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Guidance on the implementation of the Commission Recommendation 2022/C 229/01 on the definition of nanomaterial

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2023

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Abstract

A new Recommendation on the definition of 'nanomaterial' (2022/C 229/01) was adopted by the European Commission in 2022 to serve different policy, legislative and research purposes when addressing nanomaterials or issues concerning products of nanotechnologies. It is broadly applicable across a wide variety of fields. This guidance supports the implementation of the nanomaterial definition adopted in the new Recommendation. The guidance builds upon the two JRC Science for Policy Reports EUR 29647 and EUR 29942. The present guidance gives an overview of the key terms and concepts, provides a decision tree to identify nanomaterials and addresses identification of nanomaterials through measurements for the new Recommendation on the definition of nanomaterial (2022/C 229/01). Furthermore, an Annex listing documentary standards relevant for a harmonised and coherent regulatory implementation of the definition of nanomaterial at European Union and national level was added.

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Executive Summary

The European Commission adopted the new Recommendation 2022/C 229/01 on the definition of ‘nanomaterial’ on 10 June 2022, which updates the definition adopted in a previous Recommendation (2011/696/EU). The new Recommendation is based on, among others, the relevant outcomes of a review of Recommendation (2011/696/EU) between 2013 and 2021. The review noted both the broad experience by legislators and industry in applying the previous definition leading to an understanding of possibilities for improvement, as well as technical and scientific progress within the field. The new definition is broadly applicable in a wide variety of regulatory fields and may serve different policy, legislative and research purposes when addressing nanomaterials or issues concerning products containing or produced with nanomaterials. The Recommendation calls on the JRC (Joint Research Centre [of the European Commission]) to support the implementation of the definition by developing guidance, listing recommended measurement methods and best practice tools including illustrative cases. This document provides guidance pursuant to Recital (8) of the Recommendation 2022/C 229/01.

This guidance explains how terms and concepts used in the European Commission’s nanomaterial definition should be understood, and it reflects established technologies and measurement practices. In addition to new elements in the definition and to technical and scientific progress, this guidance builds upon two JRC Science for Policy Reports (EUR 29647 and EUR 29942). These two reports give an overview of the concepts and terms used in the definition of the previous Recommendation 2011/696/EU (EUR 29647) and explain how to identify nanomaterials through measurements (EUR 29942). For the new definition 2022/C 229/01 the present report substitutes EUR 29647 and furthermore provides complementary updates of EUR 29942. This report presents economic operators, as well as regulators, with detailed descriptions enabling an in-depth understanding of the nanomaterial definition and clarifies key terms and concepts. Specific terms addressed here are nanoscale, particles and external particle dimensions, material, aggregate, agglomerate and identifiable constituent particle, solid, single molecule, specific surface area by volume, particle number based size distribution and nanomaterials in products. In addition, examples of best practices for effective implementation and transparent and robust governance are presented.

This guidance does not prejudice how to incorporate a definition of nanomaterial in legislation, nor what the criteria for such a definition could be. This guidance refers to Test Guidelines (TG) published by the Organisation for Economic Co-operation and Development (OECD) and documentary standards developed by the International Organization for Standardization (ISO) and the European Committee for Standardization (CEN).

The guidance describes and explains:

- Definitions of key terms and fundamental concepts
- A decision tree to help identifying nanomaterials
- Measurement options to consider in the assessment of particulate materials

The guidance does not constitute legal advice. It will be updated as necessary in light of technical and scientific progress, as envisioned in Recital (8) of the Recommendation.



1 Introduction

On June 10, 2022, the European Commission (EC) adopted Recommendation 2022/C 229/01 [1] to further harmonise the understanding of the term ‘nanomaterial’ in a regulatory context. This Recommendation provides a definition of ‘nanomaterial’ (the ‘EC NM definition’) and it builds on and replaces the 2011 Recommendation 2011/696/EU [2] on the same topic. Annex 1 presents the Recommendation 2022/C 229/01 as published in the Official Journal of the European Union. A Recommendation allows the institutions to make their views known and to suggest a line of action without imposing any legal obligation on those to whom it is addressed.

The previous Recommendation 2011/696/EU was developed to provide a common basis for regulatory purposes across all areas of European Union (EU) policy concerning nanomaterials. Following its publication in the Official Journal of the European Union on October 18, 2011, the EU adopted corresponding nanomaterial-specific provisions in several pieces of legislation, see list of examples below.

- The Regulation concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) (EC) No 1907/2006, which was amended in 2018 through Commission Regulation (EU) 2018/1881 to address nanoforms of substances [3]
- The Biocidal Products Regulation (EU) No 528/2012 [4]
- The Medical Devices Regulation (EU) 2017/745 [5]

The legal definition of nanomaterial (nanoform) in these laws is planned to be updated and harmonised with the definition in Recommendation 2022/C 229/01. Meanwhile, the current text of these laws should be implemented. As highlighted in Table 1, the differences between Recommendation 2011/696/EU and Recommendation 2022/C 229/01 are minor. A review of the Recommendation 2011/696/EU was envisioned ‘in the light of

experience and of scientific and technological developments’ [2]. The findings and conclusions of the review are comprehensively reported in a European Commission Staff Working Document [6]. Specific details and the background for the review can be found in reports published by the JRC [7,8,9] which formed the basis for the revision of the EC NM definition.

Compared to the definition presented in the previous Recommendation, the EC NM definition of Recommendation 2022/C 229/01 introduces targeted changes to eliminate terminological ambiguity and improves the implementability of the definition by aligning it to technical and scientific progress. In addition, the new Recommendation has a stronger cross-cutting applicability within all relevant regulatory contexts in EU and national legislation, which is a major objective of the Recommendation. It defines a group of materials called ‘nanomaterials’ based on particle size and, furthermore, excludes certain materials based on their volume specific surface area. The EC NM definition applies to all materials that consist of solid particles, regardless of their origin, which can be natural, incidental, or manufactured, and independently of their agglomeration and aggregation state. The EC NM definition cannot differentiate between hazardous and non-hazardous nanomaterials as neither particle size nor specific surface area can be directly linked to hazard. Hence, identification as nanomaterial does in itself not imply any hazard. Table 1 compares the new definition adopted in Recommendation 2022/C 229/01 and the previous EC NM definition adopted in Recommendation 2011/696/EU.

The EC NM definition has been specifically worded to be easily integrated into (sectoral) legislation, where the definition may be complemented with sector-specific provisions.

The core of the EC NM definition (see also Annex 1) reflects some basic principles and

categorises a material as nanomaterial when the following two conditions are met:

- 1) It consists of solid particles
- 2) 50 % or more of its constituent particles fulfil at least one of the following conditions:
 - (a) One or more external dimensions of the particle are in the size range 1 nm to 100 nm
 - (b) The particle has an elongated shape, such as a rod, fibre or tube, where two external dimensions are smaller than

1 nm and the other dimension is larger than 100 nm

- (c) The particle has a plate-like shape, where one external dimension is smaller than 1 nm and the other dimensions are larger than 100 nm

The EC NM definition specifies one additional property, the volume specific surface area (VSSA), which can be used to demonstrate that a given particulate material is not a nanomaterial. The corresponding exclusion criterion is a VSSA of less than 6 m²/cm³.

Table 1. Comparison of the new and the previous EC NM definitions.

	2022-Recommendation (2022/C 229/01)	2011-Recommendation (2011/696/EU)
Origin of the material	Natural, incidental and manufactured	Natural, incidental and manufactured
Objects addressed	Particles	Particles
State of matter	Solid	Not specified (but 'solid' stated in 'Questions and answers' ^(a))
Relevant particles	Present on their own or as identifiable constituent particles in aggregates or agglomerates	In an unbound state or as an aggregate or as an agglomerate (including particles in agglomerates or aggregates)
'Size' criterion	Particles fulfil at least one of the conditions: <ul style="list-style-type: none"> • one or more external dimensions are in the size range 1 nm to 100 nm • For elongated shapes: two external dimensions are smaller than 1 nm and the other dimension is larger than 100 nm • For plate-like shapes: one external dimension is smaller than 1 nm and the other dimensions are larger than 100 nm 	One or more external dimensions is in the size range 1 nm-100 nm
Explicit inclusion	None (replaced by generic inclusion of all elongated particles with a diameter smaller than 1 nm and length above 100 nm and of plate-shaped particles with a thickness below 1 nm and lateral dimensions above 100 nm, in the relevant size fraction)	Fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials
Upper size limit of particles to be counted	Particles with at least two orthogonal external dimensions larger than 100 µm need not be considered	Not included
VSSA as proxy for PSD to identify nanomaterials	Not included	VSSA > 60 m ² /cm ³
VSSA as exclusion criterion to identify materials which are not nanomaterials	VSSA < 6 m ² /cm ³	Not included
Flexibility of the 50 % threshold	Not included	Lower threshold between 1 % and 50 % possible

^(a) https://ec.europa.eu/environment/chemicals/nanotech/faq/questions_answers_en.htm

This document provides guidance pursuant to Recital (8) of Recommendation 2022/C 229/01. It explains how terms and concepts used in the EC NM definition should be understood. It provides economic operators and regulators with detailed descriptions enabling an in-depth understanding of the EC NM definition. In addition, it includes examples of best practices for effective implementation/application and transparent and robust governance. This guidance does not prejudice how to incorporate a definition of nanomaterial in legislation, nor what the criteria for such a definition could be, which will be addressed in detail in the course of drafting the legal text.

This guidance is developed and published by the European Commission's Joint Research Centre (JRC) on the basis of established technologies and measurement practices. It builds upon the guidance provided in two JRC Science for Policy Reports [10,11] that give an overview of the concepts and terms used in the Recommendation 2011/696/EU [2] and explain how to identify nanomaterials through measurements [11]. This new guidance is associated to Recommendation 2022/C 229/01 for which it replaces the previous guidance [10] concerning concepts and terms used. Regarding the identification of nanomaterials through measurements, the present guidance provides relevant updated information and, furthermore, refers to specific sections in ref. [11] which remain relevant

for Recommendation 2022/C 229/01. It is recommended to consult the two JRC Science for Policy Reports [10,11] in matters related to the previous Recommendation 2011/696/EU, in particular when relevant for legislation in force. Furthermore, this guidance refers to two Test Guidelines (TGs) published by the Organisation for Economic Co-operation and Development (OECD) [12,13] and documentary standards developed by the International Organization for Standardization (ISO) and the European Committee for Standardization (CEN). The TGs and the documentary standards provide supplementary technical and practical information that may help business operators and laboratories in selecting appropriate measurement procedures and techniques for specific materials. Annex 1 provides a non-comprehensive overview of relevant standards.

The guidance describes and explains:

- Definitions of key terms and fundamental concepts used in Recommendation 2022/C 229/01
- A decision tree to help identifying nanomaterials
- Measurement options to consider in the assessment of particulate materials

The guidance does not constitute legal advice. It will be updated in light of technical and scientific progress, as envisioned in Recital (8) of the Recommendation.

Basic principles of the EC NM definition

- The material can be natural, incidental or manufactured
- The EC NM definition applies only to materials in the form of solid particles.
- The EC NM definition is based on the only feature that is common to all nanomaterials: the nanoscale external dimensions of particles. It categorises a material as nanomaterial if 50 % or more of its constituent particles fulfil at least one of the following conditions:
 - one or more external dimensions of the particle are in the size range 1 nm to 100 nm;
 - the particle has an elongated shape, such as a rod, fibre or tube, where two external dimensions are smaller than 1 nm and the other dimension is larger than 100 nm;
 - the particle has a plate-like shape, where one external dimension is smaller than 1 nm and the other dimensions are larger than 100 nm.
- The EC NM definition specifies that a material with a VSSA of less than 6 m²/cm³ is not a nanomaterial.
- The EC NM definition alone cannot differentiate between hazardous and non-hazardous nanomaterials as a definition based only on size cannot differentiate between such materials.

2 Key terms and concepts

The classification of materials according to the EC NM definition is based upon the external dimensions of the particles (of which the material consists) and on the median value of the corresponding particle number-based particle size distribution. If 50 % or more of the particles have external dimensions in the nanoscale, i.e. the range of 1 nm to 100 nm, then the material is classified as nanomaterial. The EC NM definition is generally in line with other approaches worldwide to define nanomaterials, but it is more precise and quantitative than most other existing definitions. For instance, ISO defines nanomaterial as a 'material with any external dimension in the nanoscale', where the term 'nanoscale' is defined as the 'length range approximately from 1 nm to 100 nm' [14]. The ISO definition is developed for general application and it can therefore use forward-looking words such as 'approximately' in order to allow some degree of flexibility for the size limits, which may be desired for certain applications and processes. Conversely, regulatory definitions, such as the EC NM definition, serve legislation, and such flexibility is therefore generally not allowed.

While other nanomaterial definitions might not specify the kind of particle, the EC NM

definition only applies to solid particles that are present on their own and as identifiable constituent particles in agglomerates and aggregates. In addition, the EC NM definition contains one exclusion criterion: materials with a VSSA below $6 \text{ m}^2/\text{cm}^3$ are not nanomaterials. These precise criteria set out the basis for the regulatory implementation of the EC NM definition. The EC NM definition does not provide criteria for hazard or risk assessment.

The EC NM definition is based on objective criteria. The assessment of a material against these criteria should be based on measurements following internationally accepted standards (if available), best practices and the principles of good laboratory practice (GLP). When implementing the EC NM definition in practice, it is necessary to understand the meaning of the core terms and their underlying concepts. This section explains the key terms and relevant concepts and provides illustrative examples, where required, complementary to the Commission Recommendation 2022/C 229/01.

2.1 Nanoscale

The Recommendation refers to the term 'nanoscale' as the size range 1 nm to 100 nm, where one nanometre (nm) is 10^{-9} metre or 0.000000001 m and as stated above it is in line with the ISO definition of 'nanoscale'. There is general scientific consensus that the most prominent size-related changes of material properties occur when the materials' structural sizes are reduced to, or close to, this size range.

The EC NM definition is intended to be used for regulatory purposes within the EU's jurisdiction and, therefore, any degree of flexibility that is not rigorously defined must be

avoided as it can inherently imply ambiguity. Consequently, the Commission adopted a fixed size range of 1 nm to 100 nm in the EC NM definition. It also has a material-independent extension for the lower size limit to ensure that elongated and plate-like shaped particles (see Section 2.2) having one or two external dimensions smaller than 1 nm (and at the same time the other orthogonal dimension(s) larger than 100 nm but smaller than 100 μm) are not excluded.

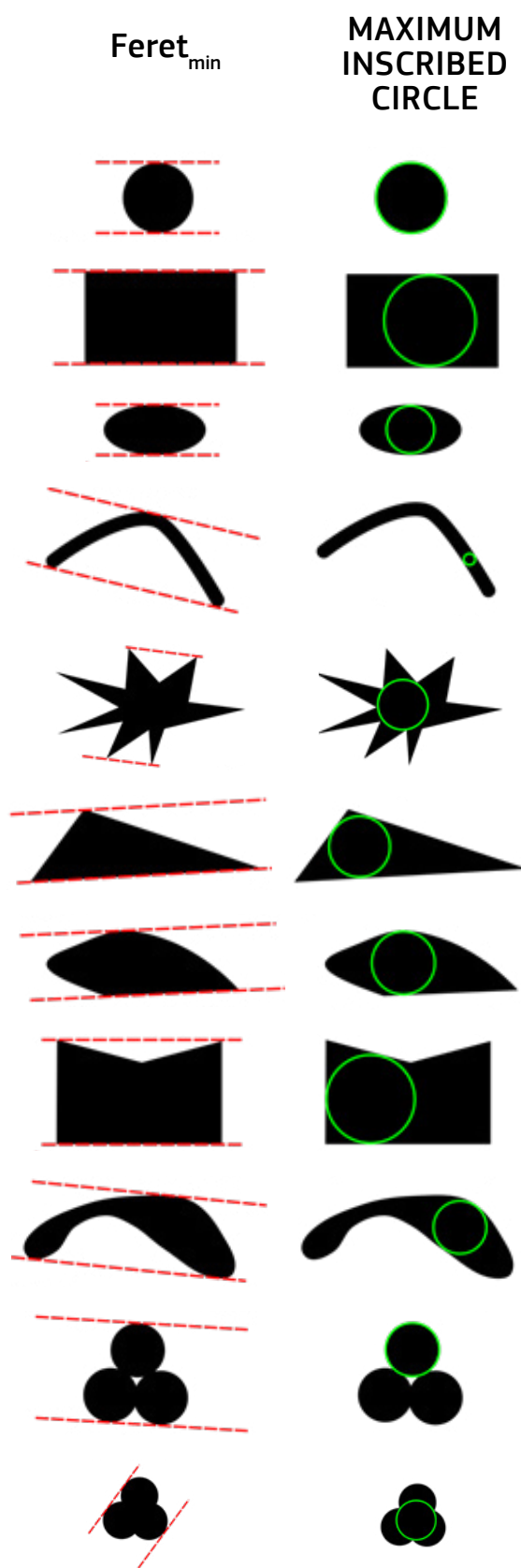
2.2 External dimensions (or ‘particle size’)

The external dimensions (of particles) are the outer dimensions that one can assess. They can, for example, be represented as a Feret diameter, which is the distance between two parallel tangents, or as the diameter of the largest circle that fits inside the virtual envelope of the boundaries of the particle on a 2D image (Figure 1). For macroscopic objects, this would be equivalent to measuring the external dimensions of a particle with a calliper. When representing the external dimension as a Feret diameter, it is clear that only spheres can be represented by a single external dimension, whereas any other particle shape can be characterised by a large number of external dimensions, which depend upon the orientation of the particle (Figure 1). When representing the external dimension as Feret diameter, the associated minimum external dimension is the minimum Feret diameter, i.e. the closest possible distance between two parallel tangents on an object (particle). The EC NM definition requires that at least one of the external dimensions shall be in the range of 1 nm to 100 nm.

The term ‘external dimension’ used in the EC NM definition is more precise than the term ‘particle size’. Different techniques can be used to determine the particle size of materials (see Section 4.2). Most of these techniques do not directly measure the external dimension of the particles. They measure other properties which are correlated with the external dimensions of the particle [15]. Such results are referred to as the ‘equivalent sphere diameters’ (or radii). Thus, the equivalent sphere diameter is the computed diameter of a virtual sphere that creates the same signal response in the particle size measurement process as the particle in question. For example, the output of sedimentation analysis is a distribution of equivalent diameters of spheres that would sediment at the same speed as the particles in the sample.

FIGURE 1

Illustration of two-dimensional projections of particles having different shapes and their external dimensions probed by two morphological parameters.



Source: Modified from Rauscher, H., Mech, A., Gibson, N., Gilliland, D., Held, A., Kestens, V., Koeber, R., Linsinger, T.P.J., Stefaniak, E.A., Identification of nanomaterials through measurements, JRC Science for Policy Report EUR 29942 EN, doi:10.2760/053982, 2019. © European Commission

Note: The last two shapes at the bottom represent agglomerates and aggregates, respectively, for which the minimum Feret diameter ($Feret_{min}$) reflects the entire agglomerate/aggregate, whereas the maximum inscribed circle refers to the constituent particles of the agglomerate/aggregate and not to the entire aggregate.

Other aspects that can have a significant impact on the outcome of particle size measurements are sample preparation (see Section 4.1) and data analysis algorithms. Especially the latter often makes assumptions about specific physical properties such as particle shape, density and refractive index. These assumptions may lead to results that could misrepresent the real particle size distribution.

While the concept of equivalent sphere diameter can be meaningful for particles with a relatively regular shape, the concept loses its meaning when particles have a pronounced elongated or plate-like shape (Figure 2). For this reason, the scope of the EC NM definition does refer neither to ‘particle size’ nor to ‘equivalent sphere diameter’ but uses the term ‘external dimension’.

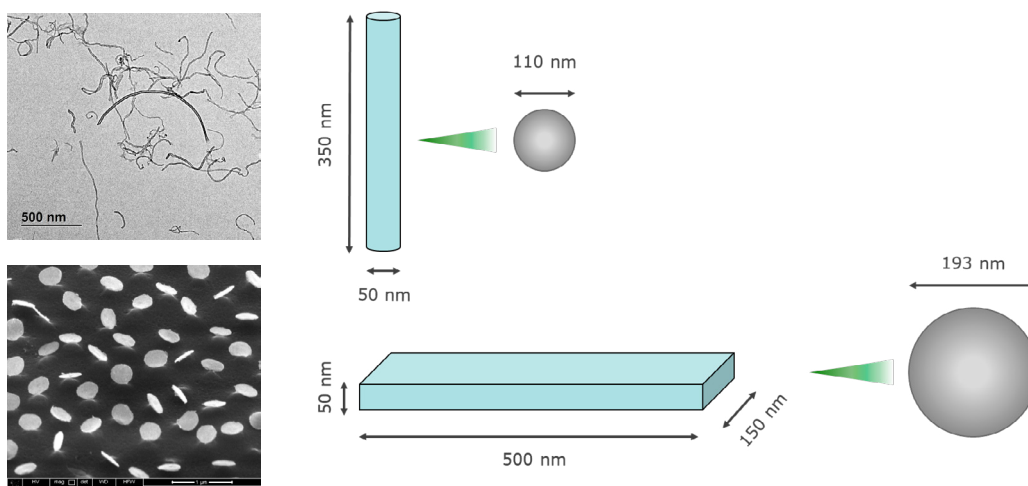
Particles having one out of three orthogonal external dimensions much smaller than the other two are called plate-like particles or platelets, whereas particles with two external dimensions much smaller than the third one may be called fibres, rods or elongated particles. In the case of fibre-like particles the length of the fibres can be ignored when determining the size distribution. In most cases, the only relevant external dimension will be their cross-sectional dimension. For plate-like particles, the dimension defining the thickness will determine whether the material is a nanomaterial.

In the above explanations and examples, the term Feret diameter is used because this parameter is straightforward to visualise and understand, and it meets the requirements of the EC NM definition. If one chooses to use

the Feret diameter to establish a number size distribution, then the most relevant Feret diameter is the minimum Feret diameter, as it is the smallest possible Feret diameter when probed in multiple orientations. However, for the implementation of the EC NM definition, it is not always necessary to establish the minimum Feret diameter of each particle. Any Feret diameter value below 100 nm is sufficient to show that the particle has at least one external dimension smaller than 100 nm.

Another size parameter that can be used for identifying nanomaterials is the maximum inscribed circle. This is the diameter of the largest circle that can be contained within the 2D projection of a particle. As Figure 1 shows, for some (but not all) particle shapes, the maximum inscribed circle diameter can be representative for the external dimension.

Both the minimum Feret diameter and the maximum inscribed circle diameter are direct assessments of the external dimensions of particles on which the EC NM definition is based. For many particles, the two shape parameters will provide similar results. However, for certain types of irregularly shaped particles (e.g. see boomerang- and star-like particle shapes in Figure 1), the minimum Feret parameter may not always probe the minimum external dimensions and, as a consequence, obtained results can be significantly biased. Therefore, especially for particles of irregular shapes, it is recommended to demonstrate the absence of possible shape constraints by acquiring results for both the minimum Feret and the maximum inscribed circle parameters [10].



Source: Modified from Rauscher, H., Mech, A., Gibson, N., Gilliland, D., Held, A., Kestens, V., Koeber, R., Linsinger, T.P.J., Stefaniak, E.A., Identification of nanomaterials through measurements, JRC Science for Policy Report EUR 29942 EN, doi:10.2760/053982, 2019. © European Commission

Note: The illustrative examples show that the volume equivalent sphere diameter concept may not be the best proxy for particles that have highly non-equiaxial shapes.

FIGURE 2

Electron micrographs of multi-wall carbon nanotubes (top) and plate-like gold particles ‘nanocoins’ (bottom) and corresponding simplified particle shapes with illustrative dimensions and their corresponding calculated volume-equivalent sphere diameters.

Equivalent sphere diameters must be used cautiously as they can significantly overestimate the minimum external dimension and thus may result in classifying a material as conventional material (i.e. not a nanomaterial) while it is in fact a nanomaterial (false negative assessment). It is, for example, not meaningful to measure the smallest external dimension of an elongated particle (e.g. nanotube) with a technique providing an equivalent sphere diameter. On the other hand,

measured equivalent diameters in the range 1 nm to 100 nm of particles with compact, near-symmetric (near-equiaxial) shapes may often reflect reality, and it is very likely that at least one of their external dimensions will also be within this range. When it can be shown, or when it is known, that the particles have near-equiaxial shapes, then the measured equivalent sphere diameters can be used to estimate the smallest external dimensions.

2.3 Material and particulate material

The term 'nanomaterial' consists of the prefix 'nano' attached to the noun 'material'. The prefix denotes a group of materials that have external dimensions in a particular size range. In order to define 'nanomaterial', it is necessary to also clarify the term 'material'.

The term 'material' is used in many different fields and disciplines, but there is no agreed universal definition, nor is it defined in legislation. The ISO Online Browsing Platform indicates numerous documentary standards that define 'material' as a 'single substance or a uniformly dispersed mixture of substances'. There is often no clear distinction in the use of the terms 'material' and 'substance', and in fact they are often used interchangeably. However, in a legal context, the term 'material' must not be interpreted as a synonym for 'substance' as the latter can have a significantly different legal definition (see e.g. REACH [16]^a). The term 'material' is general since it should be usable independently from specific legislation and be adaptable to different sectors. It allows straightforward and flexible amendment of specific legislation. When legislation is amended by using the EC NM definition, possibly with additional requirements according to sector-specific needs, legislators can replace the term 'material' by a corresponding term that describes what is covered and regulated by the specific legislation. The legislation will in any case need to include definitions of the terms used in it.

In the context of the EC NM definition, 'material' is used as an overarching term to

describe any type of matter (specifically particulate matter, i.e. matter consisting of particles) without making assumptions about, for instance, its origin, chemical composition and morphology. The words 'consisting of', used in the definition, indicate that the particles are the main compound/component of the material. Other non-particulate components such as impurities, solvents, suspension medium, stabilisers, additives and single molecules, might be present, but must not be taken into account when assessing whether a material is a nanomaterial. Powders and particles in suspension are examples of 'particulate materials', whereas most (consumer) products, such as articles (e.g. fabrics, sports equipment, electronics) and formulations (e.g. sunscreen, paint, inks), are not 'particulate materials' (see Section 2.10). They may contain particles with external dimensions in the nanoscale, but they cannot be classified as nanomaterial as a whole. According to legislation, ingredients, which consist of particles, will be assessed regarding whether they are nanomaterials or not.

The EC NM definition does not address the chemical composition of the material. A particulate material may consist of particles which are all of the same type, i.e. they have essentially the same chemical and structural composition, or it may consist of particles that differ in chemical and/or structural compositions [17]. When assessing a particulate material against the criteria of the EC NM definition for a specific regulatory purpose, the user must be aware of and take into

^a REACH definition of substance: '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'.

account the specific regulatory requirements, i.e. whether the particle type in terms of composition and structure is irrelevant for the analysis or whether individual size distributions must be obtained for different particle types present. A general rule cannot be provided here; the user must follow the requirements of applicable legislation.

There are materials (either particulate or not) that have internal structures at the nanoscale (one example would be highly porous materials such as zeolites). These materials are 'nanostructured materials' [10], but the internal structures are not relevant for the EC NM definition. This means that nanostructured materials are not nanomaterials

according to the EC NM definition, unless the external dimensions of the particles of the material fulfil the requirements of the EC NM definition.

The size of the external dimensions of the particles is the decisive evaluation criterion (except when the material is evaluated to not be a nanomaterial based on a VSSA value below $6 \text{ m}^2/\text{cm}^3$), independently of whether the particles were formed naturally, incidentally or whether they were manufactured intentionally. In that respect, natural materials can exhibit the same properties as those that are manufactured and vice versa.

Nanomaterial and material

- 'Material' is a generic term.
- 'Nano' is a prefix which denotes a group of materials that have external dimensions in the nanoscale size range.
- A nanomaterial consists of a large ensemble of particles (i.e. particulate matter).
- Non-particulate components can be present in a material but are not part of its assessment against the criteria of the EC NM definition.
- The origin, chemical composition and morphology of the material/particles is not relevant.
- Legislators can replace the term 'material' by a corresponding term that describes what is regulated in the specific legislation.
- External dimensions can be represented in various ways, e.g. the Feret diameter

2.4 Particle

The EC NM definition limits its scope explicitly to materials consisting of solid 'particles', i.e. particulate matter. In defining the term 'particle', the EC NM definition is in general agreement with the ISO definition which states that a particle is a minute piece of matter with defined physical boundaries [18].

The word 'minute' in the definition provides a relative indication that the particle or the piece of matter is very small or miniscule. For the interpretation of the EC NM definition, the word 'minute' only provides a qualitative indication that the material should consist of particles which have very small or miniscule

external dimensions. The EC NM definition states that 'in the determination of the particle number-based size distribution, particles with at least two orthogonal external dimensions larger than $100 \mu\text{m}$ need not be considered'. In practice, the size threshold of $100 \mu\text{m}$ is an arbitrary cut off value which laboratories may use as the upper limit of the number-based size distribution.

To be considered as a particle the piece of matter must have a defined physical boundary or interface. For particles which are 'present on their own', the boundary forms the perimeter that exactly contains the entire particle.

It provides a clear demarcation between the particle and the continuous surrounding bulk matter which has a physical state (i.e. gas, liquid) that is different from that of the particle. In the case of 'constituent particles in aggregates or agglomerates', the 'other side' of the boundary may be a continuous phase (i.e. gas, liquid) or e.g. another particle. A particle can consist of more than one chemical compound, for example a core with a firmly attached surrounding surface layer. For such particles the particle boundary coincides with the edge of the most outer layer (Figure 3).

Defined physical boundaries also separate crystallites in polycrystalline solid matter but these pre-existing intergranular boundaries do not constitute 'particle boundaries'. These crystallites are called grains, and they are usually formed during solidification of a liquid. Polycrystalline solid matter may be used as a basis for the preparation of nanomaterials,

e.g. comminution processes by milling or grinding. The presence of grains alone does not make these materials particulate materials, and therefore grains are not considered particles. Only the external dimensions of the particles newly created in the comminution process are particle boundaries.

The boundary of a particle is the main property for identifying a constituent particle. This identification may be challenging for a heavily sintered aggregate where constituent particles may be partly fused. In such cases, the knowledge of the production process can serve to distinguish polycrystalline materials (which are not nanomaterials) from heavily aggregated materials (which may be nanomaterials as their constituent particles may be smaller than 100 nm). Please consult section 2.5 for further information.

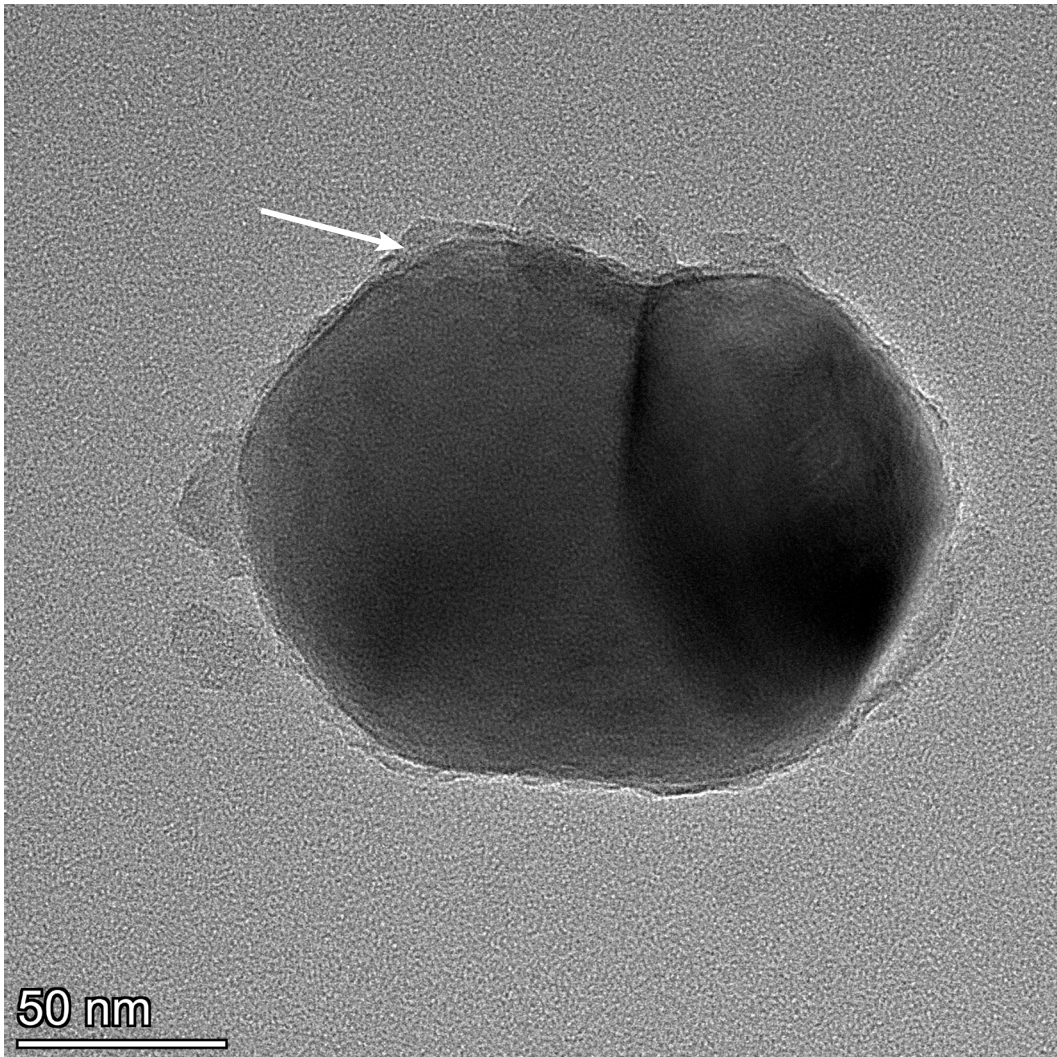


FIGURE 3

High-resolution TEM micrograph of a single titanium dioxide particle.

Source: Own elaboration. © European Commission

Note: The titanium dioxide particle (dark area) is surrounded by a thin (~4 nm) layer of aluminium oxide (white arrow); the aluminium oxide layer forms an integral part of the particle.

2.5 Aggregate, agglomerate and identifiable constituent particle

In the EC NM Definition, the term 'aggregate' means a particle comprising of strongly bound or fused particles. A distinction is made with an 'agglomerate' which refers to 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. Both explanations in the definition are based on the definitions given in ISO 26824 [18] that also contains two informative notes. One note explains that aggregates are held together by strong forces such as for example resulting from covalent bonds or because of sintering. The other note makes a distinction between the original source particles (also called primary particles in ISO 26824) and ensembles such as aggregates that are formed from these (secondary particles).

Agglomerates and aggregates are thus secondary structures that are made up of smaller particles. In the context of the EC NM definition, the smallest indivisible unit of an agglomerate/aggregate is called a 'constituent particle'^b. Agglomerates can consist of a mix of weakly bound constituent particles and smaller aggregates. To be able to include the constituent particles in the number of particles identified in the material, they must be (morphologically) identifiable in order to determine their external dimensions.

The main difference between agglomerates and aggregates is determined by the strength of the bonds between constituent particles. Agglomerates are loosely bound and may disintegrate, or be split, into their individual constituent particles, whereas it is often impossible to break up aggregates without destroying the constituent particles. Aggregates and agglomerates themselves can also interact and form larger agglomerates/aggregates. The secondary structure of this mix of aggregates and agglomerates can be complex and dynamic in size: the number

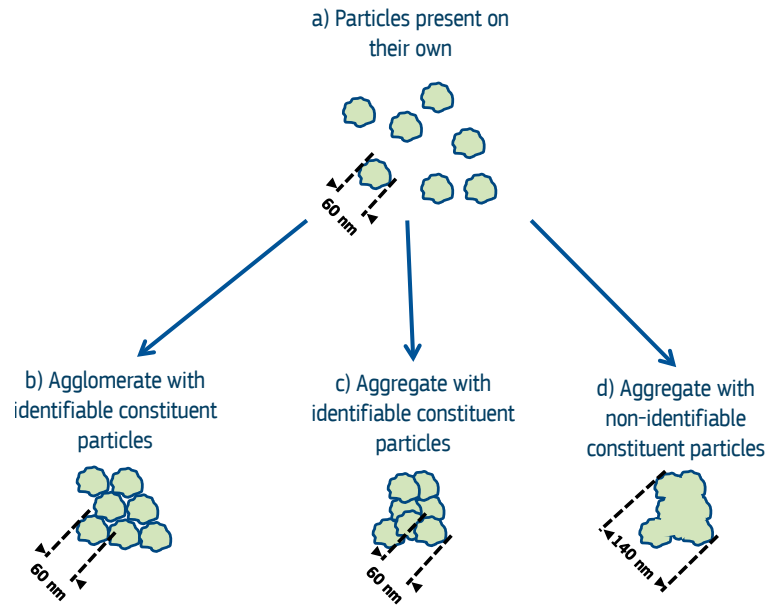
of constituent particles in a larger unit can change, especially in agglomerates. This is the main reason why the EC NM definition is based on the external dimensions of the constituent particles, which is a more stable feature, even if the constituent particles may sometimes be difficult to measure. Therefore, while the implementation of the EC NM definition does not require distinguishing between aggregates and agglomerates, the difference between aggregates and agglomerates can influence the selection of suitable measurement methods.

The external dimensions of the constituent particles of agglomerates may be determined most easily when the bonds between the constituent particles of a representative amount of the agglomerates are broken thereby yielding dispersed particles that are present on their own. Breaking the secondary structures requires that the constituent particles are held together by weak forces only and that a sufficient amount of energy is applied, for example, by means of sonication (see Section 4.1). A validated dispersion protocol is one way to ensure reproducibility when dispersing agglomerates. Figure 4 shows a graphical representation of four hypothetical scenarios of different types of particles: a) constituent particles present on their own, b) identifiable constituent particles present in agglomerates, c) identifiable constituent particles in aggregates, d) non-identifiable constituent particles in aggregates. If it can be demonstrated that after application of a dispersion protocol the material consists primarily of de-agglomerated particles that are present on their own, then any measurement method that produces meaningful number size distributions can be used for the material assessment. More information on the verification of the effectiveness of dispersion protocols can be found in [11].

^b It should be noted that the term 'primary particle', which is not used in the EC NM definition, is nevertheless often used in this context in an incorrect way. Primary particles are the original seeds from which particles grow and are therefore an unrelated concept to constituent particles, aggregates and agglomerates. Therefore, the term 'primary particle' is irrelevant for the EC NM definition.

FIGURE 4

Schematic illustration of different hypothetical particle structures.



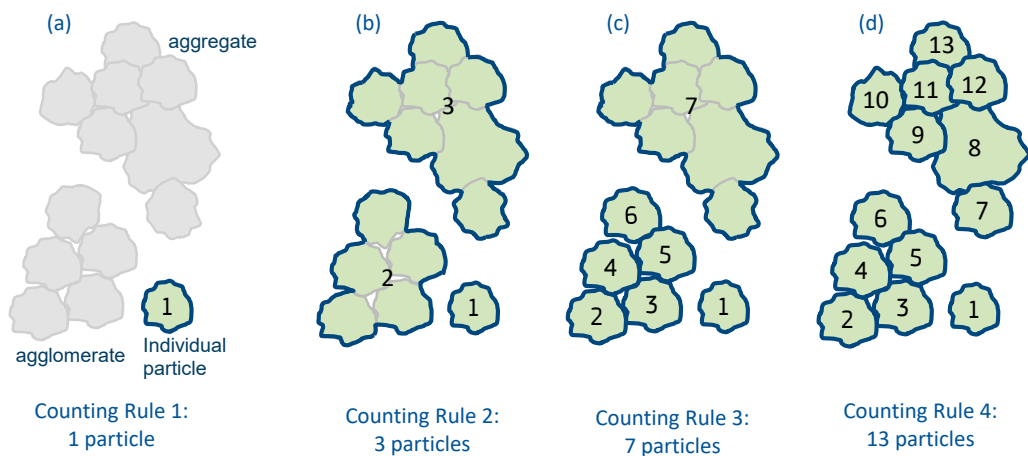
Source: Own elaboration based on figures from Bresch, et al., Counting small particles in electron microscopy images – Proposal for rules and their application in practice, *Nanomaterials* 12 (2022) 2238.

In practice, many samples contain a heterogeneous mixture of different particle structures, which can have important implications for the material assessment. An example is industrial fine powders, which are often heavily aggregated. For this type of materials, even the application of high-energy sonication will not be sufficient to achieve a complete disintegration of the secondary structures and the only solution to reliably assess such materials is by measuring the constituent particles within the agglomerates/aggregates using high-resolution imaging methods

such as transmission electron microscopy (TEM). However, sometimes the constituent particles in aggregates may be so strongly bound or fused together that their boundaries, or interfaces, cannot be discerned anymore. In this case, the aggregate does not consist of ‘identifiable’ constituent particles and the relevant external dimensions are those of the aggregate. Figure 5 illustrates four commonly encountered counting rules for particles. Counting rule 4 is the one applied in Recommendation 2022/C 229/01.

FIGURE 5

Overview of how agglomerates, aggregates and their constituent particles are counted according to different particle counting rules (CR)



Source: Rauscher et al., Is it a nanomaterial in the EU? Three essential elements to work it out, *NanoToday* 49 (2023) 101780.

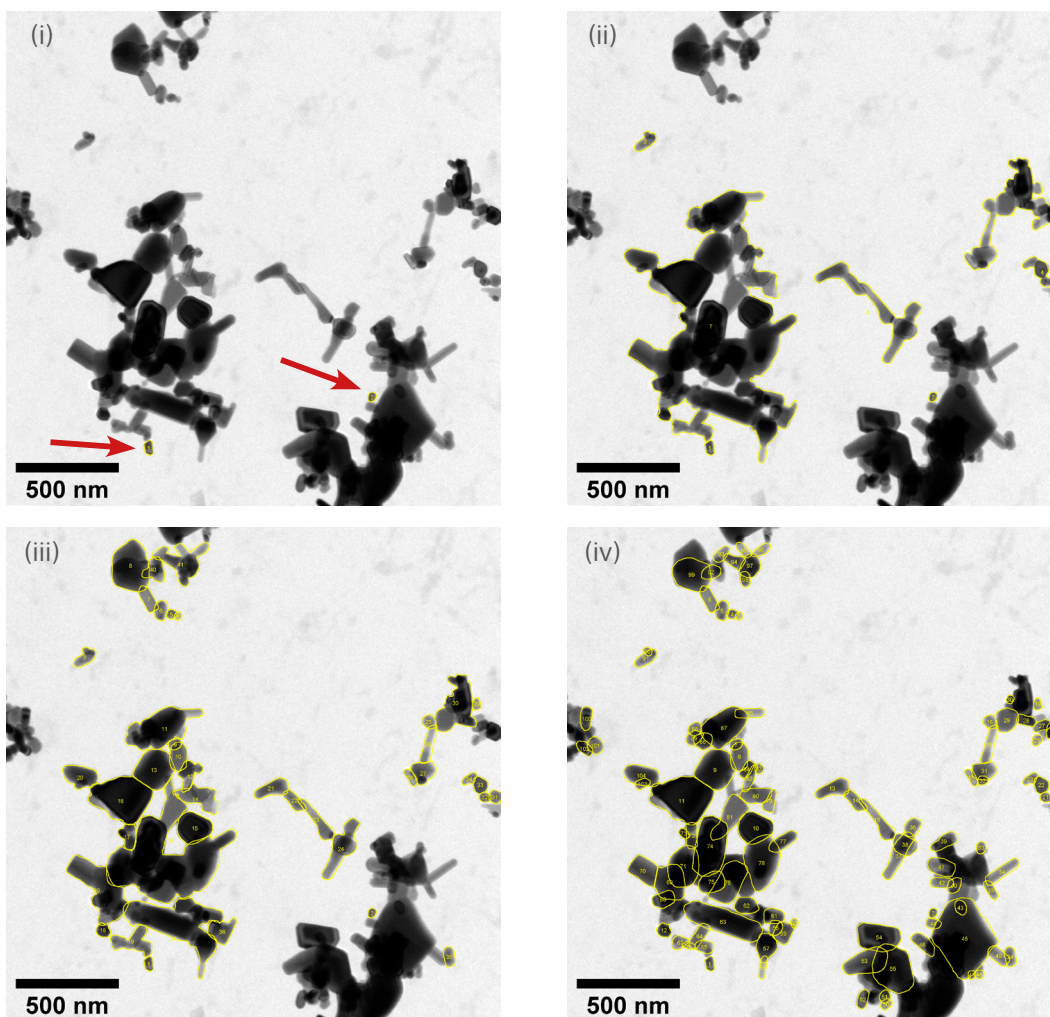
Note: The counted particles are delineated by a bold outline. One of the common CRs excludes agglomerates and aggregates (a), one CR counts them as one particle (b), one CR counts constituent particles inside agglomerates but counts aggregates as one particle (c) and one CR counts all constituent particles of agglomerates and aggregates (d). Individual particles are always counted as one particle. The number of particles counted in the hypothetical example of one individual particle, one agglomerate and one aggregate varies from 1 to 13.

A practical example of identifying and counting particles present on their own, constituent particles, agglomerates and aggregates is shown in Figure 6, which illustrates TEM image analysis and implications of correctly identifying and counting particles. In the example, the same TEM micrograph is analysed and four different rules for counting particles are applied.

In counting rule (1), only constituent particles present on their own are identified and counted, see Figure 6(i). When the material is agglomerated and aggregated, this approach typically identifies only few constituent particles on their own. For counting rule (2), Figure 6(ii), each identified agglomerate and aggregate is counted as one particle, as are also particles present on their own. For counting rule (3), Figure 6(iii), the constituent particles on their own and present in agglomerates are identified and counted individually whereas aggregates are counted as one particle. Counting rule (4), Figure 6(iv), identifies and counts constituent

particles present on their own and present in agglomerates and in aggregates. Counting rule (4) is the one that must be used for counting particles when identifying nanomaterials according to the Recommendation. Details on the evaluation of this image can be found in reference [19]. Please note that when attempting identification of constituent particles in agglomerates or aggregates, a detailed report on the approach and methodology applied for the identification of the constituent particles should always be provided to facilitate and underpin a correct assessment of the validity of the obtained number-based particle size distribution by a regulator or applicant.

Further building on the discussion on the term 'particle' (see Section 2.4), individual components of composite particles, e.g. core-shell structures, must not be considered as identifiable constituent particles, and neither are grains in polycrystalline matter considered as identifiable constituent particles.



Source: edited from Bresch, et al., Counting small particles in electron microscopy images – Proposal for rules and their application in practice, *Nanomaterials*, Vol. 12, Issue 13, 2022, Number 2238.

Note: Indicated by yellow boundaries, and according to the counting rules outlined in the text are (i) Constituent particles present on their own (2 particles, indicated by the red arrows), (ii) Constituent particles present on their own and agglomerates and aggregates (7 particles), (iii) Constituent particles present on their own and in agglomerates, aggregates (41 particles), (iv) Constituent particles present on their own, in agglomerates and in aggregates (104 particles).

FIGURE 6

Analysis of one TEM micrograph of zinc oxide particles that illustrates the differences in number of particles identified in that micrograph when following different counting rules.

During storage, handling, and use of nanomaterials, particles may form agglomerates or aggregates, resulting in the loss, or partial loss, of the originally desired or intended nanomaterial-specific properties. The disappearance of these properties does not transform the original nanomaterial into a conventional material, unless the transformed material consists mainly of particles (or becomes non-particulate) with external dimensions larger than 100 nm in which the constituent particles are no longer identifiable. Similarly,

materials may be dissolvable in specific solvents and/or under specific conditions, which is, however, not a valid criterion for declaring such a material to be a conventional material without assessing it against the EC NM definition. Only after demonstrated dissolution, the original particulate material will no longer consist of particles and therefore an assessment against the criteria of the EC NM definition is no longer applicable.

Agglomerates, aggregates and identifiable constituent particles

- Constituent particles are the smallest possible identifiable individual particles inside an agglomerate and aggregate.
- Agglomerates consist of weakly bound particles.
- Aggregates consist of strongly bound and/or fused particles.
- Agglomerates may be broken down into their constituent particles, but not always.
- Aggregates of fused particles cannot be broken down without altering the constituent particles.
- The particle boundary or interface can be used to identify constituent particles in agglomerates and aggregates.
- For the implementation of the EC NM definition, it is not necessary to distinguish between aggregates and agglomerates. The determining factor is the external dimension of the identifiable constituent particles.

2.6 Solid

The EC NM definition is explicitly restricted to solid particles. In general, the term 'solid' has several meanings. In the EC NM definition it refers to the physical state of matter that is characterised by structural rigidity and resistance to a force applied to its surface.

The term 'solid' is also used to describe an object that has no internal holes or pores, but this is not the intended use in the EC NM definition. For the Recommendation, 'solid' is one of the three classical physical states of matter, the others being liquid and gaseous. Most materials can exist in any of these three states, depending on the external conditions (temperature and pressure).

In contrast to liquid and gases, matter in solid state (at a temperature of 25 °C / 298.15 K, and a pressure of 1 atm / 101,325 kPa) has a definite shape; it does not flow or deform under applied shear stress to take the shape of its container nor does it expand to fill an entire available volume. In contrast to a solid, the atoms or molecules in a liquid (or gas) do not have fixed average positions relative to each other.

These criteria are in most cases sufficient to decide whether a material is solid. In practice, it is sufficient to assess whether a bulk material (i.e. amount of material within which component parts are not initially distinguishable on the macroscopic level [20]) is solid.

The restriction to solid particles ensures that non-solid (i.e. liquid and gaseous) particles, which are easily deformable and thus have external dimensions that are highly dynamic, are excluded from the scope of the EC NM definition. Well-known examples of non-solid particles are, amongst others, fog droplets, micelles, liposomes, vesicles, air bubbles, and droplets in emulsions, including nano-emulsions [21].

From the above classification it is also evident that single molecules are neither solid nor liquid, because the classification can only be applied to ensembles or systems large enough to form a phase for which the physical state (solid, liquid, gaseous) can be assessed; single macromolecules should not be considered as a particle. This is one reason why single molecules (see Section 2.7) do not fall under the EC NM definition.

Solid particles

- The EC NM definition of a nanomaterial covers only particles that are solid.
- Atoms or molecules in a solid have fixed average positions relative to each other, in contrast to a liquid or gas.
- Classification as a solid can only be applied to ensembles or systems large enough to form a phase for which the physical state (solid, liquid, gaseous) can be assessed.

2.7 Single molecule

A 'molecule' is an electrically neutral entity of two or more atoms that are generally held together by covalent bonds. When many molecules of the same kind are in close proximity they may (depending on the temperature and pressure) spontaneously cluster, hold together under influence of attractive forces, and may rearrange into ordered systems thereby forming a piece of solid matter (see Section 2.4) if its physical state at 25 °C / 298.15 K and 1 atm / 101,325 kPa is solid. Single molecules are neither solid nor liquid or gaseous as these states of matter can be attributed only to ensembles large enough to form a physical phase. A phase is a form of matter that is uniform throughout in chemical composition and physical state. Thus, a single molecule on its own cannot form a piece of matter (e.g. a solid particle) and, as a result, it does not fall under the scope of the EC NM definition. Single molecules consist of a well-defined and possibly large number of atoms. However, if a physical phase can

be attributed to a piece of matter, then that piece of matter is not a single molecule. This general argument does not only distinguish single molecules from particles, it also provides evidence that a given piece of matter is not a single molecule. These two discriminators, a well-defined number of atoms and the attribution of a physical state only to an ensemble of particles differentiate single molecules and nanoparticles, are useful in understanding for example synthetic polymers. Individual synthetic polymer molecules have a well-defined number of atoms, however the polymer molecules are almost never available individually, but form an ensemble (consisting of more molecules) that is a piece of matter, which can be at the nanoscale.

The exclusion of 'single molecules' implies that fullerenes are not considered particles as they are well-defined molecules (e.g. C₆₀, C₇₀).

Single molecules

- Single molecules are neither solid nor liquid or gaseous as these states of matter can be attributed only to ensembles large enough to form a physical phase.
- Single molecules are therefore not considered (solid) particles.

2.8 Specific surface area by volume

The EC NM definition identifies nanomaterials based on the median value of the number size distribution of the external dimensions of solid particles. For many materials it may be relatively easy to demonstrate that a material is a nanomaterial but at the same time it can be challenging to demonstrate that a material is not a nanomaterial. This is because from the number-based particle size distribution one can never completely rule out the possibility of not having detected significant number fractions of particles, especially when those particles have sizes close to, or beyond, the lower limit of quantification of the applied method. To provide economic operators with an easy solution to

demonstrate that a given material is not a nanomaterial, the Recommendation contains an exclusion criterion that states that a material with a specific surface area by volume of less than 6 m²/cm³ is not a nanomaterial.

The specific surface area (SSA) of a particulate material (in m²/g) is derived from the value of the surface area SA (in m²) of a powder sample and its mass m (in g): SSA = SA/m. The International Union of Pure and Applied Chemistry (IUPAC) defines the SSA in the following way: 'When the area of the interface between two phases is proportional to the mass of one of the phases (e.g. for a solid adsorbent, for an emulsion, or for an

aerosol), the specific surface area [...] is defined as the surface area divided by the mass of the relevant phase' [22].

In 2022, ISO published a revised standard for the experimental determination of the overall SSA of either powders or porous solid materials by measuring the amount of physically adsorbed gas according to the Brunauer, Emmet and Teller (BET) method [23]. The overall SSA includes all accessible internal and external surfaces. The EC NM definition does not specify the method to be used for determining the SSA. However, because of its normative nature, BET remains by far the method most commonly used. In BET, nitrogen is often the probe gas (adsorptive) of choice, but other gases, such as argon, can be used as well. Interlaboratory comparisons on nanomaterials have demonstrated that BET measurements can be reproducible [24,25].

The VSSA is very similar to the SSA; the only difference is that the surface area is normalised against the volume of the powder sample instead of its mass. One can therefore adjust the IUPAC definition of SSA to obtain the following definition for VSSA: 'when the area of the interface between two phases is proportional to the volume of one of the phases (e.g. for a solid adsorbent, for an emulsion or for an aerosol), the volume specific surface area, or VSSA, is defined as the surface area SA divided by the volume V of the relevant phase: $VSSA = SA/V$. For a non-porous particulate material this means that the VSSA is equal to the sum of the surface areas s_{a_i} of all the particles divided by the sum of the volumes v_i of all the particles [26], and also that $VSSA = SSA \times \rho$ where ρ is the material density. Thus:

$$VSSA = \frac{SA}{V} = \frac{\sum_i s_{a_i}}{\sum_i v_i} = SSA \times \rho \quad (1)$$

The density value used to convert SSA to VSSA is the (average) density of the 'relevant phase'. For example in the case of a solid non-porous TiO₂ powder, the density value used to convert the SSA to VSSA should be the density of the TiO₂ phase, not the overall 'bulk powder density'. In practice, the density value used is often the 'skeletal density' as determined by the helium pycnometry method [27,28]. In many cases this will be equal or close to the known 'true density' available for most materials, for example, in

the Handbook of Chemistry and Physics [29]. It should be noted that open particle porosity (particles have pores not totally enclosed by their walls and open to the surface either directly or by interconnecting with other pores), while increasing the surface area and decreasing overall particle density (or 'envelope density'), do not in principle affect the skeletal density. The latter is affected if there is a significant volume fraction of non-gas-accessible voids (or 'closed pores') present within the particles [27]. It may be argued that this would be unlikely for very fine particulate materials.

It is possible to estimate the VSSA from transmission electron tomography studies [30] for non-porous, non-aggregated particles that have a reasonably uniform shape and size, but this method is highly impractical or unfeasible for materials consisting of porous or very irregularly shaped particles.

Further details on VSSA [31] and the method used for assessing the VSSA are available in the OECD TG 124 [13].

The specific surface area by volume, or volume specific surface area (VSSA), is a property of powders that is closely related to the mean particle size. A powdery material consisting of perfectly monodisperse non-porous spherical particles of 100 nm diameter has a VSSA of 60 m²/cm³; larger spherical particles have lower VSSA values. Because of the way it is measured, VSSA is not strongly affected by agglomeration, so that agglomerates will have a VSSA similar to the sum of their constituent particles. This is, however, different for aggregates, which have a significantly smaller VSSA than the sum of their constituent particles. A very low VSSA value of 6 m²/cm³ implies a mean particle diameter of 1000 nm. Adding an equal number of particles (or slightly more) with a diameter of less than 100 nm will not have a significant effect on the measured VSSA value. For this reason, a VSSA value of less than 6 m²/cm³ is an appropriate criterion for identifying conventional materials (i.e. materials that are not nanomaterials).

The EC NM definition does not provide for the use of VSSA as proxy for particle size, i.e. this means that VSSA cannot be used to identify materials as nanomaterials. The VSSA of a powder does not only depend on the size of the particles, but also on the particle shape and porosity. As explained in Section 2.2,

many industrially relevant particles have shapes that are not spherical. A sphere is the shape with the lowest VSSA and any deviation from sphericity leads to an increase in the VSSA. Therefore, the VSSA value of $60 \text{ m}^2/\text{cm}^3$, which is a characteristic value for 100 nm spheres, would need to be corrected in accordance with the effective shape, and porosity, of the particles, in order to reliably

estimate the external particle dimensions. This approach is not only complicated, it also poses large measurement uncertainties which can make an unambiguous material assessment difficult.

Volume specific surface area

- For a particulate material the VSSA is equal to the sum of the surface areas of all particles divided by the sum of the volumes of all particles.
- A material with a VSSA of less than $6 \text{ m}^2/\text{cm}^3$ is not a nanomaterial.

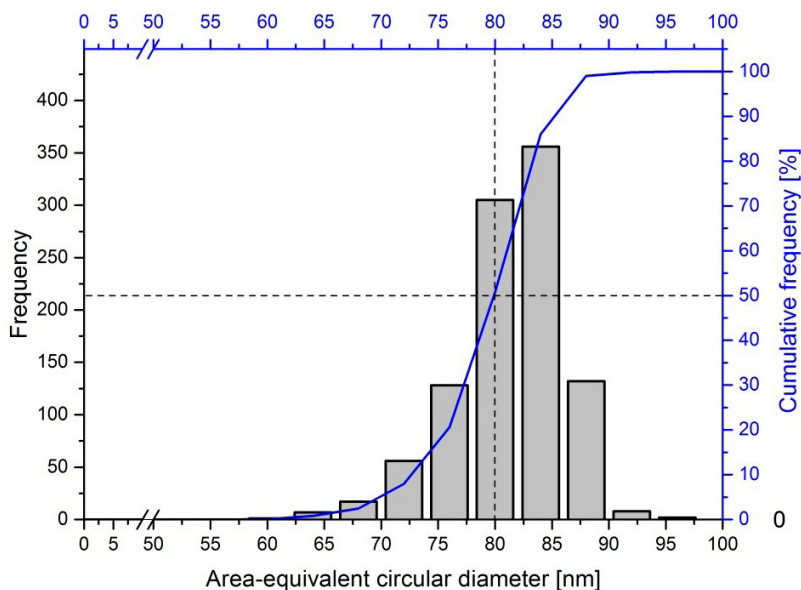
2.9 Particle number-based particle size distribution

The term ‘number size distribution’ is a short term for ‘particle number-based (or -weighted) particle size distribution’.

The simplest representation of a number size distribution is a histogram, which is a graph that shows how the size of particles in a powder, or in dispersion, is distributed between a lowest and a highest size value. Typically, the abscissa (x-axis) corresponds to the particle size information, and the ordinate (y-axis) shows the information on the number of particles of a certain size. To construct the histogram (Figure 7), particles are grouped in ‘bins’ (small sections or particle size intervals on the x-axis).

Particle size data are also often represented as a cumulative distribution (Figure 7 blue line), which can be deduced from the information shown in a histogram. The cumulative distribution is very convenient to determine the median value, x_{50} , of the number size distribution (from the intersection of the two dashed lines in Figure 7). It is the size of the particles that divides the particle population in two groups containing an equal number of particles. One group contains all particles smaller than the median size and the other group all those larger than the median size, x_{50} .

FIGURE 7
Number size distribution for the certified reference material ERM-FD101b by TEM [32]: histogram (grey) and cumulative distribution (blue line).



Source: Rauscher, H., Roebben, G., Mech, A., Gibson, N., Kestens, V., Linsinger, T.P.J., Riego Sintes, J., An overview of concepts and terms used in the European Commission’s definition of nanomaterial, EUR 29647, Publications Office of the European Union, Luxembourg, 2018, doi:10.2760/459136. © European Commission

Note: The median value of the number-based size distribution (x_{50}) is given by the intersection of the two dashed lines. In this example it is 80 nm.

For a number size distribution, the amount of particles in each size bin is simply the number of particles in the bin. This corresponds with the most direct way of counting particles, for example with a microscope. However, most other particle size analysis techniques produce other types of size distributions, based on the raw or converted measured signal produced by the particles of a certain size. This signal can be proportional to their mass, or to their surface area, or to the intensity of their scattered light, or a range of other parameters, usually covered with the term 'intensity'. It is generally not straightforward, and usually significantly amplifies errors, to transform the resulting 'mass based size distribution' or 'surface area based size distribution' or 'scattered light intensity based size distribution' into a 'number based size

distribution' [33,34]. Only in the case where the shape and material properties of the particles are regular, accurately known and do not vary between particles and where the initially measured size distribution is known with sufficient precision (sufficient number of size bins, sufficient number of particles per size bin) and where the difference in size between largest and smallest particles is small, can such transformations lead to reliable number size distributions. The uncertainty of measurement will be reflected as well in the result; and in the example shown in Table 2 for a certified reference material illustrates that in addition to an uncertainty associated to a specific method, there is also a variation in the result depending on which method is applied.

Table 2. Example of a certified reference material, giving combinations of measurement methods and measured values, as well as the associated uncertainty (data from ref 32).

SILICA NANOPARTICLES IN AQUEOUS SOLUTION			
	Size distribution parameter: Weighting / Averaging	Certified value ⁷⁾ [nm]	Uncertainty ⁸⁾ [nm]
Hydrodynamic diameter from DLS ¹⁾ (cumulants method)	Scattered light intensity -weighted / harmonic mean	89.5	2.3
Hydrodynamic diameter from DLS ²⁾ (distribution calculation algorithms)	Scattered light intensity-weighted / mean (arithmetic, harmonic, geometric) and modal	93	4
Hydrodynamic diameter from PTA ³⁾	Number-weighted / modal	82	4
	Number-weighted / arithmetic mean	87	4
	Number-weighted / median	82	4
Stokes diameter from CLS ⁴⁾ (turbidimetry)	Light extinction-weighted / modal	87	8
Area-equivalent diameter from EM ⁵⁾	Number-weighted / modal	83.7	2.2
	Number-weighted / median	83.5	2.2
Mean particle diameter from SAXS ⁶⁾ (model fitting)	Scattered X-ray intensity -weighted / modal	82.5	1.8
	Volume-weighted / modal	81.7	1.8
	Number-weighted / modal	80.9	1.7

Table 2, continued

- ¹⁾ As obtained with dynamic light scattering (DLS) according to ISO 22412:2008 applying the cumulants method described in ISO 13321:1996 at a sample temperature of 25 °C.
- ²⁾ As obtained with dynamic light scattering (DLS) applying distribution calculation algorithms such as non-negative least square (NNLS) and CONTIN for data analysis, using various averaging approaches and a sample temperature of 25 °C.
- ³⁾ As obtained with particle tracking analysis (PTA) according to ISO 19430:2016 at a sample temperature of 25 °C.
- ⁴⁾ As obtained with centrifugal liquid sedimentation (CLS) according to ISO 13318-1:2001, using an effective particle density of 2.0 g/cm³ at sample temperatures between 25 °C and 36 °C.
- ⁵⁾ As obtained with transmission and scanning electron microscopy (EM), counting only particles with an equivalent diameter larger than 60 nm.
- ⁶⁾ As obtained with small-angle X-ray scattering (SAXS) according to ISO 17867:2015 at sample temperatures between 23 °C and 25 °C, using 'model fitting' assuming homogeneous spheres and a Gaussian size distribution.
- ⁷⁾ Unweighted mean value of the means of accepted sets of data; each set being obtained in a different laboratory and/or with a different method of determination. The certified value and its uncertainty are traceable to the International System of Units (SI).
- ⁸⁾ The uncertainty of the certified value is the expanded uncertainty with a coverage factor $k = 2$ corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008.

It should be noted that a particle fraction of 50 % with one or more external dimensions smaller than 100 nm in a number size distribution is always less than 50 % in any other

usual size distribution metric, such as intensity, or particle volume or particle mass. In fact it can correspond to a tiny fraction of the total mass of the material.

Particle number based size distribution

- If 50 % or more of the particles of a material in the number size distribution have one or more external dimension in the size range 1 nm to 100 nm (the nanoscale), then the material is a nanomaterial.
- The fraction of particles with one or more external dimensions in the nanoscale can (depending on the underlying metric of the particle size distribution) be negligible when using the metric total mass of the material and at the same time be significant using the total number of particles of the material as the metric.
- 50 % of particles with one or more external dimensions smaller than 100 nm in a number size distribution are, for the same material, always less than 50 % in any other size distribution metric, such as intensity, or particle surface, volume or mass.

2.10 Nanomaterials in products

A variety of products are regulated in the EU under product-specific legislation. Some of these products contain or have been produced with nanomaterial ingredients and some of them may also have internal structures or surface components in the nanoscale, for example coatings or computer chips.

The EC NM definition is not intended to cover products as defined in EU legislation [35] or components, even if they contain nanomaterials or have an internal structure at the nanoscale. The EC NM definition applies only to the nanomaterial in terms of particulate matter itself. It does not apply to products and articles that contain a fraction of nanomaterial(s). When identifying a material as a nanomaterial or not, only the particulate material, and not the final product integrating the particulate material, should be assessed (unless the product is a particulate material in and of itself). Therefore, even in the

presence of an ingredient(s) that is a nanomaterial(s), a consumer product, article or an end product as a whole cannot be regarded as a nanomaterial. For instance, tyres can contain carbon black and silica nanomaterials as ingredients, but the tyre in itself does not become a nanomaterial. Even if a product is designed to release nanomaterials, or releases nanomaterials as wear debris during use or ageing, the original product does still not become a nanomaterial.

It should be noted that identifying an ingredient of a product as nanomaterial does not allow any conclusions on the actual concentration of particles fulfilling criteria 1(a), 1(b) or 1(c) of the EC NM definition in that product. For this, one needs to know (i) the complete quantitative composition of the product and (ii) the complete number size distribution of the ingredient.

Products containing nanomaterials

- The EC NM definition does not apply to (consumer) products or components in which nanomaterials are integrated.
- Even if a product contains nanomaterials as ingredients, or when it releases nanomaterials during use or ageing, the product itself is not a nanomaterial, unless it exists as a particulate material that meets the criteria of the EC NM definition.



1

2

3 Decision tree (flow chart) to identify nanomaterials

A flow chart, which is a graphic representation of processes, can be a useful tool to produce robust decisions based on a systematic assessment of different system conditions and their expected impacts, and help documenting the assessment of the material. The flow chart, which is a decision tree and presented in Figure 8, outlines a possible effective and cost-efficient strategy for classifying a material according to the EC NM definition. The flow chart starts with a basic sorting of the materials, followed by selecting the route of analysis. The selection

involves obtaining and using information on the material, which is also important for the choice of the analysis method. The decision criteria used in the subsequent nodes directly stem from criteria and thresholds included in the EC NM definition (i.e. median size, x_{50} , smaller than or equal to 100 nm, VSSA smaller than $6 \text{ m}^2/\text{cm}^3$). When following the steps in the flowchart, existing data, when available, representative of the material under evaluation, and appropriate for the evaluation, should be taken into account in order to minimise the testing.

3.1 Basic classification: exclusion based on material properties

The EC NM definition explicitly excludes certain materials. Non-particulate materials, single molecules, materials consisting of non-solid particles (e.g. emulsions and aerosols of liquid droplets) and nanostructured materials that are not agglomerates or aggregates, are not nanomaterials. This basic

classification step is thus a key feature of the flow chart that provides a quick exit strategy for certain types of materials based on specific material properties. Materials, which are not explicitly excluded, can proceed in the flow chart, where they are evaluated using screening and/or confirmatory methods.

3.2 Selection of the analysis route: information on the material and method matching

Materials which cannot be conclusively identified as conventional materials during the basic classification step require evaluation by following the method-specific branches in the flow chart. In the step for selecting the analysis route, the user first needs to select one out of three available routes where materials are analysed using either a screening method

or a confirmatory method. The choice of an appropriate route largely depends on the state of the material (powder or dispersion) and on the availability of specific physico-chemical data (e.g. effective density, refractive index, solubility) which may be crucial for the accurate analysis by the selected screening or confirmatory measurement method(s).

For many dispersion methods it is also important to know whether the particles have a nearly equiaxial or near-spherical shape, since highly non-symmetrical particles can yield significantly biased equivalent sphere diameters (see Section 2.2). If required, the general shape [36,37] of most particles can be determined qualitatively using electron microscopy (EM) analysis. Finally, a thorough knowledge of the measurement technique and the preparation of dispersions and samples is needed to choose the most suitable method(s) for the analysis of a given material. Detailed information on the applicability

of methods and techniques, as well as general protocols and practices, is widely available in handbooks [38,39,40] and documentary standards (see Annex 2) dealing with the characterisation of particles, nanoparticles, and fine powders. In addition, the NanoDefine Methods Manual [41] and the articles published by Gaillard et al. [17] and Mech et al. [42] provide information and illustrative cases on how to decide on the most suitable characterisation method for analysing a specific material, in relation to the requirements of the EC NM definition.

3.3 Screening

The flow chart distinguishes screening techniques/methods (Section 4.2.1) that can characterise a material in a powder state ('dry route') or in dispersion ('dispersion route'). Depending on several criteria, such as the material's dispersibility and physicochemical properties, the availability of suitable techniques, and regulatory requirements, the most suitable path is selected. For instance, a wrong dispersion procedure or inappropriate conditions, may degrade or dissolve the particles. Great care should be taken when dispersing the material, and in general the appropriateness of the dispersion route should be evaluated. If the material is in powder form and can be dispersed in a suitable liquid medium, then the analysis may be performed by either route. If the material cannot be dispersed, then the dry route should be followed.

The selection of the technique will often depend on its availability and in-house skills and expertise; furthermore, appropriate data might already be available and its actual representativeness of the test sample should then be verified, as should its usefulness and validity. Sometimes, regulatory authorities require data from a specific technique or

method, hence obliging economic operators to carry out assessments according to a specific path in the flow chart. When selecting a screening method, it is of utmost importance that the material properties and the applied method's measurement capabilities match (see Section 4). The availability of information on material synthesis/production and provenance can be of great help when selecting a suitable screening method. It has also to be kept in mind that most screening methods require additional material information to allow a reliable (accurate) calculation of the equivalent median particle size, x_{50} , (e.g. optical properties, effective densities, particle shape). If a material is available as suspension (e.g. colloidal material), the user should follow the dispersion route; one exception is that of the preparation of EM specimens which typically require drying of a suspension on a grid/stub.

For some materials, the outcome of a screening method may be inconclusive (see Section 4.2.1). In this case, the material needs to be further analysed using a suitable confirmatory method (Section 4.2.2).

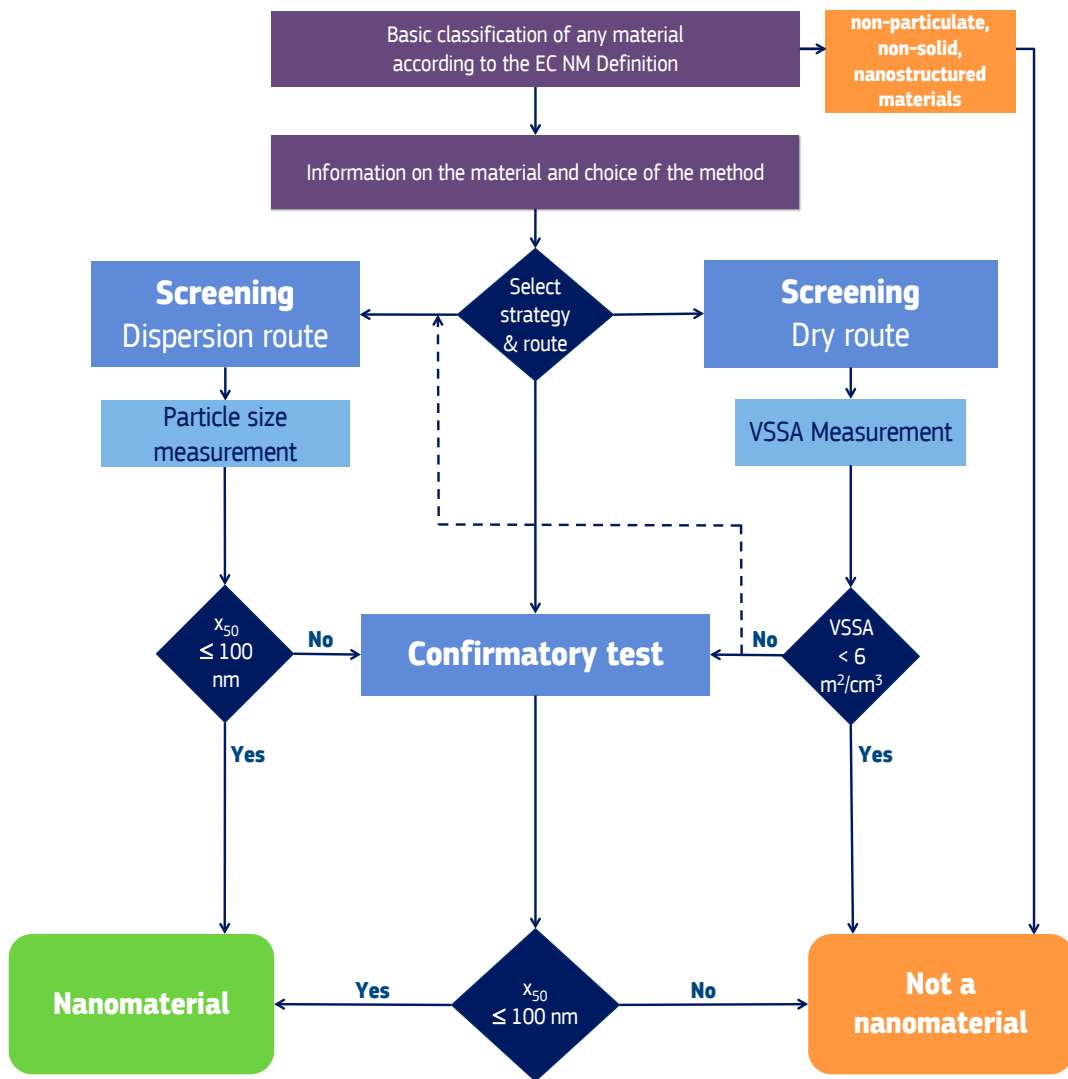


FIGURE 8

Flow chart (decision tree) for possible material classification according to the EC NM definition.

3.3.1 Screening: dispersion route

The dispersion route is applicable to materials which are either provided as a suspension or which need to be dispersed in a liquid phase prior to analysis.

After measurements performed with an appropriate method, compatible with the material (see section 4), the user continues as outlined below, depending on the outcome of the screening.

- If the material has an x_{50} smaller than or equal to 100 nm and if the results are evaluated to be acceptable, this material is a nanomaterial and there is no need for additional confirmatory methods or plausibility checks. However, if the reliability

of the result is questionable the user may perform further measurements either using other screening techniques to check the plausibility of the results, or by using a suitable confirmatory method.

The result of a screening method may be significantly biased due to particle aggregation/agglomeration, a high polydispersity, or when the particles have a non-equiaxial shape. Under such conditions, the validity of the physical measurement principles of the screening technique can be hampered, hence increasing the risk of false negative classifications.

- If the material has an x_{50} larger than 100 nm the use of a suitable confirmatory method is necessary

At any stage of analysis via the dispersion route, the user can decide to switch to a confirmatory method.

3.3.2 Screening: dry route

The definition includes a criterion that allows the exclusion of a material from the EC NM definition based on measurement of the VSSA. If the VSSA of a powdery material is less than $6 \text{ m}^2/\text{cm}^3$, then the material is not a nanomaterial and no further analysis is necessary. The VSSA may be determined following OECD TG 124 [13] or by a suitable alternative procedure (e.g. electron tomography) that includes a reliable measurement of the SSA. If appropriate data are already available and considered reliable, valid and representative, they may be used at this stage. If the VSSA is equal to or larger than

$6 \text{ m}^2/\text{cm}^3$, then the user should proceed to the confirmatory step or switch to the dispersion route (dashed arrow in the flow chart) provided the powder can be dispersed effectively in a suitable liquid medium.

When using the gas adsorption-based BET method for determining the VSSA of powders, care must be taken to select an adsorptive gas (e.g. N_2 , Ar, Kr) that is suitable for the specific material. More details and instructions on selecting an appropriate adsorptive gas can be found in reference [23].

3.4 Confirming

An analysis using confirmatory methods ('confirmatory test') is required when the results from screening methods are inconclusive or when those methods cannot be applied to a given material. Alternatively, confirmatory methods may also be chosen from the very beginning or at every step in the classification process.

The confirmatory step (see Section 4) always involves the analysis of micrographs acquired with microscopy-based methods (e.g. EM, AFM). Almost any particulate material can be analysed by EM unless it is sensitive to the high energy electron beam and/or to high vacuum conditions. Such sensitive materials may be analysed by variable pressure and/or low current EM, but even with those methods, sample preparation may remain an issue.

If image analysis gives x_{50} larger than 100 nm, then the material is classified as a

conventional material. If the resulting x_{50} is smaller than or equal to 100 nm, the material is classified as nanomaterial, see Figure 8.

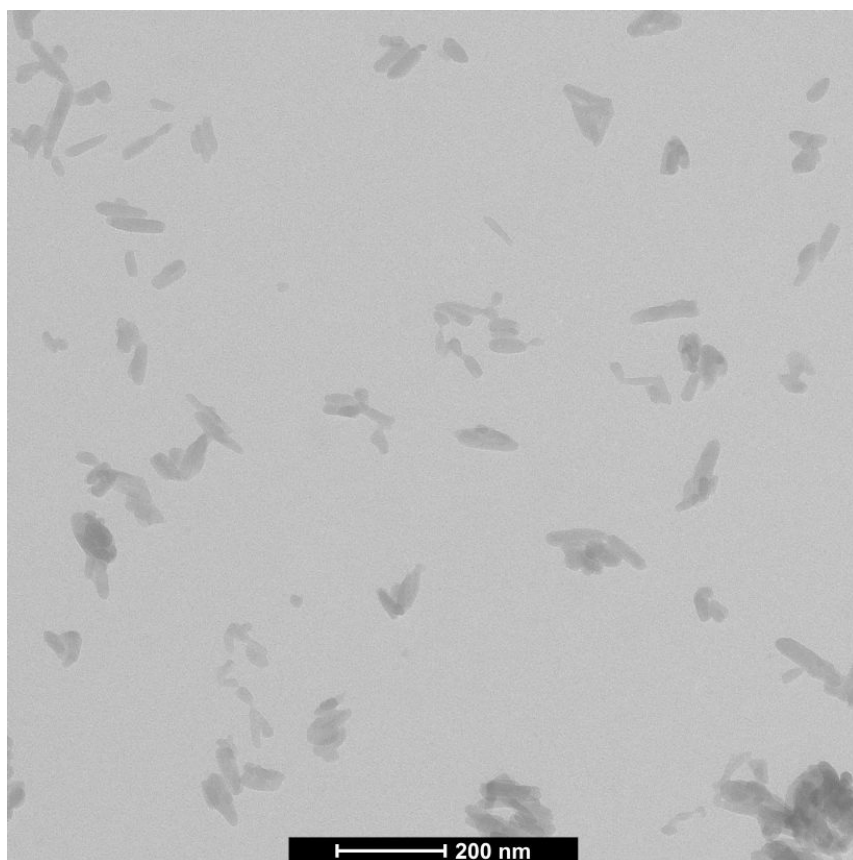
Note that according to the criteria specified in Article 1 (b) and Article 1 (c) of the Recommendation, (i) elongated particles with two external dimensions smaller than 1 nm and the other dimension longer than 100 nm and (ii) plate-like particles, where one dimension is smaller than 1 nm and the other dimensions are larger than 100 nm, have to be taken into account in the analysis of the number size distribution.

It is, however, not necessary to include particles with at least two orthogonal dimensions larger than 100 μm (i.e. particles with approximately equiaxial shape and plate-like particles) in the particle size distribution.

3.5 Practical example: Flow chart (decision tree) applied to Pigment Yellow 83

In this section, the application of the flow chart (decision tree) is explained for a specific material using publicly available data from the EU FP7 research project NanoDefine (Grant Agreement No. 604347) [43]. The material is the organic Pigment Yellow 83 (IRMM-380), a representative EM image along with

basic physicochemical properties is presented in Figure 9. Details of the methods, sample preparation, applied standard operating procedure, measurement uncertainty considerations and obtained size distribution can be found in Babick et al. [44].



Source: EU FP7 project NanoDefine, Deliverable D1.3, EU Grant Agreement No. 604347

The path followed for this material in the flow chart (decision tree) is indicated by the red arrows in Figure 10. Entering the flow chart (decision tree) the material cannot be conclusively identified as conventional material, because the material is particulate, solid and not a nanostructured material. Therefore the decision is made to perform a screening following the dry route. BET measurement and evaluation of the results gives a VSSA value of $93 \text{ m}^2/\text{cm}^3$. As this value is larger than the value of $6 \text{ m}^2/\text{cm}^3$, it cannot be

concluded that the material is not a nanomaterial. Hence, the x_{50} of the material is confirmed using TEM. As this material consists of elongated particles, the appropriate external dimension to be evaluated has to be carefully selected keeping in mind the definition of nanomaterial. In this case the minimum Feret diameter is selected as appropriate external dimension. The analysis and evaluation of the TEM images gives an x_{50} of 39 nm, thereby classifying the material as nanomaterial.

FIGURE 9

TEM image of Pigment Yellow 83 particles and basic physicochemical properties

Trade name:
Pigment Yellow 83

Trade form:
powder

Particle shape:
elongated

Skeleton density (by He-pycnometry):
 $1.484 \text{ g}/\text{cm}^3$

Specific surface area by volume:
 $93 \text{ m}^2/\text{cm}^3$

Material class:
organic

Sum formula:
 $\text{C}_{36}\text{H}_{32}\text{Cl}_4\text{N}_6\text{O}_8$

x_{50} by TEM:
39 nm

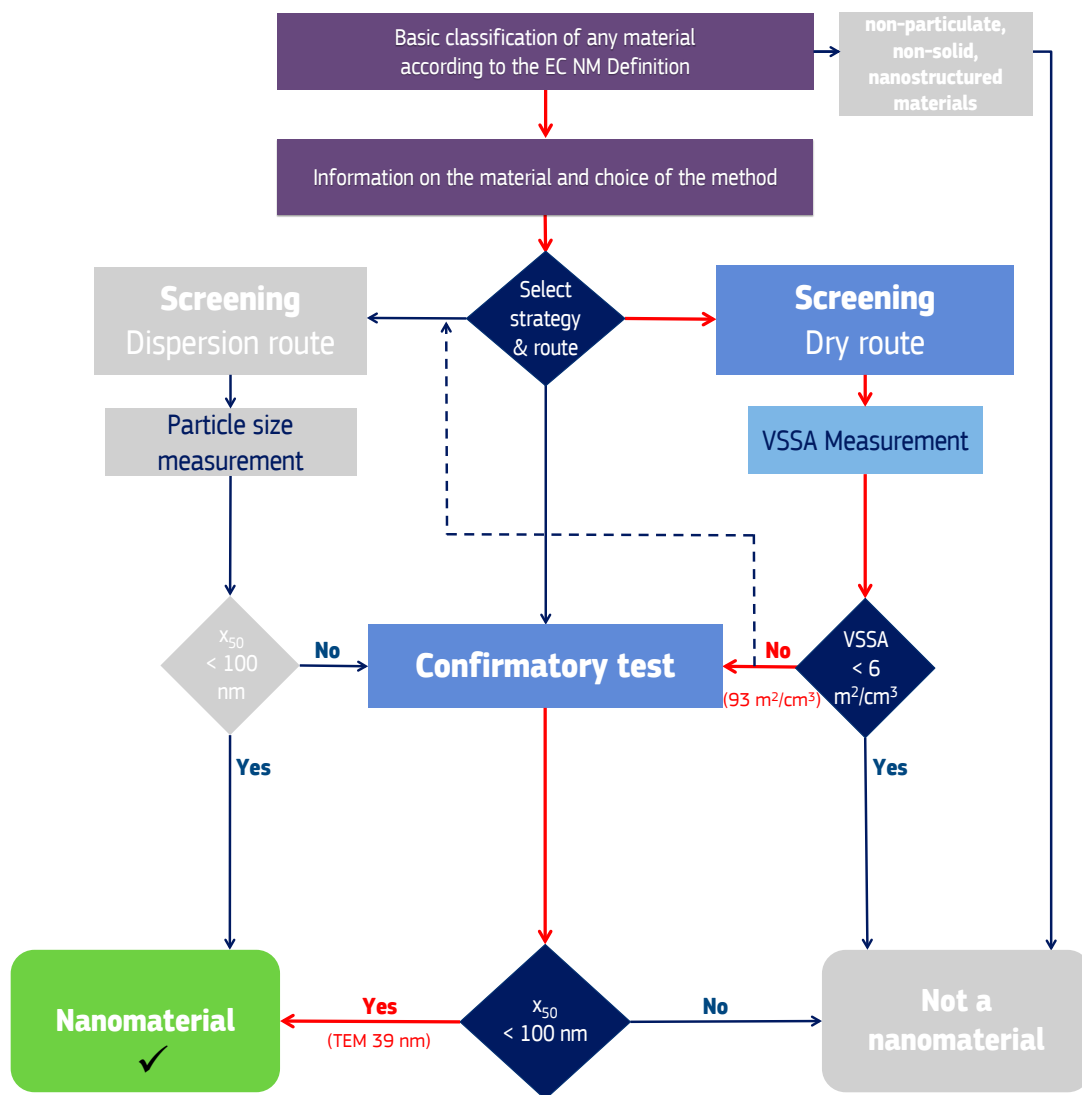


FIGURE 10

Flow chart (decision tree) used for Pigment Yellow 83

Source: based on data from the EU FP7 project NanoDefine [43]

4. Identification of nanomaterials through measurements

The identification of nanomaterials through measurements is based on measured data on the external dimensions of the constituent particles. Furthermore, the VSSA can be used to classify a material as a conventional material. Data might already be available and their appropriateness, usefulness, validity and representativeness should be verified. Any studies should generally adhere to good measurement and laboratory practice [45], including using validated methods, (certified) reference materials and standardised procedures [46]. Well-maintained instruments, appropriately trained staff, documented methods and transparent reporting is a prerequisite for measurement results to be meaningful.

A measurement consists of different consecutive steps, of which sample preparation and quantification are known to contribute significantly to the accuracy of the final measurement results. The quantification step includes the actual generation of the experimental data by an applied method, as well as all related aspects (e.g. calibration, operation and performance qualification) that are necessary to guarantee correct and accurate performance of the method.

The following sections explain important aspects of sample preparation (Section 4.1) and give an overview of mainstream methods considered applicable for assessing the particle size distribution and the VSSA of solid particles (Section 4.2). For detailed complementary technical information, the

reader may consult JRC Reports [e.g. 11,41], documentary standards and test guidelines (Annex 2).

Nanomaterials are identified via their particle number size distribution, the measurement of which can be performed, for instance, according to OECD TG 125 [12]. From the methods listed in OECD TG 125, laboratories may select a screening or confirmatory method that is appropriate for a specific material taking into account the properties of the material and the measurement performance characteristics of the method. Although electron microscopy will in most cases be the preferred method for providing regulatory relevant information within the EU, the following questions may help to select a suitable method for a certain material.

- **Are the particles in a dry state or in a suspension?**

Some methods require the particles to be in a suspension, others measure them in their dry state. While it is possible to disperse powder particles in a liquid [27] and (to a lesser degree) to obtain dried powder particles from a suspension, sample conversion may alter the properties of the particles and their agglomeration state.

- **What type of particles need to be measured?**

Particulate materials can vary widely with respect to particle size and particle size distribution, shape, concentration, and composition.

Some methods can measure a large variety of particle types whereas others have a much narrower working range that is, for instance, limited to particles of a certain size class, density, refractive index or chemical composition. Certain instruments such as electron microscopes operate under extreme measurement conditions, which can compromise the integrity of certain types of particles. Techniques equipped with element-sensitive or light scattering detection systems can lack sensitivity to quantify particles consisting of light elements (silicon, boron, etc.).

• Do the constituent particles appear in an unbound state or as parts of agglomerates or aggregates?

Small particles such as nanoparticles differ from their larger counterparts by an increasing tendency to form agglomerates and/or aggregates that is inversely proportional to their size. Only few methods can address the requirement of the EU recommendation to measure the external dimensions of constituent particles within agglomerates and aggregates. Most (screening) methods record an agglomerate or an aggregate as one individual particle.

• Are the resolution and size range of the desired method appropriate for measuring the size of the particles expected to be present in the sample?

Methods can significantly differ in size resolution (being able to distinguish, for example, a 60 nm diameter particle from a 70 nm particle) and working range (minimum and maximum size of particles that can be measured). With respect to the working range, nearly all methods are limited to roughly one order of magnitude for any single measurement. This often means that for a given measurement, for instance, particles from 5 nm to 50 nm or from 50 nm to 500 nm can be measured, but not from 5 nm to 500 nm.

• How many particles need to be measured?

A reliable experimental determination of particle size distributions requires a sufficiently large number of data points (or particles). Ensemble methods such as dynamic light scattering (DLS), centrifugal liquid

sedimentation (CLS), small-angle X-ray scattering (SAXS) measure thousands of particles simultaneously and their composite signal is used to calculate average particle size distributions (PSD); their measurement principles do not allow retrieving information on individual particles. The main advantage of these methods is the high measurement speed and the statistical robustness of the obtained PSD. Counting methods such as electron microscopy (EM) or particle tracking analysis (PTA) investigate particles individually and construct the PSD from these particle-by-particle based measurement results. Whereas the obtained PSDs are intrinsically number-based, the minimum number of particles that has to be measured in order to yield a PSD that is representative of the material can be an issue. For image analysis based methods, ISO 13322-1 provides a specific procedure to estimate the minimum number of particles to be measured for a given level of accuracy [48]. Other method-specific guidelines for determining the optimal number of particles (and fibres) are available in the OECD TG 125 [12].

• Are documentary standards available?

Documentary standards are developed by standardisation bodies. They harmonise the application of analytical instruments/techniques and methods, and thus lead to a better reliability and comparability of measurement results. Some documentary standards only describe the design and use of a specific instrument; others describe complex sets of operations for measuring specific material properties. These operations typically include aspects such as sampling, sample preparation, calibration, data acquisition and analysis. For the analysis of collected data, documentary standards may advise to use a counting rule (see section 2.5) that is not compatible with the EC NM definition, so this should be carefully checked. Furthermore, the OECD develops regulatory recognised test guidelines. Testing performed according to these test guidelines, and following Good Laboratory Practice, is covered by the OECD agreement of Mutual Acceptance of Data in the Assessment of Chemicals (<https://www.oecd.org/env/ehs/mutualacceptanceofdata/amad.htm>).

4.1 Sample preparation

Many materials that may be subject to classification as nanomaterial consist of dry powders of aggregated and/or agglomerated particles. As explained in Section 4.2 many methods used for particle size analysis require samples to be in the form of liquid-phase dispersions (i.e. suspensions); even imaging methods such as EM strongly benefit

from having samples available as stable colloidal suspensions. The transformation of a powdery sample into a stable suspension involves different successive steps which are briefly explained in the following paragraphs, and in more detail in the JRC Science for Policy Report 'Identification of nanomaterials through measurements' [11].

4.1.1 Sampling and subsampling

The assessment of a material is based on measurement results obtained on (sub)samples that are aliquots of the original material. Given the possible regulatory implications, it is of utmost importance that the tested samples are fully representative of the material. To ensure the representativeness of the

tested samples, one should apply a sampling plan that is based on internationally recognised procedures (e.g. ISO 14488 [49]). A sampling plan should include a strategy that helps deciding where and how to retrieve a small portion from the original material.

4.1.2 Wetting and pre-dispersion

The first stage in producing a stable suspension from dry particles requires the displacement of the solid-air interface with a solid-liquid interface. For hydrophilic particles and aqueous solutions, mixing may occur spontaneously with mechanical stirring being sufficient to achieve a solid-liquid interface between the solid particles and the dispersant liquid. In other cases, the powder itself may be intrinsically hydrophilic but through the production process or during storage a thin contamination layer of hydrophobic material may accumulate on the particle surface through atmospheric deposition. In such cases, bringing the particles in contact with

a small amount of a polar solvent such as methanol or ethanol, or any other suitable wetting agent or surfactant, prior to mixing with aqueous dispersion media may be sufficient to ensure an adequate wetting behaviour. Should this fail to give satisfactory results, it may be necessary to use a specific wetting agent or surfactant before adequate pre-dispersion can be achieved prior to the de-agglomeration. All chemicals used for wetting and pre-dispersion of powder particles should be non-reactive, particle-free and fully compatible with the material, measurement method and measuring instrument.

4.1.3 De-agglomeration

Screening methods (Section 4.2.1) are unable to measure the external dimensions of constituent particles in agglomerates and aggregates. As a consequence, they measure agglomerates and aggregates as single particles. Confirmatory methods (Section 4.2.2), on the other hand, are known for their capability to identify and measure constituent particles in agglomerates and aggregates.

However, even these methods may work effectively only when the constituent particles are not too strongly agglomerated/aggregated so that the boundaries of the constituent particles can be distinguished. Consequently, for both screening and confirmatory methods, the occurrence of coarse agglomerates and aggregates present in a colloidal sample will significantly bias the number-based

particle size distribution and may result in false negative assessments (i.e. classifying a material as conventional material while in reality it is a nanomaterial).

As far as possible, one should attempt to break up agglomerates and aggregates thereby aiming for a homogeneous sample of mainly individual constituent particles or, when this is not feasible, at least for obtaining smaller agglomerates/aggregates. Since agglomerates and aggregates differ by the strength of the forces keeping the constituent particles together, it can be expected that agglomerates can be broken up more easily than aggregates.

A successful de-agglomeration requires the input of external energy into the suspension,

most commonly by mechanical mixing and/or sonication. The amount of energy delivered to a given sample suspension must be recorded and be sufficient to overcome the adhesive forces amongst the constituent particles. At the same time, care has to be taken that the amount of power is not too high as this could destroy the constituent particles and/or lead to re-agglomeration. A suitable protocol for estimating the effective acoustic power output from probe-type sonicators when operating at a variety of instrument settings has been presented by Taurozzi et al. [50]. When using probe-type sonicators, attention must also be paid that the probe tip does not generate excessive amounts of wear particles, thereby contaminating the sample.

4.1.4 Stabilisation

The breakage of agglomerates (and possibly of aggregates) does not alter the original adhesive or attractive forces and, as a result, de-agglomerated particles will over time have the tendency to grow again into larger agglomerates. To ensure that the de-agglomerated particles remain sufficiently stable against re-agglomeration, different steps, such as surface charge-induced electrostatic stabilisation or steric stabilisation by adsorption of macromolecules, or a combination of both, can be applied.

The stability and agglomeration state of the sample should be verified either experimentally using EM, or empirically using an appropriate available screening method (e.g. DLS, CLS) that is sufficiently sensitive to reveal any relative changes in the size distribution due to the presence of small amounts of large aggregates/agglomerates. A suspension should be stable from the moment it is prepared until it is measured.

4.1.5 Verifying the stability of a suspension

In order to check the effective stability (resistance to re-agglomeration) of suspensions, periodic verifications can be made of the apparent particle size distribution from stock dispersions using at least one technique which is sensitive to changes in particle size and quantity. The accuracy of the measurement is not critical provided it is reproducible. Hence, a prime candidate (measuring both size and quantity) for such verification would be centrifugal liquid sedimentation (CLS) - assuming the expected particle size range and density are compatible with the lower size limit of the CLS instrument being used. CLS is a sensitive method that can reveal subtle changes in the size distribution. Some dispersions may change over time due

to gravitational sedimentation but in many cases, this does not involve re-agglomeration and may be easily reversed. It is recommended that before verifying size distributions with CLS, or other appropriate alternatives, the sample dispersion should be re-homogenised by a short period (~few minutes) of low energy sonication (e.g. using a bath-type sonicator). Suspensions should be stable at least for the duration of the measurement.

4.1.6 Verifying the effectiveness of a dispersion protocol

For the implementation of the EC NM definition, a dispersion protocol can be considered effective if it yields samples which mainly consist of non-agglomerated/non-aggregated particles. To monitor the effectiveness of a protocol, analytical methods are required which can reliably distinguish constituent particles from agglomerates and aggregates. Suitable methods are those based on EM techniques (e.g. SEM, TEM). Where such methods are available and suitably prepared samples (specimens) are possible, the effectiveness of a dispersion protocol may be evaluated by direct imaging to determine the proportion of free (non-touching) particles compared to aggregates and agglomerates. As EM methods require dry or dried samples, care must be taken to optimise the EM specimen preparation to minimise re-agglomeration of particulates during specimen drying [51,52,53]. The use of protocols involving powders directly transferred onto a stub (without bringing them first in suspension) is not recommended as the constituent particles will be too strongly overlapping.

In the case where it is not possible to verify the agglomeration and aggregation state with EM it is recommended that efforts be made to empirically verify that the protocol has been optimised to produce the minimum mean particle size possible with the facilities available for dispersion. To do this, the dispersion steps should be applied systematically using different sonication times and fixed power settings with the mean particle size being measured by methods CLS or DLS. The most suitable sonication time and power should be chosen as the time when further incremental increases in sonication time and/or in power do not result in significant further changes in mean size. It should be noted that for many materials sonication produces a stable minimum size beyond which further sonication is ineffective while other materials may reach a minimum after which the mean size begins to increase due to undesirable fusion of small particles into larger aggregates.

Sample preparation

- Validated sample preparation and dispersion protocols are essential in the process of making unbiased particle size measurements.
- The procedures should be effective, efficient and reproducible and must not change the number-based constituent particle size distribution of the original material.
- The stability of a suspension and the effectiveness of dispersion protocols should be carefully verified, in terms of de-agglomeration/re-agglomeration.

4.2 Measurement methods

4.2.1 Methods used for material screening

The purpose of material screening is to perform a fast and relatively cheap but reliable material classification without the need for more expensive confirmatory testing (see Section 4.2.2).

Because of their underlying physical measurement principles, most screening methods do not determine the particles' actual external dimensions and do not always yield number-based PSDs. Nevertheless, their results may lead to a positive identification of nanomaterials provided they establish a reliable correlation with the PSD and with the measured property (or measurand). It should be noted that none of the screening methods can identify constituent particles within agglomerates/aggregates, because agglomerates/aggregates will be measured as being single particles. Screening methods based on particle size determination can only be used for identifying nanomaterials; they cannot be used to demonstrate that a material is not a nanomaterial. The NanoDefine methods manual [41] gives additional information on the methods.

Methods (Table 3) that may be employed for material screening include:

- dynamic light scattering (DLS)
- centrifugal liquid sedimentation (CLS)
- analytical ultracentrifugation (AUC)
- tunable resistive pulse sensing (TRPS)
- particle tracking analysis (PTA)
- single particle inductively coupled plasma mass spectrometry (sp-ICP-MS)
- small-angle X-ray scattering (SAXS)
- asymmetrical-flow field-flow fractionation coupled with multi-angle light scattering (AF4-MALS)
- differential electrical mobility analysis on sprayed suspensions (spray-DEMA)

Generally, the Brunauer, Emmett and Teller (BET) method to measure the specific surface area is also considered as screening method. However, unlike the other screening methods, the BET method should only be used to identify conventional materials based on their VSSA.

Materials that do not fulfil the exclusion condition (i.e. VSSA smaller than $6 \text{ m}^2/\text{cm}^3$) should be further tested either with another suitable screening method or with a confirmatory method.

In deciding whether a certain screening method is appropriate for a given material the following questions and aspects should be considered. These questions complement the material-related questions discussed earlier.

• What is the lower effective size limit of the method?

Some screening methods such as DLS, CLS, AUC, PTA, sp-ICP-MS, spray-DEMA and BET can theoretically measure particles that are as small as 10 nm in diameter. However, the lower effective size limit of the working range strongly depends on the physicochemical properties of the material being analysed. For DLS and PTA, a lower limit of 10 nm may be achieved for nanoparticles that scatter the light intensely, but it can be challenging or not feasible at all for particles that scatter the light weakly (e.g. silica). The lower effective size limit of analytical centrifugation methods (e.g. CLS, AUC) depends on the combination of the particles' optical properties and their effective density. Small nanoparticles with an effective density close to that of the surrounding liquid medium cannot be easily separated and measured, compared to nanoparticles of the same size but of higher density (e.g. gold). The lower size limit of sp-ICP-MS depends strongly on the mass of the measured element in a nanoparticle. Metallic nanoparticles as small as 10 nm can be measured, whereas the lower size limit for metal-oxide and polymeric nanoparticles is about 50 nm.

• What is the resolution of the method?

DLS and SAXS are ensemble methods and they lack the necessary resolution to distinguish and analyse particle size populations that differ slightly in size. In comparison, superior resolutions can be obtained by methods that measure individual particles (e.g. PTA, sp-ICP-MS, TRPS, spray-DEMA) or that apply particle fractionation prior to detection (e.g. CLS, AUC, AF4-MALS).

• Does the method directly yield number size distributions?

Counting or particle-by-particle methods such as PTA, sp-ICP-MS, TRPS and spray-DEMA directly determine number-based PSDs while other methods measure either particle volumes or masses, or the intensity of a signal generated by the particles. It is known that volume- and intensity-based PSDs will underestimate the contribution of the smallest particles. Therefore, this type of screening methods can be used to demonstrate that a

material is a nanomaterial (i.e. positive identification), but the results cannot prove that a material is not a nanomaterial. One may also convert the underlying metric of the PSD but the algorithms used for such conversion introduce high measurement uncertainties that can complicate the classification of borderline materials (i.e. materials identified as both nanomaterial and conventional material depending on the method applied) and materials which consist of particles of non-equiaxial shapes.

An overview of the screening methods that may be used for the positive identification of nanomaterials according to the EC NM definition is given in Table 3. It should be noted that the reported working ranges reflect optimal measurement conditions and should, therefore, be regarded as theoretical ranges. The effective particle size working ranges can be significantly smaller depending on the type of material being analysed.

4.2.2 Methods used for material confirmation

Confirmatory methods are needed when screening methods do not yield conclusive results for nanomaterial classification. In particular for borderline materials, i.e. materials with an x_{50} (median of the number size distribution) close to 100 nm, confirmatory methods are recommended as they can provide a reliable classification.

Methods that may be used for material confirmation are scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM). For standard operating conditions, these methods require dried samples; the sample preparation step usually involves dispersing the test sample in a liquid medium, placing a drop of the obtained suspension on a suitable flat substrate, and then drying. If it is expected that sample drying will significantly alter the particle size distribution, then wet/non-dried samples should be analysed using advanced techniques (e.g. cryo-TEM, environmental SEM, liquid AFM). The use of sample preparation protocols involving powders directly transferred onto a stub or grid (without bringing them first in dispersion) is not recommended.

An overview of the confirmatory methods that may be used for the identification of

nanomaterials according to the EC NM definition is given in Table 3.

For a conclusive classification of materials, confirmatory methods should fulfil the following criteria:

- applicable to particles of non-equiaxial shapes
- able to identify constituent particles within agglomerates and aggregates
- measure the external dimensions of (constituent) particles
- documentary standards and fit-for-purpose certified reference materials are available
- exhibit nanometre resolution
- compatible with automated image processing and analysis to increase the statistical significance
- yield the particle number-based distribution of the external particle dimensions as raw data (to avoid systematic biases and large uncertainties resulting from data conversion)

Currently EM and AFM are available confirmatory methods.

According to Article (1) of the Recommendation, the EC NM definition applies to 'solid particles that are present, either on their own or as identifiable constituent particles in aggregates or agglomerates'. Unlike the screening methods, confirmatory methods have the intrinsic capability to identify and measure the external dimensions of 'constituent particles' in agglomerates and aggregates. As explained in Section 2.5 of this guidance, constituent particles are distinguished by observing their interparticle boundaries or interfaces. Since the measurement of constituent particles in agglomerates and aggregates require a significant effort, laboratories should first employ appropriate sample preparation and liquid-phase dispersion protocols to minimise the number of agglomerates and aggregates in the final sample (see Section 4.1). In particular for agglomerates, where the constituent particles are mainly connected by their corners, it is known that a sufficient amount of external

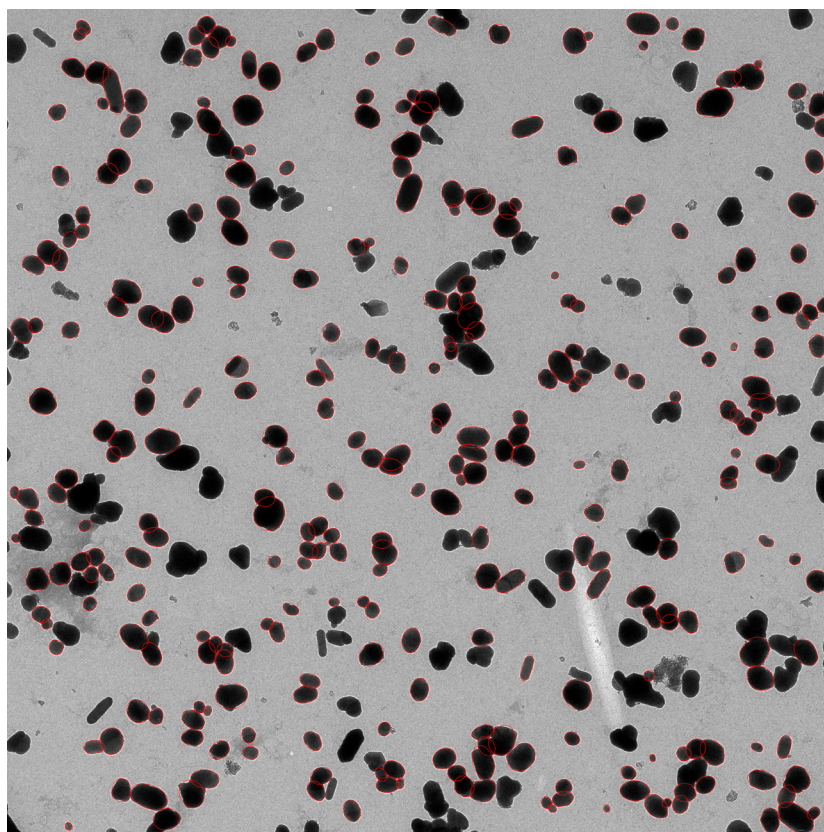
energy (e.g. through sonication) can overcome the weak attractive forces (e.g. van der Waals forces), thereby releasing the constituent particles from the agglomerates.

In Figure 11, agglomerated titanium dioxide and isolated particles (i.e. present on their own) are shown. The morphologically observable interparticle boundaries, in combination with the high contrast difference between the dark particles and the brighter substrate background, makes it relatively straightforward to identify and measure the constituent particles accurately.

The constituent particles in aggregates are held together by strong forces. As these forces are often too strong to overcome, a correct material assessment will require an accurate identification of the constituent particles within the aggregate. In contrast to agglomerates, the constituent particles of aggregates are connected by their surfaces and are overlapping, making it much more difficult to morphologically identify the boundaries and delineate the constituent particles.

FIGURE 11

TEM micrograph of titanium dioxide particles present on their own and present as constituent particles in agglomerates.



Source: Own elaboration. © European Commission

Note: Constituent particles identified by the image analysis software are surrounded by red perimeter lines.

Figure 6 (iv) depicts zinc oxide aggregates/agglomerates composed of partially overlapping constituent particles that significantly vary in size and shape. To assess whether the given material is a nanomaterial, one needs first to accurately determine the shapes and perimeters of the constituent particles including those partially overlapping. This is best achieved using the particles' mass-thickness contrast obtained with TEM and in combination with image processing software that employ specialised deconvolution algorithms [54]. For aggregates made of constituent particles that are sufficiently homogeneous (or monodisperse) in size and shape, SEM methods may be used provided a sufficient number of constituent particles are fully visible for measuring their external dimensions.

When using (semi-)automated image analysis software for measuring the external dimensions of constituent particles in aggregates and agglomerates, attention must be paid that the constituent particles are correctly identified. For an accurate identification it is crucial to use the right algorithm that is able to ascertain the exact boundaries of the constituent particles. Inappropriate algorithms may identify small clusters of constituent particles as a single particle or cut a single constituent particle into two or more entities. It is obvious that a significant number of wrongly identified constituent particles will result in a biased particle number size distribution.

Table 3. Overview of screening and confirmatory methods and their main characteristics in relation to the implementation of the EC NM definition.

	Method	PSD weighing	Type of sample	Type of particles ⁽¹⁾			Can deal with challenges of specific types of particles ⁽¹⁾				Documentary standards ⁽²⁾
				Inorg	Org	Comp	Elong	Flat	PD	Const	
Screening methods	AF4-MALS	Int	Susp	++	++	+	--	--	++	--	ISO/TS 21362
	AUC	Mass	Susp	++	0	+	--	--	++	--	N/A
	BET	N/A	Powder	++	-	+	--	--	--	--	ISO 9277
	DLS	Int	Susp	++	++	+	--	--	-	--	ISO 22412
	CLS	Ext	Susp	++	0	+	--	--	++	--	ISO 13318 series
	PTA	Number	Susp	++	+	++	--	--	+	--	ISO 19430
	SAXS (Guinier)	Mass	Susp	++	0	+	-	-	0	--	ISO 17867
	sp-ICP-MS	Number	Susp	+	-	0	--	--	0	--	ISO/TS 19590
	Spray-DEMA	Number	Susp	++	++	++	--	--	+	--	ISO 15900
	TRPS	Number	Susp	++	++	+	--	--	0	--	ISO 13319-2 (under development)
Confirmatory methods	AFM	Number	Powder	++	0	+	+	+	+	--	N/A
	SEM	Number	Powder	++	+	+	++	0	+	+	ISO 19749 ISO 13322
	TEM	Number	Powder	++	+	+	++	0	+	++	ISO 21363 ISO 13322

⁽¹⁾ Scale: ++, very well, +, well, 0, moderate, -, not well, --, not possible

⁽²⁾ Methods included in OECD TG 125

Note: Int, light intensity; Ext, light extinction; N/A, not applicable or not available; Susp, suspension; Inorg, inorganic; Org, organic; Comp, composite (solid material where each particle consists of two or more phase-separated constituents, e.g., core/shell, multi-layered, functionalised particles); Elong, elongated; PD, polydispersity; Const, constituent particles within agglomerates/aggregates.

Source: Own elaboration, reproduced from Rauscher, H., Mech, A., Gibson, N., Gilliland, D., Held, A., Kestens, V., Koeber, R., Linsinger, T.P.J., Stefaniak, E.A., Identification of nanomaterials through measurements, EUR 29942, Publications Office of the European Union, Luxembourg, 2019, doi:10.2760/053982.

5 Conclusions

The new European Commission Recommendation on the definition of nanomaterial 2022/C 229/01 is the result of a review of the previous definition 2011/696/EU followed by a revision process, which were carried out between 2013 and 2021. The revision addressed the objective, scope, clarity, and use and also intended to improve the implementability of the definition. Improvements of the clarity and the underpinning concepts as well as available relevant standards and best practices enable the application of the definition in practice across different regulatory fields.

- The EC NM definition is horizontal and not sector-specific. It is a Recommendation and thus does not impose any legal obligation on those to whom it is addressed. It is sufficiently specific and quantitative to enable its implementation in regulatory frameworks.
- The EC NM definition is based on the only feature common to all nanomaterials: their nano-scale external dimensions. It categorises a material as nanomaterial if 50 % or more of its constituent particles fulfil at least one of the following conditions:
 - one or more external dimensions of the particle are in the size range 1 nm to 100 nm;
 - the particle has an elongated shape, such as a rod, fibre or tube, where two external dimensions are smaller than 1 nm and the other dimension is larger than 100 nm;
 - the particle has a plate-like shape, where one external dimension is smaller than 1 nm and the other dimensions are larger than 100 nm.
- The EC NM definition applies to all solid particulate materials regardless of their origin, which can be natural, incidental or manufactured. The chemical composition and morphology of the particles is not relevant for the application of the definition. Nanomaterials can be hazardous, or not, and a definition based only on size

properties cannot differentiate between hazardous and non-hazardous materials.

- The EC NM definition applies only to materials in the form of solid particles (i.e., materials which are solid at normal temperature and pressure, i.e. 298.15 K and 101,325 Pa). Only ensembles or systems large enough to form a phase for which the physical state (solid, liquid, gaseous) can be assessed, can be classified as a solid.
- Single molecules are neither solid nor liquid or gaseous as these states of matter can be attributed only to ensembles large enough to form a physical phase. Single molecules are therefore not considered (solid) particles.
- The EC NM definition specifies that a material with a VSSA of less than $6 \text{ m}^2/\text{cm}^3$ is not a nanomaterial. In a particulate material, the volume specific surface area (VSSA) is equal to the sum of the surface areas of all particles divided by the sum of the volumes of all particles. It can be calculated if the particle size distribution and the particle shape(s) are known in detail. The reverse (calculating the size distribution from the VSSA value) is unfeasible.
- The term 'material' is generic and independent from specific legislation, and in specific legislation it can be replaced with terms used in that legislation, e.g. substance, ingredient, etc.
- A nanomaterial consists of a large ensemble of particles (i.e. particulate matter). Non-particulate components can be present in a material but are not part of its assessment with respect to the criteria of the EC NM definition.
- The EC NM definition is a definition of 'nanomaterial' and not 'nanostructured material'. The latter is generally taken to include materials that have nanoscale internal or surface structures, whereas the EC NM definition is only based on particle external

dimensions. Some nanostructured materials may fall under the definition if they are particulate and external particle size criteria are met.

- A 'particle' is a minute piece of matter with defined physical boundaries. Grains in polycrystalline materials are not to be considered as particles. Constituent particles are the smallest possible identifiable individual particles inside an agglomerate and aggregate.
- External particle dimensions can be represented in various ways. With regard to the EC NM definition, the external dimensions of particles with irregular shape should normally be assessed by the minimum Feret diameter and/or the maximum inscribed circle diameter.
- For the implementation of the EC NM definition, it is not necessary to distinguish between aggregates (consisting of strongly bound and/or fused particles) and agglomerates (consisting of weakly bound particles). The particle boundary or interface can be used to identify constituent particles in agglomerates and aggregates and the determining factor is the external dimension of the identifiable constituent particles.
- The 50 % threshold criterion for a nanomaterial refers to the particle number based particle size distribution.
- The fraction of particles with one or more external dimensions in the size range 1 nm to 100 nm can (depending on the details of the particle size distribution) be a very small fraction of the total mass of the material and at the same time be a majority in the total number of material particles.
- 50 % of particles with one or more external dimensions smaller than 100 nm in a number based size distribution are always less than 50 % in any other normally-used size distribution metric, such as surface, volume or mass.
- The EC NM definition does not apply to (consumer) products or components in which nanomaterials are integrated. Even if a product contains nanomaterials as ingredients, or when it releases nanomaterials during use or ageing, the product itself is not a nanomaterial, unless it exists as a particulate material that meets the criteria of the EC NM definition.

- Suitable sample preparation, appropriate measurement methods, a robust reference system and an integrated flow chart (decision tree) are key elements for a reliable identification of nanomaterials.

As explained in this report, it is important to understand the terms and concepts used in the definition in a harmonised way to enable a coherent implementation of the definition across regulatory sectors. Based on this understanding, relevant amendments to the definition of nanomaterial can be made when implementing it in sector-specific legislation. These amendments should on the one hand respect sector-specific requirements while not compromising the fundamental concepts underpinning the definition. This results in an overarching and harmonised understanding of the term nanomaterial and its regulatory implementation.

This guidance explains the key terms and fundamental concepts used in the EC NM definition. The guidance includes specifically the terms: nanoscale, particles and external particle dimensions, material, aggregate, agglomerate and identifiable constituent particle, solid, single molecule, specific surface area by volume, particle number based size distribution and nanomaterials in products. Measurement options as described in the report should be considered in the assessment of particulate materials, and the decision tree provided here may further help to identifying nanomaterials. The JRC report EUR 29942 provides complementary information on how to identify nanomaterials through measurements. An introduction to how nanomaterials are addressed in EU legislation is given by the EU academy course 'Nanomaterials in EU Legislation' (<https://academy.europa.eu/courses/nanomaterials-in-eu-legislation>).

The initiatives and work undertaken by the OECD and international standardisation bodies greatly support the implementation of the definition, by the development of test guidelines and documentary standards. In addition to Good Laboratory Practice, and whenever possible, these test guidelines and standards should be applied when assessing a material with respect to the criteria of the EC NM definition.

Stakeholders now have the tools and instruments available which are needed to implement the EC NM definition.

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List of abbreviations

2D	two-dimensional
AF4-MALS	asymmetrical-flow field-flow fractionation coupled with multi-angle light scattering
AFM	atomic force microscopy
AUC	analytical ultracentrifugation
BET	Brunauer, Emmett and Teller
CEN	European Committee for Standardization
CLS	centrifugal liquid sedimentation
Comp	composite (solid material where each particle consists of two or more phase-separated constituents, e.g., core/shell, multi-layered, functionalised particles)
Const	constituent particles within agglomerates/aggregates
DLS	dynamic light scattering
Elong	elongated
EC	European Commission
EM	electron microscopy
EU	European Union
Ext	light extinction
Inorg	inorganic
Int	light intensity
ISO	International Organization for Standardization
JRC	Joint Research Centre (of the European Commission)
N/A	not applicable or not available
NM	nanomaterial
OECD	Organisation for Economic Co-operation and Development
Org	organic
number size distribution	number-based particle size distribution
PD	polydispersity
PSD	particle size distribution
PTA	particle tracking analysis
SAXS	small-angle X-ray scattering
SEM	scanning electron microscopy
sp-ICP-MS	single particle inductively coupled plasma mass spectrometry
spray-DEMA	differential electrical mobility analysis on sprayed suspensions
SSA	specific surface area
Susp	suspension
TC	technical committee
TEM	transmission electron microscopy
TRPS	tunable resistive pulse sensing
VSSA	volume specific surface area

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Annexes

Annex 1. The text of the new Commission Recommendation

14.6.2022 EN Official Journal of the European Union C 229/1

COMMISSION RECOMMENDATION

of 10 June 2022

on the definition of nanomaterial

(Text with EEA relevance)

(2022/C 229/01)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union, and in particular Article 292 thereof,

Whereas:

- (1) Commission Recommendation 2011/696/EU ⁽¹⁾ has been applied as a reference for determining whether a material should be considered a 'nanomaterial' for legislative and policy purposes in the Union, supporting efficient and consistent implementation across sectors. Recommendation 2011/696/EU refers to a later review of the definition of nanomaterial in the light of experience and of scientific progress.
- (2) Between 2013 and 2021, the Commission carried out such a review of Recommendation 2011/696/EU, addressing the objective, scope, clarity and use of its definition of nanomaterial. The review in particular focused on whether the particle number-based size distribution threshold of 50 % should be increased or decreased and whether to include materials with internal structure or surface structure in the nanoscale such as complex nanocomponent materials including nanoporous and nanocomposite materials that may be used in specific sectors.
- (3) Technical and scientific elements underpinning the review of the definition of nanomaterial in Recommendation 2011/696/EU were summarised and published in the Commission's Joint Research Centre's (JRC) Science for Policy reports 'Towards a review of the EC Recommendation for a definition of the term 'nanomaterial' Part 1 ⁽²⁾, 2 ⁽³⁾ and 3 ⁽⁴⁾ on the experience of stakeholders with the implementation of the definition and with the identification of possible points of revision. In addition, JRC published two reports providing guidance on the implementation of the definition ⁽⁵⁾, ⁽⁶⁾ including relevant developments in standardisation by the International Organization for Standardization (ISO) and the European Committee for Standardization (CEN), results of the NanoDefine project of the Commission's 7th Framework Programme for Research ⁽⁷⁾, and further information available in the public domain.
- (4) Elements for possible modifications of the definition were the subject of a targeted stakeholder consultation between 6 May and 30 June 2021. Information received during that consultation was considered in the Commission's review of the definition of nanomaterial.
- (5) The results of the review and the stakeholder consultation, the description of the modifications made and their rationale are explained in the Commission staff working document (SWD(2022)150) accompanying this Recommendation.
- (6) A definition of nanomaterial that is appropriate in the general context of Union policy and legislation ('the definition') should be recommended, covering natural, incidental or manufactured materials.
- (7) The definition should be based on the relative fraction of particles in a defined range within the particle number-based distribution of the external dimension of the constituent particles of a material, irrespective to its potential inherent hazardous properties or risks to human health and the environment.
- (8) The definition and its core terms should, where applicable, be based on existing scientifically defined and standardised terms adopted by the international communities (ISO, CEN). The core terms used in the definition should remain sufficiently specific and should enable practical implementation of the definition within the Union regulatory context. Implementation should be supported by guidance that should be developed by the JRC and kept updated with evolving science and technical progress, listing recommended measurement methods and best practice tools⁷.
- (9) The term nanomaterial should address materials consisting of particles in solid state, present on their own or bound as constituent parts of aggregates or agglomerates. The term 'consist of' rather than 'contain' should be used to acknowledge that the particles are the principal component of the material. Other non-particulate components potentially present (e.g. additives necessary to preserve its stability or solvents that may be separated without affecting the particle size distribution) are part of the (nano-) material but should not be taken into account when assessing whether a material is a nanomaterial.

- (10) The definition should exclude non-solid (i.e. liquid and gaseous) particles. This should ensure that the highly dynamic nature of the external dimensions of non-solid particles, such as micelles or nanoscale droplets in emulsions or sprays, does not prevent the use of the external dimension as the defining qualifier in the definition.
- (11) The definition should not cover large solid products or components, even when they have an internal structure or a surface structure at the nanoscale, such as coatings, certain ceramic materials and complex nanocomponents, including nanoporous and nanocomposite materials. Some of these products or components may have been manufactured by using nanomaterials and may even still contain them.
- (12) The definition should continue to follow the 2010 opinion of the Commission's Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) ⁽⁸⁾ by defining 'nanoscale' as the size range from 1 nm to 100 nm.
- (13) The review of the definition did not identify scientific evidence that the default threshold of 50 % of particles with external dimensions at the nanoscale should be increased or decreased to address particular concerns or cover or exclude specific types of materials. The flexibility of the default threshold value in specific cases, provided in Recommendation 2011/696/EU, should be removed to ensure regulatory consistency and coherence, and to avoid that a specific material is considered a nanomaterial under one regulatory framework but not under another, hence avoiding legal uncertainty for economic operators, consumers and regulators.
- (14) The definition should cover both particles on their own and identifiable constituent particles in agglomerates or aggregates. The review of the definition highlighted that the identification and measurement of constituent particles in aggregates can be very challenging. Thus, the qualifier 'identifiable' is bound by practical considerations pertaining to their identification. These considerations should be further elaborated in guidance.
- (15) The term 'particle' should be defined as a minute piece of matter with defined physical boundaries, thus pursuant to the definition of 'particle' adopted in ISO 26824:2013. Any technical aspects of the definition of particle, e.g. with regard to its mobility, should be further clarified in guidance.
- (16) A single molecule, including a macromolecule such as a protein that may be larger than 1 nm, should not be considered as a particle. In very specific cases, the distinction may depend on a precise understanding of the term 'single molecule'. Illustrative cases and explanations should be presented in guidance.
- (17) SCENIHR indicated that setting the 1 nm to 100 nm range may lead to a limited number of materials, such as (nano)tubes that would have a diameter smaller than 1 nm and a length exceeding 100 nm, not to be considered as nanomaterials. To address this potential omission, Recommendation 2011/696/EU included in the definition fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm as nanomaterials. However, other materials may have the same size characteristics as these carbon-based materials. It is also likely that scientific progress and innovation will result in more similar materials, requiring regular and continuous updates of the scope of the definition. To avoid this, the definition should therefore include all solid particles with at least one external dimension smaller than 1 nm in the tally of particles at the nanoscale to be compared against the 50 % threshold, if at least one of the other dimensions of these particles exceeds 100 nm.
- (18) Due to their much smaller number in all reasonably foreseeable and relevant situations, particles with at least two orthogonal external dimensions larger than 100 µm do not significantly influence the relative contribution of 1 nm to 100 nm particles in the total number of particles, and therefore do not affect to a significant extent the classification of materials. The definition should allow to restrict the determination of the particle number-based size distribution only to constituent particles with at least two orthogonal external dimensions below 100 µm, provided that the choice is documented by appropriate measurement results. Practical application of this option should be presented in guidance.
- (19) Experience has shown ⁽⁹⁾ that use of specific surface area as a proxy indicator when identifying a nanomaterial can lead to interpretation and technical difficulties, for example, as a high specific surface area may be due to an internal nanostructure rather than indicate the presence of a large number of small constituent particles. Therefore, the review of the definition identified that the related option provided in point 5 of Recommendation 2011/696/EU was not appropriate and should be removed from being a qualifier in the definition of a nanomaterial.
- (20) The NanoDefine³ project demonstrated, based on a large set of different industrial materials, that there were no inconsistencies in classification of non-nanomaterials, based on the median value determined from the particle number-based size distributions and on the volume specific surface area being less than 6 m²/cm³ (even if particle shape is unknown), respectively. Therefore, a material with a volume specific surface area less than 6 m²/cm³ should not be considered a nanomaterial.
- (21) The nanomaterial definition in Recommendation 2011/696/EU should therefore be updated.
- (22) Scientific and technical progress continues and may affect the rationale behind the elements used for identifying a nanomaterial. A review of the definition should therefore be considered whenever new scientific evidence or regulatory experiences demonstrate that the definition is no longer adequate.
- (23) The definition should neither affect nor reflect the scope of application of any instrument of Union legislation or of any provisions establishing, for a group of materials, additional or specific requirements (including those for safety). It may in some cases be considered necessary to exclude certain materials from the scope of application of specific legislation or legislative provisions even if they are nanomaterials according to this Recommendation. It may likewise be considered necessary to develop regulatory requirements for additional materials not falling under the definition of the present Recommendation, in the scope of application of specific Union legislation or legislative provisions targeting nanomaterials. Such legislation should, however, aim to differentiate between a 'nanomaterial' and a member of such subgroup as to maintain consistency with the definition and consequently other legislation.
- (24) The definition in this Recommendation may serve different policy, legislative and research purposes when addressing materials or issues concerning products of nanotechnologies. It may even be used in another act providing a definition of nanomaterial for horizontal policy and legislative use adopted by the Commission or Union legislator, in which case such act would replace this Recommendation.

HAS ADOPTED THIS RECOMMENDATION:

1. 'Nanomaterial' means a natural, incidental or manufactured material consisting of solid particles that are present, either on their own or as identifiable constituent particles in aggregates or agglomerates, and where 50 % or more of these particles in the number-based size distribution fulfil at least one of the following conditions:
 - (a) one or more external dimensions of the particle are in the size range 1 nm to 100 nm;
 - (b) the particle has an elongated shape, such as a rod, fibre or tube, where two external dimensions are smaller than 1 nm and the other dimension is larger than 100 nm;
 - (c) the particle has a plate-like shape, where one external dimension is smaller than 1 nm and the other dimensions are larger than 100 nm.

In the determination of the particle number-based size distribution, particles with at least two orthogonal external dimensions larger than 100 µm need not be considered.

However, a material with a specific surface area by volume of $< 6 \text{ m}^2/\text{cm}^3$ shall not be considered a nanomaterial.

2. For the purposes of point 1, the following definitions apply:
 - (a) 'particle' means a minute piece of matter with defined physical boundaries; single molecules are not considered 'particles';
 - (b) 'aggregate' means a particle comprising of strongly bound or fused particles;
 - (c) '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.
3. It is recommended that the definition of the term 'nanomaterial' set out in the latest recommendation or other act providing a definition of nanomaterial for horizontal policy and legislative use adopted by the Commission or Union legislator is used when addressing materials or issues concerning products of nanotechnologies:
 - (a) by the Commission, when preparing legislation, policy programmes or research programmes and when implementing such legislation or programmes also with other Union institutions and agencies;
 - (b) by Member States, when preparing legislation, policy programmes or research programmes and when implementing such legislation or programmes;
 - (c) by economic operators, when preparing and conducting their own policies and research.

4. This Recommendation updates Recommendation 2011/696/EU.

Done at Brussels, 10 June 2022.

For the Commission
Virginijus SINKEVIČIUS
Member of the Commission

⁽¹⁾ Commission Recommendation 2011/696/EU of 18 October 2011 on the definition of nanomaterial (OJ L 275, 20.10.2011, p. 38).

⁽²⁾ 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; EUR 26567 EN; doi:10.2788/36237 (2014).

⁽³⁾ 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; EUR 26744 EN; doi: 10.2787/97286 (2014).

⁽⁴⁾ 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; doi:10.2788/678452 (2015)

⁽⁵⁾ An overview of concepts and terms used in the European Commission's definition of nanomaterial; EUR 29647 EN; doi:10.2760/459136 (2019)

⁽⁶⁾ Identification of nanomaterials through measurements ; EUR 29942 EN; doi:10.2760/053982 (2019)

⁽⁷⁾ The NanoDefine Methods Manual ; EUR 29876 EN; doi:10.2760/79490 (2020)

⁽⁸⁾ http://ec.europa.eu/health/scientific_committees/emerging/docs/scenih_r_o_032.pdf

⁽⁹⁾ NanoDefine, Evaluation report on the applicability ranges of the volume specific surface area (VSSA) method and the quantitative relation to particle number-based size distribution for real-world samples, Deliverable number 3.5, 2015 and Reliable nanomaterial classification of powders using the volume-specific surface area method", J Nanopart Res 19, 61 (2017); DOI: 10.1007/s11051-017-3741-x

Annex 2. Documentary standards and guidance documents

Table A1. Non-exhaustive list of documentary standards and guidance documents related to vocabulary and terms of nanomaterials, nano-objects and methods.

Standardisation body and technical committee	Reference number	Title
ISO/TC 229	ISO/TS 80004-1:2015	Nanotechnologies – Vocabulary – Part 1: Core terms (Revision under development)
ISO/TC 229	ISO/TS 80004-2:2015	Nanotechnologies – Vocabulary – Part 2: Nano-objects
ISO/TC 229	ISO/TS 80004-4:2011	Nanotechnologies – Vocabulary – Part 4: Nanostructured materials (Revision under development)
ISO/TC 229	ISO/TS 80004-6:2021	Nanotechnologies – Vocabulary – Part 6: Nano-object characterization
ISO/TC 229	ISO/TR 18196:2016	Nanotechnologies – Measurement technique matrix for the characterization of nano-objects (Revision under development)
ISO/TC 24/SC 4	ISO 26824:2022	Particle characterization of particulate systems – Vocabulary
ISO/TC 202/SC 1	ISO 22493:2014	Microbeam analysis – Scanning electron microscopy – Vocabulary

Table A2. Non-exhaustive list of documentary standards and guidance documents related to sampling, sample splitting and sample preparation.

Standardisation body and technical committee	Reference number	Title
ISO/TC 24/SC 4	ISO 14488:2007 ISO 14488:2007/Amd 1:2019	Particulate materials – Sampling and sample splitting for the determination of particulate properties
ISO/TC 24/SC 4	ISO/TS 22107:2021	Dispersibility of solid particles into a liquid
ISO/TC 24/SC 4	ISO 14887:2000	Sample preparation – Dispersing procedures for powders in liquids
ISO/TC 24/SC 4	ISO/TR 19997:2018	Guideline for good practices in zeta-potential measurement
ASTM C09.20	ASTM C702 / C702M - 18	Standard practice for reducing samples of aggregate to testing size
ASTM D04.30	ASTM D75 / D75M - 19	Standard practice for sampling aggregates
BSI LBI/37	BS 3406-1:1986	Methods for determination of particle size distribution – Guide to powder sampling
OECD	ENV/JM/MONO(2012)40	Guidance on sample preparation and dosimetry for the safety testing of manufactured nanomaterials (Revision under development)
OECD	Test Guideline No. 318	Dispersion stability of nanomaterials in simulated environmental media

Table A3. Non-exhaustive list of documentary standards and guidance documents related to screening methods and techniques used in conjunction.

Technique	Standardisation body and technical committee	Reference number	Title
Multiple techniques	OECD	Test Guideline No. 125	Nanomaterial particle size and size distribution of nanomaterials
Brunauer–Emmett–Teller (BET)	ISO/TC 24/SC 4	ISO 9277:2010	Determination of the specific surface area of solids by gas adsorption – BET method (Revision under development)
	OECD	Test Guideline No. 124	Determination of the volume specific surface area of manufactured nanomaterials
Pycnometry	ISO/TC 24/SC 4	ISO 12154:2014	Determination of density by volumetric displacement – Skeleton density by gas pycnometry
Differential mobility analysis (spray-DEMA)	ISO/TC 24/SC 4	ISO 15900:2020	Determination of particle size distribution – Differential electrical mobility analysis for aerosol particles
Differential mobility analysis (DMA)	ISO/TC 24/SC 4	ISO 27891:2015	Aerosol particle number concentration – Calibration of condensation particle counters
	ISO/TC 146/SC 2	ISO 28439:2011	Workplace atmospheres – Characterization of ultrafine aerosols/ nanoaerosols – Determination of the size distribution and number concentration using differential electrical mobility analysing systems
Light scattering aerosol spectrometer (LSAS)	ISO/TC 24/SC 4	ISO 21501-1:2009	Determination of particle size distribution – Single particle light interaction methods – Part 1: Light scattering aerosol spectrometer
Laser diffraction (LD)	ISO/TC 24/SC 4	ISO 13320:2020	Particle size analysis – Laser diffraction methods
Single particle inductively coupled plasma mass spectrometry (sp-ICP-MS)	ISO/TC 229	ISO/TS 19590:2017	Nanotechnologies – Size distribution and concentration of inorganic nanoparticles in aqueous media via single particle inductively coupled plasma mass spectrometry (Revision under development)

Technique	Standardisation body and technical committee	Reference number	Title
8 Dynamic light scattering (DLS)	ISO/TC 24/SC 4	ISO 22412:2017	Particle size analysis – Dynamic light scattering (DLS) (Revision under development)
	ISO/TC 24/SC 4	ISO/TR 22814:2020	Good practice for dynamic light scattering (DLS) measurements
	ASTM, Subcommittee E56.02,	ASTM E3247-20	Standard test method for measuring the size of nanoparticles in aqueous media using dynamic light scattering
Particle tracking analysis (PTA)	ISO/TC 24/SC 4	ISO 19430:2016	Particle size analysis – Particle tracking analysis (PTA) method (Revision under development)
	ISO/TC 24/SC 4	ISO 13322-2:2021	Particle size analysis – Image analysis methods – Part 2: Dynamic image analysis methods
	ASTM, Subcommittee E56.02	ASTM E2834-12(2018)	Standard guide for measurement of particle size distribution of nanomaterials in suspension by nanoparticle tracking analysis (NTA)
Static multiple light scattering (SMLS)	ISO/TC 229	ISO/TS 21357:2022	Nanotechnologies – Evaluation of the mean size of nano-objects in liquid dispersions by static multiple light scattering (SMLS)
Centrifugal liquid sedimentation (CLS)	ISO/TC 24/SC 4	ISO 13318-1:2001	Determination of particle size distribution by centrifugal liquid sedimentation methods – Part 1: General principles and guidelines (Revision under development)
	ISO/TC 24/SC 4	ISO 13318-2:2007	Determination of particle size distribution by centrifugal liquid sedimentation methods – Part 2: Photocentrifuge method
	ISO/TC 24/SC 4	ISO 13318-3:2004	Determination of particle size distribution by centrifugal liquid sedimentation methods – Part 3: Centrifugal X-ray method
	ISO/TC 24/SC 4	ISO 18747-1:2018	Determination of particle density by sedimentation methods – Part 1: Isopycnic interpolation approach
	ISO/TC 24/SC 4	ISO 18747-2:2019	Determination of particle density by sedimentation methods – Part 2: Multi-velocity approach

Technique	Standardisation body and technical committee	Reference number	Title
Small-angle X-ray scattering (SAXS)	ISO/TC 24/SC 4	ISO 17867:2020	Particle size analysis – Small-angle X-ray scattering
	ISO/TC 24/SC 4	ISO 20804:2022	Determination of the specific surface area of porous and particulate systems by small-angle X-ray scattering (SAXS)
Field-flow fractionation (AF4)	ISO/TC 229	ISO/TS 21362:2018	Nanotechnologies – Analysis of nano-objects using asymmetrical-flow and centrifugal field-flow fractionation (Revision under development)
Ultrasonic spectroscopy (USSp)	ISO/TC 24/SC 4	ISO 20998-1:2006	Measurement and characterization of particles by acoustic methods – Part 1: Concepts and procedures in ultrasonic attenuation spectroscopy
	ISO/TC 24/SC 4	ISO 20998-2:2022	Measurement and characterization of particles by acoustic methods – Part 2: Linear theory
	ISO/TC 24/SC 4	ISO 20998-3:2017	Measurement and characterization of particles by acoustic methods – Part 3: Guidelines for non-linear theory
Tuneable resistive pulse sensing method (TRPS)	ISO/TC 24/SC 4	ISO 13319-1:2021	Determination of particle size distribution — Electrical sensing zone method — Part 1: Aperture/orifice tube method
	ISO/TC 24/SC 4	ISO/DIS 13319-2	Determination of particle size distribution — Electrical sensing zone method — Part 2: Tuneable resistive pulse sensing method (Under development)

Table A4. Non-exhaustive list of documentary standards and guidance documents related to confirmatory methods and techniques used in conjunction.

Standardisation body and technical committee	Reference number	Title
OECD	Test Guideline No. 125	Nanomaterial particle size and size distribution of nanomaterials
ISO/TC 24/SC 4	ISO 13322-1:2014	Particle size analysis – Image analysis methods – Part 1: Static image analysis methods
ISO/TC 201/SC 7	ISO/TR 14187:2020	Surface chemical analysis – Characterization of nanostructured materials
ISO/TC 201/SC 9	ISO 11952:2019	Surface chemical analysis – Scanning-probe microscopy – Determination of geometric quantities using SPM: Calibration of measuring systems

Standardisation body and technical committee	Reference number	Title
ISO/TC 202/SC 3	ISO 29301:2017	Microbeam analysis – Analytical electron microscopy – Methods for calibrating image magnification by using reference materials with periodic structures
ISO/TC 202/SC 4	ISO 16700:2016	Microbeam analysis – Scanning electron microscopy – Guidelines for calibrating image magnification
ISO/TC 202/SC 4	ISO/TS 24597:2011	Microbeam analysis – Scanning electron microscopy – Methods of evaluating image sharpness
ISO/TC 229	ISO 19749:2021	Nanotechnologies – Measurements of particle size and shape distributions by scanning electron microscopy
ISO/TC 229	ISO 21363:2020	Nanotechnologies – Measurements of particle size and shape distributions by transmission electron microscopy
BSI LBI/37	BS 3406-4:1993	Methods for determination of particle size distribution. Guide to microscope and image analysis methods
DIN	DIN SPEC 52407	Nanotechnologies – Methods for preparation and assessment for particle measurements with atomic force microscopy (AFM) and transmission scanning electron microscopy (TSEM)
ASTM E56.02	ASTM E2859 - 11(2017)	Standard guide for size measurement of nanoparticles using atomic force microscopy
ASTM E04.11	ASTM E766 – 14 (2019)	Standard practice for calibrating the magnification of a scanning electron microscope

Table A5. Non-exhaustive list of documentary standards and guidance documents related to representation of measurement data, quality of data and reporting requirements.

Standardisation body	Reference number	Title
ISO/TC 24/SC 4	ISO/TS 4807:2022	Reference materials for particle size measurement – Specification of requirements
ISO/TC 24/SC 4	ISO 9276-1:1998 ISO 9276-1:1998/Cor 1:2004	Representation of results of particle size analysis – Part 1: Graphical representation
ISO/TC 24/SC 4	ISO 9276-2:2014	Representation of results of particle size analysis – Part 2: Calculation of average particle sizes/diameters and moments from particle size distributions
ISO/TC 24/SC 4	ISO 9276-3:2008	Representation of results of particle size analysis – Part 3: Adjustment of an experimental curve to a reference model
ISO/TC 24/SC 4	ISO 9276-5:2005	Representation of results of particle size analysis – Part 5: Methods of calculation relating to particle size analyses using logarithmic normal probability distribution
ISO/TC 24/SC 4	ISO 9276-6:2008	Representation of results of particle size analysis – Part 6: Descriptive and quantitative representation of particle shape and morphology

Standardisation body	Reference number	Title
ISO/TC 229	ISO/TS 16195:2018	Nanotechnologies – Specification for developing representative test materials consisting of nano-objects in dry powder form
ISO/TC 229	ISO/TS 23302:2021	Nanotechnologies – Requirements and recommendations for the identification of measurands that characterise nano-objects and materials that contain them
ISO/CASCO	ISO/IEC 17025:2017	General requirements for the competence of testing and calibration laboratories
ISO/TMBG	ISO/IEC Guide 98-3:2008 ISO/IEC Guide 98-3:2008/Suppl 1:2008/Cor 1:2009 ISO/IEC Guide 98-3:2008/Suppl 2:2011	Uncertainty of measurement – Part 3: Guide to the expression of uncertainty in measurement (GUM:1995)
ISO/TC 201/SC 2	ISO 20579-4:2018	Surface chemical analysis – Guidelines to sample handling, preparation and mounting – Part 4: Reporting information related to the history, preparation, handling and mounting of nano-objects prior to surface analysis
ASTM E29.02	ASTM E1617-09(2019)	Standard practice for reporting particle size characterization data

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