incidental and manufactured materials and is based solely on the size of the constituent particles of a material, without regard to specific functional or hazard properties or possible risks. It serves as a reference that is broadly applicable across different regulatory sectors and can be adapted to specific product legislation.

The European Commission recommends the following definition of the term 'nanomaterial':

'Nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm.

In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %.

The Recommendation additionaly specifies:

By derogation [...], fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials.

[...] 'particle', 'agglomerate' and 'aggregate' are defined as follows:

(a) 'particle' means a minute piece of matter with defined physical boundaries;

(b) 'agglomerate' means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components;

(c) 'aggregate' means a particle comprising of strongly bound or fused particles.

Where technically feasible and requested in specific legislation, compliance with the definition [...] may be determined on the basis of the specific surface area by volume. A material should be considered as falling under the definition [...] where the specific surface area by volume of the material is greater than $60 \text{ m}^2/\text{cm}^3$. However, a material which, based on its number size distribution, is a nanomaterial should be considered as complying with the definition [...] even if the material has a specific surface area lower than $60 \text{ m}^2/\text{cm}^3$.

The *EC NM Definition* is not legally binding and does not entail a direct obligation for Member States or stakeholders. Therefore it can be assumed that its implementation will happen through different pieces of specific product legislation. In this process the overarching broad definition can be adjusted to the scope and precise needs of a specific regulation. Examples for this are the Biocidal Products Regulation⁵, the Cosmetic Products Regulation⁶ and the Regulation on the Provision of Food Information to Consumers⁷. The latter two are currently (in 2015) being amended with the intention to harmonise the

legally binding definitions of nanomaterials with the *EC NM Definition*. This way the *EC NM Definition*, although being non-binding – has an effect on specific legislation.

1.3. Legal status of nanomaterials in the EU (REACH, CLP and product specific legislation).

Currently in the European Union there is no dedicated nano-specific regulation. However, horizontal and sector specific legislation provides a binding framework for manufacturers, importers and users to ensure the safety of substances and products on the market.

In EU chemical substances are regulated under the Regulation (EC) No 1907/2006 concerning Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). REACH provides an overarching legislation applicable to the manufacturing, placing on the market and use of substances on their own, in preparations or in articles. Another horizontal regulation related to chemical substances in Europe is the CLP Regulation (Regulation (EC) No 1272/2008), that is the Regulation on Classification, Labelling and Packaging of chemical substances and mixtures. The legislation introduces, throughout the EU, a system for classifying and labelling chemicals, based on the United Nations' Globally Harmonised System (UN GHS). Both legislations use the same terminology and are coherent in requirements.

There are no provisions in REACH referring specifically to nanomaterials nor are they explicitly mentioned in the legal text. However, REACH deals with substances, in whatever size, shape or physical state. Substances at the nanoscale are therefore covered by REACH and its provisions apply. It thus follows that under REACH manufacturers, importers and downstream users have to ensure that their nanomaterials do not adversely affect human health or the environment. (CA-59-2008 rev.1).

The Commission intends to introduce nanomaterial-specific modifications in some of the REACH Annexes and encourages ECHA to further develop guidance for registration of nanomaterials. Simultaneously the Commission is preparing an impact assessment of relevant regulatory options, in particular possible amendments of REACH Annexes, to ensure further clarity on how nanomaterials are addressed and safety demonstrated in REACH registration dossiers.

Currently several pieces of sector specific EU legislation explicitly address NMs. This includes the Regulation on the Provision of Food Information to Consumers (1169/20119), the Regulation on Plastic Food Contact Materials and Articles (10/2011), the Regulation on Active and Intelligent Materials and Articles (450/2009), the Biocidal Products Regulation (528/2012) and the Cosmetic Products Regulation (1223/2009). Other pieces of legislation are currently under revision to better address NMs, e.g. the Novel Food Regulation (258/97).

Basic information on the EU regulatory framework considering nanomaterials as well as presentation of nanomaterial definitions present in EU specific product legislation are included in Annex I of this document.

It is worth to notice that several member states like e.g. France or Belgium has created they own registration scheme and have put an obligation on the producers and importers of nanomaterials to notify any foreseen use of these materials on the national market.

1.4. REACH and CLP definition of substance, mixture and article

In this document the terminology used by REACH was applied as that regulation, along with CLP, is an overarching regulation addressing chemical substances in Europe. The terms which are most important for the discussions in this document are summarized in the following.

A **substance** is defined in REACH 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.

According to REACH a **mixture** is composed of two or more substances and an **article** is defined as an object which, during production, is given a special shape, surface or design which determines its function to a greater degree than does its chemical composition.

In line with REACH Article 10 'Information to be submitted for general registration purposes' a registration shall contain a technical dossier including the identity of the substance as specified in Section 2 of REACH Annex VI. The 'Guidance for identification and naming of substances under REACH' (ECHA 2007)² focusses on the appropriate identification of substances that fall under the legal definition of a substance in REACH and provides guidance on the substance identification parameters of Section 2 of Annex VI.

The guidance states that substance identification should use at least the substance identification parameters listed in REACH Annex IV, item 2. A substance is usually identified by its chemical composition, the chemical identity and the content of each constituent in the substance. While such straightforward identification is possible for most of the substances, it is recognised that this is not feasible or not adequate for certain substances. In those cases, other or additional substance identification information is required.

1.5. ECHA guidance: approach to different substances

In the 'Guidance for identification and naming of substances under REACH and CLP' (ECHA 2007)³ document, substances are divided into two main groups; substances of well-defined composition and substances of Unknown or Variable Composition, Complex reaction products or Biological materials, so-called UVCB substances. It is recognised that there will be borderline cases between well-defined substances (reaction products with many constituents, each within a broad range) and UVCB substances (reaction products with variable and poorly predictable composition).

² http://guidance.echa.europa.eu/docs/guidance_document/substance_id_en.pdf

³ http://guidance.echa.europa.eu/docs/guidance_document/substance_id_en.pdf

Well-defined substances: Well-defined substances can be mono or multi-constituent substances depending on the constituent concentration ranges. A mono-constituent substance according to ECHA guidance is a substance, defined by its composition, in which the main constituent is present at least at 80 % (w/w) whereas a multi-constituent substance is a substance, defined by its composition, in which the main constituent is present in a concentration $\geq 10\%$ (w/w) and < 80% (w/w). The 80 % rule was also applied in the notification scheme of new substances under the Directive 67/5488/EEC and it is considered as a "rule of thumb", however it has to be stressed that deviation from this rule is possible if properly justified.

Both mono and multi constituent substances are substances with a defined qualitative and quantitative composition that can be sufficiently identified based on the identification parameters of REACH Annex VI section 2. However, some well-defined substances are not fully defined by chemical composition alone and other identifiers are necessary. Such substances are described in the guidance as '**sub-stances of defined chemical composition and other identifiers'**. Graphite and diamond are given as examples. The chemical composition is the same for both (i.e. carbon) and thus another identifier is necessary to uniquely identify graphite and diamond. The additional identifier is in this case the crystal morphology.

UVCB substances: Substances that are not fully defined by chemical composition and other identifiers are considered to be UVCB substances. According to the guidance, a UVCB substance has a relatively large number of constituents and/or its composition is to a significant extent unknown and/or is very variable or poorly predicable. The guidance gives examples of different types of UVCB substances. For example, for substances where the chemical composition is not fully known, the substance may be identified based on manufacturing process used and the reactants (e.g. [reaction] products of [reactant 1] and [reactant 2] and etc...

The criteria for determining if a substance should be identified according to the described routes are summarised in Table 4.1 and 4.2 of the guidance. Figure 4.1 of the guidance (Figure 1 in this report) illustrates this schematically.

It should be underlined that the guidance does not include any advice for the identification and naming of nanomaterials under REACH. Within the chapter concerning 'Substances of defined chemical composition and other main identifiers' (Section 4.2.3 of the guidance, page 28) it is stated that "the current developments in nano-technology and insights in related hazard effects may cause the need for additional information on size of substances in the future. The current state of development is not mature enough to include guidance on the identification of substances in the nanoforms in the TGD."



Figure 1: Key for various types of substances according to the 'Guidance for identification and naming of substances under REACH (ECHA 2007)⁴

Provisions of REACH and CLP apply only to the substances on their own or when used in mixtures or in articles. Mixtures and articles as such are not regulated by REACH and therefore all ingredients of a mixture or article (in which case are forseen to be released) have to be registered as separte substances.

2. Analytical challenges: well defined substances

Existing legislation in the EU explicitly or implicitly address nanomaterials, and consequently a clear definition of the term for regulatory purposes is needed. As nanomaterials are used in rather diverse regulatory sectors, the intention of policy makers was to have comprehensive, science-based definition that could be used as a reference for policy and legislation, but on the other hand it should not prejudge nor reflect the scope of application of other specific legal acts. When a legally binding definition of nanomaterial is introduced into sector-specific legislation the requirements of that sector can be taken into account by adapting the definition via the introduction of additional criteria.

⁴ http://guidance.echa.europa.eu/docs/guidance_document/substance_id_en.pdf

Being a science-based definition, the *EC NM Definition* includes certain quantitative criteria against which a material needs to be tested to decide whether it is a nanomaterial. The results of those tests need to have an acceptable uncertainty in order to be broadly accepted for regulatory purposes. Those criteria are the external dimensions and the relative number of particles with one or more external dimensions between 1 nm and 100 nm in a material. The Volume Specific Surface Area (VSSA) can be another criterion if requested in specific legislation. Notably, those criteria make it rather difficult to proof, at the present scientific-technical state of the art, that a material is not a nanomaterial. In the following sections technical challenges resulting from current analytical capabilities will be outlined that arise from the quantitative criteria of the *EC NM Definition*. Several options how to overcome these challenges, by amending the definition, based on results from the NanoDefine project, will be discussed in the following sections.

2.1. External dimensions of individual particles

Concept of "external dimension"

According to the *EC NM Definition* a nanomaterial is defined by the external dimensions (size) of its constituent particles. If, for 50% or more of the particles in the number based size distribution, one or more external dimensions are in the range 1 nm - 100 nm the material is a nanomaterial. It was earlier noted in a JRC (26744)² report that the term "external dimension" is not clearly defined and that there are many cases in which it is unclear which "external dimension" should be measured, e.g. if the particles have irregular shapes which may be characterised by a large number of external dimensions (e.g. **Figure 3**).

This issue can be illustrated with a few simple examples. **Figure 2** shows some shapes that can be interpreted as two-dimensional projections of particles. External dimensions can be defined in a straightforward manner for simple shapes (circle: diameter, rectangle: side lengths, or more generally the "length/size" of the three principal, not necessarily Cartesian, axes). However, as soon as the shapes become more irregular it is not straightforward anymore which external dimensions should be measured for the purposes of the *EC NM Definition*. Various approaches are conceivable to define the external dimension(s) of particles with such shapes to be used for the purposes of the *EC NM Definition*.

This is illustrated by two simple quantities in **Figure 2**, which can be used as size descriptors: the minimum Feret diameter (2D) and the largest internal sphere (3D) (or the largest internal circle if projected in 2 dimensions). Both quantities are well-defined even for complex shapes. It is however obvious that a quantity such as the Feret minimum diameter does not always seem to describe the external dimension which the developers of the *EC NM Definition* had in mind. For a bent rod e.g. the Feret minimum diameter does not correspond to the diameter of the rod, but the largest internal circle does. Likewise, it can be asked whether for a shape with concave elements (e.g. the star shaped particle with long spikes in Figure 2) the largest inscribed sphere (3D) or circle (2D) would be the appropriate external dimension for the NM Definition. Other approaches to define the external dimensions may become necessary for more complex shapes and should be considered at a given time.

Feret _{min}	Largest internal sphere	Solidity
		1
		1
		1
	\frown	0.31
		0.43
		1
		1
		0.9
		0.53
Å		0.8
		0.95

Figure 2: Graphical interpretation of external dimension

Consequently, a single linear measurement (e.g., Feret (minimum) diameter, largest inscribed circle), though useful for many cases, may not always be adequate to describe all types of non-spherical parti-

cle, in particular those with an irregular shape, and to associate this measurement with the external dimension for the purposes of the *EC NM Definition*. Real-world particulate materials, such as the organic pigment in **Figure 3**, have often complex shapes but rarely concave shapes, based on fundamental energetic reasons. For each of the convex shapes in **Figure 2**, it is possible to fulfil the spirit of the *EC NM Definition* by either Feret minimum diameter or largest internal circle, but a criterion for selection between the two needs to be provided. There are various shape factors available that can provide additional descriptors to a particle ensemble of interest⁸ (see also ISO 9276-6:2008). Particle shape and morphology are three-dimensional properties, but the related parameters are often used also in characterising two-dimensional projections of particles because of the widespread use of image analysis methods.

Those shape descriptors can be measured using image analysis. Examples of such shape descriptors (here in two dimensions) are

circularity =
$$\sqrt{\frac{4\pi A}{P^2}}$$
 , convexity $C_x=\frac{P_c}{P}$ and solidity $~S=\frac{A}{A_c}$

where A is the projected two-dimensional area, P is the perimeter, Pc is the convex hull perimeter and Ac is the convex hull area. The convex hull of a polygon (i.e., the two-dimensional projection of a particle) can be thought of as the polygon surrounded by a rubber band. The convex hull perimeter is the length of that band and the hull area is the area spanned by it. The solidity of the shapes in **Figure 2** is indicated as additional information.

One could for example define the minimum external dimension of particles with a solidity larger than a certain threshold (e.g., 0.5) as the Feret minimum diameter. Alternatively, the largest inscribed circle could be used for particles regardless of their solidity. The decision of whether a specific particle has one (or more) external dimensions in the range 1 nm – 100 nm would then in practice imply to measure the external dimension defined as Feret minimum diameter or largest inscribed circle, depending on the particle shape or a solidity criterion. In critical cases, other descriptors may also be used. This way, the (minimum) external dimensions are clearly defined for any shape and this can be used for the purposes of the *EC NM Definition*.

Practical implementation - available techniques

According to NanoDefine Technical Report D3.1 "Techniques evaluation report", a very limited number of techniques are potentially capable of measuring distinct and, where necessary and required, the smallest external dimensions. Often it will be enough to analyse two-dimensional projections of the particles for their external dimensions, as in most instances deposited particles are in random orientation and therefore 2D images reflects all 3 principal spatial extensions, as it is usually done in electron microscopy. If from such measurements the material can be classified as nanomaterial, the third dimension does not need to be measured. However if the dimensions in the projected (x,y) plane are non-nano (> 100 nm) an analysis of the third dimension - perpendicular plane (z-direction) (e.g rods\fibres,

needles or discs\plateless particles), would be needed to clarify the status of the material as nano or non-nano. Such detailed considerations would probably be necessary for materials that have a D50 close to the threshold of 100 nm. However, for materials with a D50 reasonably far away from the cutoff it would be much more advantageous (and economic) to adopt a "screening" strategy. In such an approach one would start the analysis with readily available, less complex (non EM) methods which for many, non-critical materials could already be sufficient to decide with reasonable confidence whether the material is a nanomaterial. For example, if DLS is used at the screening stage and the DLS results indicate a D50 below 100 nm this would be enough to classify the material as NM. Likewise, other techniques can be used for classification under certain pre-requisites and following specific procedures. The conditions under which such techniques can be used and the procedures to be followed are part of the NanoDefine decision flow scheme (NanoDefine technical report D7.5 "NanoDefiner decision support flow scheme" and D7.12 "NanoDefiner final version").

Only in cases where such a first screening step is inconclusive there would be the need to use successively more advanced methods for particle size measurements.

Techniques capable of measuring external dimensions include

- Counting (microscopy) techniques: TEM, SEM, AFM
- Ensemble techniques (for special cases): SAXS, WAXD (XRD peak width) and BET

Ensemble techniques can be reasonably used to deduce external particle dimensions if the particles have uniform, well-defined shape (and size). This is rarely the case for real-life materials which exhibit both irregular particle shape and size polydispersity. Moreover, WAXD applies only to crystalline materials and provides the dimensions of crystallites. This corresponds to the particle size only if the particles consist of single crystallites. In addition, WAXD and SAXS are limited to small particles (< 100 nm for WAXD and less than about 200 nm for SAXS).

Microscopy techniques, being direct, are the most powerful tools which are available for measuring external dimensions. However, their determination can be hampered by several issues, mainly:

- Particles may degrade under the electron beam (for SEM and TEM)
- It is generally very difficult to find an optimised dispersion protocol which achieves a full separation of particles on the microscopy grid. For routine and fast identification of constituent particles in EM pictures software is needed which was not available until recently. Hence, this task is usually done by a human operator, which may result in subjectivity and potential bias of the results like for any other human operated techniques. Progress on this issue has been made in the NanoDefine project (NanoDefine Technical Report D5.1 "Automated image analysis software, incl. algorithms for agglomerate/aggregate deconvolution and size based classification") by developing new automated image analysis software capable to derive number based size distributions based on recorded EM images. A focus of the development was to provide different splitting methods to handle agglomerates and aggregates, robust handling of different noise levels and adaptability to nonstandard images

- For complex shapes, the concepts of external and minimum dimensions need to be clarified (see previous sub-section).
- During sample preparation artefacts may be formed that lead to erroneous results
- Statistically relevant number of particles need to be counted
- They are usually more time consuming and expensive than ensemble methods

For flat shapes there may be an issue when two-dimensional projections are analysed to determine their external dimensions, particularly when the lateral dimensions are quite large (> 100 nm). Their minimum external dimension is the thickness which is difficult to access in such images since isolated particles after dispersion often lay on their basal plane. Disregarding the thickness could result in false classification of such materials as non-nano. Platelets are well-known examples of such cases (**Figure 4**), but this may be also true for any other shape for which thickness is the only dimension which would classify the material as a nanomaterial according to *EC NM Definition*. As standard electron microscopy techniques provide a two-dimensional projection of three-dimensional particle, they cannot provide the information on the third dimension (thickness) of such particles. Only special techniques, such as tomography in TEM or AFM may be successful. However, such techniques are tedious, not widespread and prone to imaging issues. Instead, ensemble techniques as mentioned above and discussed further below are a more reliable approach to measure the third dimension of the particle.



Figure 3: An example of a real-world complex shape: Opaque Organic Pigment Yellow 83 (IRMM-386), a) SEM, b) TEM.

Plausibility checks, i.e., comparing with results from other methods can provide evidence whether the evaluation of EM images was affected by any of the above mentioned issues. Examples of such comparisons can be found in the NanoDefine Technical Reports D3.2 "Technique performance characteristics sheets" (D3.2, Table 3) and D3.5 "Evaluation report on the applicability ranges of the volume specific surface area (VSSA) method" (D3.5, table 4). On the example of the Kaolin in **Figure 4** the powder image obtained by EM shows flat structures and the observed external lateral dimensions have a median of 128 nm (EM). This value is not consistent with the average crystallite size of 43 nm or with the average platelet thickness of 48 nm of the particle obtained by XRD and BET respectively. This inaccuracy between obtained results lays in the differences of physical principles applied in the measurement method, which provides the value for different dimensions, plane and thickness, respectively. In contrast, the TEM image obtained after embedding and ultramicrotomy evidences structures that are more consistent with the XRD and BET approaches to smallest dimension – albeit the image evaluation and the assignment of thickness is ambiguous and subjective. Furthermore, the rather so-phisticated sample preparation is not widely available, so that overall TEM and SEM cannot be recommended for flat materials.





Figure 4: An example of real-world platelet shape: Kaolin (IRMM-385). a) powder deposited on a carbon TEM grid from a suspension imaged by SEM. b) powder embedded in epoxy resin, hardened, cross-section prepared by ultramicrotomy, imaged by TEM.

Conclusion

Measurement of external dimensions of small particles can be very difficult. This is due to conceptual and experimental obstacles. On one hand it is not clear what exactly should be considered as an external dimension, specifically in the case of particles with irregular shapes. On the other hand even with microscopy techniques, which are considered to be at the moment the best available analytical technics for shape and size of nano-objects characterisation, particles should be dispersed reasonably well. Regular and specific shapes (approximations of a sphere, cylinder, cube or ellipsoid) can be characterised well. For other cases, certain assumptions are necessary which in the worst case may lead to false classification of a material.

There are a lot of measurement techniques that rely on the particles mobility in some degree (PTA, DLS, AF4, AC, SMPS, USSP...). The corresponding equivalent diameters reflect an external dimension, which in general is not the same as the external dimension delivered by the Min Feret diameter which in turn is not the same as the external dimension delivered by the smallest inscribed circle. This difference extends to

techniques that measure the particle mass (sp-ICP-MS, Coulter-Counter, ARCHIMEDES-counter, aerosol centrifuges).

Recommendation

The clear definition of "external dimension" would remove the ambiguity of this term which may be then be addressed either directly in the text of the *EC NM Definition* or in specific guidance.

Recommendation for changes in EC NM Definition

The term "external dimension" similarly to other terms like e.g. "aggregates", could be further defined in the part 4 of the *EC NM Definition* or a specific Technical Annex to Recommendation could specify it.

Proposal for a definition of **external dimension**:

External dimension means:

- the size of the biggest circle that is entirely inscribed into the 2D projection of the particle. If the median size of the inscribed circle is outside the range of 1-100 nm, the third dimension needs to be considered by appropriate methods, or
- the minimum Feret diameter which is the distance between two parallel tangents on opposite sides of the image of a particle (if the solidity of a two-dimensional particle projection is larger than a certain value, e.g. 0.5)

Recommendation for guidance

Rather than in the *EC NM Definition*, the term "external dimension" could be defined in dedicated guidance in which additional information related to the analytical challenges should be also addressed. This should go along with guidance on how to integrate widely used techniques in the decision process.

Due to the discussed uncertainties related to dispersion, flat shapes and automatic image evaluation, a guidance could require that the resulting histogram of external dimensions and the median size are plausible compared to the results obtained from other techniques (such as SAXS, WAXD and BET) that are less affected by the challenges described in this chapter. A negative outcome of such a plausibility check can trigger further, in-depth analysis of the sample to resolve this issue. However, in many cases it can be recommended that a decision based on an average dimension (outcome from non-counting techniques such as those mentioned above) would be acceptable, as discussed below and shown Figures 8 and 9.

For some specific products such a plausibility check is already recommended in the assessment of nanomaterials. For example EFSA in "Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain⁹ recommends that: "The size parameter should always be measured by at least two independent methods (one being electron microscopy) as the results obtained from different measurement techniques may differ because of the physical principles applied in the measurement method".

2.2. Number based particle size distribution

The *EC NM Definition* uses a threshold related to the number based size distribution of particles. Counting techniques such as EM analyse a sample particle by particle and provide a number based size distribution as direct output. Non-counting techniques such as DLS or SAXS do not provide a number based size distribution as direct output but rather yield size distributions based on scattered light intensity, mass, volume or another physical quantity. These quantities need to be converted into a number based distribution if they are to be used to characterise materials according to the *EC NM Definition*. As such methods are widespread and commonly used, it is worthwhile to give clear recommendations under which conditions they can be used to identify a material as nanomaterial.

While analysing nanomaterials three main challenges, which are discussed in the following section of this document and which in reality will often occur simultaneously could be identified:

- Quantity or metrics conversion for non-counting methods (e.g. quasi-spherical particles with narrow size distribution, but holds actually for all types of particles)
- Polydispersity (Quasi-spherical particles with broad size distribution, holds also for irregular particles with broad size distribution)
- Impact of shape (Non-spherical and irregular particles)

2.2.1. Type of quantity conversion

As mentioned already in the JRC report EUR 26567 EN¹, apart from a few integral methods, techniques for particle size determination yield size distributions which are weighted by some geometric or physical type of quantity (TOQ).

Some immediately deliver a number weighted size distribution (in particular imaging, counting techniques like PTA & nCC, spICP-MS, additionally SMPS). Others are weighted by volume, mass, surface or physical quantities like turbidity (\rightarrow extinction cross section) or X-ray extinction (= volume) or light scattering (\rightarrow scattering cross section). The correlation between these physical quantities and size is usually non-power-law and non-monotonic; for ensemble methods intrinsic TOQ is not obvious and depends on the instruments set-up (e.g. detector geometry in LD).

As explained in detail in NanoDefine Technical Reports D3.4 "Mathematical conversion algorithms for noncounting methods" and D3.6 "Report of the potentials of the transformation of non-counting methods size distributions into number weighted size distributions of the constituent particles based on instrument manufacturers algorithms", non-number weighted size distributions can be converted with appropriate models for the size dependency of physical TOQ (e.g. Mie theory for light scattering), yet this conversion amplifies the measurement uncertainties at the fine edge of a distribution function . This is particularly critical since for the fine edge of a distribution function one often observes a non-uniform sensitivity towards particles (e. g. x^6 -dependency of scattered light in the nanorange), which means that the minimum particle size cannot be exactly determined – even within the intrinsic TOQ (e.g. Q_{int}). The example in Figure 5 illustrates how minute measurement uncertainties in an intensity weighted size distribution (q_{int}) are enlarged after conversion into number weighted size distributions (q_0). In addition, the conversion is even more complex for non-spherical particles or particle aggregates.



Figure 5: Real, theoretical and converted distributions in different metrics, for alumina spheres that are in reality (blue line) monomodal in their q_0 size distribution, but are measured as q_{int} distribution by DLS. The theoretically expected (green line) and the actually measured q_{int} (yellow line) are only minimally different by noise of almost negligible intensity. However, that minor noise in the measured q_{int} is considerably magnified by the conversion and introduces artifacts of non-existing small particles (red line). For detailed discussion see NanoDefine Technical Report D3.4 "Mathematical conversion algorithms for non-counting methods", from which this figure is reproduced.

Conclusion

The type of quantity conversion generally introduces errors and amplifies measurement uncertainties dramatically, if they are not evaluated before in the original TOQ.

Recommendation for guidance

- Guidance is required, which methods (constituted by a measurement technique + quality criterion + conversion algorithm) are admissible.
- For a classification as nanomaterial based on TOQ conversion, guidance should require proof that the classification is not due to the amplification of measurement uncertainty.
- Guidance should specify that to demonstrate that a material is a nanomaterial, conversion into particle number based size distribution is not required if the intensity or mass or volume or surface-weighed median is already smaller than 100 nm, because in that case the number median is certainly smaller 100 nm.
- Preference should be given to use counting methods whenever possible

2.2.2. Polydispersity and upper size limit

Real-life materials are often polydisperse, and this leads to one of the major analytical challenges for the implementation of the *EC NM Definition*. In order to decide whether a material is a nanomaterial or not, one needs to be able to measure all particles, including those which are bigger than 100 nm. In many cases, the size ratio between nanoparticles (<100 nm) and bigger ones can exceed several orders of magnitude.

At present, only a few techniques can measure 1 nm particles (TEM, AFM, AUC-RI, SAXS, BET).¹⁰ On the other hand, a very limited number of techniques (optical microscopy, SEM and laser diffraction) can accede to the millimetre domain, while missing the lower end of the nanometre range. Moreover, laser diffraction provides number-based size distributions only after mathematical conversion. It is therefore technically impossible in practice to measure all particles in a very polydisperse material with the same technique.

Merging results from different techniques is sometimes thought of as a possible way out. However, usually this is not done for isolated (non-aggregated and non-agglomerated) spherical particles due to fundamental problems related to statistics, to the different measurement principles behind the techniques and to the difficulty in validating the merged results. These challenges increase tremendously in complexity for materials made of irregularly shaped and aggregated particles with a size range spanning several orders of magnitude.

These challenges can be illustrated be the following example of a borderline case with a broad size distribution (see Figure 6). The relative standard deviation of diameter in TEM analysis will be used in here and in the remainder of this document to quantify polydispersity. The size distribution is log-normal with a count median of 100 nm and a relative distribution width of σ =1 (red line in following Figure). It is represented by a number based maximum size x99.9%,0 of 2.0 µm (i.e., 0.1 % of the particles are larger than 2.0 µm) and the volume based value of x99.9%,3 is 40 µm. In addition, the fraction larger than 1 µm is 1.07 % by number and 75.7% by volume. The volume median size is 2 µm and the volume specific surface area Sv is 4.93 m²/cm³. A volume-based method should detect the 0.135 v% fraction smaller than 100 nm (representing 50% of the particle number).



Figure 6: Number (Q0) and volume (Q3) weighted sum functions for log-normal size distributions of different polydispersity: "narrow" ($\sigma_{ln} = 0.5$) and "broad" ($\sigma_{ln} = 1.0$) but same number weighted median (particle size in nm).

This means that in theory for polydisperse materials the requirements of the *EC NM Definition*, i.e., determination whether the relative number of particles with a size between 1 nm and 100 nm is above or below 50% can be easily beyond the capability of existing methods.

In practice however, there are ways out of such metrological problems that are associated with polydispersity and which in theory hamper the implementation of the *EC NM Definition*. Real world particular materials which need to be tested whether they are nanomaterials according to the *EC NM Definition* are usually made for a specific purpose and need to fulfil specific performance criteria. Such materials are manufactured under quite controlled conditions, which lead to a relatively small polydispersity, i.e., a narrow size distribution. As a consequence their size distribution often falls within the measurement range of a single method. For instance this is true for most of the pigments and fillers selected for the NanoDefine project (polydispersity around 0.7).

If the size distribution of a material is within the nanometre range, or better within the measuring range of one method like SMPS or TEM, it can be analysed with a single method to determine whether it is a nanomaterial. However, it has to be noted that even for TEM, the accessible size range for a given image magnification is 1:40 according to the NanoDefine experts.

In practice the combination of large agglomerates or aggregates together with some very small nanoparticles, attached to the surface of the large particles, is often encountered. This also leads to a broad size distribution. If the surface of micrometre particles is investigated by TEM, it is necessary to record thousands of images, which may be automatically done overnight. If the width of a TEM image for measuring 2 nm (and detecting about 1 nm) particles is around 180 nm a line of 100 images side by side is needed to cover a length of 18 μ m. To put the challenge into perspective, we note that the pigments and fillers in used in NanoDefine – representative for particulate materials whose performance is directly linked to well-defined

sizes – were found to have a polydispersity of around 0.7 (relative standard deviation of diameter in TEM analysis), corresponding roughly to the example above with $\sigma = 0.5$. The other example with $\sigma = 1$ is not unusual for the many particulate forms of chemical intermediates. Such materials often obtain their desired properties after melting or dissolution, and are thus not optimized for well-defined sizes. The latter are often part of the industry portfolios of "particulate" that need to be screened with respect to the *EC NM Definition*.

Another consequence of a count median of 100 nm without specifying an upper size limit for counting is the following. If there is at least one particle smaller than 100 nm attached to a large micrometre particle, this material would be classified as nanomaterial. Such a classification would however depend on whether such small, attached particles could be found or not. In practice there are additional issues like sample drift, image focus and contrast. The analytical challenge is thus intimately related to a missing guidance on upper size limits up to which particles should be considered either for determining the number based threshold (median) or for being regarded as part of the nanomaterial. Furthermore an equivocal guidance on the consideration of traces of nanoparticles in liquids and powders which would illustrate the conceptual indifference between very polydisperse substances and mixtures of differently sized forms of the same substance is still needed.

Hence the goal to bring number of particles that needs to be measured within the measurement range of a single method can also be achieved by limiting the maximum particle size that needs to be measured in a sample. Such an upper size limit could be linked to the basic definition of "particle" as "minute piece of matter with defined physical boundaries" (ISO 26824:2013, 1.1). In this context "minute" could be specified as not being visible anymore for the naked eye, i.e. smaller than around 100 micrometres. The upper size limit could therefore be chosen to be 100 micrometres on the ground that a larger piece of matter would not be minute and hence should not be called "particle" anymore.

Conclusion

Polydispersity is a challenge for the measurement of particle size distribution, particularly for the purposes of the *EC NM Definition*, but it can be handled if the polydispersity index is not too large, e. g. around or below 0.7 as for the pigments and fillers in the NanoDefine project. For materials with a broad size distribution (up to the micrometre range) and a large polydispersity index the challenges are such that the *EC NM Definition* cannot be implemented without further guidance. If the number of particles below 100 nm is high, but their mass or volume fraction is negligible, then the analytical challenges become big.

Recommendation for guidance

- For implementation of the *EC NM Definition*, guidance is needed that allows applying an upper size limit in measurements and particle statistics. However it has to be noted that applying any kind of upper size in measurements that are used to classify a material as nano or non-nano would result in an increase of the number of materials classified as nanomaterials.
- Introduce an upper size to indicate
 - the maximum size of particles in a nanomaterial; in this case the particles with a size

up to the upper size limit would constitute the nanomaterial (depending on the result of the analysis) whereas the entire material would "contain" but not "consist of" nanoparticles

- o 100 micrometres appear as acceptable upper size limit
- An analysis whether a material is a nanomaterial according to the *EC NM Definition* should always be accompanied with information on the upper and lower size limits applied for measuring the size/size distribution of particles (including justification).

2.3. Aggregates and agglomerates

The *EC NM Definition* refers to particles in an unbound state but also as constituents of aggregates or agglomerates. This is due to the concern that under certain conditions these larger entities might be disintegrated into their constituents, which may behave differently compared to aggregates and agglomerates. It is therefore necessary to identify the constituent particles and measure their size even when they are part of aggregates or agglomerates. If the latter is not possible dispersion is required to de-agglomerate agglomerates and to measure their constituent particles. Suspended, unbound nanoparticles often tend to agglomerate; therefore stabilisation during the measurement is required.

The responsible ISO Technical committees have provided specific definitions for the terms "constituent particle" (actual visible part of an aggregate) and "primary particle" (building part of an aggregate in a former state, which can change e.g. by coalescence before aggregate formation).

The general term "particle" refers to an entity, which is clearly separated from its environment (i.e. interface with the fluid continuum) by physical boundaries before aggregation. After aggregation, the primary particles do not remain necessarily separated by physical boundaries: sintering at high temperatures, chemical reaction between adjacent surfaces (e.g. siloxane bonds) and recrystallization may result in fused particle structures where the physical boundary is lost (see Figure 7).



Figure 7: *HRTEM images of silica. Source: Technical Workshop on 'Aspects to consider in the technical and scientific review of the EC Recommendation on the Definition of Nanomaterial'. Brussels, 19 March 2014. Presentation by R. Weinand, Evonik Industries AG.*

In fact the *EC NM Definition* defines aggregate as "*a particle comprising of strongly bound or fused particles*" which generates additional challenges in the very identification of the minute piece of matter for regulatory pourposes.

Recommendation for guidance

- The term "particle" should be more precisely defined for use with the EC NM Definition.
- If the particle size measurement of a sample after dispersion to individual aggregates results in a number median smaller than 100 nm, then the constituents must be smaller and no further investigation for the nanomaterial classification is necessary.
- In all other cases, one should check if the particles larger than 100 nm are aggregates which include constituent particles smaller than 100 nm. In practical terms, this check compares the measured size to the smallest size as observed by techniques that are independent of any physical separation of constituent particles, and is thus the same plausibility check as recommended for TEM above. As for TEM, a negative outcome of such a plausibility check can trigger further, in-depth analysis of the sample to resolve this issue. However, in many cases it can be recommended that a decision based on an average dimension (outcome from non-counting techniques such as those mentioned above) would be acceptable.

2.3.1. Review of the possibility of physical separation of constituent particles

The *EC NM Definition* requires identifying the constituent particles of a material and measuring their size even when they are part of aggregates or agglomerates (see previous sub-section). However, only few methods are able to probe the internal structure of agglomerates and aggregates and to deliver the required information about constituent particles and their size (e.g. HRTEM). Therefore the question arises whether it is generally possible to prepare samples such that the constituent particles are dispersed and accessible to be measured with other, less laborious and costly techniques.

It is clear that such preparation procedures should only detach the constituent particles from each other but must not change their size and the size distribution in the material. Whether this is possible depends on the specific material, as indicated by results obtained for materials in the NanoDefine project. Representative results from the NanoDefine project are summarized in Table 1.

For a material that is relatively easy to disperse (BaSO₄), the numerical values for $x_{50,0}$ from the suspension-based techniques agree well with the dispersion-independent results from SEM, and the two forms of BaSO₄ are consistently classified as nano-form and non-nano-form by all methods. However, for a material that is not easy to disperse (organic pigment, see also **Figure 3**), the internal validity checks fail for several methods, and the $x_{50,0}$ results from the remaining methods are not consistent. It should be noted that a dedicated work package in NanoDefine had previously optimized dispersion protocols for each of these materials.

		measured on suspended material after dispersion SOP							
NanoDefine Materials (selection)	TEM Median of min. Feret Diameter	SEM Median of min. Feret Diameter	VSSA (BET) Smallest dimension	Median by PTA method	Median by AC disc method	Median by AUC method	Median by AC cuv method	Median by DLS method	Median by SLS method
	nm	nm	nm	nm	nm	nm	nm	nm	nm
IRMM-380 – organic pigment (transparent)	39	40	40		47		15	68	
IRMM-386 – organic pigment (opaque)	221	157	153	114	197		80	296	158
IRMM-387 – BaSO4 (ultrafine grade)		34	41		62	24	48	76	
IRMM-381 – BaSO4 (fine grade)		214	360	283	243	207	254	285	635
IRMM-388 – coated TiO2	181	185	102	254		204	65	288	155

Table 1: Results from the NanoDefine project for a selection of the industrial real-world test materials. All values are $x_{50,0}$ except for the value from VSSA which is an ensemble average. TEM used predispersion, then transfer to TEM grid, into vacuum and histogram analysis. SEM and VSSA analyze the powder in vacuum. VSSA was evaluated with shape-specific cut-offs². All suspension-based methods used identical sample preparation, but the SOPs for that preparation were previously optimized for each material and are not the same.

For some materials (e.g. precipitated silica, pyrogenic titania) it appears to be rather difficult to disperse them "down" to the constituent particles, because the required energy is so high that once the aggregates break up it is not clear anymore whether the resulting particles can be associated with the original constituents. In other words, excessive dispersion can cause comminution (fracture & erosion) of constituent particles, and excessive ultrasonic dispersion can introduce large amounts of polydisperse contaminant particles to the sample.

One could argue that dispersing at a defined/well-controlled level of dispersion stress and specific en-

ergy input may be adopted as best practice. However, warranting comparability across different laboratories regarding specific energy input is difficult. This specifically holds true for high stress intensities of ultrasonication or high energy input (> 1MJ/m³), which would be necessary to obtain an acceptable state of dispersion. It would also go along with a temperature rise of the sample possibly leading to thermal particle degradation – particles formed in such processes would be new particles previously not present in the material.

Furthermore the issue of size determination of soluble and/or reactive materials should be carefully considered as according to the current definition such materials need to be classified although they cannot be dispersed.

Conclusion

Many wide-spread techniques for the measurement of particle size cannot distinguish between individual particles and agglomerates or aggregates and cannot determine constituent particles in agglomerates and aggregates. They are therefore not well suitable to show that a material is not a nanomaterial, because this would require dispersion of possible agglomerates and aggregates into their constituent particles. The latter may be relatively easy for some materials, for other materials it can be very difficult to achieve, as outlined above (or to validate). In doing so, potential artefacts from the dispersion energy input need to be considered.

Recommendation for Guidance

- Mobility-based techniques can be used for identification as nanomaterial, i.e., if the D50 of a material obtained with a mobility-based method is below 100 nm, this material is a nanomaterial.
- Mobility-based techniques cannot be used to demonstrate that a material is not a nanomaterial, because they cannot distinguish between individual particles and agglomerates/aggregates.
- Mobility-based techniques can be used for screening up to a D50 of roughly a factor 2 or 3 above the cut-off of 100 nm. If the result for the D50 obtained with a suspension-based method is between 100 nm and 200 nm and if mobility-independent methodology (SAXS, XRD, BET) indicates that particles with diameters smaller than the mobility-D50 are present in the material, then testing by SEM/TEM is required.
- Mobility-based techniques (if applicable) can be used as the first step towards a decision whether a material is a nanomaterial according to the definition by the EC. In such a case, the following decision scheme, which involves screening followed by conditional verification by EM, is recommended:



Figure 8: Decision scheme for dispersions starting with screening using mobility-based techniques

2.3.2. Determination of the VSSA

At present it is possible to measure a specific surface area by mass for dry solid materials or powders with the gas adsorption method ("BET-method"). If the particle density is also known, then the 'volume-specific surface area' can be calculated and may be used as a proxy to identify a potential nanomaterial. However due to sensitivity of specific surface area to the measurement method used and due to the uncertainties of the relation between the VSSA and the number size distribution, it is specified in *the EC NM Definition* that results for number size distribution should prevail and it should not be possible to use the specific surface area to demonstrate that a material is not a nanomaterial.

Volume-Specific Surface Area (VSSA, units of m²/cm³) is an ensemble property of powders. VSSA is inversely proportional to the size of the powder particles. It is obtained by multiplication of mass-specific surface area (measured by the BET technique) with the skeletal density (ASTM D3766). An ensemble of monodisperse spheres with 100 nm diameter has a VSSA of 60 m²/cm³. The EC recommendation for a regulatory definition of nanomaterials⁴, states that where technically feasible and requested in specific legislation the VSSA can be used to determine compliance with the definition of nanomaterial.

The VSSA approach has the important advantage over classifying, imaging and counting techniques that it does not involve dispersion protocols. Further, the BET technique as the basis for VSSA determination is ISO-standardized and in widespread use,¹¹ generates low costs and values are already specified for many commercial materials. Accordingly, VSSA was proposed for identification of both nano

and non-nano materials, especially for particulate materials that only have a size fraction in the nanoscale, or that contain primary nanostructures in highly agglomerated or aggregated forms.¹² The VSSA concept was refined by shape-specific cut-offs in the 2nd JRC report². In general, the values of the median diameter in number metrics and the average smallest dimension extracted from an ensemble surface measurement are not identical. Hence the VSSA cannot replace a determination of the median in number metrics, but NanoDefine explored the uncertainty margins of VSSA vs. EM on 26 real-world materials, a selection of 23 materials(shown in Table 2) which are correctly classified by VSSA (from BET), 3 are false positives, and 0 false negative. This result was not compromised by the various compositions, strong agglomeration and sizes from 10 nm to 4 µm with typically 50% polydispersity. The VSSA method mitigates the challenges of EM to assess the thickness of platelets, but worked as well on fibres and particles of irregular shapes. VSSA measures also soluble reactive or non-dispersible materials, such as the many chemical intermediates that are shipped and handled as particulates, but then further transformed after melting or dissolution. According to NanoDefine Technical Report D3.1 "Techniques Evaluation Report", VSSA (by BET) is the only technique apart from SEM and TEM to cover the entire size range from 1 nm to 10 µm diameter, with limitations of SEM and TEM to reach the lower and upper limits, respectively.

VSSA has several limitations:

1) Microporosity from coatings or internally nanostructured materials leads to an underestimation of external dimension, such as for the coated TiO_2 in **Table 2** and eventually to false positives. The t-plot evaluation of isotherms¹³ further developed by NANoREG, requires isotherm data over an extended pressure range, but was found to resolve false positives, incl. the case of the coated TiO_2 . But the t-plot evaluation led to one false negative, specifically the transparent organic pigment in **Table 2**, which did not occur with standard BET evaluation.

2) Multimodal substances and mixtures were not tested but are anticipated as not applicable to VSSA.
3) For VSSA values above 10 m²/cm³, the shape of the constituent particles from a simple SEM scan must be known for a correct classification. We find no added value from such a scan for lower VSSA.

4) Aggregates with near-complete sintering represent a "risk" of having false negatives by VSSA screening. This risk can be encountered by a low VSSA screening cut-off, e.g. a factor ten from the 100nm-equivalent cut-off. A tenfold reduction of surface marks a loss of particulate nature inside a bulk solid with internal pores, and would also solve the inconsistent treatment of fused aggregates and nanoporous materials in the current recommendation.

5) Materials with excessive polydispersity (related to mixtures) represent another "risk" of having false negatives by VSSA screening. However, for real-world materials that had around 50% polydispersity, this was only a minor concern (**Table 2**).

Taking all the above into account, NanoDefine and NANoREG deliverables provide a screening strategy and propose a VSSA screening cut-off at 6 m²/cm³, with shape-specific evaluation supported by SEM above that value. Imaging or validated counting techniques or evidence from materials with a logical size relationship are indispensable within an uncertainty factor of two around the shape-corrected

VSSA cut-off. The t-plot method is recommended only to resolve classification that may be false positives for compositions that can be anticipated to induce microporosity of nm or sub-nm dimension.

Material	D	VSSA (BET)	VSSA (BET)	D50 (EM)	VSSA OK?	Comments
		m²/cm³	% of cut- off	% of cut- off		
IRMM-380 – org. P. (tr)	2	100	40	40	ОК	
IRMM-386 – org. P. (op)	2	26	153	157	ок	acc. to D1.3 "particle", but elongated in EM. Evaluated with D=2.
IRMM-381 - BaSO4 (f)	2	11	360	214	ок	acc. to D1.3 "rhombo- hedral", but elongated in EM. Evaluated with D=2
IRMM-387 – BaSO4 (uf)	3	148	41	35	ОК	
IRMM-382 - MWCNT	2	455	9	10	ОК	
IRMM-383 – Nano Steel	1	75	27		ОК	platelets: EM false neg- ative / not measurable
IRMM-384 – CaCO3 (f)	2	15	258	158	ОК	
IRMM-385 – Kaolin	1	42	48	128	ОК	platelets: SEM meas- ured lateral dimension
IRMM-388 – coatd TiO2	3	59	102	213	false positive	surface coating porosity
BAM11 – Zeolite powder	3	767	8	133	false positive	internal pores
IRMM-389 – basic methac- rylate copolymer	3	1	4 084	2 000	ок	
Fumed SiO2	3	459	13	12	ОК	
FeOOH P. Yellow 42	2	326	12	20	ОК	
TiO2 Rutile	3	61	98	210	false positive	surface coating porosity
Cu/Zn P. metal 2	1	36	56		ОК	platelets: EM false neg- ative / not measurable
Fe2O3 P. Red 101	3	44	136	249	ок	complex shape: TEM hard to assign small'st dimens.
CoAl2O4 Al-Co-Blue	3	33	182	527	ОК	not dispersable: TEM cannot assign particles
TiO2 Anatase	3	35	171	130	ОК	
Azo P. Yellow 83 tr	3	86	70	47	ОК	
Pigment Yellow 42	2	324	12	10	ОК	
Pigment Red 101	2	419	10	9	ОК	
Pigment Yellow 139	3	43	141	150	ОК	
Pigment Red 254 (op)	3	24	245	233	ОК	
Pigment Red 254 (tr)	3	153	39	36	ОК	
Pigment Blue 15:4	2	103	39	30	ОК	
Pigment Orange 73	1	30	67		ОК	platelets: EM false neg- ative / not measurable

Table 2: Quantitative relation between VSSA (by BET) and D50 in number metrics by EM. D is the number of small dimensions (D=1 for platelets, D=2 for rods/fibers, D=3 for all other shapes). The % of cut-off are calculated according to Ref. **Error! Bookmark not defined.** or both VSSA and EM D50 to he smallest dimension being 100 nm diameter. For details see NanoDefine Technical Report D3.5 "Evaluation report on the applicability ranges of the volume specific surface area (VSSA) method and the quantitative relation to number based particle size distribution for real-world samples".

Conclusion:

In 2011 European Commission along with the *EC NM Definition* has published a series of Q&A which aimed to provide additional clarification and technical details related to the Recommendation. In this document EC explains that VSSA measurements are highly sensitive to the techniques used and are very material dependent thus the specific surface area could have not been considered to be used to demonstrate that a material is not a nanomaterial. However it also says that scientific knowledge may expand the possibility to use it the future. Considering the recently obtained results in VSSA development by two leading FP7 projects NanoDefine and NanoReg such possibility seems to arise giving an opportunity for more rapid and easier implementation of the *EC NM Definition*.

Recommendation:

VSSA should be recommended in the revised recommendation and/or guidance for use as a screening tool for monoconstituent substances to identify both nano and non-nano materials. NanoDefine and NANoREG deliverables provide a screening strategy for technical guidance documents. VSSA is not validated for multimodal distributions or mixtures, and is not applicable to suspensions, formulations, articles, consumer products.

The following decision scheme, which involves initial screening by VSSA followed by conditional verification by EM, is recommended:





Recommendation for guidance

Use of VSSA

A material with the VSSA of less than 6 m²/cm³ and with a monomodal particle size distribution (PSD) should not be considered a nanomaterial.

Comment: showing monomodality of PSD does not require measurements in number but it can be in any metrics (mass or volume), provided that modes at low sizes are captured.

Screening strategy

With the existing data from NanoDefine as training set, the following screening strategy is suggested. If applied to further data from real-world materials as validation set, this screening does achieve a correct classification, leaving only borderline materials assessment by sophisticated methods (NanoDefine methods tier 2):

- 1. Measure skeletal density and BET (outgassing conditions within thermal stability range)
- 2. Check monomodal condition
- 3. If VSSA is more than a factor x10 below cutoff (VSSA < 6 m²/cm³), classify as non-nano.
 - Reason: uncertainty factor of x3 arises from the possible reduction of the shape-adjusted VSSA cutoff. Another uncertainty factor x2 from the experimentally determined range of VSSA mismatch against EM D50 (Figure 10). Combined uncertainty is x6, hence, x10 is considered as conservative, but subject to discussion of the exact screening cutoff value.
- 4. If VSSA > 6 m²/cm³, identify the shape from a simple SEM image. Re-evaluate with pragmatic aspect ratio criteria as proposed here to select the appropriate shape-specific cutoff as proposed by JRC report²:
 - Sphere (aspect ratio <3:1) D=3 → nano, if VSSA > 60 m²/cm³
 Note: Here the aspect ratio can be taken from 2-D projection. Flat shapes are recognizable in SEM and TEM by the flat grey scales.
 - Rod (aspect ratio >3:1:1) D=2 → nano, if VSSA > 40 m²/cm³
 - Platelet (aspect ratio >3:3:1) D=1 → nano, if VSSA > 20 m²/cm³
- 5. Quit VSSA screening and escalate to Tier 2 methods (EM or other)
 - If the simple SEM image shows multimodality (except if all modes are > 100 nm)
 - If the VSSA value is within an uncertainty range x2 around the shape-corrected cut-off (Figure 9)
- 6. Options to remove false positives from inner or coating porosity
 - Acquire and evaluate adsorption isotherms by NANoREG method (requiring long evacuation, dense data points at low pressures). Re-evaluate with outer VSSA.
 - NanoDefine Tier 2 methods.
- 7. Options to reduce the x2 uncertainty range around the cut-off:
 - "baseline" EM evaluation of closely related materials with a logical relationship of processing

conditions and size distribution ¹⁴

It should be noted that the need to know the shape for step 3 will typically require a simple SEM scan, but it does not require dispersion of the material, and it does not require statistical evaluation of EM images. These are the two most time-consuming steps and sources of EM uncertainty, as agreed by both JRC report #1, NanoDefine Technical Report D3.1 "Materials Evaluation Report" and by industry. Considering additionally that BET is already known and publicly available for many materials, the above screening is a tremendous reduction of the technical hurdles to implement the EC nanomaterial defini-



tion.

Figure 10: Applicability of VSSA as screening tool.

2.4. Colloids

Colloids are particular materials, which from the regulatory point of view may be considered as both mixtures and substances on their own, depending on the technique of preparation and the applicable legislative act.

Colloids are marketed in suspension and many cannot be dried without changing their structure. To highlight the sample preparation issue, consider the inverse analogy to soluble or reactive solids that cannot be dispersed without changing their structure. Specific examples of colloids include wax emulsions or adhesive polymer dispersions. Microscopic techniques are hence in general not applicable because their results might always arise from preparation artefacts. Colloidal materials are well suited for suspension analysis methods. However, the frequent use of organic molecular additives, such as acrylic acids or surfactants, increases the hydrodynamic dimension beyond the solid particle boundary. The latter is currently often interpreted as being relevant for the external dimension according to the *EC NM Definition*. The actual extension of such a stabilizing "corona" is below 10 nm thickness, so that standard suspension techniques are well applicable. Only for borderline materials in an uncertainty range up to 120 nm median diameter, combinations of techniques with complementary measurement principles are required to determine corona thickness and solid particle diameter, e.g. by combining sedimentation and size exclusion.

A pertinent issue for colloids is the need to know if particles are "solid" because according to the view expressed in the Commission Staff working document of 2012 the *EC NM Definition* applies only to solid particles. Specification should be made whether this refers to the glass temperature or to the melt temperature. Polymers are considered as not solid above glass temperature. However, a cut-off on melt temperature has the advantage that it applies to all chemical compositions. For practical purposes a cut-off at room temperature (25°C) is recommended. This approach is consistent with recent drafts from US-EPA.¹⁵

Recommendation

- It should be clarified that the general scope of the EC NM Definition is not (and should not be) limited to solid materials, since it defines a particle as "minute piece of matter with defined physical boundaries". Only later, this was interpreted as "solid" in the COM Staff Working Document. However, also soft nanomaterials may be of concern in specific regulatory contexts.
- Determining the size distribution poses much fewer analytical difficulties if the particles are solid rather than consisting of soft or liquid matter. Hence the definition should refer only to solid particles.
- It should be clarified in the *EC NM Definition* or in specific guidance that the *EC NM Definition* refers to a material at a temperature of 298 K and a pressure of 101 kPa.

2.5. Proving that a material is not a nanomaterial

The *EC NM Definition* describes criteria for classification of a material as nanomaterial. For many stakeholders and purposes it is also important to reliably know when a material is not a nanomaterial. The latter fact is very difficult to prove because most of the available measurement methods do not detect particles at the lower size range of the *EC NM Definition* and will therefore not account for such particles for the number median of a sample. Moreover, the presence of larger particles will also be problematic for those methods that detect particles in the lower nanometre size range. The necessary dynamics of the measured size range which needs to cover 4 or more decades and the fact that no method can cover this range in one measurement with a reasonable statistics make a reliable proof that a material is not a nanomaterial extremely difficult. It would be therefore very helpful to (i) have a screening strategy that allows avoiding very sophisticated and time consuming methods under certain circumstances and (ii) to have the possibility to classify, with acceptable effort and certainty, that a material is not a nanomaterial.

Recommendation for guidance

Screening measurements and, for borderline cases, confirmatory measurements can be performed by a range of methods. It is the responsibility of the analyst to choose appropriate methodology. Methodical guidance, Nano- and Non-nano- Reference Materials are emerging from the NanoDefine project.

Screening can be performed by methods (such as FFF, CLS) that are well-established to deliver mass or volume metrics of the particles dispersed to a liquid suspension, under the following conditions:

- For a classification as non-nanomaterial, proof is required that the particles larger than 100 nm do not consist of constituent particles smaller than 100 nm. In practical terms, this proof compares the size measured in suspension to the size measured by techniques that are independent of any physical separation of constituent particles, e.g., EM.
- For a classification as nanomaterial based on conversion towards number metrics, proof is required that the classification is not due to the amplification of measurement uncertainty.

Screening can alternatively be performed by VSSA with shape-specific cut-offs:

- For a classification as non-nanomaterial, JRC report (EU27240 EN)³ considers a VSSA cut-off at 5m²/cm³, which is in good agreement with NanoDefine results.
- NanoDefine results further suggest that VSSA with (qualitative) SEM support is applicable for screening purposes in the range above 6m²/cm³, but not below 200 nm minimum external dimension (NanoDefine Technical report D3.5)
- For a classification as nanomaterial, the potential contribution of microporosity should be considered, e.g. by the t-plot evaluation (NANoREG)

Borderline cases remain after either screening approach. NanoDefine results (**Table 1**) suggest that minimum external dimensions from 50 nm to 200 nm as measured by screening methods are borderline cases and require confirmatory measurements, often by electron microscopy (e.g. SEM, TEM).

3. Additional analytical challenges: Mixtures

The *EC NM Definition's* scope covers nanomaterials when they are substances or in mixture. However, as clarified by EC¹⁶ the inclusion of a nanomaterial as one of ingredients of a formulation does not turn the final product into a nanomaterial. Consequently, for the legal purposes only the ingredient present in the nano-form has to be characterised, unless specific legal act states otherwise. This creates a great challenge in the analytical characterisation of mixtures specifically if they have to be analysed by an importer and downstream user, whose obligation is to correctly register and label nano-ingredient is such formulation.

Currently there is no limitation in the quantities of material which has to be classified as being nano or non-nano material, which forces analytical laboratories to detect and quantify even vanishingly low traces of nanomaterial in a mixture, limited only by the limits of detectability. The lower the total content of particles (in a "pure" liquid, or in a suspension or formulation), the bigger is the challenge as the limits of the detection of chemical entity advance over time from currently ppm level for many methods, and most probably ppt level in the coming decades, making a material's classification even more uncertain as the nanomaterial characterisation methods are not yet on so advanced stage.

Recommendation:

Currently there is an uncertainty how non-powder forms (e.g. suspensions) with potential contents of nanomaterials should be measured. One way out would be to determine the particle size distribution if the material has an EC or a CAS number, and the outcome of such an analysis should be applied to that material. For example, colloidal silica has a CAS number, hence it may or may not be a nanomaterial, depending on the results of the particle size analysis. On the other hand, a formulation does not have such a number and hence it cannot be a nanomaterial in its own right. This is in line with the current view of the EC that the inclusion of a nanomaterial as ingredient into a mixture is not turning whole mixture into a nanomaterial.

Mixed powders

As for the regulatory purposes each ingredient of the mixture has to be registered (according to the tonnage), classified and labelled separately one of the most significant challenges in the analysis of mixtures of different powders, may actually be the physical separation of the constituent chemical entities for further analysis. Currently there is no method capable to separate physically particles of different chemicals to analyse them one by one although there has been some progress made in the last years in the development of fractioning techniques.

On the other hand for the analysis of the mixed powders one could question the need of the physical separation of the chemical entities as some of the available methods may offer possibilities to identify single particles by chemical composition directly in the mixture as e.g SEM coupled with EDX,^{17,18,19} High resolution Z-contrast Scanning Transmission Electron Microscopy^{20,21,22} or TEM coupled with EDX or EELS^{,23,24,25,26} when a contrast can be used to map different chemical species, as well as spICP-MS. However these techniques are still in the development stage and may not be suitable for all types of materials, moreover their application comes across additional encounters. One specific challenge is to find inert dispersing liquids for all ingredients. If that succeeds, the next challenge lies in the analytical methods in case that it is a mixture of different chemical entities or mixed substances with very large size range for which separate specific analytical methods have to be applied. Such circumstances are to be expected for mixtures (as the complementary properties are the motivation to mix substances in order to combine their functionalities and thus to achieve a specific performance).

The issue of an inert dispersing liquid can be critical already for TEM analysis, but especially for all suspension-based techniques. For nearly all measurement methods the limits of detection (in terms of size range and concentration) depend critically on the substance of investigation, especially in the size range below 1µm. It is thus in general not possible to correctly measure a mixture of powders.

The technique with least dependence on the substance(s) of investigation is SEM (see an example of its application to a mixture in Figure 5). However, the lower size range below 10nm and the often challenging image evaluation of SEM are limits to the practical implementation.

Recommendation

The size distribution of the individual ingredients before mixing should be determined and used, whenever available and necessary

4. Analytical challenges: nano-ingredients and components of articles and consumer products

The EC recommendation on the definition of nanomaterial covers nanomaterials when they are a substance or a mixture. As already mentioned before, when they are added to a product they remain a nanomaterial, but their addition does not necessarily turn the final product into a nanomaterial. Still a nanomaterial ingredient included in the final product has to be registered, self-classified and labelled following the EC regulatory framework. Therefore the stakeholder putting the final product on the EU market has to face very challenging analysis of a complex material in which nanomaterial may be only a very small fraction.

A range of product-specific regulations are currently established or in preparation (see Annex I). These regulations require either the indication of contained nanomaterials on the label or restrict the use of nanomaterials. Compliance with the regulations has to be controlled by the respective enforcement authorities and will in practice be monitored by statutory analytical laboratories. These need to have methods available for the detection, identification and quantification of nanomaterials present in products. Requirements for the minimum performance characteristics of the respective methods need to be established (as they are for conventional analytes, e.g. residues and contaminants). As shown in NanoDefine deliverable report D7.4 such requirements do not yet exist for methods for the analysis of nanomaterials in products.

This chapter thus focusses on the measurement of nanomaterials in products, e.g. in the framework of the enforcement of labelling obligations or (not yet established) maximum residue/content limits for nanomaterials.

4.1. Nanomaterials in articles and consumer products

This chapter addresses nanomaterials that are contained in products, i.e. forming an integral part of the product. Presence of nanomaterials in products can result from: (i) addition of ingredients specifically designed as nanomaterials (e.g. CNT in composite materials, TiN in food packaging plastics, nano-TiO₂ in sunscreens), (ii) addition of conventional ingredients with size distribution into the nano-range (e.g. fillers and pigments), (iii) incidental nanomaterials (e.g. debris from milling, impurities, contamination), (iv) natural nanomaterials (e.g. proteins in food) and (v) internal nanostructures (e.g. semicrystalline polymers or cementitious systems).

While most of the above mentioned final products are not in scope of the EC NM definition recommendation they still present a major challenge as the nano-ingredient is a subject for different legislative acts (e.g labelling obligation).

Analytical techniques cannot in general differentiate between these cases without additional knowledge on the composition and processing of a specific specimen to be tested. Analysis of incidental nanomaterials is particularly challenging as without further guidance, EU legislation forces analytical labs to detect and quantify traces thus raising the issue of trace analysis and technically feasible detection limits (minimum required performance limits – MRPL). Natural nanomaterials and nanostructured materials make it extremely challenging to analyse a product as a whole by imaging or counting methods due to the enormous background from materials that are not particulate (e.g. polymers) or otherwise excluded (e.g. proteins) from the definition recommendation. The second JRC report² on the review of the EC nanomaterial definition discussed the solution to classify instead by each ingredient, which is also in agreement with the recommendations given in section 4.

In the further considerations the focus will be on deliberately added nanomaterials to products (case i) and ii)). An overview of current and expected presence of potential nanomaterials in products, very approximately ranked by market volumes²⁷ is included in the Annex III.

4.2. Specific challenges related to analysis of NM in products

In addition to the general challenges (size, counting methods, agglomerates/aggregates) that analysis of nanomaterials faces, there are a number of challenges specific to the analysis of nanomaterials in the final products. These include:

(a) need for chemical selectivity

The particles of the targeted nanomaterial have to be distinguished from other possibly present nanomaterials in the product such as natural nanomaterials, other types of engineered nanomaterials, incidental nanomaterials etc. Any analytical techniques for the determination of nanomaterials in products thus have to be chemically specific and capable of unambiguously identifying the chemical identity of the respective NM. This excludes a range of non-specific characterisation techniques (e.g. CLS, DLS, PTA, EM without analytical functions etc.)

(b) sample matrix

In products, particles are usually embedded in the sample matrix. This has several implications:

- Microscopic techniques generally fail to analyse a product as a whole due to the dominating background nanostructures from materials that are not particulate (e.g. polymers) or otherwise excluded (e.g. proteins) from the definition recommendation. An example (sunscreen) is shown in 9 and demonstrates the complexity.

- For most characterisation techniques other than electron microscopy the target particles have to be extracted from the sample without changing the properties and the size distribution of the particles.

- Components of the product matrix may adhere to the particles. This will lead to an apparently larger size as compared to the uncovered particle unless the sample preparation removes all matrix components from the particle surface.

- simple screening techniques cannot be applied either because they are not chemically specific (see above) or they rely on surface (VSSA) which is not accessible because the particles are incorporated in the product matrix. Even after extraction a film may remain on the surface, inhibiting its proper determination.

c) detection limits

In contrast to pure materials/substances, NM in products makes only a fraction of the sample. Low fractions may exclude characterisation techniques the detection limit of which does not match the NM concentration in the sample

d) presence of different species of a chemically identical material

Products may contain different particles of the same chemical composition. In this case the presence of the non-nano materials (be it larger particles or molecules/ions of same composition) may obscure the presence of the nanoparticles



Figure 11: SEM image of a sunscreen sample containing different types of particles (BAM-13a). To enable imaging, the original water/oil matrix with its additional nanoscale structures was already removed by dilution, sonication and ozone etching.

4.3. Technical feasibility of the analysis of NM in products

In principle, it is possible to analyse nanomaterials in products. The effort for method development is usually higher as for pure NM and for certain types of NM (e.g. fragile NM, very small sizes) or specific particle/matrix combinations this may be very challenging. Currently, only a limited number of laboratories is capable of measuring NM in products. For the widespread enforcement of NM legislation for products it is recommended to build up infrastructure and expertise on European and national level in the form of an European reference laboratory and a network of national reference laboratories for the analysis of NM in products. This would include the establishment of reference methods and materials as well as a regular proficiency testing scheme.

The issue of minor content of nano-materials in a mixture or product has to be addressed (chapter 4.1). Some regulations that refer to the definition (e.g. cosmetics regulation) require that ingredients are classified each by each (not as the mixture), this approach should be integrated into the dedicated guidelines for definition harmonisation.

5. Summary of the Recommendations

This chapter briefly summarises all recommendations discussed in this document. The recommendations refer to clarifications or changes in the definition itself or to the preparation of guidance.

5.1. The term "external dimension"

Currently *EC NM Definition* refers to "external dimension" of the particles without further clarification of its meaning. The clear definition of "external dimension" would remove the ambiguity of this term which may be then be addressed either directly in the text of the *EC NM Definition* or in specific guidance.

Recommendation for changes in EC NM Definition

The term "external dimension" similarly to other terms like e.g. "aggregates", could be further defined in the part 4 of the EC NM Definition or a specific Technical Annex to Recommendation could specify it.

Proposal for a definition of **external dimension**:

External dimension means:

- the size of the biggest circle that is entirely inscribed into the 2D projection of the particle. If the median size of the inscribed circle is outside the range of 1-100 nm, the third dimension needs to be considered by appropriate methods, or
- the minimum Feret diameter which is the distance between two parallel tangents on opposite sides of the image of a particle (if the solidity of a two-dimensional particle projection is larger than a certain value, e.g. 0.5)

Recommendation for guidance

Rather than in the *EC NM Definition*, the term "external dimension" could be defined in dedicated guidance in which additional information related to the analytical challenges should be also addressed. This should go along with guidance on how to integrate widely used techniques in the decision process.

Due to the discussed uncertainties related to dispersion, flat shapes and automatic image evaluation, a guidance could require that the resulting histogram of external dimensions and the median size are plausible compared to the results obtained from other techniques (such as SAXS, WAXD and BET) that are less affected by the challenges described in this chapter. A negative outcome of such a plausibility check can trigger further, in-depth analysis of the sample to resolve this issue. However, in many cases it can be recommended that a decision based on an average dimension (outcome from non-counting techniques such as those mentioned above) would be acceptable, as discussed below and shown Figures 8 and 9.

For some specific products such a plausibility check is already recommended in the assessment of nanomaterials. For example EFSA in "Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain"²⁸ recommends that: "The size parameter should always be measured by at least two independent methods (one being electron microscopy) as the results obtained from different measurement techniques may differ because of the physical principles applied in the measurement method".

5.2. Particle size distribution based on particle numbers

The *EC NM Definition* uses a threshold related to the number based size distribution of particles. Noncounting techniques do not provide a number based size distribution as direct output but rather yield size distributions based on scattered light intensity, mass, volume or another physical quantity. These quantities need to be converted into a number based distribution if they are to be used to characterise materials according to the *EC NM Definition*. As such methods are widespread and commonly used, it is worthwhile to give clear recommendations under which conditions they can be used to identify a material as nanomaterial.

Type of quantity conversion

Apart from a few integral methods, techniques for particle size measurement yield size distributions which are weighted by some geometric or physical type of quantity (TOQ). The type of quantity conversion generally introduces errors for non-spherical particles and amplifies measurement uncertainties dramatically, if they are not evaluated before in the original TOQ. Thus an equivocal guidance with further explanation would improve the implementation of *EC NM Definition*.

Recommendation for guidance

- Guidance is required, which methods (constituted by a measurement technique + quality criterion + conversion algorithm) are admissible.
- For a classification as nanomaterial based on TOQ conversion, guidance should require proof that the classification is not due to the amplification of measurement uncertainty.
- Guidance should specify that to demonstrate that a material is a nanomaterial, conversion into particle number based size distribution is not required if the intensity or mass or volume or surface-weighed median is already smaller than 100 nm, because in that case the number median is certainly smaller 100 nm.

• Preference should be given to use counting methods whenever possible

5.3. Polydispersity

For materials with a broad size distribution (up to the micrometre range) and a large polydispersity index measuring PSD is very challenging which without further detailed guidance creates difficulties in implementation of *EC NM Definition*.

Recommendation for guidance

- For implementation of the EC NM Definition, guidance is needed that allows applying an upper size limit in measurements and particle statistics. However it has to be noted that applying any kind of upper size in measurements that are used to classify a material as nano or non-nano would result in an increase of the number of materials classified as nanomaterials.
- Introduce an upper size to indicate
 - the maximum size of particles in a nanomaterial; in this case the particles with a size up to the upper size limit would constitute the nanomaterial (depending on the result of the analysis) whereas the entire material would "contain" but not "consist of" nanoparticles
 - o 100 micrometres appear as acceptable upper size limit
- An analysis whether a material is a nanomaterial according to the EC NM Definition should always be accompanied with information on the upper and lower size limits applied for measuring the size/size distribution of particles (including justification).

5.4. Aggregates and agglomerates

The *EC NM Definition* refers to particles in an unbound state but also as constituents of aggregates or agglomerates. Consequently the constituent particles have to be identified and measured even when they are part of aggregates or agglomerates. The general term "particle" refers to an entity, which is clearly separated from its environment by physical boundaries before aggregation. Furthermore *EC NM Definition* defines aggregates as "a particle comprising of strongly bound or fused particles" in which case physical boundaries are lost. This generates additional challenges in the identification of the minute piece of matter for regulatory purposes which could be supported by the appropriate guidance document.

Recommendation for guidance

- The term "particle" should be more precisely defined for use with the EC NM Definition.
- If the particle size measurement of a sample after dispersion to individual aggregates results in a number median smaller than 100 nm, then the constituents must be smaller and no further

investigation for the nanomaterial classification is necessary.

In all other cases, one should check if the particles larger than 100 nm are aggregates which include constituent particles smaller than 100 nm. In practical terms, this check compares the measured size to the smallest size as observed by techniques that are independent of any physical separation of constituent particles, and is thus the same plausibility check as recommended for TEM above. As for TEM, a negative outcome of such a plausibility check can trigger further, in-depth analysis of the sample to resolve this issue. However, in many cases it can be recommended that a decision based on an average dimension (outcome from non-counting techniques such as those mentioned above) would be acceptable.

Another perplexing issue that many of wide-spread techniques for the measurement of particle size cannot distinguish between individual particles and agglomerates or aggregates. They are therefore not well suitable to show that a material is not a nanomaterial, because this would require dispersion of possible agglomerates and aggregates into their constituent particles. A dedicated guidance would improve the implementation of *EC NM Definition*.

Recommendation for Guidance

- Suspension-based techniques can be used for identification as nanomaterial, i.e., if the D50
 of a material obtained with a suspension-based method is below 100 nm, this material is a
 nanomaterial.
- Suspension-based techniques cannot be used to demonstrate that a material is not a nanomaterial, because they cannot distinguish between individual particles and agglomerates/aggregates.
- Suspension-based techniques can be used for screening up to a D50 of roughly a factor 2 or 3 above the cut-off of 100 nm. If the result for the D50 obtained with a suspension-based method is between 100 nm and 300 nm and if dispersion-independent methodology (SAXS, XRD, BET) indicates that particles with diameters smaller than the suspension-D50 are present in the material, then testing by SEM/TEM is required

5.5. Colloids

Colloids are very particular class of materials in which only a part of the substance is a particulate phase. Although colloids are not explicitly mentioned in the *EC NM Definition* its provisions applies and additional clarification should be provided in the dedicated guidance on how to approach their classification.

Recommendation

- It should be clarified that the general scope of the EC NM Definition is not (and should not be) limited to solid materials, since it defines a particle as "minute piece of matter with defined physical boundaries". Only later, this was interpreted as "solid" in the COM Staff Working Document. However, also soft nanomaterials may be of concern in specific regulatory contexts.
- Determining the size distribution poses much fewer analytical difficulties if the particles are solid rather than consisting of soft or liquid matter. Hence the definition should refer only to solid particles.
- It should be clarified in the *EC NM Definition* or in specific guidance that the *EC NM Definition* refers to a material at a temperature of 298 K and a pressure of 101 kPa.

5.6. Specific Surface Area

The EC NM Definition states that "Where technically feasible and requested in specific legislation, compliance with the definition [...] may be determined on the basis of the specific surface area by volume. A material should be considered as falling under the definition [...] where the specific surface area by volume of the material is greater than 60 m^2/cm^3 . However, a material which, based on its number size distribution, is a nanomaterial should be considered as complying with the definition [...] even if the material has a specific surface area lower than 60 m^2/cm^3 . However the EC in Q&A that accompanies the Reccomandation stays that scientific knowledge may expand the possibility to use it the future.

Considering recently obtained by two leading FP7 projects NanoDefine and NanoReg results such possibility seems to arise giving an opportunity for more rapid and easier implementation of the *EC NM Definition*.

Recommendation:

VSSA should be recommended in the revised recommendation and/or guidance for use as a screening tool for monoconstituent substances to identify both nano and non-nano materials. NanoDefine and NANoREG deliverables provide a screening strategy for technical guidance documents. VSSA is not validated for multimodal distributions or mixtures, and is not applicable to suspensions, formulations, articles, consumer products.

Recommendation for guidance

Use of VSSA

A material with the VSSA of less than $6 \text{ m}^2/\text{cm}^3$ and with a monomodal particle size distribution (PSD) should not be considered a nanomaterial.

Comment: showing monomodality of PSD does not require measurements in number but it can be in any

metrics (mass or volume), provided that modes at low sizes are captured.

Screening strategy

With the existing data from NanoDefine as training set, the following screening strategy is suggested. If applied to further data from real-world materials as validation set, this screening does achieve a correct classification, leaving only borderline materials assessment by sophisticated methods:

- 8. Measure skeletal density and BET (outgassing conditions within thermal stability range)
- 9. Check monomodal condition
- 10. If VSSA is more than a factor x10 below cutoff (VSSA < 6 m²/cm³), classify as non-nano.
 - Reason: uncertainty factor of x3 arises from the possible reduction of the shape-adjusted VSSA cutoff. Another uncertainty factor x2 from the experimentally determined range of VSSA mismatch against EM D50 (Figure 10). Combined uncertainty is x6, hence, x10 is considered as conservative, but subject to discussion of the exact screening cutoff value.
- 11. If VSSA > 6 m²/cm³, identify the shape from a simple SEM image. Re-evaluate with pragmatic aspect ratio criteria as proposed here to select the appropriate shape-specific cutoff as proposed by JRC report²:
 - Sphere (aspect ratio <3:1) D=3 → nano, if VSSA > 60 m²/cm³
 Note: Here the aspect ratio can be taken from 2-D projection. Flat shapes are recognizable in SEM and TEM by the flat grey scales.
 - Rod (aspect ratio >3:1:1) D=2 → nano, if VSSA > 40 m²/cm³
 - Platelet (aspect ratio >3:3:1) D=1 → nano, if VSSA > 20 m²/cm³
- 12. Quit VSSA screening and escalate to Tier 2 methods (EM or other)
 - If the simple SEM image shows multimodality (except if all modes are > 100 nm)
 - If the VSSA value is within an uncertainty range x2 around the shape-corrected cut-off (Figure 9)
- 13. Options to remove false positives from inner or coating porosity
 - Acquire and evaluate adsorption isotherms by NANoREG method (requiring long evacuation, dense data points at low pressures). Re-evaluate with outer VSSA.
 - NanoDefine Tier 2 methods.
- 14. Options to reduce the x2 uncertainty range around the cut-off:
 - "baseline" EM evaluation of closely related materials with a logical relationship of processing conditions and size distribution¹⁴

It should be noted that the need to know the shape for step 3 will typically require a simple SEM scan, but it does not require dispersion of the material, and it does not require statistical evaluation of EM images. These are the two most time-consuming steps and sources of EM uncertainty, as agreed by both JRC report #1, NanoDefine Technical Report D3.1 "Materials Evaluation Report" and by industry. Considering additionally that BET is already known and publicly available for many materials, the above screening is a tremendous reduction of the technical hurdles to implement the EC nanomaterial definition.

5.7. Proving that a material is not a nanomaterial

The *EC NM Definition* describes criteria for classification of a material as nanomaterial. For many stakeholders and purposes it is also important to reliably know when a material is not a nanomaterial. The latter fact is very difficult to proof because of limitation in the available methods. Therefore a dedicated guidance should be created which will describe how to approach this question.

Recommendation for guidance

Screening measurements and, for borderline cases, confirmatory measurements can be performed by a range of methods. It is the responsibility of the analyst to choose appropriate methodology. Methodical guidance, Nano- and Non-nano- Reference Materials are emerging from the NanoDefine project.

Screening can be performed by methods (such as FFF, CLS) that are well-established to deliver mass or volume metrics of the particles dispersed to a liquid suspension, under the following conditions:

- For a classification as non-nanomaterial, proof is required that the particles larger than 100 nm do not consist of constituent particles smaller than 100 nm. In practical terms, this proof compares the size measured in suspension to the size measured by techniques that are independent of any physical separation of constituent particles, e.g., EM.
- For a classification as nanomaterial based on conversion towards number metrics, proof is required that the classification is not due to the amplification of measurement uncertainty.

Screening can alternatively be performed by VSSA with shape-specific cut-offs:

- For a classification as non-nanomaterial, JRC report (EU27240 EN)³ considers a VSSA cut-off at 5m²/cm³, which is in good agreement with NanoDefine results.
- NanoDefine results further suggest that VSSA with (qualitative) SEM support is applicable for screening purposes in the range above 6m²/cm³, but not below 200 nm minimum external dimension (NanoDefine Technical report D3.5)
- For a classification as nanomaterial, the potential contribution of microporosity should be considered, e.g. by the t-plot evaluation (NANoREG)

Borderline cases remain after either screening approach. NanoDefine results (**Table 2**) suggest that minimum external dimensions from 50 nm to 200 nm as measured by screening methods are borderline cases and require confirmatory measurements, often by electron microscopy (e.g. SEM, TEM).

6. Conclusions

This report presents recommendations on a revision of the *EC NM Definition* of nanomaterial based on analytical possibilities. The NanoDefine project makes these recommendations based on (a) a comprehensive review of currently available techniques for the analysis of particle size distributions according to uniform technical criteria and (b) extensive practical experiences made in the first two years of the project in analysing real world materials which represent a large variety of different types of materials for which the need of a regulatory a classification as nanomaterial or non-nanomaterial is expected.

The work performed in the project has revealed a number of elements of the definition that should technically better be specified or conceptually clarified. Some of the recommendations could be adopted by modification of the *EC NM Definition* itself, others may be used to develop specific guidance to implement the definition. Implementing the definition in practice should be done in a tiered approach involving screening and in-depth techniques as appropriate and required. With regard to legal consequences of NM classification there is the need for uniform/standardised procedures for conducting measurements, data analysis and reporting all steps, in particular since a tiered approach means that different measurement techniques are involved.

The most important issues which the NanoDefine project recommends to address concern

- the definition of particle external dimensions
- issues around the conversion of measured quantity into a number based distribution and how to handle polydispersity to determine the number based particle size distribution
- a clear definition of constituent particles and the use of the volume specific surface area to handle aggregates and agglomerates
- clarification how to treat non-powder materials (e.g. colloids)
- the implementation of a screening strategy involving mobility-based techniques and/or the specific surface area
- the possibility to proof that a material is not a nanomaterial

In view of the technical and regulatory progress it is also recommended to review the definition again after 7 years.

ANNEXES

Annex 1: Non exhaustive list of the nano-specific provisions and guidelines in EC regulatory frameworks

Table 3: Non exhaustive list of the nano-specific provisions and guidelines in EC regulatory frameworks

Regulatory	Nano-specifi	c provisions in the leg	al text in relation to:	Guidance		
framework	Definition	Approval procedure	Information re- quirements	Separate assess- ment	Labelling require- ments	Recommen- dations
REACH						x
Regulation 1907/2006						
Biocidal Products	x	x		x	x	
Regulation 528/2012						
Cosmetic Products	x	x	x	x	x	X
Regulation 1223/2009						
Novel food					X*	X
Regulation 258/1997						
Novel food	x			x		
Revised Regula- tion 258/1997						
Food additives				x	X*	X
Regulation 1333/2008						
Plastic FCM		X		X		X
Regulation 10/2011						
Active & Intelligent FCM Regulation 450/2009		X		X		X
Food Info to Con- sumers	X				X*	
Regulation 1169/2011						
Medical Devices COM(2012) 542	X	X		X	X	

* Labelling of novel foods and food additives containing nanomaterials is required under FIC Regulation 1169/2011

Annex 2: Definitions of nanomaterials present in specific product legislations

In the EU, legally binding nanomaterial definitions were adopted as part of sector-specific legislation. While the EC NM Definition contains a broad description of the term nanomaterial, including also naturally occurring nanoparticles, definitions adopted in product specific legislation often restrict their field of applicability to *intentionally manufactured* or *engineered nanomaterials*. These differences are mainly due to the necessity to limit the definition to the type of compounds the specific legislation is address-ing.²⁹

As of October 2015 the EC NM Definition is used in the newly adopted Regulations on Biocides and Medical Devices and the Cosmetics and Food Information to Consumer (FIC) Regulations are in the advanced stages of the modification of the existing nanomaterial definition to become consistent with the Recommendation. Moreover the Commission is looking at ways to use the definition in the context of any potential nanomaterial specific provisions related to REACH. In addition, the definition was recommended for use by EU agencies such as ECHA and EFSA that have already started to apply it in their work.

Legislation	Size range	Solubility	Considers Aggre- gates and Ag- glomerates	Distribution Threshold	Only Intentionally manufactured/ Engineered	Novel properties	Considers VSSA
European Com- mission recom- mendation for a definition	1-100	No	Yes	50% by number	No	No	Yes
European Union Cosmetic Prod- uct Regulation (new proposed definition, 2013)	1-100	Yes	Yes	50% by number	Yes	No	
Food information to Consumer Regulation (FIC) (new proposed definition, 2013)	1-100	No	Yes	50% by number	Yes	No	
Biocides Regula- tion No 528/2012	1-100	No	Yes	50% by number	No	No	
Medical Devices Regulation	1-100	No	Yes	50% by number	No	No	
New Novel Food Regulation 258/1997 (ref to FIC)	1-100	No	Yes	50% by number	Yes	No	

Table 4: Summary of the regulatory nanomaterials definition used in the EU

Biocides Regulation No 528/2012

Article 3 of the new Biocides Regulation provides a definition of nanomaterial which was adapted from the EC Recommendation.

Medical Devices Regulation (draft document: COM(2012) 542 final)

Article 2.1 (15) of new Medical Devices Regulation provides a definition which was adapted from the EC Recommendation.

Medicinal Product Regulation

No specific provisions for nanomaterial are included in the medicinal product legislation (Directive 2001/83/EC).³⁰ The European Medicinal Agency published in 2006 a Reflection Paper (EMEA, 2006)³¹ which states that the *nanometre scale ranges from the atomic level at around 0.2 nm (2 Å) up to around 100 nm*. This definition differs in the lower limit (0.2 nm instead of 1 nm) from the *EC NM Definition*. On the Agency website,³² nanotechnology is instead defined as follows: **nanotechnology is the use of tiny structures - less than 1,000 nanometres across - that are designed to have specific properties. The upper limit in this definition differs from the ones specified in the EC recommended definition, some medicinal products considered by the Agency as nanomedicines, i.e. liposomes, can have in fact dimensions larger than 100 nm.**

European Union Cosmetic Product Regulation No 1223/2009

The nanomaterial definition included in the Cosmetic Product Regulation No 1223/2009, differently from the EC NM Definition, limits the term nanomaterial to *insoluble* or *biopersistent* and *intentionally manufactured* materials, therefore excluding all soluble and/or naturally occurring materials with dimensions at the nanoscale. An adaptation to the EC Recommendation definition is however foreseen (particle size distribution, particle, agglomerate and aggregate definitions integrated). The term *intentionally manufactured*, used in the actual Cosmetic Regulation definition is proposed to be substituted with *manufactured to perform/fulfil a specific function or purpose*.

Food Information to Consumer Regulation No 1169/2011

Article 3, Chapter 1 of the Food Information to Consumer Regulation No 1169/2011 provides a definition of engineered nanomaterial. An adaptation of this definition to that provided in the Recommendation 2011/696/EU is however foreseen (draft DG SANCO), with sector specific provisions.³³

Regulation on plastic materials and articles intended to come into contact with food The Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food provides some specifications for engineered nanomaterials without explicit definition of the term: (23)"New technologies engineer substances in particle size that exhibit chemical and physical properties that significantly differ from those at a larger scale, for example, nanoparticles. These different properties may lead to different toxicological properties and therefore these substances should be assessed on a case-by-case basis by the Authority as regards their risk until more information is known about such new technology. Therefore it should be made clear that authorisations which are based on the risk assessment of the conventional particle size of a substance do not cover engineered nanoparticles".

Article 9 "Specific requirements on substances" provides that "Substances in nanoform shall only be used if explicitly authorised and mentioned in the specifications in Annex I."

Novel food (Revised) Regulation 258/1997

The proposal for a revision of the Novel Food Regulation³⁴ does not include its own definition of "nanomaterial" but makes reference to the FIC Regulation and its definition of "engineered nanomaterial". Any change of the definition in the FIC Regulation would therefore automatically apply to novel food.

Guidance on REACH

In its guidance documentation on registration of nanomaterials for the purposes of REACH, the European Chemicals Agency (ECHA) explicitly refers to the EC Recommendation 2011/696/EU. Hence, the EC Recommendation is currently used as working definition for the purposes of REACH.

Annex 3: Overview of current and expected presence of potential nanomaterials in products

Table 4 presents an overview of current and expected presence of potential nanomaterials in products, very approximately ranked by market volumes²⁷. For most of these materials the size distribution can extend into the nano range, depending on the product requirements, production process and measurement metrics.

Product group	Possible nanomaterials	Specific "nano" enforcement
Rubber	Carbon Black, Silica, (in future possibly: mwCNTs),	
Plastics, paints and coatings	Fillers (CaCO ₃ etc), pigments (organic or inorganic), fibres (mwCNTs), polymer particles	
Construction materials	Intrinsically nanostructured, but addition of e.g. silicates,	
Paper and packaging	Sheet silicates, polymer parti- cles	
Food	particulate food additives, e.g.	food labelling (nano)
	silica, titania, others,nano- encapsulated ingredi- ents/supplements (e.g. vita- mins, antioxidants, trace ele- ments, preservatives)	Food Information to Con- sumer Regulation No 1169/2011
Food contact materials	barrier property enhancers (TiN, nano-polymers, nano- organoclays, TiO ₂), biocides (Ag, ZnO)	
Cosmetics	nano-TiO ₂ , pigments, abrasives (e.g. silica, alumina), active in- gredients (e.g. fullerenes, hy- droxy apatite)	cosmetics ingredients labelling (nano) European Union Cosmetic Product Regulation No 1223/2009
Household products, cleaners	Silicates, Ag	
Medicinal products	Ag	
Biocidal products	Ag, ZnO, CuCO ₃	
Electronics	Metals, quantum dots, swCNT	ROHS

Table 5: Products with current and expected presence of nanomaterials

Annex 4: Availability of standardized, validated analytical methods and reference materials

The practical value of the definition is determined by its relevance for further particle and product properties (e. g. product performance, toxicology) and by the availability of analytical tools that allow for material classification according to it. Such analytical tools comprise measurement techniques (MTs), adequate procedures for sample preparation and conducting measurements as well as data analysis, a harmonised theoretical frame for representing particle size measurements (terms, mathematical definitions) and reference materials for MT validation and routine performance checks. This chapter addresses analytical which apply to the relevant size range of the definition (1 nm to 10 μ m).

Standards for describing particle size (and shape) and for representing results of particle characterisation

In principle, there is a common understanding of terms like "particle" or "particle size" and on the way of representing results of particle characterisation. Yet, in practice (real-life) there is still a lot of confusion, caused by the different scientific and national background of people dealing with particle characterisation in general and particularly in the field of NM characterisation. In addition, even the existing international standards are not always consistent with respect to terms, definitions, symbols and graphical representation. For this reason, there are ongoing efforts by ISO committee TC24/SC4 to harmonise the "language" of particle characterisation. Independent from this, the main issues regarding particle characterisation are already described in the ISO 9276 (all parts).

Availability of standardised and validated measurement techniques

Techniques for particle size measurement can be either grouped by the principle of size determination (for instance based on imaging – e. g. TEM or SFM, based on particle mass or volume– e. g. sp-ICP-MS or TRPS, based on particle mobility – e. g. DLS & AC, based on scattering patterns – e. g. ALS & SAXS) and by the principle of quantifying the different size fractions (by counting measurement results at single particle; by quantification after fractionating the particle system, by evaluating spectroscopic; i. e. frequency, time or spatially resolved, signals of the original or a representative sample; by obtaining an integral size value). Both kinds of grouping are relevant for the definition since they determine the MT's performance of finding the number weighted median of constituent particles or at least a similar size value (cf. discussions in chapter 3).

In principle there are a lot of potentially interesting MTs, which may be employed for the implementation of the definition. Yet, not all techniques can be considered as *validated* (i. e. successfully validated in at least one published inter-laboratory comparison, which at best included instruments of different manufacturers) and *standardised* (i. e. standardised for particle size measurement by an ISO or CEN standard). In this regard it is possible to distinguish among:

- fully validated and standardised MTs (e. g. DLS, ALS for micrometre particles, USSP for submicron suspension, BET)
- standardised MTs (e. g. SEM, TEM, PTA, cuvette and disc AC, DEMA for aerosol particles, TRPS for x > 0.4 μm, ...)
- partially validated MTs (i. e. accuracy of size measurement shown in scientific papers; e. g. PTA, AUC, AF4)
- MTs with verified poor performance (e. g. ALS-LD for submicrometre particles)
- commercially available, yet non-validated and non-standardised MTs (e. g. TRPS for NPs, DEMA for sprayed suspensions)
- available MTs, used mainly in research context (e. g. UV/VIS for size determination of colloidal gold or silver)

The non-exhaustive list of available international standards for techniques to determine particle size is listed below.

Availability of standardised and validated procedures for sample preparation

Sampling and sample preparation (incl. dilution, dispersion and stabilisation) are inevitable steps for particle size measurements. Yet, the requirements on both are as manifold as the different types of particle systems, the involved materials and the considered MTs. For this reason there are only few international standards that provide some general rules for sampling and sample preparation, while other standards give advice for specific types of materials or specific MTs.

General rules:

- ISO 14488: Particulate Materials Sampling and sample splitting for determination of particulate properties
- ISO 14887: Sample preparation Dispersing procedures for powders in liquids

Specific rules:

- ISO 8780, Pigments and extenders Methods of dispersion for assessment of dispersion characteristics.
- ISO 14703, Fine ceramics (advanced ceramics, advanced technical ceramics) Sample preparation for the determination of particle size distribution of ceramic powders.
- ISO/TS 12025:2013 Nanomaterials Quantification of nano-object release from powders by generation of aerosols
- see also: ASTM B 821-92 and ASTM B 859-95 for metal powders

Apart from this there are several scientific publications, which deal with sampling and sample preparaton. They form a sound basis on which protocols for suspending powders, dispersing suspended particles or stabilising suspension samples can be developed.

Reference materials for particle characterisation

Preliminary remarks on RMs for particle characterisation

Reference materials (RMs) are required for the general validation of measurement techniques and for routine checks of instrument performance. For the purpose of particle characterisation, RMs are particle systems with defined size distribution and well-known shape of the particles; there are also suspension RMs with defined particle number concentration. Since the available MTs for particle characterisation differ considerably with respect to probed particle property and intrinsic type of quantity, reference cannot be given to a universally valid true particle size, but to MT-specific values of the size distribution (e. g. median size x50,0 for electron microscopy and modal value xmod,3 for discAC).

Particulate RMs can be distinguished with regard to particle shape, polydispersity and material. Most frequently RMs with spherical particles are preferred because the term "size" is unambiguously defined (thus referenced values are usually provided for more than one MT). Yet there are also RMs consisting of particles with regular, non-spherical shape (e. g. rods; then uniform in size) and of particles with irregular, varying shape (e. g. grinding powders; then also polydisperse). Concerning the size distribution one can distinguish among quasi-monodisperse samples (i. e. narrowly monomodal), which are employed for testing the accuracy of size measurements, multi-disperse sample (i. e. with multiple narrow modes based on quasi-monodisperse samples), which allow to check the quantification accuracy and size resolution, and broad monomodal samples, which apart from the size and quantification accuracy and can resolve size detection limits of the MTs. Additional to the effects of shape and size distribution, one has to consider the possible impact of material on the performance of a MT. This is particularly important, when light scattering and extinction properties are relevant (e. g. in PTA, discAC), when atomic weight or mass density determine signal strength (e. g. in RPS or EMS).

Particulate RMs for nanoparticle range (≤ 100 nm)

Quasi-monodisperse RMs ≤ 100 nm

- Nanoparticulate, quasi-monodisperse RMs are provided as stabilised suspensions. The particles are frequently spherical or at least of convex grain-like shape. Typical materials are:
- polystyrene latex (various size fractions \geq 40 nm, few thousand ppmw)
- colloidal silica (various size fractions \geq 20 nm, few thousand ppmw)
- colloidal sols of gold (Au) or silver (Ag); ≥ 5 nm, few ppmw
- Few RMs in the nano-range consist of non-spherical particles (gold-rods).

Please note the following rule of thumb: The finer the average particle size is, the larger is relative distribution width.

Multi-disperse RMs ≤ 100 nm

Since such RMs can be easily generated from quasi-monodisperse samples, they are usually not available commercially. Lately there are some attempts to provide at least bimodal nanoparticle suspensions as RM.

In any case, such mixtures are prepared based by defined volumes or weights of each component; i. e. the referenced quantities of each mode are volume or mass. There is no multi-disperse RM based on number concentration in the nano-range.

Broad monomodal RMs ≤ 100 nm

Some nano-RMs that are sold as quasi-monodisperse are in fact rather broadly distributed. A reliable indication of such an event is a strong variation among the different equivalent diameter. The lack of Nano-RMs with a deliberately high polydispersity corresponds to the fact that most frequently only mean values (or median or modal sizes) are referenced, but none parameters of polydispersity.

Particulate RMs for sub- and low micrometre range (0.1 µm ... 10 µm)

The shift from the nano- to the submicrometre region coincides with some changes regarding the available RMs: Such RMs are provided as suspension or as powder (especially for >1 μ m), polydisperse RMs of spherical or even irregularly shaped particles are available, the relevant materials are partly different (e. g. colloidal Au-sols belong to nano-range, while > 100 nm glass beads become available).

Quasi-monodisperse RMs for 0.1 µm ... 10 µm

Quasi-monodisperse RMs for 0.1 µm ... 10 µm are made of

- polystyrene latex (various size fractions ≥ 40 nm, few thousands ppmw)
- colloidal silica (various size fractions ≥ 20 nm, several thousand ppmw)
- glass beads (\geq 1 µm ; powder).

The particles are frequently spherical. RMs of non-spherical particles with uniform size and morphology are under discussion.

Multi-disperse RMs for 0.1 µm ... 10 µm

As above: Such RMs have to be generated out of quasi-monodisperse samples. Usually this leads to volume-/mass-based mixture. Since there are RMs for particle number concentration in the micrometre range, it is even possible to produce number-based mixtures; yet not below 1 µm.

Broad monomodal RMs for 0.1 µm ... 10 µm

For the size range of 100 nm to 10 µm there are several commercial RM that are composed of nonspherical (irregularly shaped) particles with a monomodal, but broad size distribution (e. g. grinded quartz or silicon nitride). In addition there also RMs consisting of broadly distributed glass beads.

• polystyrene latex (various size fractions \geq 40 nm, few thousand ppmw)

- colloidal silica (various size fractions \geq 20 nm, few thousand ppmw)
- colloidal sols of gold (Au) or silver (Ag); ≥ 5 nm, few ppmw
- Few RMs in the nano-range consist of non-spherical particles (gold-rods).
- Please note the following rule of thumb: The finer the average particle size is, the larger is relative distribution width.

RM materials for particle number concentration

There are few measurement techniques that additional to size measurement yield the number concentration for each size class (e. g. DEMA, PTA, sp-ICP-MS, TRPS). In these cases it would be in principle helpful to have RMs for particle number concentration. Also, mixtures of RMs with defined particle size and number concentration would be useful for testing the counting accuracy of counting MTs or the accuracy of modal weights (in number) for multi-disperse RMs.

In spite of this need, there are no real RMs for particle number concentration with $x < 1 \mu m$. Such kind of materials are only available for the micrometre range and are e. g. used for testing optical particle counters.

Discussion

Even though there are several MTs for the determination of particle size in the relevant range of 1 nm to 10 µm only few some of them are standardised by ISO and CEN and even less have been validated by published interlaboratory comparisons (high scientific standards are presumed). In particular there is a lack of validated and standardised counting MTs for suspension samples: PTA does not cover the whole range is not fully validated; similar applies to spray-DEMA and TRPS. Hence, the only route to reliably counting are imaging techniques (e. g. SEM, TEM), which require the deposition of particles on a suitable substrate. The main issue of such MTs with respect to the definition is to ensure representativeness of the deposited particles. Imaging techniques have the further advantage of detecting the constituent particles within aggregates and agglomerates. Other MTs that also probe the constituents are SAXS (under good circumstances: XRD) and BET. A lot of MTs are based on the particle's mobility (e. g. DLS, AC); they typically yield equivalent diameters that are similar to the external (aggregate) dimension or the dimension of large "pores", in any case they are larger than the constituent particles. This holds also true for MTs that are based on particle mass.

For validation and routine checks, there are several commercially available RMs. Most of them are quasi-monodisperse systems of spherical particles. Typical materials for the nano-range are PSL, gold and amorphous silica, yet for the size range < 10 nm it is reduced to noble metals (then with relatively broad size distribution). The range above 100 nm (to 10 μ m) offers several commercial RMs which are already sold for years. Beside systems with spherical particles (PSL, silica, glass beads) there are also well-accepted RMs of irregularly shaped particles with relatively high polydispersity (grinded quartz and silicon nitride). In general there is a need for more multi-disperse RMs in the relevant size range and a clear lack of multi-disperse systems with referenced mixing ratio based by number. This coincides with

a lack for RMs on particle size and number concentration.

In conclusion, the current set of analytical tools already allows for an implementation of the definition in practice, yet there remain significant gaps with regard to its comprehensive validation and standardisation:

- standardised procedures for preparing representative samples for imaging techniques
- validation of promising MTs (not only imaging techniques, but also those considered for tier 0 & 1) in large scale interlaboratory comparisons (high standards on sample handling and data evaluation; with different kinds of RMs)
- further development of RMs for x < 10 nm, of multi-disperse RMs with defined number-based mixing ratio and of RMs for particle number concentration

The non-exhaustive list of available international standards for techniques to determine particle size is listed below.

International standards on particle size analysis

This section includes a non-exhaustive list of international standards available with hyperlinks for particle sizing techniques.

Size analysis

ISO 9276-1:1998, Representation of results of particle size analysis – Part 1: Graphical representation.

ISO 9276-1:1998/Cor 1:2004

<u>ISO 9276-2:2014</u>, Representation of results of particle size analysis - Part 2: Calculation of average particle sizes/diameters and moments from particle size distributions.

<u>ISO 9276-3:2008</u>, Representation of results of particle size analysis - Part 3: Adjustment of an experimental curve to a reference model.

<u>ISO 9276-6:2008</u>, Representation of results of particle size analysis – Part 6: Descriptive and quantitative representation of particle shape and morphology.

ISO 26824:2013, Particle characterization of particulate systems – Vocabulary.

<u>ISO/TS 11888:2011</u>, Nanotechnologies – Characterization of multiwall carbon nanotubes – Mesoscopic shape factors.

Sampling and sample preparation

<u>ISO 14887:2000</u>, Sample preparation – Dispersing procedures for powders in liquids.

<u>ISO 8780-1:1990</u>, Pigments and extenders – Methods of dispersion for assessment of dispersion characteristics – Part 1: Introduction.

<u>ISO 8780-2:1990</u>, Pigments and extenders – Methods of dispersion for assessment of dispersion characteristics – Part 2: Dispersion using an oscillatory shaking machine.

<u>ISO 8780-3:1990</u>, Pigments and extenders – Methods of dispersion for assessment of dispersion characteristics – Part 3: Dispersion using a high-speed impeller mill.

<u>ISO 8780-4:1990</u>, Pigments and extenders – Methods of dispersion for assessment of dispersion characteristics – Part 4: Dispersion using a bead mill.

<u>ISO 8780-5:1990</u>, Pigments and extenders – Methods of dispersion for assessment of dispersion characteristics – Part 5: Dispersion using an automatic muller.

<u>ISO 8780-6:1990</u>, Pigments and extenders – Methods of dispersion for assessment of dispersion characteristics – Part 6: Dispersion using a triple-roll mill.

<u>ISO 23900-1:2015</u>, Pigments and extenders – Methods of dispersion and assessment of dispersibility in plastics – Part 1: General introduction.

<u>ISO/TS 16176:2011</u>, Rubber compounding ingredients – Carbon black – Determination of the aggregate-size distribution at ultimate dispersion.

Electron microscopy

<u>ISO 13322-1:2014</u>, Particle size analysis – Image analysis methods – Part 1: Static image analysis methods.

<u>ISO/TS 10797:2012</u>, Nanotechnologies – Characterization of single-wall carbon nanotubes using transmission electron microscopy.

<u>ISO/TS 10798:2011</u>, Nanotechnologies – Characterization of single-wall carbon nanotubes using scanning electron microscopy and energy dispersive X-ray spectrometry analysis

ISO 22493:2014, Microbeam analysis – Scanning electron microscopy – Vocabulary

ISO 15932:2013, Microbeam analysis – Analytical electron microscopy – Vocabulary

PTA/DUM

ISO/DIS 19430, Determination of particle size distribution – Particle tracking analysis.

TRPS / ESZ / n-Coulter-counter

ISO 13319:2007, Determination of particle size distributions – Electrical sensing zone method.

ICP-MS

<u>ISO/TS 16965:2013</u>, Soil quality - Determination of trace elements using inductively coupled plasma mass spectrometry (ICP-MS).

<u>ISO 17294-1:2004</u>, Water quality - Application of inductively coupled plasma mass spectrometry (ICP-MS) – Part 1: General guidelines.

<u>ISO 17294-2:2003</u>, Water quality: "Application of inductively coupled plasma mass spectroscopy (ICP-MS) – Part 2: Determination of 62 elements.

<u>ISO 30011:2010</u>, Workplace air – Determination of metals and metalloids in airborne particulate matter by inductively coupled plasma mass spectrometry.

<u>ISO/TR 17276:2014</u>, Cosmetics – Analytical approach for screening and quantification methods for heavy metals in cosmetics.

DMAS / DEMS = SMPS for aerosol particles

<u>ISO 15900:2009</u>, Determination of particle size distribution – Differential electrical mobility analysis for aerosol particles.

ISO 27891:2015, Aerosol particle number concentration – Calibration of condensation particle counters.

ISO 12025:2012, Nanomaterials -- Quantification of nano-object release from powders by generation of aerosols

AC

<u>ISO 13318-1:2001</u>, Determination of particle size distribution by centrifugal liquid sedimentation methods – Part 1: General principles and guidelines.

<u>ISO 13318-2:2007</u>, Determination of particle size distribution by centrifugal liquid sedimentation methods – Part 2: Photocentrifuge method.

<u>ISO 13318-3:2004</u>, Determination of particle size distribution by centrifugal liquid sedimentation methods – Part 3: Centrifugal X-ray method.

<u>ISO 15825:2004</u>, Rubber compounding ingredients – Carbon black – Determination of aggregate size distribution by disc centrifuge photosedimentometry.

DLS

ISO 13321:1996, Particle Size Analysis – Photon Correlation Spectroscopy.

ISO 22412:2008, Particle size analysis – Dynamic light scattering (DLS).

ALS – laser diffraction

ISO 13320:2009, Particle size analysis - Laser diffraction methods.

<u>ISO 24235:2007</u>, Fine ceramics (advanced ceramics, advanced technical ceramics) – Determination of particle size distribution of ceramic powders by laser diffraction method.

ISO 8130-13:2001, Coating powders – Part 13: Particle size analysis by laser diffraction

SAXS

ISO/TS 13762:2001, Particle size analysis – Small angle X-ray scattering method. WITHDRAWN

ISO 17867:2015, Particle size analysis – Small-angle X-ray scattering.

USSp

<u>ISO 20998-1:2006</u>, Measurement and characterization of particles by acoustic methods — Part 1: Concepts and procedures in ultrasonic attenuation spectroscopy.

<u>ISO 20998-2:2013</u>, Measurement and characterization of particles by acoustic methods — Part 2: Guidelines for linear theory.

<u>ISO/CD 20998-3</u>, Measurement and characterization of particles by acoustic methods — Part 3: Guidelines for non-linear theory.

XRD

<u>EN 1330-11:2007</u>, Non-destructive testing - Terminology - Terms used in X-ray diffraction from polycrystalline and amorphous materials.

<u>EN 13925-1:2003</u>, Non-destructive testing - X-ray diffraction from polycrystalline and amorphous material - Part 1: General principles.

<u>EN 13925-2:2003</u>, Non-destructive testing - X-ray diffraction from polycrystalline and amorphous materials - Part 2: Procedures.

ISO 24095:2009, Workplace air – Guidance for the measurement of respirable crystalline silica.

BET

ISO 9277:2010, Determination of the specific surface area of solids by gas adsorption – BET method.

<u>ISO 18757:2003</u>, Fine ceramics (advanced ceramics, advanced technical ceramics) – Determination of specific surface area of ceramic powders by gas adsorption using the BET method

Bibliography

¹ Rauscher H., Roebben G., Amenta V., Boix Sanfeliu A., Calzolai L, Emons H., Gaillard C., Gibson N., Linsinger T., Mech A., Quiros Pesudo L., Rasmussen K., Riego Sintes J., Sokull-Klüttgen B., Stamm H., Towards a review of the EC Recommendation for a definition of the term "nanomaterial" Part 1: Compilation of information concerning the experience with the definition, JRC Scientific and Policy Report, EUR 26567 EN, Eds. H. Rauscher, G. Roebben, 2014

² Roebben G., Rauscher H., Amenta V., Aschberger K., Boix Sanfeliu A., Calzolai L., Emons H., Gaillard C., Gibson N., Holzwarth U., Koeber R., Linsinger T., Rasmussen K, Sokull-Klüttgen S., Stamm H., Towards a review of the EC Recommendation for a definition of the term "nanomaterial" Part 2: Assessment of collected information concerning the experience with the definition, JRC Scientific and Policy Report, EUR 26744 EN, Eds. G. Roebben, H. Rauscher, 2014

³ Rauscher H., Roebben G., Boix Sanfeliu A., Emons H., Gibson P., Koeber R., Linsinger T., Rasmussen K, Riego Sintes J., Sokull-Kluettgen B., Stamm H., Towards a review of the EC Recommendation for a definition of the term "nanomaterial": Part 3: Scientific-technical evaluation of options to clarify the definition and to facilitate its implementation, EUR 27240 EN, Eds. H. Rauscher, G. Roebben, 2015

⁴ European Commission, Commission Recommendation of 18 October 2011 on the definition of nanomaterial, Official Journal of the European Union. 2011/696/EU: 38-40, 2011

⁵ REGULATION (EU) No 528/2012 concerning the making available on the market and use of biocidal products. <u>http://echa.europa.eu/regulations/biocidal-products-regulation/legislation</u>

⁶ REGULATION (EC) No 1223/2009 on cosmetic products.

http://ec.europa.eu/growth/sectors/cosmetics/legislation/index_en.htm [Accessed 28th August 2015]

⁷Regulation (EU) No 1169/2011, <u>http://eur-lex.europa.eu/legal-</u> <u>content/EN/ALL/?uri=CELEX:32011R1169</u>

⁸ Olson, E., Particle Shape Factors and Their Use in Image Analysis–Part 1: Theory, Journal of GXP Compliance, 15, 3, 85-96, 2011.

⁹ <u>http://www.efsa.europa.eu/en/efsajournal/pub/2140</u> [Accessed 28th August 2015]

¹⁰ From NanoDefine Technical Report D3.2

¹¹ Hackley, V.A., Stefaniak, A. B. (2013) Real-world precision, bias, and between-laboratory variation for surface area measurement of a titanium dioxide nanomaterial in powder form. J Nanopart Res 15:1-8

¹² Kreyling W, Semmler-Behnke M, Chaudhry Q (2010) A complementary definition of nanomaterial. Nanotoday. 5:154:168

¹³ de Boer, J. H., Linsen B. G. and Osinga T. J. (1965). Studies on pore systems in catalysts: VI. The universal t curve. Journal of Catalysis 4(6): 643-648.

¹⁴ Gilliland D., Gibson P., Hempelmann U., Vicent Pena J.B., Kund K., Wohlleben W., Koch T., Burke A., Mcnulty G., Hartl-Gunselmann A., Knobl S., Reisinger M., .Sokull-Kluettgen B., Stamm H., Liewald H., Basic comparison of particle size distribution measurements of pigments and fillers using commonly available industrial methods, EUR 26916, doi: DOI 10.2788/21024, 2014

¹⁵ U.S. Environmental Protection Agency, Chemical substances when manufactured or processed as nanoscale materials; TSCA reporting and recordkeeping requirements, Fed. Regist. 2015, 80 (65), 18330-18342.

¹⁶ http://ec.europa.eu/environment/chemicals/nanotech/faq/questions_answers_en.htm [Accessed 28th August 2015]

¹⁷ http://www.sensore-electronic.com/en/SEMEDX ChemicalElementalAnalysis.html [Accessed 28th August 2015]

http://www.oxford-instruments.com/products/microanalysis/energy-dispersive-x-ray-systems-edsedx/eds-for-sem/eds-software-aztec/eds-element-mapping-autolayer-and-trumap [Accessed 28th August 2015]

¹⁹ http://wings.buffalo.edu/faculty/research/scic/sem-eds.html [Accessed 28th August 2015]

²⁰ Pennycook, S.J., Z-contrast stem for materials science, Ultramicroscopy, 30, 1, 58-69, 1989

²¹ http://www.nrel.gov/pv/measurements/scanning_transmission.html [Accessed 28th August 2015]

²² Sales, D.L, Beltran, A. M, Lozano, J.G., Manuel, J.M. ,Guerrero-Lebrero, M. P., Ben, T., Herrera, M. Morales, F. M., Pizarro, J. Sanchez, A. M., Galindo, P. L. Gonzalez, D., Garcia, R. Sergio, Molina, S.I. High-Resolution Electron Microscopy of Semiconductor Heterostructures and Nanostructures, Chp 2, 150, 23-62, Online ISBN978-3-642-23351-7, Eds A. Patane, N. Baltan 2012

²³ http://www.innovationservices.philips.com/sites/default/files/materials-analysis-tem.pdf [Accessed 28th August 2015]

²⁴ http://www.nanolabtechnologies.com/stem-eels-eds [Accessed 28th August 2015]

25 http://www.edax.com/Products/EDS/TEAM/TEAM-EDS-for-TEM-Analysis-Phase-Mapping-Spectra-Collection.aspx [Accessed 28th August 2015]

26

http://www.oxfordinstruments.com/OxfordInstruments/media/nanoanalysis/brochures%20and%20thumbs/TEM-Explained.pdf [Accessed 28th August 2015

²⁷ Stark, W. J., Stoessel, P. R., Wohlleben, W., Hafner, A, Industrial applications of nanoparticles, Chem. Soc. Rev. 2015,44, 5793-5805.

²⁸ http://www.efsa.europa.eu/en/efsajournal/pub/2140 [Accessed 28th August 2015]

²⁹ Bleeker, E. A. J. et al, Considerations on the EU definition of a nanomaterial. Science to support policy making. Regulatory Toxicology and Pharmacology 65, 119-125, 2013

³⁰ European Parliament and Council, Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use, 2001

³¹ European Medicinal Agency, Reflection Paper on Nanotechnology-based Medicinal Products for Human Use, EMEA/CHMP/79769/2006, 2006

32

http://www.ema.europa.eu/ema/index.jsp?curl=pages/special topics/general/general content 000345.i sp&mid=WC0b01ac05800baed9

³³ Commission Delegated Regulation amending Regulation EU 1169/2011 of the European Parliament and Council on the Provision of food information to consumer as regards the definition of "engineered nanomaterials", SANCO 11616/2013.

³⁴ European Commission (2013). Proposal for a Regulation of the European Parliament and of the Council on novel foods. COM(2013) 894 final