

Nano or Not Nano? A Structured Approach for Identifying Nanomaterials According to the European Commission's Definition

*Agnieszka Mech, Wendel Wohlleben, Antoine Ghanem, Vasile-Dan Hodoroaba, Stefan Weigel, Frank Babick, Raphael Brüngel, Christoph M. Friedrich, Kirsten Rasmussen, and Hubert Rauscher**

Identifying nanomaterials (NMs) according to European Union legislation is challenging, as there is an enormous variety of materials, with different physico-chemical properties. The NanoDefiner Framework and its Decision Support Flow Scheme (DSFS) allow choosing the optimal method to measure the particle size distribution by matching the material properties and the performance of the particular measurement techniques. The DSFS leads to a reliable and economic decision whether a material is an NM or not based on scientific criteria and respecting regulatory requirements. The DSFS starts beyond regulatory requirements by identifying non-NMs by a proxy approach based on their volume-specific surface area. In a second step, it identifies NMs. The DSFS is tested on real-world materials and is implemented in an e-tool. The DSFS is compared with a decision flowchart of the European Commission's (EC) Joint Research Centre (JRC), which rigorously follows the explicit criteria of the EC NM definition with the focus on identifying NMs, and non-NMs are identified by exclusion. The two approaches build on the same scientific basis and measurement methods, but start from opposite ends: the JRC Flowchart starts by identifying NMs, whereas the NanoDefiner Framework first identifies non-NMs.

1. The Need to Identify Nanomaterials

Nanotechnologies hold the promise to revolutionize our style of living and nanotechnology products can be found in a vast and increasing number and broad range of consumer products from cosmetics to tires. Despite significant efforts to acquire knowledge concerning the biological effects and environmental impact of nanomaterials (NM) and possible adverse effects there are still many open questions related to safety of these materials.^[1] In principle, the introduction of new technology often creates new challenges for legislators, especially when its functionalities, commercially promoted as benefits, raise concerns in other fora about health and environmental risks. In such cases, new legislation or the adaptation of the existing one may be necessary, in which case the legislators have to define what needs to be regulated.

Dr. A. Mech, K. Rasmussen, Dr. H. Rauscher
European Commission
Joint Research Centre (JRC)
Ispira 21027, Italy
E-mail: hubert.rauscher@ec.europa.eu

Dr. W. Wohlleben
BASF SE
Material Physics Research
Ludwigshafen 67056, Germany

Dr. A. Ghanem
Solvay–Research and Innovation Centre Brussels
Brussels 1120, Belgium


Dr. V.-D. Hodoroaba
Federal Institute for Materials Research and Testing (BAM)
Berlin 12205, Germany

Dr. S. Weigel
German Federal Institute for Risk Assessment (BfR)
Berlin 10589, Germany

Dr. F. Babick
Technische Universität Dresden
Institute of Process Engineering and Environmental Technology
Dresden 01069, Germany

R. Brüngel, Prof. Ch. M. Friedrich
Department of Computer Science
University of Applied Sciences and Arts Dortmund (FH Dortmund)
Dortmund 44227, Germany

Prof. Ch. M. Friedrich
Institute for Medical Informatics
Biometry and Epidemiology (IMIBE)
University Hospital Essen
Essen 45122, Germany

 The ORCID identification number(s) for the author(s) of this article can be found under <https://doi.org/10.1002/sml.202002228>.

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In 2008, the European Commission (EC) analyzed the European Union (EU) legislation and how it addressed nanotechnology and nanomaterials, resulting in a communication to the European Parliament on regulatory aspects of nanomaterials.^[2] The review concluded that although the EU regulatory framework in principle is applicable to nanomaterials it may need some amendments to fully cover all safety aspects of nanotechnologies.

In response to the conclusions of this first regulatory review, the European Parliament called upon the Commission to establish a “comprehensive science-based definition of nanomaterials in Community legislation as part of nanospecific amendments to relevant horizontal and sectoral legislation.”^[3] Hence, the EC adopted a recommendation on the definition of nanomaterial (EC NM Definition) for regulatory purposes in 2011.^[4] The EC NM Definition applies to particulate materials and is broadly applicable to all regulatory sectors in the EU. As it is a recommendation, it is not legally binding. Until now, it has been integrated, wholly or partly, into the text of several pieces of legislation addressing, e.g., biocides,^[5] chemicals,^[6,7] and medical devices,^[8] whereas the regulations on cosmetic products^[9] and novel foods^[10] have different nanomaterial definitions, which were introduced before the EC NM Definition was published.

The EC NM Definition is horizontal and is, in general, in line with other definitions worldwide, e.g., by the International Organization for Standardization (ISO),^[11] but it is more specific and quantitative than other definitions. The quantitative aspect of the EC NM Definition enables its implementation in a regulatory framework. The EC NM Definition is based on the only feature that is common to all nanomaterials: their nanoscale external dimensions.^[12] Identification of nanomaterials through this criterion involves the measurement of the external particle dimensions from a few nanometers to well into the micrometer range. The EC NM Definition states that if 50% or more of the particles in a material have one or more of their external dimensions between 1 and 100 nm, the material is a nanomaterial. As additional criterion, the EC NM Definition states that “[...] A material should be considered as falling under the definition [of nanomaterial] [...] where the specific surface area by volume of the material is greater than 60 m²/cm³. However, a material which, based on its number size distribution, is a nanomaterial should be considered as complying with the definition [of nanomaterial] [...] even if the material has a specific surface area lower than 60 m²/cm³.”^[4] Hence, the EC NM Definition uses the specific surface area by volume (VSSA) as a proxy approach to the particle size distribution criterion to identify nanomaterials, but does not use it to identify a material as not being a nanomaterial.

Whether a material is a nanomaterial or not a nanomaterial (in the following also referred to as non-nanomaterial) has regulatory consequences. Some legislation has specific data requirements for nanomaterials, requires a specific safety assessment or notification to authorities. The regulation on Registration, Evaluation, Authorization and Restriction of Chemicals (EC) No 1907/2006 (REACH),^[6] for example, requires separate registration and a specific risk assessment of substances in the nanoform.^[7] Therefore, identification for regulatory purposes of a material as a nanomaterial or

not a nanomaterial has major implications and the outcome of the identification exercise should therefore be accurate and robust, and accepted by all involved parties, i.e., regulators and industry. The identification should be economically viable and at the same time unambiguous as it serves to assure the safety of products placed on the EU market. The identification of nanomaterials should rely on methods that are readily available and fit for the purpose.^[13] The successful implementation of the EC NM Definition therefore relies on appropriate analytical methods that can distinguish nanomaterials from non-nanomaterials. However, the identification of nanomaterials is challenging, as there are tens of thousands of different materials with different particle shapes, chemical compositions, size polydispersity or agglomeration/aggregation state, which have to be characterized.

Much effort has been invested in developing these analytical methods, but even when using state-of-the-art methods, it is still challenging^[14] to determine accurately particle number-based particle size distributions in the range 1–100 nm. All methods have their range of applicability that depend on their measurement principles but also on the physico-chemical properties of the materials. Only few of the currently available methods directly yield the size distribution by particle number, as required by the EC NM Definition, and no single method is currently available that can establish for all materials whether they fulfil the EC NM Definition or not.^[13,14] Particle size is a method- or operationally-defined measurand as different methods measure different quantities, and thus the results obtained with one method cannot be directly compared with those obtained by another method.^[14,15] In view of the multitude of possible nanomaterials and the availability of different methods for particle size analysis, a strategy for assessing particulate materials against the EC NM Definition in a scientifically rigorous and economically efficient manner is urgently needed.

In 2013, the project NanoDefine “Development of an integrated approach based on validated and standardized methods to support the implementation of the EC recommendation for a definition of nanomaterial,” funded through the EU’s 7th Framework Programme for research, was launched to tackle these challenges in a joint effort of regulators, metrology institutes, industry and academia. NanoDefine explicitly supported the governance challenges associated with the implementation of legislation concerning nanomaterials by addressing relevant issues such as the availability of suitable measurement techniques, reference materials and validated methods to find solutions that would be acceptable to all stakeholders (authorities, policy makers, and industry). The project developed an integrated and interdisciplinary approach to unambiguously identify nanomaterials and non-nanomaterials for regulatory purposes.

To support the implementation of the EC NM Definition, the NanoDefine consortium has developed a comprehensive decision-making framework to match the analytical challenges triggered by the EC NM Definition.^[16] The NanoDefiner Framework follows a tiered approach, which allows reliably identifying a material as a nanomaterial, and it includes robust criteria that help users classifying materials based on volume-specific surface area and/or median particle size. A significant

part of the Decision Support Flow Scheme (DSFS) is devoted to excluding materials as NM while the criteria and requirements laid down in the EC NM Definition follow a conceptually opposite approach. Being able to experimentally demonstrate that a material is not a nanomaterial is certainly important in view of nano-related regulatory requirements, especially if those measurements can be performed in a time- and cost-efficient manner.^[6,17,18] The NanoDefiner Framework consists of a comprehensive methodology and measurement strategy that supports practitioners in industry, regulatory bodies and enforcement laboratories in implementing the EC NM Definition.^[16,19] The NanoDefine Methods Manual^[16] describes the NanoDefiner Framework and its tools. It provides guidance on the practical implementation of the EC NM Definition throughout the material characterization process, and on the characterization techniques employed including their working ranges and limitations. The NanoDefine Methods Manual assists the user in identifying the most appropriate measurement method(s) to assess any substance or mixture according to the EC NM Definition of a nanomaterial. The Methods Manual also outlines how to assess a material against the criteria of the definition through proxy solutions, i.e., by applying measurement techniques that provide indirect identification.

In parallel, the EC's Joint Research Centre has analyzed requirements, means and methods for fulfilling regulatory requirements to identify nanomaterials (e.g., refs. [12,14,20]), as well as relevant elements to consider for a revision of the EC NM Definition.^[17,21,22] This is particularly important as the amended REACH annexes include explicit provisions for substances in nanoform.^[7] As REACH is the overarching legislation for chemicals in general, its provisions have far-reaching implications across the regulatory landscape in the EU and beyond. It was therefore appropriate to elaborate and provide points to consider in the assessment of particulate materials according to the EC NM Definition in order to identify nanomaterials through measurements.^[14] Large parts of these considerations are based on and derived from the results of the NanoDefine project. However, the decision criteria follow a more conservative regulatory approach with the emphasis on how to positively identify nanomaterials.

This article describes the comprehensive approach developed within the NanoDefine project on how to identify nanomaterials for regulatory purposes. It focuses on the core of the NanoDefiner Framework, which is a DSFS for accelerated and reliable identification of materials according to the EC NM Definition that guides the user through several steps with the objective to come to a regulatory relevant and transparent identification of a material as a nanomaterial or non-nanomaterial. The latter identification is not required by legislation, but it is important for industry for deciding how the material should be registered, for controlling of existing and new applications and for health, safety and environment related issues. Furthermore, when enforcement laboratories analyze substances and products placed on the market they need to be able to identify non-nanomaterials. Considering the number of substances and products which need to be assessed fast, economic and reliable identification of non-nanomaterials is essential. Finally, the article compares the more rigorous regulatory approach, identifying which materials are nanomaterials, suggested by the JRC

as described in^[14] with the broader NanoDefiner Framework, outlining the boundaries of the two approaches.

2. The Challenge

Under the EU chemicals legislation^[6] the legislator expects that the registrant knows whether the material to be registered is a nanomaterial or not. Obtaining this knowledge is, however, not always a trivial issue; it can be a true challenge to determine correctly that a certain material is indeed a nanomaterial, but it may be even more demanding to decide that a material is a non-nanomaterial.

The defining assessment criterion for a nanomaterial according to the EC NM Definition, that external dimensions are in the nanoscale, implies determination of the median value of the number-based distribution of the external dimensions of the particles. The particles to be measured are the constituent particles of the material, regardless of whether these particles are parts of aggregates or agglomerates, or appear separate from one another.^[4] A detailed and unambiguous specification of the quantity that is intended to be measured (the measurand) is required to reliably assess and compare particle size measurement results.^[15] The specification includes several parameters such as the physical principle of the measurement technique, the sample preparation protocol and the data analysis procedure. Furthermore, the measurand must allow a reliable classification of a material as a nanomaterial or non-nanomaterial according to the EC NM Definition. The measured particle size results should always be reported together with the size range covered by the measurement; this size range must be sufficiently broad so that it allows reliable conclusions on the particle size distribution of the analyzed material.

Currently, no single method is available that can measure all materials and all relevant particle sizes.^[20] Hence, a combination of different complementary methods relying on different measurement principles and measurands should be ideally employed to investigate the material's particle size distribution. As already stated, size is an operationally defined measurand, and thus the measurements and applied methods need to be carefully considered. Ideally, the application of standardized methods is preferable. However, while some methods are standardized for specific materials, this is far from common for many other materials and methods. It is also recognized that sample preparation is fundamental,^[23] as all particle size analysis techniques require that samples are prepared in a specific and adequate way (e.g., by dispersion) before actual measurement. Hence, appropriate (standardized) sample preparation procedures, and in particular validated dispersion protocols, are essential in the process of performing unbiased particle size measurements. For these procedures to be acceptable, they must be effective, efficient, reproducible, robust, and they must not compromise the integrity of the constituent particle size distribution of the original material. While the issues of sample preparation is well recognized, there is still a lack of standard operation procedures (SOPs) for preparing nanomaterials for testing; the NanoDefine project proposed a number of SOPs (see part 3 of ref. [16]) for sample preparation, but there are still many issues to consider and more SOPs are needed. As

it currently does not seem to be possible to develop a SOP for each individual material, a way forward here may be to develop SOPs for groups of materials.

All these technical requirements needed for reliable assessment of particle size, and the lack of validated methodologies make the process of the identification of nanomaterials not only complex but possibly also very time-consuming and expensive. Hence a systematic and structured approach that streamlines the identification of nanomaterials according to the EC NM Definition is urgently needed.

3. The NanoDefiner Framework: An Intelligent Strategy for the Identification of Nanomaterials and Non-Nanomaterials

The NanoDefine project developed a comprehensive approach on how to identify nanomaterials and non-nanomaterials for regulatory purposes, the NanoDefiner Framework, see **Figure 1**.

It builds on three pillars: i) a Methods Knowledge Base (MKB),^[16] ii) a technique-driven Materials Categorization Scheme (MCS),^[24] and iii) the DSFS.^[16] The Methods Knowledge Base, the Material Categorization Scheme, and the DSFS are also implemented in a newly developed software solution, the NanoDefiner e-tool,^[25] which is available online.^[26] All elements of the framework are interlinked and fed by the results and knowledge acquired during the application of the other parts of the framework.

The MKB is a result of a detailed evaluation of the characterization methods,^[16] which were selected as candidates for the reliable analysis of the number-based particle size distribution of a material and to assess it against the criteria of the EC NM Definition. Only widely available, mature methods were included in the evaluation. Based on the measurement capabilities of each method, characterization methods for tier 1 (screening) and tier 2 (confirmatory) are recommended (see **Table 1**) for use within the NanoDefiner Framework; some methods can be used in general, whereas others are only

applicable to specific materials. Details of the criteria applied to evaluate the methods' applicability for the implementation of EC NM Definition can be found in refs. [13,16]. Additionally, the framework was tested on a training set of materials.^[27]

The second pillar is an MCS for particulate materials, which is driven by the capabilities of commonly available characterization techniques for particle size measurement as evaluated in the MKB. The MCS thus categorizes a material according to the particle properties that are most important for the determination of their size. The MKB shows that these are the chemical composition, number of small external dimensions and shape, expected size range, trade form and dispersibility, stability under test conditions and other properties. For example, the chemical composition of the particles strongly influences the choice of the appropriate characterization techniques as the detection sensitivity can be composition-dependent. Particle shape is another important categorization criterion as many characterization techniques assume that particles are spherical or yield an equivalent spherical size, which severely limits their applicability to particles with a nonspherical shape. Further, the expected size range can limit the choice of the techniques for obtaining an accurate result as techniques for particle size measurement have their own measurement range or are more sensitive to particles in a certain size range. Some materials may be incompatible with the conditions of certain measurement techniques, e.g., they may be sensitive to irradiation by electrons or may be stable only in a narrow temperature range. A detailed discussion of the selected properties is presented in refs. [16,24]. Categorization of a material according to the MCS can be linked to the performance profiles of characterization methods from the MKB. This way, for any particulate material the most appropriate method(s) can be identified to measure the particle size distribution with the objective to determine whether a material is a nanomaterial, by matching the capabilities of the methods from the MKB with the outcome of the categorization from applying the MCS to a specific material. Details on how to apply the MCS and the method performance tables from the MKB can be found in the NanoDefine Methods Manual.^[16]

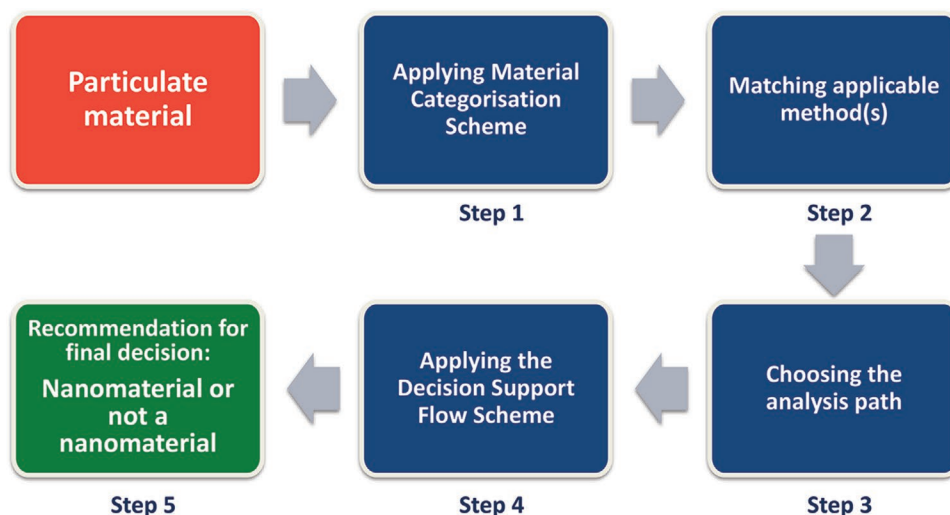


Figure 1. Sequential logical steps in the NanoDefiner Framework. Adapted with permission.^[16]

Table 1. Characterization methods for tier 1 (screening) and tier 2 (confirmatory).

| | Recommended by NanoDefine for general use (based on the results obtained on the NanoDefine Training Set materials ^[27]) | | Not applicable for general use, but potentially suitable for specific materials (based on the results obtained on the NanoDefine Training Set materials ^[27]) | | |
|--------|---|------------|---|-----------|------------|
| Tier 1 | DLS | Spray-DEMA | PTA | XRD | ALS |
| | AC | Mini TEM | LD | sp-ICP-MS | AF4-ICP-MS |
| | BET | | SAXS | USSP | AF4-LS |
| Tier 2 | TEM | | AFM | | |
| | SEM | | | | |

Abbreviations: AC, analytical centrifugation; AF4, asymmetrical flow field-flow fractionation; AFM, atomic force microscopy; ALS, angular light scattering; BET, Brunauer–Emmett–Teller method; DEMA, differential electrical mobility analysis (also spray-DEMA); DLS, dynamic light scattering; ICP-MS, inductively coupled plasma–mass spectrometry; LD, laser diffraction; LS, light scattering; PTA, particle tracking analysis; SAXS, small-angle X-ray scattering; SEM, scanning electron microscopy; sp-ICP-MS, single-particle ICP-MS; TEM, transmission electron microscopy; USSP, ultrasonic spectroscopy; XRD, X-ray diffraction.

The third pillar of the Framework is the DSFS, which starts with a particulate material and applies the MCS,^[24] after some basic classification. This then allows identifying the most suitable (applicable) method(s) to characterize that particulate material (see part 2 of ref. [16]). Based on this, the analysis path (“path” and “route” is used interchangeably) can be chosen in the DSFS. Applying the DSFS then results in a decision on whether the material should be considered as a nanomaterial or non-nanomaterial.

Figure 1 shows the main steps in identifying nanomaterials by measurements.^[16] A reliable classification result depends on carrying out each of these steps according to validated SOPs or using best practices.

When a particulate material is to be assessed for whether it falls under the EC NM Definition step 1 is to collect physico-chemical data of the material, and specifically those which are most important for the application of sizing methods, e.g. chemical nature, shape including the number of small external dimensions, expected size range, dispersibility, or stability under test conditions. A detailed discussion of the most relevant physico-chemical properties for size determination can be found in refs. [16,24]. With these data, the MCS can then be applied. Step 2 then starts with acquiring knowledge of the measurement method(s), as it is important to understand which method(s) would be pertinent for each material.^[14,16] This and the outcome of step 1 allow matching material with measurement method(s), i.e., to select the method(s) which is/are best suited to measure the particle size of a material with specific physico-chemical properties.^[24] Step 3—choosing the analysis path—is fundamental for the actual testing as it allows analyzing the material in the most appropriate, reliable and economically viable way.

A variety of methods is available for measuring and analyzing particle size distributions, and they belong to the screening or confirmatory type. Screening methods are fast and relatively inexpensive and still allow to positively identifying a material as a nanomaterial, but they do not directly measure the number-based distribution of the external particle dimensions. Confirmatory methods are usually more resource-intensive, but they may provide a more reliable material classification allowing to resolve doubts (or disputes), and they are used when screening methods do not allow a reliable classification. The measurement techniques’ capabilities can limit their applicability for certain materials. Hence, a thorough knowledge of the measurement technique to be applied

is indispensable to interpret the outcome of a measurement correctly and to understand whether a specific technique is fit for the purpose, to achieve a correct classification of a material as a nanomaterial or not according to the EC NM Definition. Moreover, all particle size analysis techniques require that samples are adequately prepared before measurement. Hence, appropriate (standardized) sample preparation procedures, or the use of best practices, are essential in the process of performing unbiased particle size measurements. Such procedures and practices should be clearly reported along with the particle size results.

With the choice of the analysis path the user has entered step 4, the DSFS, which guides the users through the decision process to arrive at a final decision whether a material is a nanomaterial or not. At the end of the DSFS the user arrives at the final step 5, the decision whether the material to be assessed is a nanomaterial or not a nanomaterial.

In the following, we outline the main elements of the NanoDefine DSFS, which is described in detail in ref. [16] with extensive reasoning for the criteria used in every decision node. An overview of the full scheme is presented in **Figure 2**.

The DSFS has a tiered structure and allows the user to start with basic methods, moving to more sophisticated methods, as necessary and relevant, and to reach a decision on whether the material is a nanomaterial or not a nanomaterial when sufficient information is obtained and then exit the DSFS. The NanoDefiner Framework is based on the assumption that many particulate materials can be classified as nanomaterials or non-nanomaterials by comparatively simple, robust and cost-efficient methods (tier 1) which are commonly used in many laboratories. Put simply, three conclusions can be reached based on tier 1 methods: i) the material is a nanomaterial, ii) the material is not a nanomaterial, and iii) confirmatory methods are needed. In a regulatory context, conclusions (i) and (iii) would be accepted as such. For conclusion (ii), the regulatory acceptance would be a case-by-case decision and would depend on factors such as the material assessed and the legislation under which the assessment takes place, as, e.g., a guidance^[28] by the European Food Safety Authority refers to the NanoDefine approach but additionally requires that size is determined by two independent methods. Only in cases where the outcomes of screening methods (tier 1) are not sufficiently reliable, more sophisticated methods (tier 2) should be applied to unequivocally assess the particle size (or rather the size distribution) of the material in question.

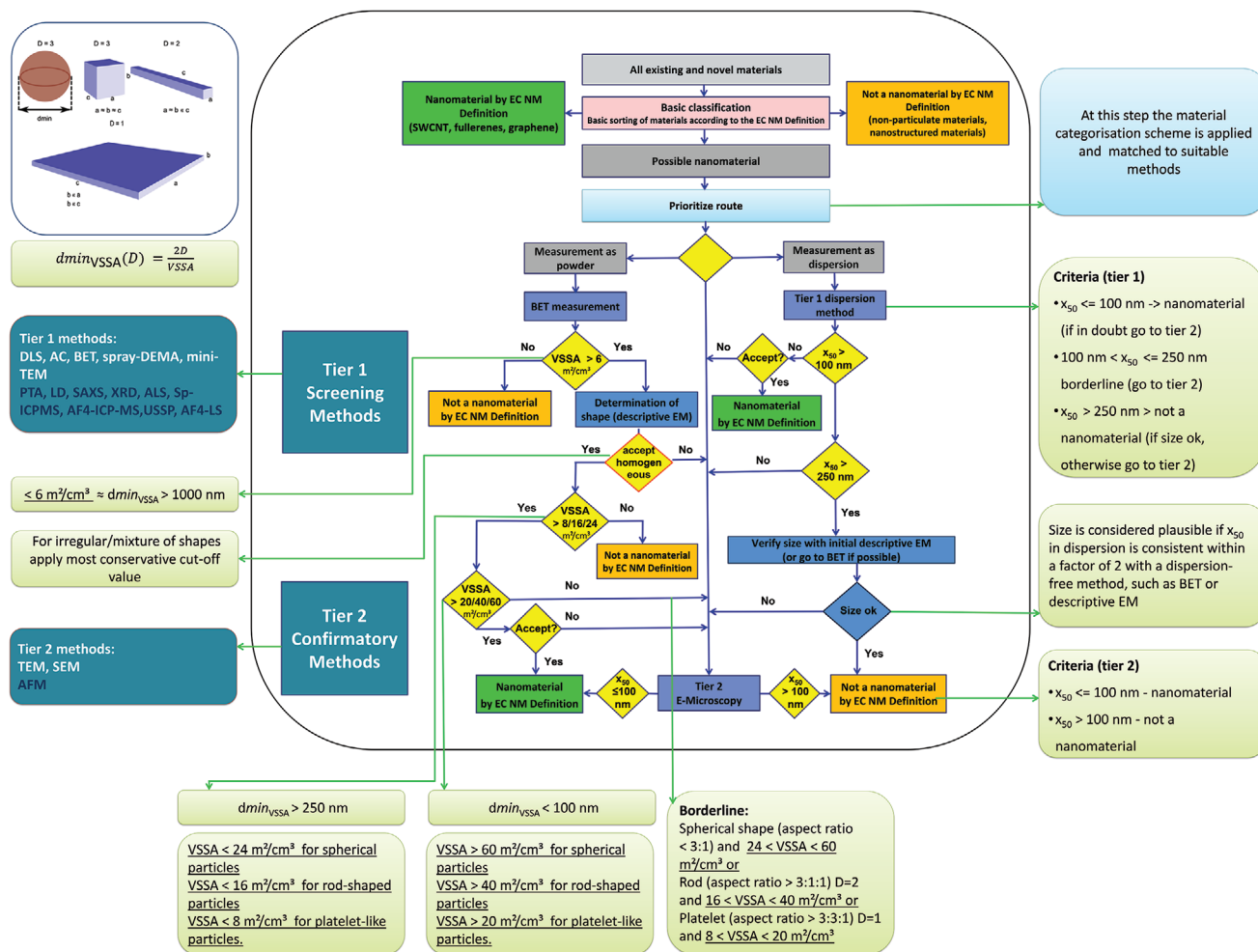


Figure 2. The NanoDefiner DSFS for material classification according to the EC NM Definition (main, box with black border and explanations outside in small boxes). Adapted with permission.^[16] The green boxes outside of the DSFS include the criteria and cutoff values for the decision nodes. In the blue boxes outside the DSFS on the left side tier 1 and tier 2 methods are specified (white: methods recommended by NanoDefine for general use; dark blue: not applicable for general use, but potentially suitable for specific materials).

Tier 2 is particularly relevant, e.g., for mixtures of different types of nanoparticles or different shapes. This concept allows i) exploiting as far as possible cost-efficient methods that are currently available/used in stakeholders' laboratories and ii) limiting the use of more work-intensive methods and high-end instrumentation to the cases where tier 1 methods fail.

The DSFS is a multistep procedure in which scientifically justified criteria are applied at each decision node. This provides guidance on the most reliable and fastest way to identify a material as a nanomaterial or non-nanomaterial according to the EC NM Definition. The first step of the DSFS consists of a basic classification whether the material is explicitly considered as a nanomaterial (fullerenes, graphene flakes, and single-wall carbon nanotubes with one or more external dimensions below 1 nm) or automatically excluded from the EC NM Definition (e.g., nonparticulate materials such as proteins or micelles^[29]). Moreover, the EC NM Definition does not cover nanostructured materials. Such materials can therefore immediately be classified as non-nanomaterials, and no further testing of them is needed.

The next step is to select the analysis route. There are three principal routes: two screening (tier 1) routes (the powder route and the dispersion route) and the direct application of confirmatory methods (tier 2). In this step, crucial information on the sample to be analyzed is collected.

At this point, the MCS and the method-material matching performance tables from the MKB^[16] should be applied to i) identify the most appropriate method(s) for measuring the particle size distribution of the material and ii) enable the correct choice of the analysis path of the DSFS. The NanoDefine Methods Manual provides detailed guidance on how to apply the MCS and method-material matching tables.^[16] This is automated in the e-tool.

Tier 1 methods can either characterize a material in powder form or in dispersion. The form in which the material is available is a key factor that affects the choice of the analysis path in the DSFS. If a material is already in dispersion, only the dispersion route, which is the right branch in Figure 2, can be chosen. The analysis of a powder obtained from a dried suspension is not reliable and thus not recommended.^[16] A

material in powder form, which can be dispersed in a liquid, can be analyzed in two ways: as powder or in dispersion. The choice of the actual particle size measurement method is directly linked to the outcomes of the prioritization route, as some of the methods may simply not be suitable for certain types or forms of nanomaterials. The choice of the route may depend on several other factors such as the availability of the mobility-based methods or a specific regulatory purpose, as some methods may be obligatory for certain legislation. Tier 1 can, in principle, also be omitted in favor of immediately applying a tier 2 confirmatory method (see Table 1). Tier 2 methods are considered more expensive, but they can be applied to most materials, even to those with complex particle shapes and/or structures.

The measurement techniques generally recommended by NanoDefine for the analysis of a dispersion in tier 1 are spray-differential electrical mobility analysis (spray-DEMA), all analytical centrifugation (AC) techniques and dynamic light scattering (DLS). For specific materials other methods may also be suitable as screening methods (see Table 1).^[16] The outcome of the screening performed with an appropriate tier 1 method should be interpreted by applying the following criteria.

- i) If the number-based particle size distribution of the material has an x_{50} (median particle diameter) smaller than or equal to 100 nm based on acceptable characterization results, the material is considered a nanomaterial without the need for tier 2 confirmation. The same conclusion can be drawn if the median diameter of a volume, extinction, or intensity-weighted particle size distribution is smaller than or equal to 100 nm because the median of such a size distribution as measured for example by AC, DLS, or asymmetrical flow field flow fractionation in combination with light scattering (AF4-LS), is always larger than the median of its corresponding number-based size distribution. If the reliability of the characterization results is doubtful, a new analysis should be performed either with a different tier 1 method to check the plausibility of the results, or by applying a tier 2 method.
- ii) If the material has an x_{50} larger than 100 nm or if the reliability of the characterization results is doubtful, the plausibility of the results should be verified by either (a) “descriptive electron microscopy (EM) analysis” or (b) the Brunauer–Emmett–Teller method (BET). “Descriptive or qualitative EM analysis” allows determining whether the general particle shape of the sample is compact (i.e., approximately spherical or equiaxial) and whether the particle size is in reasonable agreement with the results of the tier 1 method. Strong disagreement regarding the particle size between tier 1 and descriptive EM could indicate aggregation, which makes it necessary to move to tier 2. BET can be applied to verify the outcome of the tier 1 screening if the trade form of the material is a dry powder. Details on plausibility testing can be found in.^[16]

If the particle shape is not compact, tier 2 confirmatory methods should be applied. If the particle shape is i) compact and ii) the material is sufficiently dispersed and not aggregated or agglomerated (i.e., the particle size results obtained from tier 1 method and descriptive EM match reasonably well) and iii) the x_{50} is larger than 250 nm, the material can

be considered with reasonable reliability as non-nanomaterial. If the x_{50} is larger than 100 nm and below 250 nm the material is considered a borderline case and tier 2 analysis should be applied. The threshold of 250 nm is suggested for both the powder and dispersion paths based on findings made in NanoDefine that most of the materials for which tier 1 methods gives an x_{50} above 250 nm (after checks for plausibility, aggregation and particle shape) are not nanomaterials according to the EC NM definition.^[4]

The left-hand side of Figure 2 represents the powder route, which is mainly based on the well-established BET method to be used in combination with the skeletal density for determination of the VSSA of a material. According to the EC NM Definition, VSSA may be used as alternative metric for nanomaterial identification in addition to the number-based size distribution if a specific piece of legislation allows it. However, the EC NM Definition does not provide for VSSA to show that a material is not a nanomaterial and the particle size distribution remains the only means to do that. On the other hand, the possibility to classify a material as a nanomaterial or non-nanomaterial by determining the VSSA, together with the conditions of applicability to use VSSA as classification criteria, is discussed in detail in refs. [18,30]. An overview of determining VSSA with BET is given in ref. [31].

In principle, there is a relation between the particle size distribution and the VSSA. According to the findings of NanoDefine, VSSA can be used under specific conditions as a proxy to identify nanomaterials, and also materials that are not nanomaterials in agreement with the number-based criterion of the EC NM Definition. VSSA may be used as a classification criterion if the size distribution is monomodal, the particle shape can be approximated as spheres, rods or platelets and if there is no mixture of particle shapes and no severe aggregation of the particles. Otherwise, the user has to move to tier 2 and perform an EM-based analysis. The left-hand side of Figure 2 represents the detailed decision tree for classification as nanomaterial or non-nanomaterial when applying BET. The decision tree is divided into three main blocks with increasing levels of complexity for the classification process: i) screening; this allows classifying a material as non-nanomaterial if the VSSA is very low, ii) further decision making by taking into account the particle shape, and iii) verification of borderline cases leading to the conclusion that the material is a nanomaterial or to the application of tier 2 methods for a final decision. The detailed description of the criteria applied in the decision flow scheme can be found in the NanoDefine Methods Manual.^[16]

The DSFS was tested within the NanoDefine project in ten case studies that involved materials of various compositions (organic/inorganic/carbonaceous), different particle sizes (nanomaterial/borderline material/non-nanomaterial) and different shapes (particles/fibers/platelets). A further element of variation was that evaluators with different background (academia/industry/regulator) were employed.^[27] Case studies explored both tier 1 (powder route) and tier 1 (suspension route), and benchmarked the results against a tier 2 method (scanning electron microscopy (SEM) or transmission electron microscopy (TEM)). Most cases were found to be generally consistent, and only for two materials did the results not lead to a conclusive assessment due to the lack of reliability of

pre-existing data. In all cases, the results from manual assessment and by the e-tool assessment were consistent.

Detailed results can be found in ref. [27] and examples of the usage of the framework are presented in the next section. These case studies cover the entire DSFS and show how it can be applied in practice. One should, however, be clear on the limits of the DSFS: it can be only as good as the measurement methods and results which it makes use of, and it should be linked to expert knowledge. Such knowledge is valuable at every step in the DSFS, and in particular to assess and double check whether the available data are suitable and robust enough to allow a reliable decision. When using historical data, often generated for different purposes, for the assessment of whether a material is a nanomaterial or non-nanomaterial, it must be rigorously assessed whether the data are fit for the purpose. If in doubt, new data should be generated according to guidance provided in the NanoDefine Methods Manual.^[16] There may be cases when even the best available methods are unable to resolve the particle size distribution, e.g., when the particles have very irregular shapes and/or when they are aggregated or entangled in a complex way. If this is the case, an assessment of such materials against the EC NM Definition may require a special (case-by-case) approach adapted to the specific situation rather than following the predefined scheme.

4. Examples for the Application of the NanoDefiner Framework

Within the NanoDefine project, case studies were performed to test the DSFS, the MCS,^[24] and the e-tool.^[25] Details can be found in a NanoDefine Technical Report.^[27] Here, we present a summary of three case studies that illustrate the application of the NanoDefiner Framework. The selected materials have a complex morphology and thus their classification as nanomaterial or non-nanomaterial according to the EC NM Definition challenges the NanoDefiner Framework. The materials in the case studies differ in chemical composition, size polydispersity, shape and size, and the case study results demonstrate the consistency of the framework. All measured data are publicly available.^[32]

4.1. Calcium Carbonate CaCO₃ (IRMM-384)

A fine powder of calcium carbonate CaCO₃ (IRMM-384) was tested. According to the MCS,^[24] using information on the type of the material (inorganic, dry powder) and the shape of the particles (rod/cigar-like^[18]), the following methods are recommended for reliable analysis:

- 1) Tier 1: BET (if the shape is homogenous and the particle size distribution of the sample is monomodal).
- 2) Tier 2: TEM, SEM, and atomic force microscopy (AFM).

BET analysis was chosen as method for the powder route (example A).

Additionally, and to further test the NanoDefiner Framework, also the AC cuvette relying on refractive index (AC-cuv-RI) tier

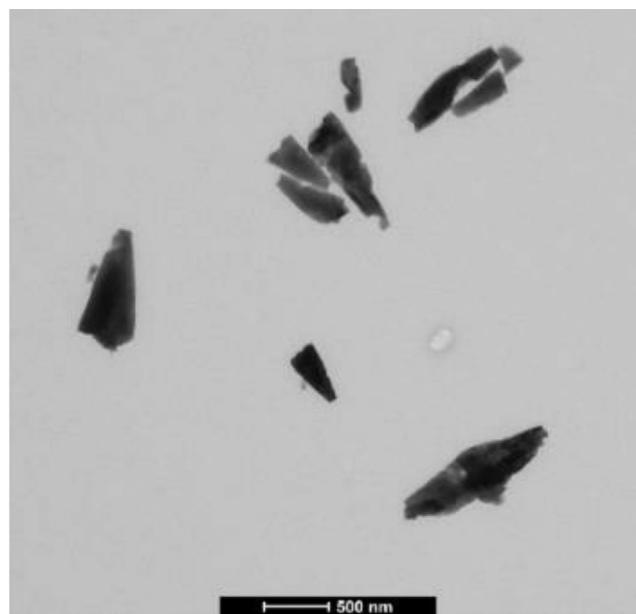


Figure 3. TSEM micrograph of CaCO₃ (IRMM-384).

1 method was applied to analyze the particle size distribution of the same material in dispersion state (example B). As no tier 1 method based on particle mobility can reliably determine the size of elongated particles this method was not recommended by the MCS.

As shown below, the DSFS resulted in the same final classification in both cases that the material is not a nanomaterial.

Example A: Dry Powder Route

Data used for the decision:

- i) Specific surface area (SSA) and considerations from ref. [18]: VSSA = 15 m²/cm³ and thus $d_{(VSSA)} = 267$ nm.
- ii) Transmission-SEM (TSEM) micrograph (see **Figure 3**; measurement by Coda-Cerva): shape (by TSEM) = rod/cigar-like.

Figure 4 gives a graphical representation of the decision path. Based on the SSA measured by BET, the VSSA was calculated to be 15 m²/cm³ and as this is lower than the VSSA criterion of 16 m²/cm³ established for elongated particles the material can be classified as non-nanomaterial.

Example B: Dispersion Route

Data used for the decision:

- i) AC-cuv-RI dispersion data:^[33] $x_{50} = 232$ nm (conversion from mass to particle number metrics).
- ii) TSEM micrograph (shown in example A): shape (by TSEM) = rod/cigar-like.
- iii) Quantitative SEM image analysis:^[18] x_{50} Feret(min) = 157 nm.

The graphical representation of the decision path is presented in **Figure 5**. Based on the AC-cuv-RI measurements the x_{50} was determined to be 232 nm (conversion from mass to particle number metrics was used) and is between the 100 and 250 nm thresholds in the DSFS. Hence, it was necessary

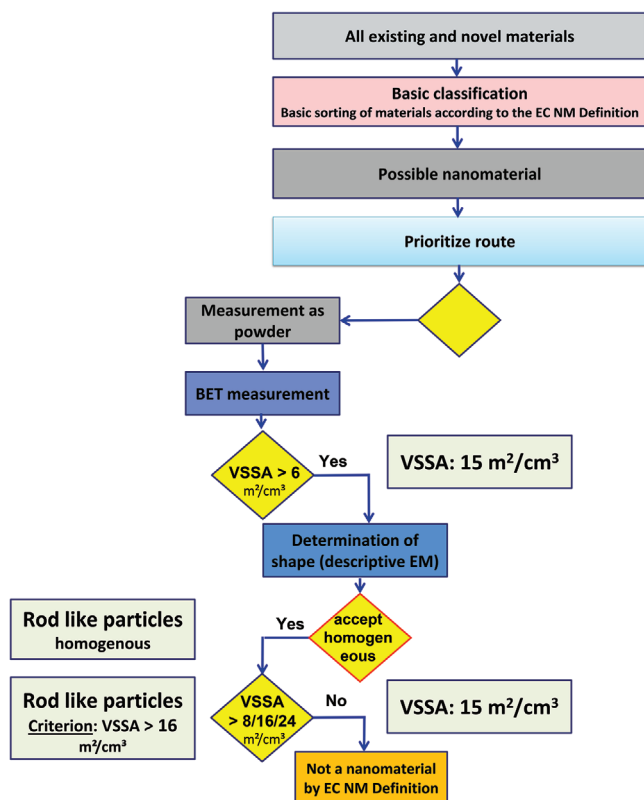


Figure 4. CaCO₃ classification path based on BET analysis.

to apply a tier 2 method. Here quantitative SEM analysis yields an x_{50} of 157 nm, based on the Feret(min) diameter. Thus, the material can be classified as non-nanomaterial which is the same classification as in the dry powder route.

It is worthwhile noting that if the x_{50} as determined by AC-cuv-RI would exceed the upper threshold of 250 nm the DSFS would require a plausibility check by descriptive EM (including particle shape determination). This would further lead to the application of a recommended tier 2 method. Alternatively, the user could proceed with BET analysis; in either case the result would be a correct classification as non-nanomaterial.

4.2. Kaolin (IRMM-385)

Kaolin is a natural aluminosilicate (dry powder) with platelet-like morphology. Based on this information (inorganic material, platelets) and applying the MCS, all tier 1 mobility-based methods were excluded, as they will overestimate the relevant particle dimension, in this case the thickness of the platelets. Tier 2 electron microscopy methods were excluded as well as they have poor access to the platelet thickness. This leaves only BET and AFM as suitable methods for particle size determination.

As the project partner performing the case studies did not have access to AFM analysis, only the dry powder route based on BET was tested.

Data used for the decision:

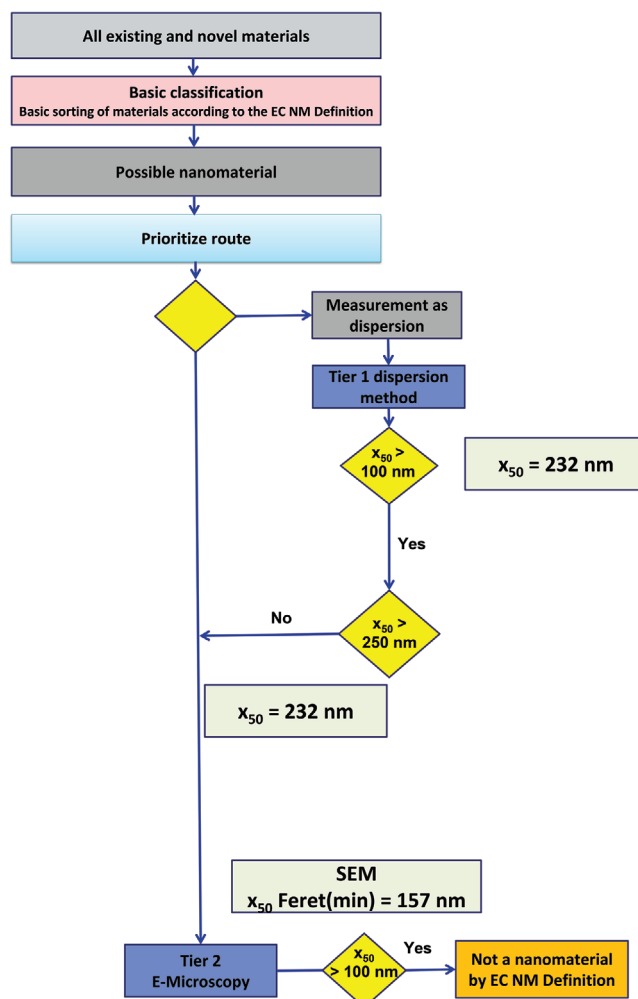


Figure 5. CaCO₃ (IRMM-384) classification path based on the AC-cuv-RI and SEM analysis.

- i) SSA measured by BET (following ISO 9277:2010) resulting in a VSSA of 42 m²/cm³. This corresponds to a platelet thickness of 48 nm.
- ii) SEM micrograph, shape: platelets (Figure 6).

The graphical representation of the decision path is presented in Figure 7.

Based on the SSA measured by BET, the VSSA was calculated to be 42 m²/cm³. The determination of shape and homogeneity of the sample by EM led to the conclusions that the particles have platelet like shape and that the sample is homogenous. The VSSA value exceeds the cutoff value of the VSSA criterion of 40 m²/cm³ established for flat particles, and the material can clearly be classified as a nanomaterial.

For the purpose of the case study and to check the consistency of the NanoDefiner Framework, a quantitative analysis of SEM micrographs was also performed, going beyond the recommended analysis. The measurements were carried out on a Zeiss Supra 40 (5 kV/10 kV/20 kV) with an inLens detector and the powder sample was blown in dry form on an Al grid in order not to orientate the platelets preferentially parallel to the



Figure 6. SEM micrograph of Kaolin (IRMM-385).

substrate. The analysis resulted in a Feret(min) x_{50} of 37 nm allowing to identify Kaolin (IRMM-385) as a nanomaterial in accordance with the decision based on VSSA.

4.3. Zinc Oxide ZnO (NM-113)

Zinc oxide ZnO (hygroscopic, white fluffy powder) from the Organisation for Economic Co-operation and Development (OECD) Working Party on Manufactured Nanomaterials sponsorship programme was tested. This case study aimed at exploring the possibility to use historical data from previous projects to classify materials as a nanomaterials or non-nanomaterial, thus no additional laboratory analysis was performed. All data used in this case study are available in ref. [34].

The MCS analysis concluded that it is possible to use all tier 1 methods and tier 2 methods. For this case study, it was decided to follow the dry powder route.

Data used for the decision:

- i) SSA measured by BET 6.2 m²/g resulting in VSSA of 34 m²/cm³
- ii) Shape determination from SEM image (see Figure 8), indicating high agglomeration of particles and heterogeneity of the material
- iii) Semi-automatic quantitative TEM analysis (26 parameters, 109 particles analyzed), in which the primary particles appeared polyhedral and with different size distributions. Qualitatively, two main morphological types appear distinguishable:
 - a) Particles with aspect ratio near to 1 (typically in the 80–100 nm range).
 - b) Particles with aspect ratio > 2 (typically in the 180–200 nm range).

During the image analysis the constituent particles were not isolated from the agglomerates/aggregates and eventually all objects were considered for final particle count (see images in Figure 9). This type of historical data analysis was not in line with NanoDefine recommendation as it does not allow to determine the constituent particle size distribution in number as requested by the EC NM Definition.

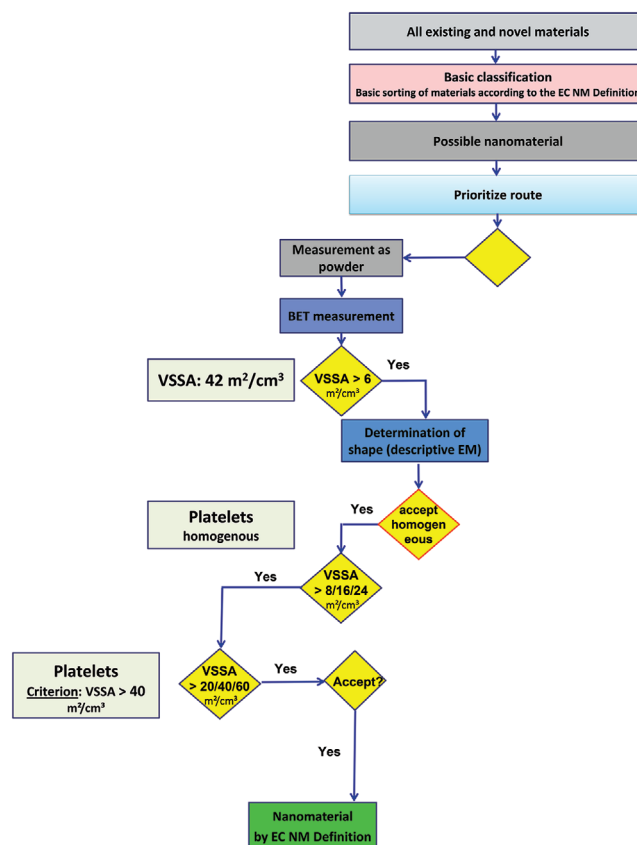


Figure 7. Kaolin (IRMM-385) categorization path based on the powder route (BET).

The graphical representation of the decision path is presented in Figure 10.

Based on the SSA measured by BET, the VSSA was calculated to be 34 m²/cm³. The determination of shape and homogeneity of the sample by EM revealed size multimodality of the sample and presence of particles with different shapes. In order to test the dry powder route on the material with these characteristics, the information on heterogeneity of the sample was ignored. For samples containing particles of mixed or irregular

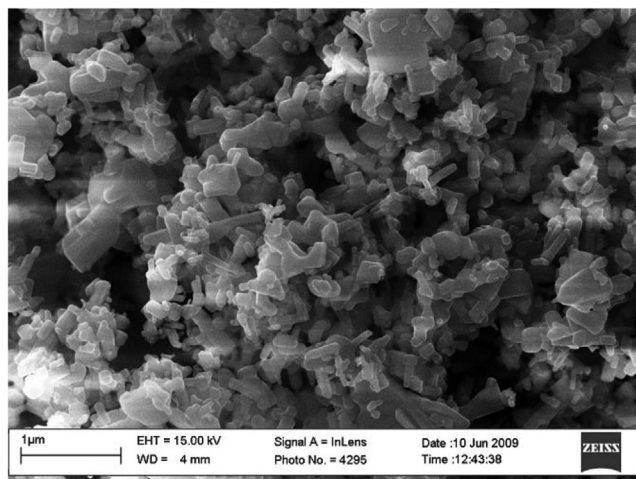


Figure 8. SEM micrograph of ZnO (NM-113).

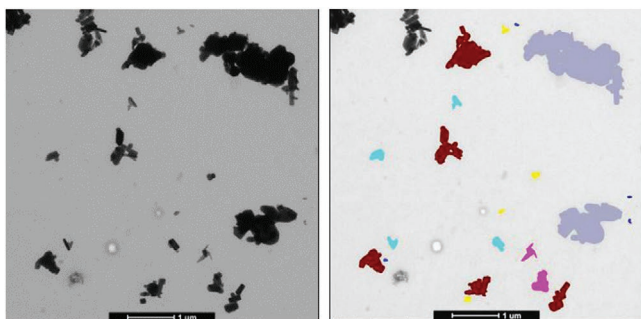


Figure 9. Left panel: TEM micrograph of ZnO (NM-113); right panel: same micrograph as in the left panel, objects marked in color are counted as single particles.

shapes the NanoDefiner Framework proposes to apply the most conservative (i.e., most sensitive to classification as nanomaterial) cutoff values which are those of platelets. The VSSA value exceeds the cutoff value of the first VSSA threshold of $8 \text{ m}^2/\text{cm}^3$ and hence this material cannot be classified as non-nanomaterial. The VSSA value exceeds the second threshold of $20 \text{ m}^2/\text{cm}^3$, which would lead to the classification of this material as nanomaterial. This classification was not accepted due to the known size multimodality of the sample, resulting in the need of using a confirmatory tier 2 method. However, the quantitative analysis of TEM images had not been performed according to the protocols established in the NanoDefiner Framework, as the TEM analysis predates the framework. Instead, it was done in a way that excludes its use for reaching a decision whether the material is a nanomaterial or not.

These results show that the reliable reuse of historical data generated for other purposes than nanomaterial classification may be very limited and needs to be done with caution. It has also to be underlined that the application of the most conservative cutoff value of $20 \text{ m}^2/\text{cm}^3$ (assuming that the constituent particles are platelets) may lead to a false-positive identification of a material as nanomaterial.^[18]

The results of all case studies confirm that the NanoDefiner Framework and its tools are tested best practice procedures that allow industrial and regulatory stakeholders to identify in a most reliable and cost-efficient way any material as nanomaterial or non-nanomaterial according to the EC NM Definition.

5. Identification of Nanomaterials through Measurements: Suggestions from the JRC

To support the implementation of the EC NM Definition^[4] which is being integrated into EU legislation, the JRC developed and published a document^[14] that describes how to identify nanomaterials through measurements. Partly based on findings of NanoDefine,^[16] it is also a follow-up of previous JRC Reports about the EC NM Definition,^[12,17,21,22,35] and it discusses options and points to consider when assessing whether a particulate material is a nanomaterial according to the EC NM Definition. Ref. [14] also recognizes the same challenges as outlined in Section 2 of this paper and describes steps to take toward identification of nanomaterials. Steps 1–3 for identifying nanomaterials by measurements are

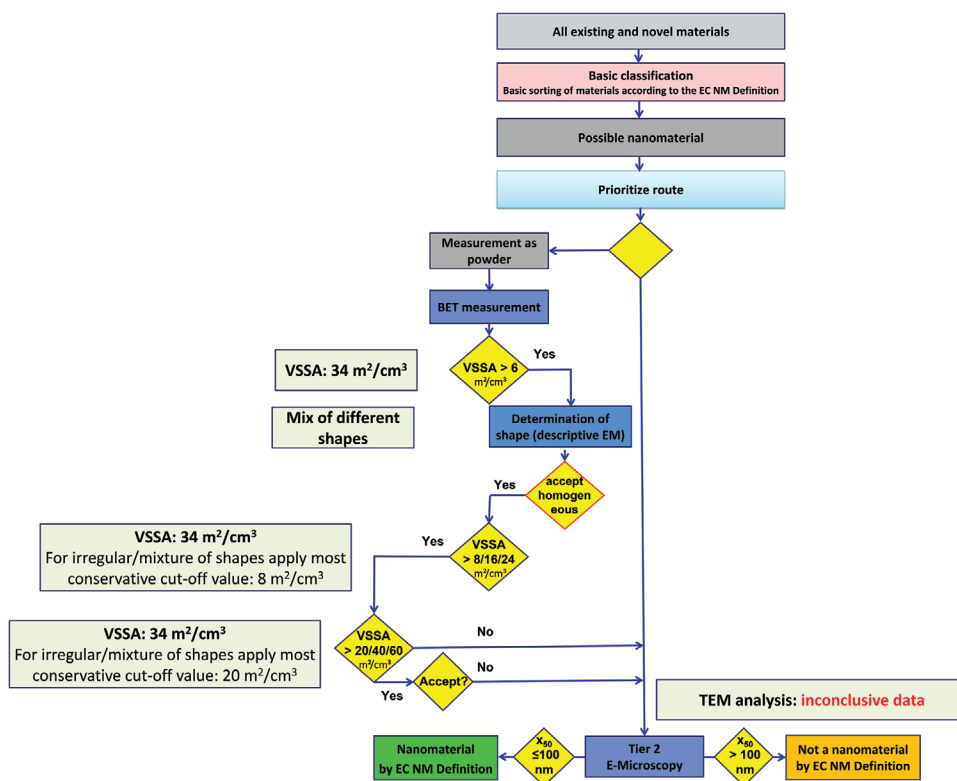


Figure 10. ZnO NM-113 classification path based on the BET and TEM analyses.

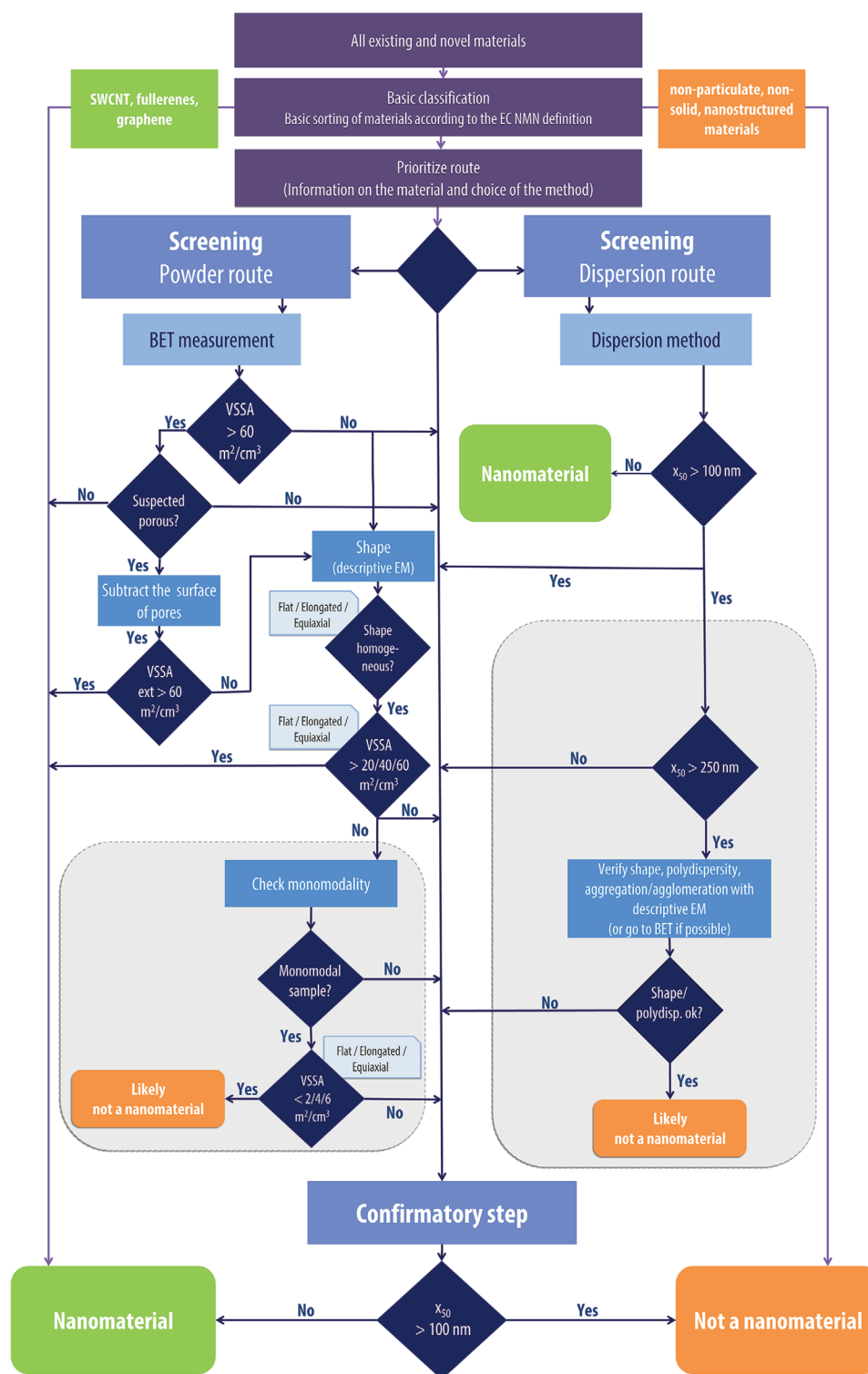


Figure 11. JRC Flowchart for material classification strictly following the EC NM definition. The gray shaded boxes with dashed lines represent empirical and theoretical criteria for classifying materials that are likely not nanomaterials. Adapted with permission.^[14]

the same as in the NanoDefiner Framework (see description in Section 3 and Figure 1). The differences are in steps 4 and 5. The JRC Flowchart, presented in **Figure 11**, strictly follows the EC NM Definition and the final decision is

based only on the criteria explicitly included in the EC NM Definition.

Both the DSFS and the JRC Flowchart first identify materials that, according to the EC NM Definition, explicitly are

considered as nanomaterials (e.g., graphene and carbon nanotubes) or are not nanomaterials (e.g., proteins and nanostructured materials). They continue with the choice of the best route for analysis, with three options: two screening routes, the powder route and dispersion route, or to apply confirmatory methods directly.

The powder route in the JRC Flowchart implements the explicit criterion from the EC NM Definition that if the VSSA (where the surface of any pores should be subtracted) is greater than $60 \text{ m}^2/\text{cm}^3$, the material is considered as a nanomaterial. In case of doubt, this can be checked also with confirmatory methods. Materials with a VSSA lower than or equal to $60 \text{ m}^2/\text{cm}^3$ may still be unambiguously identified as nanomaterials, provided they consist of platelets or rod-shaped particles and the shape of the particles is homogeneous. In such cases the material is a nanomaterial if the VSSA is greater than $20 \text{ m}^2/\text{cm}^3$ (for platelets) or $40 \text{ m}^2/\text{cm}^3$ (for rods).^[18]

In principle, for all other materials the next step would be the application of confirmatory methods. However, the left gray shaded area in Figure 11 indicates one more screening possibility, which identifies materials that most likely are not nanomaterials; materials with a monomodal size distribution and a VSSA of less than $6 \text{ m}^2/\text{cm}^3$ (spheres and cubes), $4 \text{ m}^2/\text{cm}^3$ (rods), and $2 \text{ m}^2/\text{cm}^3$ (platelets).

For comparison, the powder route in the NanoDefiner DSFS (Figure 2) starts by identifying materials with a VSSA lower than $6 \text{ m}^2/\text{cm}^3$, which are most likely not nanomaterials. For materials with a VSSA greater than $6 \text{ m}^2/\text{cm}^3$ more screening steps based on homogeneity and particle shape identify further materials that most likely are not nanomaterials, until nanomaterials or cases that need confirmatory methods remain.

In the dispersion route, the material to be measured is present in liquid dispersion, and the route includes particle size analysis methods that cannot distinguish between individual constituent particles, agglomerates, and aggregates. The methods do not yield a number-based particle size distribution as primary outcome of the measurement but require a mathematical conversion, e.g., when the primary outcome is a scattered light intensity based distribution.

The JRC Flowchart checks the particle size against the limit of 100 nm and a positive identification as nanomaterial is possible if the median (x_{50}) of the size distribution is lower than 100 nm. In principle, the next step for all other materials is the application of confirmatory methods. However, the gray shaded box at the right part of the flowchart suggests that if the x_{50} value is greater than 250 nm and where the material's particle shape, degree of polydispersity and agglomeration/aggregation state have been confirmed by descriptive EM, the material is most likely not a nanomaterial.

Also, the NanoDefine DSFS starts by checking against the limit of 100 nm in a step that identifies materials that are nanomaterials. The next step in the DSFS is to identify materials that most likely are not nanomaterials. These are materials with an x_{50} greater than 250 nm, and, depending on particle shape, polydispersity and agglomeration/aggregation state, verified by, e.g., EM, may not be nanomaterials.

Within the JRC Flowchart, results for number-based size distribution generally take precedence over an assessment based on VSSA. However, if certain requirements are fulfilled,

the VSSA can, as illustrated, serve as a proxy to identify nanomaterials, which can facilitate the classification of a material according to the EC NM Definition.

6. Discussion and Conclusions

Currently, the EU is the only geographical area in the world with a legal definition of the term nanomaterial. The EU thus probably is most advanced with regard to legislation specifically addressing NMs, but also outside the EU nanomaterials are debated in a regulatory context.^[36–39] The identification of nanomaterials is based on measurements, and the confidence in measurement results is increased by implementing a reference measurement system, which ensures that reliable results can be obtained. Typically, such a system is based on documented and validated methods and reference materials, which will provide confidence in the results and the final assessment. Documentary standards, validated methods, and reference materials are already available for some nanomaterials, but, in general, this area needs to be further developed and much work is ongoing, e.g., in OECD and in ISO, and more can be expected in the future.

When reference measurement systems are not available, best practices should be applied, such as those developed and tested by NanoDefine and collected in the NanoDefine Methods Manual.^[16] The NanoDefiner Framework (see Figure 1) was developed to help especially industry (but all stakeholders can apply it) to identify nanomaterials according to the EC NM Definition. Its starting point is to identify materials that the EC NM Definition specifies to be either nanomaterials or non-nanomaterials. Then the powder route screens for materials that in all likelihood are not nanomaterials. Continuing in tier 1, the DSFS powder route then identifies evident nanomaterials, or in case of ambiguous results, proceeds to tier 2 methods. In the dispersion route evident nanomaterials are first identified, then the remaining materials are sorted into two groups, one that should be tested with tier 2 methods under all circumstances as the measured size distribution is ambiguous, and one with results that should be confirmed with another tier 1 method; the materials in the latter group are most likely not nanomaterials.

The NanoDefiner Framework builds on three interlinked components: i) a Methods Knowledge Base, ii) a technique-driven Materials Categorization Scheme, and iii) the Decision Support Flow Scheme. The MKB is a result of a comprehensive evaluation of widely available and well-established characterization methods,^[16] selected as candidates for the reliable analysis of the number-based size distribution of a particulate material. The MKB provides an evaluation of the working range of each of the methods, as well as an overview of their limits and strengths. This information is extremely relevant both for matching a material with the most suitable method for its analysis, and for developing efficient decision procedures that allow industrial and regulatory stakeholders to identify both nanomaterials and non-nanomaterials. The technique-driven MCS for particulate materials takes into account material characteristics, e.g., the type of material (monotype/multiple types of particles, or article/formulation), chemical composition, number of small external dimensions and shape, expected size range, trade form and dispersibility, stability under testing conditions and other

specific properties. The material categorization is linked to the performance profiles of commonly used particle sizing techniques, and this considerably facilitates the selection of appropriate particle-sizing techniques. The third component, namely the DSFS, guides the user to a decision on whether the material is a nanomaterial or not based on clear and scientifically underpinned decision criteria. This paper presents the summary of three examples of applying the DSFS to materials.

The NanoDefine DSFS and the JRC Flowchart are two approaches to classifying materials as nanomaterial or non-nanomaterial. Both schemes, which take into account the nonspherical shape of most real-world materials, rely on the evaluation of the performance of measurement techniques, application of the same technique-based material categorization scheme and a decision flow scheme with a tiered approach. There are, however, important differences in scope and purpose between the flowcharts.

One of the main objectives in the development of the NanoDefine approach was to provide guidance for identification of both non-nanomaterials and nanomaterials; the strict regulatory needs are to identify nanomaterials (which is described in the JRC Flowchart), whereas the identification of non-nanomaterials goes beyond. This was requested by many stakeholders,^[17,21,22] even though the EC NM Definition provides explicit criteria only for the positive identification of nanomaterials. The NanoDefine approach applies by default the information obtained from measurements of particle size distributions and VSSA for extended conclusions on whether a material is a nanomaterial or not. Those conclusions are backed by experimental findings from testing a diversity of particulate materials with a variety of methods leading to a decision on whether they fulfil the EC NM Definition. As a result, the NanoDefine DSFS suggest certain empirical decision criteria to exit the scheme with a reasonably reliable classification (nanomaterial/non-nanomaterial) as early as possible (i.e., economically) without the need to apply confirmatory techniques, which often demand considerable resources. The criteria for these early decisions are not explicitly included in the EC NM Definition, even though there is evidence for their validity in practice.

The JRC Flowchart approach was developed strictly within the provisions of the EC NM Definition^[14] and uses only criteria which are explicitly included or rigorously derived from the basic criteria (the 20 and 40 m²/cm³ cutoff values for VSSA for specific particle shapes). The JRC Flowchart also acknowledges that since the publication of the EC NM Definition in 2011 further considerations on some parameters, e.g., particle shape and porosity,^[22,29] led to more detailed criteria for identification of nanomaterials via the powder route. Furthermore, even though the JRC Flowchart acknowledges and mentions possible empirical criteria for the identification of non-nanomaterials, it always recommends confirmatory methods for clear identification.

The NanoDefine DSFS and the JRC Flowchart share important principles, namely they are i) cost efficient (the NanoDefine DSFS even more so), as they propose a tiered approach for the selection of the most adequate analytical route to get to an identification according to the EC NM Definition, ii) easy to implement as they integrate current practice and readily

available methods, and iii) flexible as they allow for the inclusion of new technologies and can be adapted to amended regulatory requirements. Both approaches allow the use of the same analytical methods. The NanoDefiner Framework is additionally implemented in an e-tool, which is available as free and open source software and can be downloaded from a public service.^[26] The e-tool provides an extensive dossier with detailed explanation of every decision step as output.

The difference between the two approaches lies in their focus and purpose. The focus of the JRC Flowchart is on positive identification of nanomaterials while adhering to the explicit criteria provided by the EC NM Definition. The JRC Flowchart is possibly better adapted to avoid false negative classification (i.e., classifying nanomaterials falsely as non-nanomaterials) and mislabeling in a legal context, where it may be less important to capture all borderline cases. Materials that are not nanomaterials are identified by exclusion, rather than by positive identification. The NanoDefine DSFS is designed to first identify materials that are not nanomaterials, and then identify nanomaterials. The purpose of the DSFS is in supporting industry in regulatory processes, e.g., for screening a large portfolio of materials, and quickly separate those that are a lesser concern from those that may be a concern. This helps to estimate the number of materials that will require more scrutiny and, possibly, follow up under specific legislation. For industry as well as for regulators (e.g., enforcement laboratories) this facilitates budget and resource planning.

Both the NanoDefine DSFS and the JRC Flowchart support the implementation of the EC NM Definition across different pieces of legislation, however, as explained above, with a different scope. The NanoDefine DSFS is a comprehensive, economic strategy to assess materials against the EC NM Definition. It is part of the NanoDefiner Framework, which was designed by scientists with expertise in nanomaterial synthesis and characterization, in collaboration with industrial stakeholders and regulators. The empirical decision criteria that are used in addition to the regulatory criteria for identifying nanomaterials according to the EC NM Definition aim at arriving at a fast but reliable decision whether the material is not a nanomaterial or whether it is a nanomaterial. This aspect makes it particularly useful for innovators already in the material development phase to predict classification as nanomaterial or non-nanomaterial and to anticipate regulatory requirements for the final product. It supports therefore innovators who consider applying the Safe-by-Design concept for nanomaterials during the development of novel materials and products.^[40]

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

classification, definition, identification, nanomaterials, particle size, regulation

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- [1] B. Fadeel, L. Farcas, B. Hardy, S. Vázquez-Campos, D. Hristozov, A. Marcomini, I. Lynch, E. Valsami-Jones, H. Alenius, K. Savolainen, *Nat. Nanotechnol.* **2018**, *13*, 537.
- [2] Communication from the Commission to the European Parliament, The Council and the European Economic and Social Committee, Regulatory Aspects of Nanomaterials, COM (2008) 366 Final, <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=COM:2008:0366:FIN:EN:PDF> (accessed: April 2020).
- [3] European Parliament, European Parliament resolution of 24 April 2009 on Regulatory Aspects of Nanomaterials P6 TA(2009)0328, (2008/2208(INI)), <https://www.europarl.europa.eu/sides/getDoc.do?pubRef=-//EP//NONSGML+TA+P6-TA-2009-0328+0+DOC+PDF+V0//EN> (accessed: April 2020).
- [4] European Commission, Commission Recommendation of 18 October 2011 on the Definition of nanomaterial, OJ **2011**, L 275, 38.
- [5] European Parliament and Council, Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 Concerning the Making Available on the Market and Use of Biocidal Products, OJ **2012**, L 167, 1.
- [6] European Parliament and Council, Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 Concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), Establishing a European Chemicals Agency, Amending Directive 1999/45/EC and Repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/ECL, OJ **2006**, L 396, 1.
- [7] Commission Regulation (EU) 2018/1881 of 3 December 2018 Amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as Regards Annexes I, III, VI, VII, VIII, IX, X, XI, and XII to Address Nanoforms of Substances, OJ **2018**, L 308, 1.
- [8] European Parliament and Council, Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017 on Medical Devices, Amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009 and Repealing Council Directives 90/385/EEC and 93/42/EEC, OJ **2017**, L 117, 1.
- [9] European Parliament and Council, Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products, OJ **2009**, L 342, 59.
- [10] European Parliament and Council, Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on Novel Foods, Amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001, OJ **2015**, L 327, 1.
- [11] ISO/TS 80004-1:2015(en) Nanotechnologies—Vocabulary—Part 1: Core Terms, <https://www.iso.org/standard/68058.html>.
- [12] G. Loevestam, H. Rauscher, G. Roebben, B. Sokull-Kluettgen, N. Gibson, J.-P. Putaud, H. Stamm, *Considerations on a Definition of Nanomaterial for Regulatory Purposes*, EUR 24403 EN, European Union, Luxembourg **2010**, <https://doi.org/10.2788/98686> (accessed: April 2020).
- [13] F. Babick, J. Mielke, W. Wohlleben, S. Weigel, V.-D. Hodoroaba, *J. Nanopart. Res.* **2016**, *18*, 158.
- [14] H. Rauscher, A. Mech, N. Gibson, D. Gilliland, A. Held, V. Kestens, R. Koeber, T. P. J. Linsinger, E. A. Stefaniak, *Identification of Nanomaterials through Measurements*, EUR 29942 EN, European Union, Luxembourg, <https://doi.org/10.2760/053982> (accessed: April 2020).
- [15] V. Kestens, G. Roebben, J. Herrmann, Å. Jämting, V. Coleman, C. Minelli, C. Clifford, P.-J. De Temmerman, J. Mast, L. Junjie, F. Babick, H. Cölfen, H. Emons, *J. Nanopart. Res.* **2016**, *18*, 171.
- [16] A. Mech, H. Rauscher, F. Babick, V.-D. Hodoroaba, A. Ghanem, W. Wohlleben, H. Marvin, S. Weigel, R. Brüngel, C. M. Friedrich, K. Rasmussen, K. Loeschner, D. Gilliland, EUR 29876 EN, *The NanoDefine Methods Manual*, European Union, Luxembourg, **2020**, <https://doi.org/10.2760/79490> (online), <https://10.2760/58586> (print) (accessed: April 2020).
- [17] H. Rauscher, G. Roebben (Eds), *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, European Union, Luxembourg **2015**, <https://doi.org/10.2788/678452> (accessed: April 2020).
- [18] W. Wohlleben, J. Mielke, A. Bianchin, A. Ghanem, H. Freiberger, H. Rauscher, M. Gemeinert, V.-D. Hodoroaba, *J. Nanopart. Res.* **2017**, *19*, 61.
- [19] <http://www.nanodefine.eu/>.
- [20] T. Linsinger, G. Roebben, D. Gilliland, L. Calzolari, F. Rossi, N. Gibson, C. Klein, *Requirements on Measurements for the Implementation of the European Commission Definition of the Term ‘Nanomaterial’*, EUR 25404, European Union, Luxembourg, **2012**, <https://doi.org/10.2787/63490> (accessed: April 2020).
- [21] H. Rauscher, G. Roebben (Eds), *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, European Union, Luxembourg **2014**, <https://doi.org/10.2788/36237> (accessed: April 2020).
- [22] G. Roebben, H. Rauscher (Eds), *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, European Union, Luxembourg **2014**, <https://doi.org/10.2787/97286> (accessed: April 2020).
- [23] OECD, *Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials*, ENV/JM/MONO(2012)40 **2012**.
- [24] C. Gaillard, A. Mech, W. Wohlleben, F. Babick, V.-D. Hodoroaba, A. Ghanem, S. Weigel, H. Rauscher, *Nanoscale Adv.* **2019**, *1*, 781.
- [25] R. Brüngel, J. Rückert, W. Wohlleben, F. Babick, A. Ghanem, C. Gaillard, A. Mech, H. Rauscher, V.-D. Hodoroaba, S. Weigel, C. M. Friedrich, *Materials* **2019**, *12*, 3247.
- [26] <http://www.nanodefine.eu/index.php/nanodefiner-e-tool> and <https://labs.inf.fh-dortmund.de/NanoDefiner> (accessed: April 2020).
- [27] NanoDefine, Deliverable 7.13, Report on Case Studies, https://www.nanodefine.eu/publications/reports/NanoDefine_TechnicalReport_D7.13.pdf **2019** (accessed: April 2020).
- [28] E Scientific Committee, A. Hardy, D. Benford, T. Halldorsson, M. J. Jeger, H. K. Knutsen, H. Naegeli, H. Noteborn, C. Ockleford, A. Ricci, G. Rychen, J. R. Schlatter, V. Silano, R. Solecki, D. Turck, M. Younes, Q. Chaudhry, F. Cubadda, D. Gott, A. Oomen, S. Weigel, M. Karamitrou, A. Mortensen, *EFSA J.* **2018**, *16*, 5327.
- [29] Questions and Answers on the Commission Recommendation on the Definition of Nanomaterial, https://ec.europa.eu/environment/chemicals/nanotech/faq/questions_answers_en.htm (accessed: April 2020).
- [30] W. Wohlleben, *Development of an Integrated Approach Based on Validated and Standardized Methods to Support the Implementation of the EC Recommendation for a Definition of Nanomaterial*,

- NanoDefine Technical Report D3.5, http://www.nanodefine.eu/publications/reports/NanoDefine_TechnicalReport_D3.5.pdf, **2018** (accessed: April 2020).
- [31] N. Gibson, P. Kuchenbecker, K. Rasmussen, V.-D. Hodoroaba, H. Rauscher, Characterization of Nanoparticles: Measurement Procedures for Nanoparticles, 3.5.1, Volume Specific Surface Area: Gas Adsorption Analysis by the BET Method, ISBN 978-0-12-814182-3 **2020**.
- [32] NanoDefine measurement reports (access password: NanoDefine), <https://cloudstore.zih.tu-dresden.de/public.php?service=files&t=df134ce4a94b90c4a3da0a16a050ba2b>, (accessed: April 2020).
- [33] Private communication from BASF NanoDefine Project Partner, (accessed: December 2020).
- [34] C. Singh, S. Friedrichs, M. Levin, R. Birkedal, K. A. Jensen, G. Pojana, W. Wohlleben, S. Schulte, K. Wiench, T. Turney, O. Koulaeva, D. Marshall, K. Hund-Rinke, W. Kördel, E. Van Doren, P.-J. De Temmerman, M. Abi Daoud Francisco, J. Mast, N. Gibson, R. Koeber, T. Linsinger, C. L. Klein, NM-Series of Representative Manufactured Nanomaterials Zinc Oxide NM-110, NM-111, NM-112, NM-113 Characterisation and Test Item Preparation, JRC Technical Report, JRC 64075, EUR 25066 EN **2011**, <https://doi.org/10.2787/55008> (accessed: April 2020).
- [35] H. Rauscher, G. Roebben, A. Mech, P. N. Gibson, V. Kestens, T. Linsinger, J. Riego Sintes, *An Overview of Concepts and Terms Used in the European Commission's Definition of Nanomaterial*, EUR 29647 EN, European Union, Luxembourg **2019**, <https://doi.org/10.2760/459136> (accessed: April 2020).
- [36] K. Rasmussen, M. González, P. Kearns, J. Riego Sintes, F. Rossi, P. Sayre, *Regul. Toxicol. Pharmacol.* **2016**, *74*, 147.
- [37] U.S. Food and Drug Administration, Guidance for Industry. Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology, Docket number: FDA-2010-D-0530, <http://www.fda.gov/RegulatoryInformation/Guidances/ucm257698.htm> **2014** (accessed: July 2020).
- [38] U.S. Environmental Protection Agency, *Fed. Regist.* **2017**, *82*, 3641.
- [39] K. Rasmussen, B. Sokull-Klüttgen, I. J. Yu, J. Kanno, A. Hirose, M. R. Gwinn, *Adverse Effects of Engineered Nanomaterials. Exposure, Toxicology, and Impact on Human Health*, 2nd ed. (Eds: B. Fadeel, A. Pietroiusti, A. A. Shvedova), Academic Press, London **2017**, Chap. 7, pp. 160–189.
- [40] A. Kraegeloh, B. Suarez-Merino, T. Sluijters C. Micheletti, *Nanomaterials* **2018**, *8*, 239.