



European
Commission

JRC SCIENCE AND POLICY REPORTS

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

Edited by

Gert Roebben and Hubert Rauscher

Authors

Gert Roebben
Hubert Rauscher
Valeria Amenta
Karin Aschberger
Ana Boix Sanfeliu
Luigi Calzolari
Hendrik Emons
Claire Gaillard
Neil Gibson
Uwe Holzwarth
Robert Koeber
Thomas Linsinger
Kirsten Rasmussen
Birgit Sokull-Klüttgen
Hermann Stamm

2014

Report EUR 26744 EN

European Commission

Joint Research Centre
Institute for Reference Materials and Measurements

Contact information

Gert Roebben and Hubert Rauscher
Address: Joint Research Centre,
E-mail: gert.roebben@ec.europa.eu
Tel.: +32 14 571816
Fax: +32 14 571548

<http://irmm.jrc.ec.europa.eu/>

<http://www.jrc.ec.europa.eu/>

Legal Notice

This publication is a Science and Policy Report by the Joint Research Centre, the European Commission's in-house science service. It aims to provide evidence-based scientific support to the European policy-making process. The scientific output expressed does not imply a policy position of the European Commission. Neither the European Commission nor any person acting on behalf of the Commission is responsible for the use which might be made of this publication.

JRC91377

EUR 26744 EN

ISBN 978-92-79-39427-0

ISSN 1831-9424

doi: 10.2787/97286

Luxembourg: Publications Office of the European Union, 2014

© European Union, 2014

Reproduction is authorised provided the source is acknowledged.

Printed in Belgium

Abstract

This report provides the JRC assessment of feedback on the experiences of stakeholders with the EC nanomaterial definition, published in 2011 (EC Recommendation 2011/696/EU). The report is a follow-up report of the previous JRC report (EUR 26567 EN, 2014), which compiled feedback collected by JRC in 2013 and early 2014, partly through a dedicated survey. Based on the current report, JRC will prepare a set of recommendations for the revision of the EC nanomaterial definition, as part of the review process foreseen in the 2011 EC Recommendation.

JRC Scientific and Policy Report

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

Edited by

Gert Roebben and Hubert Rauscher

Authors

Gert Roebben,^{*1} Hubert Rauscher,^{*2} Valeria Amenta,² Karin Aschberger,² Ana Boix Sanfeliu,¹
Luigi Calzolari,² Hendrik Emons,¹ Claire Gaillard,² Neil Gibson,² Uwe Holzwarth,² Robert Koeber,¹
Thomas Linsinger,¹ Kirsten Rasmussen,² Birgit Sokull-Klüttgen,² Hermann Stamm²

August 2014

¹ Institute for Reference Materials and Measurements

² Institute for Health and Consumer Protection

* Corresponding authors

DISCLAIMER

Certain commercial equipment, instruments, brand names and materials are identified in this report as examples or to specify adequately an experimental procedure. In no case does such identification imply recommendation or endorsement by the European Commission, nor does it imply that the material or equipment is necessarily the best available for the purpose.

EXECUTIVE SUMMARY

This report is the second in a series of reports of the Joint Research Centre (JRC) of the European Commission (EC) “Towards a review of the EC Recommendation for a definition of the term nanomaterial”. The EC Recommendation (2011/696/EU) proposes a nanomaterial definition (in this report referred to as 'the EC definition' or 'the definition'), which was developed to provide a common basis for regulatory purposes across all areas of European Union (EU) policy.

In the present report JRC assesses the information collected between August 2013 and April 2014 from scientists, research institutes, regulatory bodies, non-governmental organisations and industry regarding implementation of the EC Recommendation. Consequently, it builds on the first report of the series entitled “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”.

After an introduction (Section 1), this second report puts the EC nanomaterial definition in perspective by comparing it with other existing nanomaterial definitions, thereby identifying the most prominent characteristics of the EC definition (Section 2).

Probably the most distinguishing aspect of the EC nanomaterial definition is the use of particle size distributions based on the numbers of particles, and not on the mass or volume of the particles, as the main classification feature. The general advantages and disadvantages of using this metric are assessed in Section 3.1. Then section 3.2 focusses on the choice of 50 % as the value for the threshold particle number fraction to distinguish nanomaterials from non-nanomaterials, and examines whether this criterion is effective as a “boundary line” between materials that are generally perceived to be nanomaterials and those that are not. Section 3.3 investigates the consequences of allowing flexibility in the choice of the threshold value on transparency, regulatory uptake and implementation.

It became obvious from the feedback collected by the JRC that there is a need to clarify a number of issues around and inside the definition. The report first reflects on apparent misunderstandings on the purpose (Section 4.1) and scope (Section 4.2) of the definition. Section 4.3 discusses the main terms in the current definition that have been identified as potential sources of confusion for its common understanding and application. Section 4.4 assesses generic questions on how to implement the definition.

Section 5 is focussed on the main implementation challenges resulting from the current definition, and on proposals about how to overcome these. Most of the challenges brought to the attention of the JRC are related to analytical methods: how can one implement the definition through measurements? Section 5.2 introduces the concept of measurement uncertainty, which is crucial to quantify this analytical challenge, to judge proposed measurement methods and ultimately to compare measurement results. Section 5.3 discusses the most demanding analytical challenge, which is the measurement of the size of constituent particles inside aggregates. Section 5.4 evaluates a number of routes that may be used as alternatives to the direct implementation of the definition (i.e. individual analysis of constituent particles), such as the use of surface area measurements (5.4.1), the conversion from other types of size distributions to number based size distributions (5.4.2), the use of simple separation or filtration methods (5.4.3), the consideration of information on manufacturing processes (5.4.4) and the extrapolation of results obtained on one material to other similar materials (*read-across*) (5.4.5). Section 5.5 summarises this chapter on analytical challenges and considers related resources.

The definition explicitly includes a limited number of specified materials which do not meet the generic definition based on characteristics of the particle size distribution. Therefore, Section 6.1 reflects on the methods available to detect and identify these materials (fullerenes, single-wall carbon nanotubes and graphene). Section 6.2 investigates the consequences of potentially expanding this 'positive list' of materials.

Finally Section 7 addresses the issue of nanostructured materials as well as possible reasons for, and consequences of, expanding the current definition – which covers only particulate matter – towards inclusion of other types of nanostructured materials.

Based on the feedback received regarding the current definition, compiled in the first report of the series, and its assessment, presented in this second report, the JRC is now working on a set of indications on how the definition could be modified to improve its clarity, effectiveness and implementability. These recommendations will be included in a final report (Part 3 of the series), which is expected to be released later in 2014.

Table of content

DISCLAIMER.....	0
EXECUTIVE SUMMARY	1
1 INTRODUCTION	8
2 THE EC NANOMATERIAL DEFINITION COMPARED WITH OTHER DEFINITIONS.....	9
2.1 COMPARISON OF INTENDED PURPOSES AND SCOPES	9
2.1.1 Legal status	9
2.1.2 Broadness of scope.....	9
2.1.3 Origin of materials	10
2.1.4 Particulate vs non-particulate matter	10
2.2 COMPARISON OF TECHNICAL ASPECTS	10
2.2.1 Size range of relevant microstructural features	10
2.2.2 Threshold for the fraction of particles in a particle size distribution	10
2.2.3 Agglomerates and aggregates	11
2.2.4 Specific surface area	11
2.2.5 Nano-specific functional properties	11
2.2.6 Solubility	12
2.3 SUMMARY: THE ESSENTIAL ELEMENTS AND CHARACTERISTICS OF THE EC NANOMATERIAL DEFINITION	12
3 THE PARTICLE NUMBER BASED PARTICLE SIZE DISTRIBUTION AS DEFINING FEATURE	13
3.1 THE CHOICE FOR PARTICLE NUMBER FRACTION AS METRIC IN THE NANOMATERIAL DEFINITION... 13	
3.1.1 General considerations on concentration metrics	13
3.1.2 Relevant concentration metrics in particle toxicology	13
3.1.3 Recommendations from national and international organisations.....	14
3.1.4 Conclusions	15
3.2 CONSEQUENCES OF CHOOSING 50 % AS THE THRESHOLD PARTICLE NUMBER FRACTION	16
3.2.1 Borderline cases.....	16
3.2.2 Materials perceived as nanomaterials but excluded from the definition	17
3.2.3 Materials not perceived as nanomaterials but included in the definition	17
3.2.4 Considerations on imposing additional criteria and limits	18
3.2.5 Conclusion	18
3.3 REGULATORY CONSEQUENCES OF A FLEXIBLE THRESHOLD VALUE	19
3.3.1 The exposure consideration	19
3.3.2 The consistency consideration	19
3.3.3 The implementation consideration	20
3.3.4 Conclusion	21
4 NEEDS FOR CLARIFICATION OF THE CURRENT EC NANOMATERIAL DEFINITION	22
4.1 CLARIFICATION OF THE PURPOSE OF THE DEFINITION.....	22
4.2 CLARIFICATION OF THE SCOPE OF THE DEFINITION	22
4.2.1 Manufactured or not	22
4.2.2 Defining properties other than size.....	23

4.2.3	Non-particulate materials	23
4.2.4	Products	23
4.2.5	Mixtures	24
4.2.6	(Volume-)specific surface area	24
4.3	CLARIFICATION OF TERMS USED IN THE DEFINITION	24
4.3.1	Particle.....	24
4.3.2	Unbound state and physical boundaries.....	25
4.3.3	Agglomerate and aggregates	26
4.3.4	Primary particles and constituent particles.....	27
4.3.5	Size and external dimension.....	28
4.3.6	Conclusion	28
4.4	CLARIFICATION ON HOW TO IMPLEMENT THE DEFINITION.....	28
4.4.1	Current implementation support and guidance.....	28
4.4.2	Guidance on the required measurement system.....	29
4.4.3	Integrated testing strategies and tiered approaches	31
4.4.4	Conclusions and major gaps	32
5	THE MAIN, ANALYTICAL IMPLEMENTATION CHALLENGES	33
5.1	INTRODUCTION	33
5.1.1	Overview of analytical challenges brought up in the JRC survey	33
5.2	CONFORMITY ASSESSMENT AND MEASUREMENT UNCERTAINTY	35
5.2.1	The role of measurement uncertainty in conformity assessment	35
5.2.2	Generic components of measurement uncertainty	35
5.2.3	Measurement uncertainty in particle size analysis: the current status	36
5.2.4	Specific uncertainty contributions from elements of the nanomaterial definition	39
5.2.5	Volume-specific surface area	41
5.2.6	Outlook.....	41
5.3	MEASURING THE SIZE OF CONSTITUENT PARTICLES IN AGGREGATES.....	42
5.3.1	Aggregates, agglomerates and their dispersion into constituent particles.....	42
5.3.2	Constituent particles and primary particles	43
5.3.3	Measurement of constituent particle size inside smallest dispersible units.....	44
5.3.4	Conclusion and outlook.....	45
5.4	ALTERNATIVE ANALYTICAL ROUTES TO IMPLEMENT THE EC NANOMATERIAL DEFINITION.....	45
5.4.1	Use of specific surface area measurements.....	45
5.4.2	Conversion of other size distributions to particle number based size distributions	52
5.4.3	Simplified binary binning methods.....	54
5.4.4	Considerations of manufacturing information	55
5.4.5	Possibilities for read-across	59
5.5	THE FINANCIAL IMPLICATIONS OF THE ANALYTICAL CHALLENGES	62
5.5.1	Estimates from JRC report EUR 26567	62
5.5.2	Feedback on the estimates from JRC report EUR 26567.....	62
5.5.3	Conclusion	63
6	MATERIALS EXPLICITLY INCLUDED IN THE DEFINITION.....	64
6.1	METHODS TO DETECT AND IDENTIFY FULLERENES, SINGLE-WALL CARBON NANOTUBES AND GRAPHENE	64
6.1.1	Fullerenes, graphene flakes and single wall carbon nanotubes in the EC definition	64
6.1.2	Standards for the identification of fullerenes, graphene flakes and single wall carbon nanotubes.....	65

6.1.3	Other measurement methods to identify fullerenes, CNTs and graphene	65
6.1.4	Conclusion	65
6.2	EXPANSION OF THE LIST OF EXPLICITLY INCLUDED MATERIALS.....	66
6.2.1	Graphene	66
6.2.2	Nanotubes	66
6.2.3	Quantum Dots	69
6.2.4	Nanoclay	69
6.2.5	General considerations.....	70
7	NANOSTRUCTURED MATERIALS.....	71
7.1	REGULATORY RELEVANCE OF NANOSTRUCTURED MATERIALS.....	71
7.1.1	Introduction	71
7.1.2	Regulatory relevance of nanostructured materials.....	71
7.2	METHODS TO CHARACTERISE NANOSTRUCTURED MATERIALS	74
7.2.1	Extension of the particle size distribution-based nanomaterial definition	74
7.2.2	Implementation of an extended nanomaterial definition.....	75
7.3	CONCLUSIONS.....	75
	REFERENCES	76

ACRONYMS

0D,1D,2D,3D	0-, 1-, 2- and 3-dimensional
a, b, c, d	dimensions of sides and cross-sections of regularly shaped particles
ACC	American Chemistry Council
AFM	Atomic Force Microscopy
ASTM	ASTM International
BAM	German Federal Institute for Materials Research and Testing
BET	Brunauer-Emmett-Teller
BAuA	German Federal Institute for Occupational Safety and Health
BfR	Bundesinstitut für Risikobewertung, Germany
CEN	European Committee for Standardization
CLP	EU Regulation Classification, Labelling and Packaging
CLS	Centrifugal Liquid Sedimentation
CNT	Carbon nanotube
CRM	Certified Reference Material
<i>D</i>	diameter of a sphere
<i>d</i>	diameter of circular cross-section
DLS	Dynamic Light Scattering
DG	Directorate-General of the European Commission
DG ENV	Environment Directorate-General
EC	European Commission
ECHA	European Chemicals Agency
EDX	Energy-dispersive X-ray Spectroscopy
EFSA	European Food Safety Authority
EM	Electron Microscopy
ETUC	European Trade Union Confederation
EU	European Union
FFF	Field-flow Fractionation
FP7	Seventh framework programme of the European Community for research and technological development including demonstration activities
FSP	Flame spray processing
ICCA	International Council of Chemical Associations
ICCR	International Cooperation on Cosmetic Regulation
ICP-MS	Inductively coupled plasma mass spectrometry
IEC	International Electrotechnical Commission
IHCP	Institute for Health and Consumer Protection
ILC	Inter Laboratory Comparison
IRMM	Institute for Reference Materials and Measurements
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
JRC	Joint Research Centre
<i>l</i>	length
LD	Laser Diffraction
LED	light-emitting diode
NIST	U.S. National Institute for standards and technology
OECD	Organisation for Economic Co-operation and Development

OEL	Occupational exposure limit
PI	polydispersity index
PNT	polymer nanotube
PSA	Particle size analysis
PSD	Particle size distribution
PTA	Particle Tracking Analysis
Q&A	Questions and Answers
r	radius
REACH	EU Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals
RIVM	Dutch National Institute for Public Health and the Environment
RM	Reference material
SAXS	Small-Angle X-ray Scattering
SC	Sub-committee (of a standards developing organisation technical committee)
SCCS	Scientific Committee on Consumer Safety
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SECO	Swiss State Secretariat for Economic Affairs
SEM	Scanning Electron Microscopy
sp-ICPMS	Single Particle Inductively Coupled Plasma Mass Spectrometry
SPM	Scanning Probe Microscopy
SSA	Specific surface area
SVHC	Substance of very high concern
SWCNT	single-walled carbon nanotube
SWD	Staff Working Document of the European Commission
TC	Technical Committee
TEM	Transmission Electron Microscopy
TNT	Titanium dioxide nanotube
TR	Technical Report
TS	Technical Specification
UBA	German Federal Environment Agency
UV-Vis	Ultraviolet and visible light
VAMAS	Versailles Project on Advanced Materials and Standards
VCI	German Chemical Industry Association
VSSA	Volume Specific Surface Area
$X_{F,min}$	minimum Feret diameter
XRD	X-ray Diffraction

1 INTRODUCTION

In 2011, the European Commission published a Recommendation (2011/696/EU) with a proposed definition for the term *nanomaterial*, specifically for regulatory use,¹ covering natural, incidental and manufactured materials and based solely on the size of the constituent particles of a material, without regard to specific functional or hazard properties or risks:

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

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

The Recommendation further specifies:

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

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

- (a) ‘particle’ means a minute piece of matter with defined physical boundaries;
- (b) ‘agglomerate’ means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components;
- (c) ‘aggregate’ means a particle comprising of strongly bound or fused particles.

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

In the same Recommendation (2011/696/EU), the EC announced that the proposed definition would be reviewed in 2014: "...*Technological development and scientific progress continue with great speed. The definition including descriptors should therefore be subject to a review by December 2014 to ensure that it corresponds to the needs.*"¹

In 2013, the Joint Research Centre of the European Commission (JRC) started collecting feedback from stakeholders and users of the EC nanomaterial definition, and earlier in 2014 JRC released a first report, with a compilation of the collected feedback and data.²

The current (second) JRC report provides an assessment by JRC of the information collected in the first JRC report, where necessary complemented with new or additional input.

This second JRC report will be followed later in 2014 by a third JRC report, in which JRC will formulate its recommendations to the EC policy services that will need to judge whether the current definition needs revision, and if yes, in which way it should be adapted.

2 THE EC NANOMATERIAL DEFINITION COMPARED WITH OTHER DEFINITIONS

The nanomaterial definition in the EC Recommendation (further in this report called *the EC definition, or the definition*) was not the first and is not the only definition of the term nanomaterial. This chapter compares parameters and properties applied in different definitions of nanomaterials. Particular emphasis is given to the *EC definition* and especially why it includes or excludes certain aspects and issues relevant for nanomaterials.

In addition to the *EC definition*, the definitions considered here are from standardisation organisations: the International Organization for Standardization (ISO), and the European Committee for Standardization (CEN)³; industrial organisations: the American Chemistry Council (ACC)⁴, the International Council of Chemical Associations (ICCA)⁵, the German Chemical Industry Association (VCI)⁶, and from regulatory sources: the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR)⁷, the International Cooperation on Cosmetic Regulation (ICCR)⁸, EU regulations (Cosmetic Product Regulation⁹, Food Information to Consumer Regulation¹⁰, Biocides Regulation¹¹, Medicinal Product Regulation¹², Medical Devices Regulation¹³, Regulation on plastic materials and articles intended to come into contact with food^{14,15}). Also (draft) national definitions of nanomaterials are included and come from countries in the EU: France¹⁶, Belgium¹⁷ and Denmark^{18,19}, as well as outside the EU: Switzerland²⁰, the United States of America²¹, Taiwan⁴, Korea²², China²³, Australia²⁴ and Canada²⁵. Details of these definitions can be found in section 2 of the recent JRC Report EUR 26567.² In addition, the ETUC concept of a regulatory definition of a substance in the nanoform was considered in this report.²⁶

2.1 Comparison of intended purposes and scopes

The definitions of nanomaterials from the sources listed above can be classified by their scope and by the type of applications that they intend to address (scientific, regulatory, industrial). For the nanomaterial definitions the following aspects are important: the legal status, the scope, the origin of the materials addressed in a definition and whether the material is particulate or nanostructured. These aspects are reviewed below.

2.1.1 Legal status

Many of the documents addressing the nanomaterial definition issued by international committees and organisations as well as some non-European countries, are advisory, non-normative and non-regulatory and give guidance or recommendations only. EU sector-specific legislation and national registries and mandatory reporting schemes in EU member states and the associated definitions are legally binding.

The *EC definition* is nominally a Recommendation only and hence not legally binding; at the same time it is an instrument explicitly aiming at the harmonisation of existing and future legislation with regard to defining what a nanomaterial is in a regulatory context. The *EC definition* was used in recently adopted Regulations on Biocides¹¹ and on Medical Devices¹³ and is currently used as a basis for the modification of the Cosmetics⁹ and Food Information to Consumers Regulations¹⁰. It is also envisaged to use the Recommendation in other EU regulations, such as REACH, and by EU agencies with regulatory roles.

2.1.2 Broadness of scope

Some of the nanomaterial definitions mentioned above have a scope that is defined in a broad, generic way, whereas others have a narrow, specific scope. Like other definitions by international organizations or national authorities, the *EC definition* does not limit its applicability to certain (chemical) compositions or to certain application fields. (An exception is the definition from the ICCR, which applies only to cosmetic ingredients.)

In contrast to the *EC definition* and its broad scope applicable across different sectors, the definitions and nanomaterial specifications from EU sector specific legislation have a scope limited to the area of the legislation in question. Although those definitions are already in line or will be aligned with the *EC definition*, the limitation of the scope emerges from the well-defined area of application of the regulations themselves.

2.1.3 Origin of materials

Nanomaterial definitions can apply to materials of all kinds of origin, or they can have a scope that is limited to (intentionally) manufactured particles.

Some of the definitions do not address this point at all (definitions from ISO, CEN, SCENIHR, Biocides and Medical Devices Regulations), but most of the definitions limit their scope to “intentionally manufactured” or “engineered” materials. The *EC definition*, however, not only includes intentionally manufactured materials, it also explicitly includes incidentally manufactured and naturally occurring particles. This means *de facto* that all materials regardless from their origin or purpose may fall under the *EC definition*. EU regulations referring to or inspired by the *EC definition* may restrict the scope of the materials effectively covered with criteria such as the origin of the material.

2.1.4 Particulate vs non-particulate matter

While several other definitions also include non-particulate, but nanostructured materials, the *EC definition* is explicitly limited to particulate materials. In this sense, the *EC definition* corresponds to the concept of atmospheric particle matter that includes for example 'PM10' (particles with an aerodynamic diameter of 10 micrometres or less) and 'PM2.5' (*fine particles* with an aerodynamic diameter of 2.5 micrometres or less). (The obvious difference between the 'PM' materials and the *EC definition* is that the latter also includes particulate matter not dispersed in the atmosphere.)

2.2 Comparison of technical aspects

2.2.1 Size range of relevant microstructural features

The defining property that all nanomaterial definitions have in common is the size of constituent microstructural features, e.g. particles. The basic size range is identical (1 nm to 100 nm) for almost all definitions. Whereas several definitions refer to both external and internal structural features, the *EC definition* is limited to the external dimensions of the constituent microstructural features. Internal structure in the *EC definition* is only relevant for the specific subset of nanostructured nanomaterials that are aggregates or agglomerates, but also here the relevant size range is that corresponding with the external dimensions of the constituent particles.

The SCENHIR opinion additionally considers materials with a median size between 100 nm and 500 nm as a nanomaterial, if a statistical extrapolation of the average size and associated standard deviation indicates that possibly 0.15 % or more of the (number of) particles are smaller than 100 nm. Similarly, the Swiss (SECO) guideline²⁰ uses the 1 nm to 100 nm range, and in addition considers a material with an average particle size below 500 nm to be a nanomaterial if the particle size distribution is not known.

The Biocides Regulation and the SECO guideline as well as the *EC definition* explicitly include fullerenes, graphene flakes and single wall nanotubes even if their relevant external dimension is below 1 nm.

2.2.2 Threshold for the fraction of particles in a particle size distribution

Compared with other definitions of nanomaterial, the feature that distinguishes the *EC definition* most explicitly is the threshold value for the fraction of particles in a particle size distribution falling inside a defined size range. The *EC definition* states a threshold value of 50 % in the number based particle size distribution; with a content of particles equal to or above this threshold the material is

defined to be a nanomaterial. Furthermore, the possibility to lower this threshold to a value between 1 % and 50 % is included.

Most definitions do not use the particle size (distribution) as the main identifying parameter (ISO, ICCR, North American and Asian national definitions), and for these definitions a threshold particle fraction is not a relevant parameter to qualify a material as a nanomaterial. For definitions referring to both internal and external features with a size in the nano-range, it is even less straightforward to set up such quantitative criterion. Only for definitions that refer to a specific property induced by the nanostructure of the material, one could set a threshold based on this specific property.

The few other definitions that contain a threshold fraction fall in two categories: particle size distribution is based on a) particle mass or b) particle number. Particle mass based particle size distributions are used in definitions of chemical industries associations (ACC, ICCA, VCI), which commonly set a threshold of 10 % mass. In the definition proposed by ICCA, an additional cut-off of 50 % (mass based) is defined if aggregates/agglomerates consist of nano-objects.

For the particle number based thresholds, the threshold value of 50 %, as in the *EC definition*, is used in legislation within the geographical EU area, such as recently adopted or adapted European Regulations and the French definition. Outside the EU area, some definitions propose a different threshold value:

- The Australian working definition for industrial nanomaterials specifies a 10 % particle number based threshold. The Swiss SECO guideline stipulates a 1 % number based limit. It is not clear whether and how these smaller threshold values are already being applied in practice.

- The SCENIHR Opinion seemingly suggests the lowest threshold value, by proposing that a material is a nanomaterial when more than 0.15 % of the particles may have a diameter below 100 nm. This value can not directly be compared with the 50 % value of the *EC definition*: the SCENIHR Opinion value of 0.15 % follows from a statistical reasoning that aims at taking into account the width of the particle size distribution: the SCENIHR definition includes materials for which the measured average particle size is above 100 nm but only if the standard deviation of this average value indicates that probably there are more than 0.15 % of the particles smaller than 100 nm. De facto, this approach also makes the upper size limit (100 nm) less rigid.

- The ETUC concept, on the other hand, proposes an 80 % threshold for the number of particles with a diameter of 100 nm or below. Interestingly, in the case of a particle size fraction below 100 nm between 10 % and 80 %, ETUC speaks of a multi-constituent substance composed of the nanoform and the bulk material.

2.2.3 Agglomerates and aggregates

Agglomerates and aggregates are not explicitly addressed in ISO, ICCR, North American and most Asian national definitions. In all other definitions assessed, including the *EC definition*, the question of agglomerates and aggregates is explicitly addressed. In particular, the *EC definition* restricts the term 'nanomaterial' to materials containing *particles*. Like particles in an unbound state, agglomerates and aggregates are explicitly included.

2.2.4 Specific surface area

Apart from the VCI and SCENIHR nanomaterial definitions, the *EC definition* is the only definition that establishes the volume specific surface area as a complementary criterion, and the *EC definition* states that a material is classified as a nanomaterial if the volume specific surface area is larger than $60 \text{ m}^2/\text{cm}^3$.

2.2.5 Nano-specific functional properties

There has been, from the earliest debates on the definition of the term nanomaterial, a discussion between stakeholders proposing to define nanomaterials based on *size* (either external size, as in the

EC definition, or the size of internal, structural features) and stakeholders emphasising that a nanomaterial should display *novel properties or properties or phenomena attributable to its [nano] dimensions, or nano-scale properties* (ACC, national definitions of USA, Taiwan, Taiwan, China, Australia and Canada). This discussion is somehow linked to the concept of “engineered” nanomaterials as described in Section 2.1.3 above (on the origin and purpose of materials).

The *EC definition* is size-based only and therefore not limited to materials produced intentionally with specific properties.

2.2.6 Solubility

Another parameter that is used in nanomaterial definitions is the solubility of particles. However, this is only applied in the case of definitions in the cosmetic field (ICCR, EU Cosmetic Product Regulation) and by the ACC. The text of the *EC definition* does not include this aspect.

2.3 Summary: the essential elements and characteristics of the EC nanomaterial definition

The *EC definition* has quite some overlap with many other nanomaterial definitions; however it is unique in the combination of its scope and the technical parameters addressed. An important feature is its legal status and the broad scope of application. Although the *EC definition* is published in a legally non-binding document, it serves as a basis for the definition of nanomaterials in specific EU legislation. On the one hand, the *EC definition* is not limited to a certain type or origin of a material, but on the other hand it only covers particulate materials with specific size features, which therefore potentially show specific properties.

In terms of the physical properties that determine whether a material is considered a nanomaterial or not, the particularity of the *EC definition* is in the combination of a size range, a threshold value of 50 % for the fraction of particles in a particle number based particle size distribution, and the additional volume specific surface area criterion.

As such, the *EC definition* is a prime example of definitions that are 'regulation and concern'-inspired:

- it is broad and inclusive (in an attempt not to exclude any potentially harmful materials for which novel properties are not (yet) demonstrated), and at the same time it is restricted to particulate materials (the materials that are realistically the only ones that can lead to direct exposure of humans and the environment),

- it is relying on external dimensions, more specifically on particle size values (providing a classical and seemingly feasible way of implementing the definition),

- it is based on the particle number based particle size distribution (because particle mass based size distributions are easily affected by the presence of a few large particles, and in line with a basic toxicological concept of the *number* of interactions that can potentially create a toxic effect).

Any proposal to revise the definition that affects these main characteristics would require a strong justification.

3 THE PARTICLE NUMBER BASED PARTICLE SIZE DISTRIBUTION AS DEFINING FEATURE

The particle size distribution (PSD) of a particulate material is a list of values or the corresponding graphical presentation or mathematical equation, which show how the particles in a material are distributed over different size classes. The PSD can be "mass-based" (when the PSD shows the combined mass of all particles per size class, compared to the total mass of material) or "number-based" (when the PSD shows the number of particles per size class compared to the total number of particles in the material). Other types of size distribution are based on volume per size class, surface area per size class, or (any kind of) signal intensity per size class.

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

This Chapter investigates three aspects: first, the choice for the particle number based fraction as the defining metric, secondly the choice of 50 % as the default threshold value, and thirdly the consequences of allowing the flexibility to deviate from this 50 % threshold.

3.1 The choice for particle number fraction as metric in the nanomaterial definition

Some of the respondents to the online survey launched by JRC in preparation of the review of the definition (see report EUR 26567 EN, report 1 of 3 in this series)² argued that the mass-based PSD should be used in the definition, because routine measurements in industry are mainly based on volume-, mass- or intensity based PSDs, while methods for measuring particle number-based PSDs have only been tested on an investigative level.

It must be noted, however, that only number-based PSDs give a clear picture of how many particles of a certain size are available in a sample. This information is missing when using mass-based distributions, especially as the latter are dominated by larger particles if there is a substantial size difference between the smaller and the larger particles in the material. This section further investigates whether it is important to know the number based PSD, and use it in the nanomaterial definition, rather than the mass based PSD.

3.1.1 General considerations on concentration metrics

For soluble chemicals, concentration is traditionally reported as 'amount-of-substance' concentration (in other words: referring to the number of moles of the substance of interest in a given volume). Neglecting potential isotopic differences, amount-of-substance and mass based concentrations of soluble chemicals are equivalent and can be converted into each other using the molecular weight.

The situation is different when considering particles as the building blocks of a material. Particles of a certain material are usually not all identical, but are characterized by a size distribution, a shape distribution and even a density distribution. Having measured their mass-based PSD is not sufficient to calculate the number of particles, as this calculation requires precise information on parameters such as particle size, shape and density.

3.1.2 Relevant concentration metrics in particle toxicology

In classical toxicology with soluble chemicals the dissolved material is present in the form of molecules or ions, which move and freely interact with biologically relevant active sites. Since every ion or molecule moves separately, they can potentially all find a toxicologically relevant active site.

Molecules and ions that are bound together in a particle cannot move independently, and thereby the number of interactions per gram of particle substance with biologically relevant active sites is limited. Size and shape of the particles (or surface area in a simplified view) are the parameters to consider when estimating the number of interactions possible for each gram of particle substance

(effective dose) and therefore determine the extent of the measured toxicological effect.²⁷ It must also be kept in mind that molecules or ions and particles may interact differently with biological entities.

Nevertheless, Occupational Exposure Limits (OEL) for particulate compounds are traditionally provided as mass concentration. Size effects are only implicitly recognised when different size fractions (e.g. respirable vs inhalable) receive different OELs. The mass concentration metric may not be very meaningful especially for ultrafine or nanoparticles. At an OEL of 1 $\mu\text{g}/\text{m}^3$, the corresponding small amount in mass could correspond with a huge number of ultrafine particles. The consequence is that exposure assessment based on mass could underestimate the toxicity of nanoparticles, as such small particles do not contribute considerably to the overall mass concentration, even though they represent the larger amount in terms of particles number. A dose-metric expressed as particle number could be much more relevant in these situations.²⁸

Particle number has indeed been used for exposure assessment of fibres, and the particle counter instruments which are used to measure exposure in occupational settings utilize the number concentration metric (particle count per air volume).²⁹ But there are other relevant metrics as well, for example when studying toxicity of ultrafine particles in lungs, which is linked to the accumulation of particles in the lungs (lung overload). The extent of the overload and therefore of the toxicological effects has been proposed to be related to the overall *volume* of the particles (particle or agglomerate density).^{30,31} In another study, by Gallagher et al.³², the lung overload condition seemed to be associated to inflammation only when the *surface area* of the particles was high, and Tran et al.³³ proposed total particles surface area as the most relevant metric to express the lung burden due to poorly soluble particles.

In several toxicological studies, surface area has been demonstrated to be the most relevant dose-metric for comparing the effects of different kinds of nanoparticles of different sizes and the best correlation between *in vitro* and *in vivo* studies has been found using such metric.^{33,34,35} Therefore, even if the relation between particles surface area and inflammation is not yet certain, for inhalation toxicity studies, mass may not be the key parameter to be considered. In fact, the issue of dosimetry is even more complex, since there is often a significant difference between the average concentration in an environment and the effective dose at the cellular level, as discussed for *in vitro* testing by Teeguarden and Oberdorster.³⁶

Since the current EC definition restricts nanomaterials to particulate matter, the choice of appropriate dose metric to be used in toxicological or exposure studies must be inspired by the general findings of particle toxicology, and be related to the mechanism of action of the nanomaterial: "*The effective dose of a nanomaterial may be smaller on a mass basis than the effective dose of larger particles of the same material if the mode of action relates to the total particle number or surface area*".^{37,38}

3.1.3 Recommendations from national and international organisations

As already stated in Chapter 2, the EC definition is developed particularly for regulatory purposes, which has certainly influenced the choice of the metric used in the definition. In this subsection we investigate how different organisations with regulatory vocation have linked their regulatory concerns with the definition or classification of nanomaterials.

In the document "*R14-4 Recommendations for nanomaterials applicable to Chapter R.14 Occupational exposure estimation*"³⁹ of the Guidance on information requirements and chemical safety assessment, ECHA asserts that all three main metrics: i) mass concentration (unit kg/m^3); ii) number concentration (unit $1/\text{m}^3$); and iii) surface area concentration (unit m^2/m^3) can be relevant when performing exposure estimation studies. ECHA's suggestion is that the right metric to choose is the one which is "*correlated with the health effect of concern, can be relatively easily measured and be both measurable and sensitive enough to detect differences in the probable ranges encountered*".

The best metric to choose is likely to be different for different nanomaterials and for the kind of health effect with which exposure studies correlate.

SCENIHR was the first organization to publish a recommendation to define the term nanomaterial using a number-based PSD. SCENIHR argued that a small mass concentration of nanoparticles in a material might still correspond with a great number of nanoparticles.^{7,40} For the same reason, also the European Trade Union Confederation (ETUC) recommends the use of a number-based PSD rather than the mass or volume based one for the definition of a substance in the nanoform.²⁶

OECD does not have its own specific nanomaterial definition based on mass or particle number based PSDs. In a document on nanomaterial sample preparation and dosimetry, OECD recommends that dose is not only reported in the traditional mass-based manner, but that also surface area- and particle number-based PSDs must be reported in toxicological studies, as these parameters may play a key role in determining the toxicity of nanomaterials.⁴¹ In the document on risk assessment of manufactured nanomaterials, OECD also states that using different metrics in the risk assessment of nanomaterials could help understanding which metric is more closely related with the mechanism of action of a certain nanomaterial.³⁷

The Scientific Committee on Consumer Safety (SCCS) agrees that, differently than for conventional chemicals, metrics such as mass or volume may not be appropriate for nanomaterials, but additional parameters such as particle number concentration and surface area must be provided in toxicological assessments.⁴² Similarly, EFSA, in its guidance document on risk assessment of nanomaterials, recommends that together with mass-based dose, additional information concerning the conversion of the mass dose metric to surface area and/or number of particles must be provided, especially when a comparison with non-nanofoms is required in the risk assessment context.⁴³ In the report "*Interpretation and implications of the European Commission Recommendation on the definition of nanomaterial*"⁴⁴ published in 2012, the Dutch National Institute for Public Health and the Environment (RIVM) highlights that the practical implication of using a number-based PSD rather than a mass-based PSD is that more materials will be classified as nanomaterial, provided that the threshold remains the same. RIVM underlines that having a definition using the number-based PSD reduces the chances to classify a material as non-nanomaterial even if the majority of the particles are below 100 nm. This situation could occur when using a mass-based particle size distribution.⁴⁴

The German Competent Authorities, BfR (Federal Institute for Risk Assessment), BAuA (Federal Institute for Occupational Safety and Health) and UBA (Federal Environmental Agency) in the report "Nanomaterials and REACH" affirm that the number-based PSD could be used as a parameter to compare different nanoforms of the same substance.⁴⁵

While mass concentration has traditionally been considered the most convenient dose metric for toxicological and exposure studies with soluble chemicals, the pertinence of using only this dose metric for particles is questioned by many.³⁴ Since at the moment no agreement has been reached in the scientific community on the appropriate exposure metric to be used for the assessment of exposure to particles, the concept of using multiple metrics (mass, surface area and number of particles) simultaneously in exposure measurements has been widely proposed and in some cases has already been applied.^{35,46} This trend to use alternative metrics such as particle number, volume specific surface area or a combination of all these parameters more often is also observed for studies performed with particulate nanomaterials.^{36, 47, 48, 49, 50} When reporting toxicological effects of nanomaterials, the number of particles of a certain size can indeed become a key parameter, as it is related to the possible number of interactions with biologically relevant sites. Similarly, surface area is also a parameter that may correlate with the effects of nanoparticles, because it determines the amount of active atoms exposed at the surface and able to interact with the environment.

3.1.4 Conclusions

A single, most pertinent dose metric to be used in toxicological studies has not been agreed at international level. The most relevant dose metric is likely to be different for different nanomaterials

and for different toxicological endpoints. The right metric to choose would be the one most closely associated with the mode of action leading to a certain effect.⁵¹ An immediate and important consequence of nevertheless using (only) particle number based PSDs as criterion in the EC definition, is that also materials are included that may have an extremely small mass fraction of nanoparticles. Therefore, it is fair to state that the choice of number-based PSDs to define nanomaterials is based largely on an attitude of precaution of the responsible regulatory authorities, as the number-based PSD is the metric which most effectively avoids that the smaller size fractions in the PSD are hidden, on purpose or not, by smaller numbers of larger particles. In the following sections and chapters, several practical difficulties associated with a definition based on particle number based PSDs will be mentioned and discussed, as well as possible ways to work around them.

3.2 Consequences of choosing 50 % as the threshold particle number fraction

This section assesses whether the 'right' materials are 'captured' by choosing 50 % as the value for the threshold in the number based PSD. Relevant data have been derived from chapter 10 ("Information on actually measured particle size distributions for a representative set of materials ...") and from chapter 7 ("Summary of experiences from relevant actors in the implementation of the definition including best practices and open challenges") of JRC report 1.²

It should be noted beforehand that in any provision with a quantitative threshold, borderline cases are expected and should not come as a surprise. Examples can be found in the assessment of water quality or the presence of substances of very high concern (SVHC) in articles, and differences in conclusions may come both from sampling and measurement uncertainty (see also section 5.2). It can also be noted that, as 'nanogrades' of a certain substance can be registered and identified under REACH under one registration number, it will be quite normal that some grades of the same declared substance would be classified as nanomaterial according to the EC definition and others do not.

3.2.1 Borderline cases

The online survey conducted by JRC has provided opinions and comments on the application and consequences of the definition of nanomaterial. The participants to the questionnaire that had experience in measuring the size distribution of particulate materials were asked to provide information about borderline cases, i.e., materials for which it was difficult to decide whether they are nanomaterials according to the EC definition. Of the 42 respondents who make use of size distribution measurements, about 70 % claimed to have encountered problematic borderline cases, while about 30 % had not. The addressees were asked to provide specific borderline cases rather than a theoretical discussion on hypothetical cases. However, the many respondents who mentioned that there are borderline cases, did not provide an actual description of such cases. Therefore, while the qualitative information suggests that more than two thirds of organizations with experience in measuring particle size distribution of samples have encountered cases where it was difficult to decide whether they are nanomaterials according to the EC definition, the lack of data unfortunately does not allow estimating the frequency of such borderline cases.

Real borderline cases reported in section 10 of the first report illustrate the challenge in proper classification of nanomaterials. An example is the material "Red iron oxide" reported in section 10.5, which is a specific commercial product used for a wide variety of application in building materials, paint and plastics. It has an average hydrodynamic diameter (measured by DLS) of 222 nm, a volume-specific surface area (VSSA) of 51 m²/cm³ (equivalent to monodisperse spheres with an average diameter of approximately 117 nm), an average minimum Feret diameter (by SEM) of 107 nm, an average equivalent circle diameter (by SEM) of 124 nm, an average minimum Feret diameter (by TEM) of 94 nm, and an average equivalent circle diameter (by TEM) of 110 nm, thus rendering the classification dependent not only on the measurement technology chosen (e.g. electron microscopy vs. dynamic light scattering), but also on the measured parameter (minimum Feret diameter vs. equivalent circle diameter, with the former being closer to the 'smallest external dimension' required in the definition). This example shows that "borderline" cases may only be revealed when comparing

results obtained with different techniques (measuring different quantities) or with different data evaluation methods. The number of such cases may be reduced by referring to specific measurement techniques and data evaluation methods, e.g., specifying what external dimension is actually reported. It is also noted that if there are concerns regarding the measurement of size and size distributions they seem to be associated primarily with the upper size limit of 100 nm and much less with the lower size limit of 1 nm.

3.2.2 Materials perceived as nanomaterials but excluded from the definition

The term nanomaterial has been used for many years now, and a wide variety of materials have become known as nanomaterials, in particular materials which are shown to have specific properties because of their nanoscale structure. It is the aim of the EC definition to capture these materials with a single, common and verifiable approach. This however may be difficult to achieve, given the vast diversity of materials generally perceived as nanomaterials. A pragmatic solution is to expand the core definition with a list of explicitly mentioned materials, if they would not be captured by the core definition. Such a list is also discussed in Section 6.2. In this section, the focus is on the effect of the 50 % threshold value on the unwanted exclusion (and inclusion, see next section) of materials in the definition.

The issue of inclusion or exclusion of materials that are generally perceived as nanomaterials is a subtle one, as different organizations, scientists and other stakeholders probably have different and varied opinions of what should generally be considered a “nanomaterial”. Some indications can be extracted from the comments to the questionnaire (reported in sections 7.2.1 through 7.2.8, ref. 2).

Only a few comments are related to materials not covered by the definition, even if they are perceived to be nanomaterials. They all refer to engineered materials. In particular the following comments were made:

- Nanotubes in general should be included.
- The reference to the fullerenes, single wall nanotubes and graphene flakes in point 3 of the EC Recommendation should be extended to also encompass other shapes/forms of graphene materials, such as cones and ribbons.
- It is suggested that single wall nanotubes, flakes and spheres made from other materials than carbon should also be considered.

Furthermore, bigger structures that have internal nanoscale features which give them a unique functionality are often perceived as nanomaterials and marketed as such, but they are not included in the EC definition. Examples are nanocellulose sponges that can be used to immobilize oil spills or as filters.

3.2.3 Materials not perceived as nanomaterials but included in the definition

The issue of including materials which often are not perceived to be nanomaterials has attracted many more and varied comments.

Most respondents from trade and industry associations are concerned that many materials produced for a long time and used safely would now fall under the definition of nanomaterial. Several of these comments relate to a “general feeling” on nanomaterials and its current connotations for the general public, and claim that the definition is not viewed as neutral as it is intended and stated in the definition text. These industry associations, their members and their customers are concerned that the definition is rather linked to a perceived hazard associated with nanomaterials in general.

In other comments it was mentioned that the majority of insoluble particulate materials could become nanomaterials according to the EC Recommendation, since they may easily contain a significant number fraction of particles with external dimensions in the nanoscale, even if the

volume/mass fraction is very low. While this claim has yet to be verified, it is conceivable that high-resolution electron microscopy of common particulates would reveal the presence of “incidental” nanoparticles, possibly originating from post-production contamination or handling, or perhaps due to residual nanoparticles arising from a production process designed to create much larger grained materials. One reply claimed that with the 50 % threshold the definition would indeed encompass too many substances that should not be considered as nanomaterial (e. g., sand, pigments). There are several suggestions that the definition should not include these materials, but only materials that are developed to exhibit novel characteristics, such as improved physical or chemical properties compared to the same material without nanoscale features.

3.2.4 Considerations on imposing additional criteria and limits

Currently the EC definition is based on 2 quantitative criteria: the size of the individual constituent particles (with the lower and upper size limits of the nanoscale, 1 nm and 100 nm) and the 50 % threshold in the particle number based particle size distribution. In view of the above paragraphs on borderline cases and the difference between materials perceived and defined as nanomaterials, one may consider refining the definition with one or more additional criteria and corresponding limits.

For example, the definition does not specify an upper limit for the size of particles that should be counted to determine the fraction of particles with external dimensions at the nanoscale: in any given ensemble of particles, all particles should be counted regardless of their size. As a consequence, a material consisting of centimetre-sized pebbles mixed with an equal amount of nanoparticles is a nanomaterial. To avoid such situations, and without harming the purposes of the EC definition of nanomaterial, it could be considered to establish additional criteria:

- (i) One possibility is to define an additional cut-off size "C" well above 100 nm, e. g. $C = 10 \mu\text{m}$. Particles with minimum external dimensions larger than C would not be taken into account when determining the fraction of nanosized particles in a given material. Thereby the material is divided into a fraction S (particles smaller than C) and a fraction L (particles larger than C). Only fraction S would be evaluated whether it fulfils the definition of nanomaterial. Without further provisions, this would increase the number of materials meeting the nanomaterial definition.
- (ii) Another possibility would be to define, in addition to the threshold in the particle number based particle fraction, a minimum particle mass based particle fraction (X %, e.g. 1 %); in other words: a material would be a nanomaterial only if at least 50 % of its constituent particles have a minimum external dimension between 1 nm and 100 nm (as in the current definition), and if in addition this fraction represents more than X % of the mass of the material. Imposing this additional criterion would necessarily reduce the number of materials meeting the definition, to an extent that depends strongly on the choice of the value 'X'.
- (iii) The additional limits proposed under (i) and (ii) could also be combined: one could impose a minimum mass fraction of the particles in the fraction S, for the material as a whole to be a nanomaterial. Depending on the choice of the values C and X, the number of materials meeting the definition may be smaller or larger than for the current definition.
- (iv) A different scenario would start with the same analysis as scenario (i), i.e. the introduction of fractions S and L with the cut-off value C. Again, only fraction S would be evaluated whether it fulfils the definition of nanomaterial. Then, and in contrast to scenario (i), according to the result of the particle size distribution analysis, fraction S would, or would not, be called a nanomaterial, whereas fraction L would not be part of that nanomaterial at all. This scenario would effectively split up a material into a mixture of a nanomaterial and a non-nanomaterial. (This is a scenario that has similarities to the proposal made by ETUC.²⁶)

3.2.5 Conclusion

Choosing a single scale and metric (at least one external dimension between 1 nm to 100 nm) and a single threshold value (50 % in the particle number based PSD) in the nanomaterial definition creates

a sharp cut between nanomaterials and non-nanomaterials. Given the diversity of materials generally perceived as nanomaterials, it is inevitable that this singular cut in the definition has the consequence that on the one hand a number of materials generally not considered as nanomaterials will be covered by the definition and, on the other hand, certain materials generally considered as nanomaterials will not be covered by the definition. Choosing a different value than the current 50 % value will not significantly reduce the combined risk. This observation strengthens the view that the threshold should be based on a value of 50 % (or more) to limit the nanomaterial definition to materials for which the majority component has a nanoscale character.

As a possibility to further reduce the number of materials not intended to be covered by the definition of nanomaterials, the introduction of one or more additional thresholds can be considered. Several possible scenarios were presented. In order to prevent the opposite (true nanomaterials not being covered by the definition) the definition already uses a short list of explicitly included materials. In Section 6.2, the expansion of this list of materials is discussed as another alternative to improve the match between the nanomaterial definition and the materials considered nanomaterial in daily practice.

3.3 Regulatory consequences of a flexible threshold value

The EC definition contains the following clause: '...In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %...'.¹ This section investigates the advantages and disadvantages of this flexibility.

3.3.1 The exposure consideration

The above-mentioned clause of the EC definition is inspired by the fundamental risk assessment paradigm that RISK is a combination of HAZARD and EXPOSURE, as well as to the precautionary principle. For certain uses, e.g. resulting in high exposure (and thus concern), the flexible threshold opens the possibility to define for regulatory purposes also a material containing a percentage between 1 % and 50 % of nanoparticles as a nanomaterial (the EC definition already defines that materials containing more than 50 % nanoparticles are nanomaterials). Thus, by lowering the threshold in selected legislations, the nanomaterial-specific provisions of those legislations would apply and e.g. submission of specific and/or additional information, and performance of a separate nanospecific risk assessment etc., would be required.

An example of legislative areas for which a lower threshold (than the default 50 %) could be relevant is legislations addressing applications that are designed to result in exposure to nanomaterials in products such as food and cosmetics where respectively oral and dermal exposure is intentional. However, it must be noted that exposure is not only determined by the relative content of nanoparticles in the nanomaterial ingredients, but also by the relative content of these ingredients in the consumer product. Therefore, the reduction of the threshold in the definition does not necessarily imply an effective improvement in product safety.

3.3.2 The consistency consideration

It is assumed that applying the same definition of nanomaterial with the possibility of a flexible threshold would enhance regulatory uptake of the definition in all areas of legislation. However, if the same material (with the same PSD) is used in different applications covered by different legislations, the situation may arise that different thresholds are applied to the same material. Thus a material containing less than 50 % nanoparticles may be regarded as a nanomaterial for regulatory purposes within certain EU legislative areas (using lower thresholds) but not within others (that use the default threshold). Nevertheless, the flexible threshold means that for particular uses a lower threshold can be applied whereas applications with a lower level of concern would not need the lower end of the threshold range.

Such a situation may cause confusion and create regulatory uncertainty. Moreover, nanomaterial definitions in different Member States may deviate from the EC Recommendation. Such deviations may concern for example the threshold, which is the case for France^{16,52} and Belgium¹⁷ in which the definitions are applied to substances intentionally manufactured at the nanoscale and exclude some areas. A possible solution could be the co-ordination between legislators in different areas to agree on a common, lower threshold across legislations so that the same material would always be categorised in the same way for regulatory purposes. However, based on experience from implementing the chemicals legislation before REACH, it may be cumbersome to harmonise a threshold different from the default one across different areas of legislation. A common, lower threshold across all legislation could have considerable consequences on the marketing of materials and products (price, change of ingredients etc.).

3.3.3 The implementation consideration

The implementation of a legal definition of a material based on quantitative criteria, such as the EC nanomaterial definition, requires measurements to check whether materials are classified correctly.

For this purpose, it is preferable to have standardised measurement methods; however, there is no absolute requirement, for example under REACH, to use standardised methods for determining the identity and chemical compositions of substances. This is due to the intrinsic diversity of materials that are covered by the definition of substance: standardisation bodies cannot provide specific standard measurement methods for each substance. Although in many cases standardised methods are not available, substances are nevertheless identified, compositions are reported and relevant classifications applied. In this absence of standardised methods, a larger responsibility is bestowed on the individual laboratories to ensure the reliability of their results.

For nanomaterials the issue is complicated due to the absence of validated, reliable methods to measure the PSDs of materials, especially if they consist of particles with a wide distribution from the nanorange to micrometre size (or larger). The identification of the smallest nanoparticles in such broad PSDs is not yet possible. Another issue is that the publications that analysed how well currently available methods could possibly address the lower end of the flexible range of threshold values (i.e. closer to 1 % than to 50 %), agree that the relative measurement uncertainty increases when the threshold is lowered. Currently available methods may not be able to identify these small quantities or only do this with considerable difficulty and lack of accuracy; thus currently available methods are not capable of reliably implementing the current definition with a threshold lower than the 50 % default value. Detailed information on measurement uncertainties associated with the measurement of particle sizes and size distributions as well as a discussion on specific uncertainty contributions from elements of the EC definition can be found in Section 5.2 of this report.

JRC Report 1² lists the *pros and cons* of common size measurements techniques. Only about half of these techniques give a number-based result directly. (See also Section 4.3 where this is discussed in further detail.) JRC Report 1 also states "... *In the broadest sense, a single measurement method is only valid for a full assessment of the EC nanomaterial definition if it has been shown to provide particle number based size distributions in the size range around 100 nm, preferably from 1 nm to several micrometres. One of the conclusions of JRC Reference Report 25404⁵³ was that no such method exists and it is not expected that it will be developed in the near future. On the other hand, a method that provides particle number based size distributions from 1 nm to several micrometres for all types of materials is not necessary, and several methods have been validated for the measurement of the particle number based particle size distribution of specific types of nanomaterials. ...*".

Clearly, measurement methods need further development to ensure that the number based size distribution can be reliably measured. One way to address the PSD measurement issues could be to provide guidance documents that reflect the state of the art and that include the limits to currently available methods. But it is more important to continue the efforts already undertaken to develop, improve and validate accurate methods for PSD measurements and identification of the nanofraction in materials.

3.3.4 Conclusion

The flexible approach impacts negatively on the transparency of the legislation addressing nanomaterials due to the fact that materials may be regarded as nanomaterials or not, depending on the legislation. It counteracts the intention that the EC definition should guarantee that a material which would be regarded as nanomaterial in one sector will be given the same classification if used in another one. However, the flexible approach is assumed also to promote the regulatory uptake of the same definition, where only the number based percentage of nanosized particle content in the materials threshold varies. In this context we note that another option is that the 50 % number-based threshold value could be fixed and implemented as the only threshold for defining nanomaterials, and each specific area of legislation could then define, as relevant, application limits for content of nanomaterial in products addressed by the specific legislation or define limits specific to certain nanomaterials. Regarding the implementation, there is a need to continue to develop and improve the measurement methods; the lower the threshold, the larger is the improvement needed. Actually, current methods would not allow reproducible and valid measurements at the lower end of the flexible threshold range.

4 NEEDS FOR CLARIFICATION OF THE CURRENT EC NANOMATERIAL DEFINITION

A survey was carried out by the JRC in preparation and support of the review of the EC definition of nanomaterial to collect feedback from key actors on their experience with the implementation of the definition. An overview of recurring elements which, in the opinion of the respondents to that survey, need clarification was provided in the preceding report in its chapter 9.3.2.² This section gives an assessment of these clarification needs.

4.1 Clarification of the purpose of the definition

Some survey respondents were confused about the purpose of the EC Recommendation. For them it is not clear whether the aim is to propose a scientific definition or a tool for legislation. Actually, it was precisely stated in the text that "the definition in this Recommendation should be used as a reference for determining whether a material should be considered as a 'nanomaterial' for legislative and policy purposes in the Union" (recital 3).¹ On the latter issue, the 2012 Staff Working Paper on the nanomaterials on the market⁵⁴ is more explicit and it is mentioned that "the purpose of the Recommendation is to ensure consistency across legislative areas as well as across guidance and other technical documents by the European Commission. In addition, the Commission invites Member States, the EU agencies and economic operators to use this definition, for example, in the adoption and implementation of legislation and policy and research programmes concerning products of nanotechnologies". Based on these clear statements further clarification on the purpose of the EC definition does not seem to be necessary.

It is argued by some respondents that there is a lack of harmonisation between existing regulations in national laws, or also in different sectors. Actually, one of the purposes of the EC Recommendation is to ensure conformity across legislative areas, as the same materials are often used in different contexts; the EC Recommendation was developed to provide a coherent cross-cutting reference here. Consequently the EC Recommendation should guarantee as far as possible that a material which would be regarded as a nanomaterial in one sector will be given the same classification if used in another one. The harmonised definition will improve the transparency of the use of nanomaterials.

4.2 Clarification of the scope of the definition

Clarification on the relationship between the EC Recommendation and legislation in EU Member States, and the differences in scope, is also requested.

4.2.1 Manufactured or not

In the scope of the French Decree⁵² establishing a national registration scheme for nanomaterials as well as in the scope of the Belgian Nanomaterials Declaration Scheme¹⁷ or in the definition used by the Danish Environmental Protection Agency,¹⁸ certain activities or products are in part or totally excluded. Firstly, in these national regulations, only manufactured products are considered. Moreover, in the French decree,⁵² for example, the minimum proportion of the number size distribution is specified to be 50 % but it is also stated that "in specific cases and where warranted by concerns for the environment, health, safety or competitiveness, this minimum proportion may be reduced". In the Belgium scheme,¹⁷ quite a number of materials are excluded from the registration requirement: nanoproducts sold between businesses and products that fall under specific regulations (e.g. food, biocides, feed, pharmaceuticals, medical devices, cosmetics, pesticides and waste) and also pigments.

It seems that the definitions used in different legislative provisions that exist in various countries are derived from a basic definition of the term "nanomaterial", by restricting its scope (e.g. by origin, or to certain groups of substances and products). Due to the different scopes, the concerned nanotechnology stakeholders need to be careful in their declarations of nanomaterials in different

countries, resulting in an additional workload. However, if indeed a common basic definition is used, then this minimises the risk of contradicting national and sectorial definitions. The EC definition can serve as such a 'basic' definition and facilitates an overall consistent approach and implementation.

Some survey respondents indicate that they would prefer if only manufactured nanomaterials were covered by the EC definition. However, for the purpose of harmonization, the Commission has chosen to identify a nanomaterial only on the basis of its particle size, regardless of its origin or the intention to produce such a material. Properties or possible risks posed by a nanomaterial do not depend on whether an object is natural, produced incidentally, or the result of an engineering process with or without the explicit intention to manufacture a nanomaterial. In that respect, natural materials can exhibit similar properties as those that are manufactured and vice versa. For a general definition of a certain class of materials, it would consequently not be coherent to ignore certain types of materials just on the basis of their origin. However, if needed, the scope of the definition can be adapted to specific areas; e.g., the Biocidal Products Regulation,¹¹ which uses the EC Recommendation, is only applicable to "manufactured" nanomaterials.

4.2.2 Defining properties other than size

Some survey respondents suggest that other specific properties should be considered instead of or in addition to the size. However, referring to other properties specific to nanomaterials would likely be detrimental to the legal clarity of the definition. The specific properties to be considered could vary, depending on the focus of specific sectors. Furthermore, it would be necessary to define (i) which properties should be considered and (ii) for each property, in a quantitative way, when it should be considered as being different from the same property of the non-nano form of the same material. This would certainly introduce more complexity and subjectivity into the definition. Size is the only universally applicable, clear and measurable criterion which could be used to define materials in a sector-independent way with a minimum of arbitrariness in the choice of the defining criteria.⁵⁵

4.2.3 Non-particulate materials

Some types of materials are not matching the EC definition, even if they are covered by the corresponding ISO nanomaterial definition. The EC definition is limited to materials consisting of particles (and, according to the EC's Q&A documentation,⁵⁶ excluding non-particulate materials such as proteins or micelles as present for example in mayonnaise), and excludes nanostructured materials (i.e. solid products, films, parts or components) with an internal or surface structure in the range between 1 nm and 100 nm, such as computer chips). According to this, and taking into account the current EC position on particulate material (see also the discussion on the term "particle" below), nano-emulsions, nanoporous materials, solid and liquid nanofoams, and particles with an engineered nanoscale internal structure are not covered by the current EC definition if their external dimensions are larger than 100 nm.⁵⁴ All these exclusions could be explicitly specified in the EC Recommendation in order to avoid misunderstandings and provide more clarity.

4.2.4 Products

According to the EC Recommendation, "nanomaterial" means a natural, incidental or manufactured material containing particles.¹ The definition uses the verb "contain" instead of "consist of". The term "contain" can induce some misunderstandings. Indeed, any material containing particles and where more than 50 % of those particles have external dimensions in the nanoscale, can be considered as nanomaterial according to the EC definition. On the other hand in the Q&A documentation,⁵⁶ it was specified "that if a nanomaterial is used amongst other ingredients in a formulation the entire product will not become a nanomaterial". If one strictly follows the text of the EC Recommendation,¹ the Q&A document is not accurate. Using the term "consist of" instead of "contain" could help to avoid this kind of misunderstanding because it restrains the limit of concerned materials.

Notably, the different language versions of the EC Recommendation are inconsistent with respect to the wording "contain" vs. "consist of". For example, the French, Spanish, German, Italian, and

Swedish versions use words which are equivalent to "contain", whereas the Dutch and Danish versions use words equivalent to "consist of". Possibly, this is one of the reasons why some survey respondents indicated that it is not clear whether a material which contains nanoparticles is considered as a nanomaterial or not, even if this point was clarified in the existing Q&A document (Questions 3, 10, 11 and 18),⁵⁶ a link to which was provided with the invitation to the survey.

4.2.5 Mixtures

The Recommendation's scope includes nanomaterials when they are, in the terms of REACH, substances or mixtures, but a consumer- or end-product will not become a nanomaterial itself if it contains nanomaterials as ingredients.⁵⁶ In order to avoid misunderstandings this could be specified in the EU Recommendation itself.

The Recommendation does not make any specification regarding the chemical nature or identity of the material. If, for example, the material in question is a mixture consisting of particles of two or more different substances, then there is in theory the possibility to test each of the substances individually and decide whether they are nanomaterials (option 1). This would imply that the particles need to be distinguished by their chemical identity. The second option (option 2) is to apply the criteria of the definition to the mixture as a whole and count all particles regardless of their chemical identity, and without the need to distinguish them chemically.

Both options are conceivable and therefore it depends on intent: if one is interested in product ingredients, or the constituents of a mixture, then each material should be assessed individually, according to option 1. If one is interested in the nano-nature of the mixture as a whole (which may well be the case) then option 2 would be applicable.

The Recommendation itself can be reasonably applied following both options. However, it should be clearly indicated which option is to be applied.

4.2.6 (Volume-)specific surface area

Clarification is also requested about the use of VSSA. The Recommendation clearly indicates "where technically feasible and requested in specific legislation, compliance with the definition in point 2 may be determined on the basis of the specific surface area by volume". However, this characterisation cannot be applied to all material types. Therefore, VSSA is not an obligatory analysis method. Moreover, to some survey respondents it is also not clear what to do with materials with a VSSA above the limit but with particles that are not in the nanoscale. Highly porous materials such as zeolite granulate with particles larger than 100 nm might fall under this type. It could be classified as a "false" nanomaterial, as the definition introduces VSSA not as a screening tool but as additional criterion. It could be more explicitly specified whether in such a case the particle size criterion overrules the VSSA criterion or vice versa.

4.3 Clarification of terms used in the definition

There are a number of terms used in the EC Recommendation that need to be clarified in order to avoid certain misunderstandings pointed out during the survey.

4.3.1 Particle

An important issue to consider in the context of nanostructured materials is the definition of "*particle*" and its interpretation in different contexts.

CEN ISO/TS 27687:2008⁵⁷ (Nanotechnologies - Terminology and definitions for nano-objects -- Nanoparticle, nanofibre and nanoplate) quotes the following definition for particles from ISO 14644-6:2007⁵⁸:

"Particle - minute piece of matter with defined physical boundaries"

This definition is also used for the EC definition.¹ CEN ISO/TS 27687:2008(en)⁵⁷ further specifies that a physical boundary can also be described as an interface and that a particle can move as a unit. This definition is also referred to in the JRC Reference report⁵⁵ on Considerations on a Definition of Nanomaterial for Regulatory Purposes (EUR 24403). ISO further specifies that the general particle definition applies to nano-objects.

ISO 21501-1:2009(en)⁵⁹ (Determination of particle size distribution — Single particle light interaction methods — Part 1: Light scattering aerosol spectrometer) provides the following slightly different definition of the term "particle": "*Particle - discrete element of the material regardless of size*" whereas ISO 29464:2011(en)⁶⁰ (Cleaning equipment for air and other gases — Terminology) defines a particle as "*Particle - small discrete mass of solid or liquid matter*".

None of these definitions provides a size limit above which a discrete piece of matter would not be called a particle anymore, "minute" and "small" being unprecise qualifiers. ISO 21501-1:2009⁵⁹ explicitly excludes size as criterion for the term "particle".

There is also a debate whether certain types of discrete elements of matter match the EC definition of nanomaterial. This discussion is related to the interpretation of the term "particle". In the Staff Working Document (SWD)⁵⁴ accompanying the EC's Second Regulatory Review on Nanomaterials⁶¹ it is argued for example that "*nano-emulsions consist of liquid nano-objects suspended or dispersed in a liquid phase. They are not covered by the EU nanomaterial definition, because the term particle as defined in the Commission Recommendation is intended to cover only nano-objects with a defined, rigid shape, thus in essence solid nano-objects.*" Here the SWD⁵⁴ refers to the Q&A documentation⁵⁶ of the Commission which explains that "*... the Commission definition of "nanomaterial" is limited to materials consisting of particles (excluding non-particular materials such as proteins or micelles as present for example in mayonnaise) ...*"

The remark in the SWD⁵⁴ (section 3.1) that the EC definition is intended to cover only nano-objects with a defined, rigid shape, thus in essence solid nano-objects, narrows the term "particle" specifically for that definition and implies that only a selection of particles should be covered by the EC definition. Such a limitation of the scope of the term "*particle*" could be mentioned explicitly in the EC definition, e.g., in a recital.

Another issue to be clarified is whether (clusters of) molecules, proteins, fats, starch and other macromolecules with external diameters above 1 nm should be considered as nanomaterials. The definition itself is not entirely clear in this respect. As one possibility, one could consider entities as particles if their nature would not change drastically if one such entity is divided in two new entities. As a consequence, if their external dimensions are in the nanoscale they would be considered as nanomaterial. For example, if a polystyrene nanoparticle is broken into pieces, it will lead to two polystyrene nanoparticles. In this case, it could be considered as a nanomaterial. In contrast if a protein is separated into two parts, two materials with different nature will be obtained. Hence it would not be called a particle or considered as a nanomaterial. On the other hand, protein clusters which can be disintegrated into individual, equivalent constituents would consequently fall under the scope of the definition. Excluding certain materials would then be an option when the definition is implemented in specific sectors, by defining additional, sector-specific criteria for such materials, for example biodegradability or solubility.

4.3.2 Unbound state and physical boundaries

According to the EC Recommendation (e.g. in paragraph 2),¹ "Nanomaterial" means a natural, incidental or manufactured material containing particles, in an *unbound* state or as an *aggregate* or as an *agglomerate*.... Furthermore, a particle is defined as "a minute piece of matter with defined *physical boundaries*".

In the context of the EC Recommendation (e.g. paragraphs 2 and 4a),¹ the terms "**unbound state**" and "**physical boundaries**" refer to individual particles which are defined as "a minute piece of matter with defined *physical boundaries*". Survey respondents pointed out that there is ambiguity in

the use of these terms and they consider that it is not obvious to determine whether the EC Recommendation is only limited to particulate materials, even if, implicitly, the EC definition clearly considers nanomaterials as particulate materials with a particle size distribution.⁵⁴

Referring back to the ISO definition(s) of particle one could ask what defined physical boundaries (or interfaces) actually are. In physics and chemistry a region of space throughout which all physico-chemical properties of a material are essentially uniform or at least do not change abruptly, is called a "phase". Different phases are separated by phase boundaries. A phase boundary can hence be defined as an interface where one or more physico-chemical properties change abruptly. In other words, at a phase boundary the chemical potential

$$\mu = \left(\frac{\partial G}{\partial N} \right)_{T,p} \text{ (here expressed for constant temperature } T \text{ and pressure } p)$$

'jumps', or, when going spatially from one phase to the neighbouring one, $\Delta\mu \neq 0$. The basic *physical boundary* is therefore a phase boundary. Such a phase boundary is well defined but need not to be rigid. Phase boundaries can also change or disappear with time. Hence, from a physico-chemical viewpoint a defined physical boundary is a phase boundary, and therefore a particle *with defined physical boundaries* need not consist of solid material; it could be a liquid droplet or consisting of soft matter.

The term "unbound state" may also be specified more precisely. A particle (according to ISO 26824:2013⁶² a particle can move as a unit), which is in a region of a potential without minimum, or has enough kinetic energy to escape from such a potential minimum could be regarded as unbound. If an unbound particle is in translational movement, its vector of momentum and the vector of translational movement of any part of that particle point at the same direction.

4.3.3 Agglomerate and aggregates

The terms "unbound state" and "physical boundaries" are employed above to contrast with the "aggregate" and "agglomerate" states, which are held together by weak or strong interactions between smaller particles. Unfortunately, both terms are often used incorrectly or not in accordance with the ISO definition or with the interpretation of the EC Recommendation.

Agglomerate is understood by the EC Recommendation (paragraph 4b)¹ as 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. In the source definition, provided by ISO,³ a note is also added to specify that '*the forces holding agglomerates together are weak forces, for example van der Waals forces or simple physical entanglement*'. A second note to the ISO definition specifies that agglomerates are also termed secondary particles and the original source particles are termed primary particles.

Aggregates are defined as *particles comprising of strongly bound or fused particles* (Recommendation paragraph 4c).¹ The ISO terminology⁶² describes *aggregate* as '*particle comprising strongly bonded or fused particles where the resulting external surface area is significantly smaller than the sum of surface areas of the individual components*'. A note to the ISO definition explains that '*the forces holding an aggregate together are strong forces, for example covalent bonds, or those resulting from sintering or complex physical entanglement*'.⁶² A second note was provided by ISO which explains that aggregates are also termed secondary particles and the original source particles are termed primary particles.⁶² On the one hand, the additional precision provided in the notes of ISO may help to clarify the EC definition. On the other hand, in a regulatory context they could also be a source of unclarity as it is not obvious how to make the difference between simple (agglomerate) and complex (aggregate) physical entanglement.

With respect to the distinction between "agglomerates" and "aggregates", the EC Recommendation is more succinct than the ISO definition⁶² and can be ambiguously interpreted. However, the Q&A document⁵⁶ provided by the EC specifies that agglomerated and aggregated particles may exhibit the

same properties as unbound particles and adds that there can be cases during the life-cycle of a nanomaterial where the particles are released from weakly bound agglomerates or under certain conditions (e.g., strong shear forces) also from more strongly bound aggregates. Therefore, as long as both aggregates and agglomerates are included in the definition, it is not necessary to distinguish between these two types of particle ensembles for the purposes of the definition.

Survey respondents (especially industrial associations or individual companies) pointed out that considering aggregates as well as agglomerates as nanomaterials includes too many materials. Considering the challenges that come with the inclusion of aggregates in the scope of the definition of nanomaterials, and which have been already pointed out in the JRC reports EUR 26567² and EUR 25404⁵³, a discussion on whether aggregates or certain types of aggregates should be included in the definition, seems useful. If this discussion would lead to a revision of the definition introducing a distinction based on the terms aggregate and agglomerate, then it would be necessary to develop precise criteria for agglomerates and aggregates to distinguish them from each other.

Another point to consider is the difference between an aggregate and a microparticle that is built as an assembly of nanoparticles in a bottom up approach. The term aggregate is usually reserved for the assemblies of particles of the same nature originating from a single production process, and not for the bottom up assembly of multiple particles of different nature and with different functions, or, e.g., the production of multi-layered core-shell particles. It could be helpful to clarify this point by explicitly indicating, for example, whether or not these 'aggregates' are considered as nanomaterials, also if they cannot be separated into nanoparticles, or if they do not release nanoparticles.

One way to tackle the above two issues may be to define criteria that help to determine whether parts of the 'aggregates' are bound so strong that the energy required to separate them into their constituent particles (for counting and measuring their external dimensions) would likely change the constituent particles themselves. These very strongly bound constituents of aggregates, for example fused primary particles, or intentionally and strongly bonded core-shell assemblies of different substances, could be called "smallest dispersible units" and the definition could be based on their size distribution. To follow this 'smallest dispersible unit' approach option would make the definition more complex. For example, when defining protocols to determine smallest dispersible units specific conditions for specific materials would need to be considered. Furthermore, as the smallest dispersible unit might have an irregular shape, considerations on how the external size of such a unit is defined remain necessary, as for the constituent particles (see section 4.3.5). However, it may relieve some of the analytical challenges discussed later (see section 5.3).

4.3.4 Primary particles and constituent particles

Certain terms were pointed out as confusing by the survey respondents, even if the terms were not used in the EC definition, for example the term "**primary particle**". This term was not employed in EC Recommendation, but it is used in ISO terminology where it is defined as "original source particle of agglomerates or aggregates or mixtures of the two".⁶² Despite having undergone a growth process, fusion, covalent binding or coalescence with other particles, sometimes primary particles can still be inferred from the shape and structure of a larger particle. However, these inferred primary particles have often lost their individual existence.

Instead of "primary particles", the EC Recommendation uses the term "constituent particles", in the following way: "...definition of the term "nanomaterial" in Union legislation should be based solely on the size of the *constituent particles* of a material" (recital 4), but no definition of that term was provided. The term "constituent particles" is also used in Recital 12 of the EC Recommendation: "... the definition in this Recommendation should therefore also include particles in agglomerates or aggregates whenever the constituent particles are in the size range 1 nm-100 nm ..."¹

It would be useful to have a clear definition of the term **constituent particle** for the purposes of the EC definition. The term "constituent particle" is already used in the ISO definition of the term 'primary particle', but is not defined yet as a separate term. A draft proposal by ISO defines the term

as an 'identifiable, integral component of a larger particle'.⁶³ This is why primary particles are often not constituent particles.

4.3.5 Size and external dimension

The definition clearly states that a decisive criterion for a nanomaterial is the size of the constituent particles, regardless of the size of the aggregates or agglomerates. Some survey respondents were very concerned that it would be not possible to characterise *primary particles*. In the terms of the definition, they were probably referring to *constituent particles*. It is indeed not easy to identify constituent particles within an aggregate. This measurement issue is discussed in section 5.3.

Moreover, if a particle has an irregular shape, it is not straightforward to decide which *size* should be measured.⁵³ There are a number of more specific external particle dimensions defined in the field of image analysis, that are related to the *size* used in the EC definition, such as the minimum Feret diameter or the diameter of the largest circle that can be drawn inside the contour of a 2D image of the particle. Unfortunately, these more specific dimensions are not generally applicable across the diversity of nanomaterial shapes.

In any case, a clarification of the terms size and external dimension may be necessary. For example, it is not clear to all stakeholders that the average constituent particle sizes measured for aggregated pigments, metal oxides, silicas etc, that are reported on the corresponding datasheets supplied to customers, are usually not of direct use for the assessment of the EC definition, since they do not correspond to the median value of a particle number based particle size distribution. Nevertheless, such data give an insight into what is considered as the size of constituent particles by commercial operators.

4.3.6 Conclusion

A number of issues and terms could be clarified to reduce the risk of misinterpreting the definition:

- the terms "particle", "physical boundary", "unbound state", "constituent particle", "external size" and "aggregate" in the context of the EC definition can be clarified.
- the use of the verb "contain" in the EC definition can be reconsidered.

4.4 Clarification on how to implement the definition

4.4.1 Current implementation support and guidance

Implementation support and guidance to the people and organisations that have to work with the definition can take different forms.

In a previous section (Section 4.2.3) of this report, the existing Q&A document, available from the website of DG Environment,⁵⁶ was already mentioned and discussed. This document, answering general questions from stakeholders and the public at large, can continue to be refined and updated, based on the feedback received by the EC services. This feedback will also be used in the review and possible revision of the definition itself.

A different type of support is related to the practical implementation of the definition through *measurements*. Since the majority of the guidance documents collected in Section 6 of JRC Report 1 (EUR 26567)² are measurement-related, this measurement aspect will be the focus of this chapter.

Some of the guidance documents listed in JRC Report 1² were written in preparation of a nanomaterial definition (e.g. ref. 7). Other documents provide guidance on what to do with nanomaterials in a specific regulatory context (e.g. refs 39, ⁶⁴). A third category of documents are standard methods describing how to determine (nano-)material properties, in particular particle size, which is of obvious relevance for the EC definition (e.g. CEN or ISO standards or OECD test guidelines). It is noted that only one of the documents listed in ref. 2 is written explicitly about how

to determine, in the sense of the EC definition, whether a material is a nanomaterial or not. This is in the JRC Reference Report EUR 25404⁵³ and it highlights the generic requirements on measurements for use in the implementation of the EC definition. Acknowledging these generic requirements is the first step in the development of more specific guidance, if needed.

4.4.2 Guidance on the required measurement system

It takes time before reliable measurement results are obtained in a new measurement area. The reliability of a measurement result strongly depends on the reliability of the *measurement system* in which the measurements were made. In general terms, a measurement system consists of:

- 1) validated **methods**,
- 2) **laboratories** with the required expertise to implement the methods, and
- 3) **materials for quality assurance**, to calibrate and verify the performance of methods and laboratories.

As with any measurement problem, expertise is required to select the most appropriate measurement system. Non-experts need to team up with measurement experts to identify the relevant methods and tools to solve their specific problem. This collaboration is usually a part of the contract review to be performed by the laboratory selected to perform a measurement. An experienced analyst should be aware of the available parts of a reliable measurement system and can use tools and databases not suitable for laypersons. With this respect, the situation in nanotechnology does not differ from any other measurement area. Bearing this in mind, the following presents the picture emerging for the three main elements of a measurement system.

4.4.2.1 Validated methods

Available standard methods for particle size analysis

When searching for an existing validated method, it is useful to start the search among the standard measurement methods. A set of standard measurement methods that is highly relevant for the implementation of the EC definition has been developed in ISO/TC 24/SC 4 'Particle characterisation'.^A These standards address a number of generic particle size analysis issues (presentation of measurement data, the issue of sampling, the dispersion of powders, image analysis) and a number of specific instrumental techniques. However, these methods are not written with the specific purpose for implementing one or another nanomaterial definition.

The value of standard measurement methods is that they are broadly accepted consensus methods. Typically they have been in use for some time, and during this time they have been validated for use with specific types of materials. This however does not imply that they are suitable or valid for use with any kind of potential nanomaterial when assessing it against the EC definition.

Several documents like the JRC Reference Report EUR 25404⁵³ or the OECD 'Review of the applicability of the OECD test guidelines to manufactured nanomaterials'⁶⁵ contain a snapshot of the status of standardisation at the time of publication. Similarly, ISO is developing a Technical Report entitled 'Measurement method matrix for manufactured nano-objects'. However, because of the wide variety of nanomaterials, and because of the large number of relevant measurement methods

^A http://www.iso.org/iso/home/standards_development/list_of_iso_technical_committees.htm
(accessed 31/7/2014)

(see also Section 4 of ref. 2), and because all ISO documents undergo a permanent process of periodic revision and improvement, such review publications are rapidly outdated.

Therefore, the on-line searchable databases containing lists of and links to documents are more interesting sources for guidance on which methods to choose and use. Well-known are the databases provided by the standard development organisations themselves, such as ISO and ASTM, which have search engines that make identification of standardised methods straightforward, even if the limitations and scope of the standard method are not always clearly indicated in the database. Another, more horizontal, example is the American National Standards Institute's Nanotechnology Standards Panel (ANSI-NSP) Nanotechnology Standards Database, launched in 2013.^B The purpose of this database is '... to capture information about standards and associated documents (standards, best practices, guidelines) that directly relate to nanomaterials and nanotechnology-related processes, applications and products'. It does not contain the guidance documents themselves, but provides a short scope or description of the documents and a link to the relevant website.

Validation of measurement methods for use with the EC definition

It is a mere fact that the resources of international standardisation bodies, in particular the time available to their experts, are insufficient to develop and maintain an ever expanding range of standard measurement methods applicable for answering very specific questions.⁶⁶ For example, the range of nanomaterials is too broad to have a prescriptive standard document to establish for each kind of material whether it is a nanomaterial or not, according to the EC definition.

This implies that individual laboratories (or groups of laboratories) will have to perform in-house validation studies, to check whether their method of choice can be used to reliably assess whether the materials they are investigating are nanomaterials or not. Such validation efforts are required for newly developed methods, but also for most (if not all) of the methods already standardised, as the standard documents are typically not sufficiently detailed to judge whether they are applicable to a specific or new type of nanomaterial. The validation may require more or less effort depending on how specific the method is designed for, and dedicated to, a specific measurement challenge.

An important element of every validation study is its experimental design, which has to match the customer expectations about the accuracy of the results to be produced. Therefore, validation studies start with making a choice of method-performance criteria. In a regulatory context, it is not unusual that such criteria are defined in the legislation itself. Currently, the EC definition does not specify quantitative method performance criteria (for example, a requirement that the expanded measurement uncertainty of the results produced by a method shall be smaller than 10 %).

4.4.2.2 Proficient laboratories

Proficient laboratories are laboratories that have a documented expertise with a specific measurement method or measurement challenge. During the preparation of this report, it has been suggested that it would be useful to have a list of proficient laboratories. This is currently not available. However, one must bear in mind that the publication of such a list would strongly discriminate against all laboratories not included in this list. Given the fact that the field is still in flux, it is not likely that any list could ever depict the current situation without distorting the market.

On the other hand, several laboratories are already accredited according to ISO/IEC 17025⁶⁷ for one or several specific measurement on nanomaterials. The contact data and their scope of accreditation can be retrieved from the databases of the various accreditation bodies, thus allowing speedy

^B <http://nanostandards.ansi.org/tiki-index.php> (accessed 31/7/2014)

identification of laboratories. While of course accreditation alone does not guarantee correct results, the fact that a laboratory has undergone accreditation is a good sign. Furthermore, any laboratory is free to undergo accreditation, so relying on databases on accredited laboratories generates a level playing field for a common European market.

A typical way to prove one's proficiency is through successful participation in an interlaboratory proficiency test. There are no published proficiency studies yet with the specific aim of checking whether a laboratory can reliably assess whether a material is a nanomaterial according to the EC definition or not. This specific type of interlaboratory study may need to be organised in future.

4.4.2.3 *Materials for quality assurance*

The best known analytical quality assurance tools are the so-called *reference materials*, which basically consist of a material for which one or more properties of interest are known with a suitable accuracy and degree of confidence for a specific intended use in a measurement process.

Several publications and documents have provided an overview or summary of available reference materials relevant for nanotechnology (see section 4.2.4 of ref. 2). However, no continuously updated guidance document exists that allows identification of the available reference materials. As with proficient laboratories or standardised methods, the lack of such document is not a problem: several databases exist that allow searching for suitable reference materials. Furthermore, a whole industry of small service providers has developed that identify and re-sell reference materials. Laboratories therefore have the possibility to identify and obtain the necessary reference materials, if available.

One example of a relevant database is the BAM Nanoscale Reference Materials Database.^C It was established to also cover the non-certified reference materials; non-certified reference materials do not come with a fully traceable and accurate assigned property value, as certified reference materials (CRMs) do. The latter are indeed not yet available in great abundance. The existing relevant CRMs are often highly monodisperse suspensions of spherical nanoparticles, mainly designed for calibration of the size-scale of a particle size analysis instrument. With these materials one cannot assess whether a method or laboratory is capable of tackling a more complex measurement challenge, such as the assessment of whether a polydisperse, possibly heterogeneous particulate material matches the EC definition or not.

4.4.3 **Integrated testing strategies and tiered approaches**

One could also envisage developing a generic, high-level 'integrated testing strategy', as for example proposed by ECHA (for the specific case of dealing with nanomaterials in REACH),³⁹ or by ETUC (to deal with materials of different morphology, spherical or not),²⁶ or by SCENIHR (to deal with materials with an average particle size near to or above the 100 nm limit value).⁷ Making use of measurement standards such as the ones developed in ISO/TC 24/SC4, the ISO/TC 229 'Nanotechnologies' has also started studying the possibility of developing a document describing a tiered approach to determine whether or not a material is a nanomaterial, relying on a combination of screening and confirmatory measurement methods. However, the link between this future ISO document and the EC definition is unclear, at this moment. Of more direct relevance is NanoDefine, the FP7 project^D developing a tiered measurement approach for the EC definition. Possibly this

^C <http://www.nano-refmat.bam.de/en/> (accessed 31/7/2014)

^D www.nanodefine.eu (accessed 31/7/2014)

approach will find its way to CEN/TC 352 with the aim of turning it into a standard document. Also, it will be used in the NANoREG project on regulatory testing of nanomaterials.^E

Two caveats to the tiered approach or integrated testing strategy can be made here:

- A proposed testing strategy should not be interpreted in a restrictive way, as this would stifle innovation into more efficient methods.
- It is difficult to finalise and standardise a testing strategy now, during the review of the EC nanomaterial definition. It is expected that at least a number of issues with the current EC nanomaterial definition will be clarified by the EC. Until then, the researchers working on guidance documents have to make a number of assumptions which not necessarily will be in line with the outcome of the revision process.

4.4.4 Conclusions and major gaps

The above paragraphs indicate that guidance on the required measurement system that specifically deals with the implementation of the EC definition is not available. At the same time, it is observed that the available measurement infrastructure (searchable databases for standards, accredited laboratories and reference materials) eliminates the need for dedicated guidance documents in this area, as called for by some stakeholders. This is especially true as any specific and prescriptive guidance document will most likely be outdated already on the day of its release.

Nevertheless, a number of relevant gaps have been identified, in terms of available validated methods and method performance criteria, proficiency tests and reference materials. This situation cannot be improved by writing additional guidance documents, but requires organising and performing analytical work and analysing data obtained within and between laboratories, with the help and support of interested stakeholders.

^E www.nanoreg.eu (accessed 31/7/2014)

5 THE MAIN, ANALYTICAL IMPLEMENTATION CHALLENGES

5.1 Introduction

In the survey carried out by the JRC in 2013 in support to the review for the EC definition, the participants were asked whether their organisation had been 'facing issues in implementing the definition's specification on size distribution' (see section 7.2.4 of ref. 2). Several respondents mentioned the ambiguity of some parts of the definition, which indeed is an issue to be solved if one aims at a harmonised implementation of the definition. This issue was already addressed in Chapter 4 of this report. Other respondents referred to the difficulty of measuring particles inside products. This is an interesting subject as well (and specifically addressed in section 17 of ref. 2), but the question is not of immediate relevance for the implementation of the EC definition as such, as this definition is about nanomaterials themselves, and not about the products in which nanomaterials are used as ingredients.

Most of the remaining relevant survey answers are related to analytical challenges. An overview and summary of these challenges is described in the following section 5.1.1. A few important analytical challenges are discussed in more detail in sections 5.2 and 5.3. In addition, and this was especially a point of discussion in the March 2014 Technical Workshop^F discussing a draft version of ref. 2, the industrial stakeholders raised the issue of the economic costs associated with the analytical work required to implement the EC definition. After investigating a number of alternative, less expensive, or faster, or more convenient, analytical routes in section 5.4, the financial implications of the analytical challenges are discussed in section 5.5.

5.1.1 Overview of analytical challenges brought up in the JRC survey

5.1.1.1 Relevant analytical challenges mentioned in the responses to the JRC survey

Among others, the following measurement-related implementation issues were mentioned or claimed by the survey respondents, mainly by trade and industry associations and private companies:

- As regards the VSSA criterion, the pore structure of some materials is not considered adequately.
- Companies have no experience/instruments for measuring number based PSDs.
- Representativeness of the sampling and of the measurement is an issue. Sample preparation in itself is seen as a problem, because it can change the size distribution of a material.
- Aggregates (and their constituent particles) are regarded as an issue; e.g. iron oxide pigments are obtained as agglomerated and cannot be measured as such. How to ensure that the degree of dispersion was sufficient?
- Measurements are difficult for polydisperse materials or non-spherical particles.
- Most of the methods that are readily available to companies produce mass based PSDs. However, conversion from mass- or volume-based to number-based PSD is problematic and error-prone.

^F Technical Workshop 'Aspects to consider in the technical and scientific review of the European Commission Recommendation of 18 October 2011 on the Definition of Nanomaterial', Brussels, 19 March 2014

- Specific measurement methods are applicable only for a narrow size range. This creates a problem if the material has a broad size distribution.
- Product manufacturers must often rely on the certificates of analysis provided by raw material vendors. Because no number-based PSA methods are currently validated, contradictory information is often obtained. As a result, in borderline cases, the same raw material may be considered a nanomaterial or not depending upon the test method and assumptions selected by a vendor.
- It is often impossible to recognise the constituent particles in an aggregate particle (e.g. in partly amorphous materials).
- Provided with sufficient energy, materials that would not normally be considered as nanomaterials could be broken apart into smaller particles of nanoscale dimensions, thus leading to a false classification of the material as a nanomaterial.

5.1.1.2 Discussion of the analytical challenges mentioned in the responses to the JRC survey

The received comments are rather diverse, but the lack of standardised, validated analytical methods is probably seen as the major drawback as regards the implementation of the definition. The respondents mentioned several times that any definition for regulatory purposes must be accompanied by validated analytical methods. The added value of a validated method is the quantitative understanding of the main contributions to the measurement uncertainty of the results it produces. (Section 5.2 explains the relevance of measurement uncertainty.) As long as no method (including sample preparation) is defined, discrepancies between the methods may lead to different results and finally to a non-uniform classification of one and the same material.

In Report 1,² JRC already tried to sort and identify within the received comments what precisely are the relevant analytical challenges brought up by the survey respondents and via other feedback routes. From the list of generic and specific measurement issues presented in section 4 of ref. 2, JRC considers the following as the most relevant challenges in the implementation of the definition:

- The EC definition is based on the *minimum external dimension*. The vast majority of non-microscopy based techniques for particle size measurement measure equivalent spherical diameters, which are average particle dimensions, and not a minimum dimension. This is an important obstacle for the assessment of materials for which the particle's shapes deviate from spherical.
- The definition requires the *counting of constituent particles in aggregates or agglomerates* as individual particles. The majority of the materials to be tested and classified as nanomaterial or not, will consist of mixed aggregates and agglomerates and most analytical techniques for particle size determination measure aggregates and agglomerates as if they were individual particles without internal structure. Many aggregates also cannot be disaggregated or dispersed into their constituent particles without damaging these constituent particles. In this frame, best practices for dispersion (here understood as 'breaking up into constituent particles')⁶ and sample preparation should be developed. The lack of standardised/harmonised dispersion protocols generates doubts as regards the achievement of complete dispersion, and legal insecurity as regards the classification of the material as being a nanomaterial or not. This issue is discussed in detail in section 5.3.

⁶ The term 'to disperse' also has a second meaning: that of 'to bring into dispersion'. For example: one can disperse a powder in a liquid medium, or one can re-disperse particles settled from a liquid, at the bottom of a recipient, by shaking the recipient. Ultrasonication of a liquid containing aggregated and agglomerated particles, may result in both: dispersion in the sense of breaking up agglomerates and some of the aggregates and dispersion in the sense of bring the particles into a (more or less uniform) state of suspension in the liquid.

- The definition specifies a 50 % threshold value for the fraction of particles in a *particle-number based PSD*. The analytical techniques for particle counting in the nano-range are limited and may be not capable of accurately identifying materials as being nanomaterial based on the particle number based PSD requirement. Indeed, most common commercial PSA instruments determine the PSD of materials based on mass or volume fractions from which a number distribution is mathematically generated. The accuracy of converting a mass- or volume-based measurement distribution to a number distribution is questionable,⁶⁸ and discussed in more detail in section 5.4.2.

5.1.1.3 Summary of the analytical challenges mentioned in the responses to JRC survey

The combination of the three analytical challenges mentioned above presents an obstacle to the implementation of the EC definition except for those materials that are clearly a nanomaterial or clearly not a nanomaterial. Indeed, at the moment there is no single reliable method able to fulfil all requirements of the definition, i.e. determine the minimum external dimension number distribution of an otherwise unknown material and count at the same time particles within aggregates or agglomerates as individual particles. In addition there are no particle count reference materials in the nano-range, making it difficult to develop, cross-correlate and validate new methods. While this situation is unfortunately not uncommon for new or even established regulatory areas, it is important that this observation is taken into account when revising the EC definition.

5.2 Conformity assessment and measurement uncertainty

5.2.1 The role of measurement uncertainty in conformity assessment

Measurement uncertainty has been briefly presented in Section 3.4.2 of JRC Reference Report EUR 25404,⁵³ which concludes as follows: '...measurement uncertainty values are the quantitative expression of the reliability of the results of a validated measurement method...'.

It is important to understand why one should even worry about measurement uncertainty. The interested reader is referred to ISO/IEC Guide 98-1,⁶⁹ which gives an extensive introduction to the concept of measurement uncertainty; but essentially, reporting a measurement result without its measurement uncertainty is like telling half the story. Indeed, assessing and reporting the measurement uncertainty is a matter of:

- honesty (one should not hide one's uncertainty about a measurement result),
- transparency (one cannot compare the equivalence of two reported measurement results without knowing their respective measurement uncertainty),
- accountability (those who take decisions based on measurement results must be able to take into account the measurement uncertainties in the assessment of the risk of taking a wrong decision).

In the regulatory context of the EC definition, the latter argument of accountability is crucial. The specific role of measurement uncertainty in conformity assessment is acknowledged in several EC policy areas, as documented for example in the 'Report on the relationship between analytical results, measurement uncertainty, recovery factors and the provisions of EU food and feed legislation'.⁷⁰ To summarise these documents: one cannot compare a measured value with a legally defined threshold or limit, unless one takes into account the uncertainty of the measurement result. A basic example is shown in Section 3.4.3 of ref. 53.

5.2.2 Generic components of measurement uncertainty

To make meaningful estimates of measurement uncertainty, one must acknowledge that measurement uncertainty is made up of different main components.

A first component stems from the **measurement method**, and this includes both instrumental and sample preparation aspects of the method. Both (within-laboratory) repeatability and (between-laboratory) reproducibility of most measurement results depend on inherent aspects of the

measurement method. These elements of measurement uncertainty are assessed in in-house and in interlaboratory studies, and are, ideally, reported in documents describing the (standardised) measurement method.

The second aspect is the **human factor**, varying between operators and laboratories, depending on their expertise and proficiency. This aspect is the reason why the correct application of previously validated or even standard methods should also be validated in every laboratory wishing to use the method, at least to a certain extent. Such method validation studies typically assess a number of elements in a quantitative manner, for example by performing series of tests in controlled conditions to assess *precision*, and by testing a reference material, to assess *trueness*.

The third main aspect is that of the test **material**. The application of a certain method to a new, previously untested material may create additional measurement uncertainty. This can be minimised by including multiple materials in the method validation studies, and by clearly indicating and respecting the working range over which a method is validated. Furthermore, most materials are inevitably heterogeneous to some extent. This implies that the test sample is not necessarily representative for the material to be evaluated. This sampling and representativity issue can at least partly be dealt with in statistical terms and by using intelligent sampling designs.⁷¹ While increasing the number of samples and tests will typically reduce this part of measurement uncertainty, a compromise will always have to be found between the number of tests that can reasonably be performed (given the associated cost), and the resulting reduction of the measurement uncertainty.

The reader will notice that the majority of uncertainty estimates presented in the following sections are derived from **interlaboratory studies**. The variance of results between qualified laboratories using an appropriate method for a defined measurement purpose is often a more reliable estimator of the measurement uncertainty of a particular method, than the uncertainty estimates produced by individual laboratories. Of the three main uncertainty components listed above, the interlaboratory studies can help to assess the components intrinsic to the method, and the human factor. Most often, the interlaboratory studies are performed on well-defined, homogeneous materials, and the material-specific uncertainty component is small. The interlaboratory reproducibility shall therefore be considered as a lower limit of the uncertainty values that will occur in daily practice.

5.2.3 Measurement uncertainty in particle size analysis: the current status

In this section relevant examples are given, representative for the current status of the understanding of measurement uncertainty in PSA. Where possible, measurement uncertainty values will be quoted at the 95 % confidence level. This means that with 95 % confidence the difference between the measured and the true value is smaller than the quoted measurement uncertainty.^H

5.2.3.1 Measuring size distributions of particles larger than 100 nm

The EC definition does not require the measurement of the size of a single, individual particle; it requires an assessment of the distribution of particle sizes of a large number of particles. Establishing the required PSD is a measurement with 2 aspects: size of particles and number of particles. The measurement uncertainty of a point on the PSD curve therefore is a combination of the

^H Often variability in measurement results is reported at the standard uncertainty level, corresponding with a confidence level of about 65 %, but this is an insufficient confidence level for most regulatory applications. In many cases the 'expanded' uncertainty at 95 % confidence level can be approximated as 2x the value of the standard uncertainty.

measurement uncertainty associated with the measurement of the size of a particle (the x-axis of a PSD), and the measurement uncertainty associated with the counting of particles in the different 'size bins' (the y-axis of a PSD).

Yoshida et al. have systematically investigated these uncertainty components via numerical simulations and experimental studies, also for the case of bimodal and polydisperse powders of spherical silica particles (e.g. ref. 72). They have also reported results for electron microscopy based PSA on very large numbers of perfectly spherical but polydisperse glass spheres in the range between 1 micrometre and 100 micrometre. Variations within and between number-based particle sizing methods (electron microscopy, electrical sensing zone method and sedimentation balance method) indicate an expanded uncertainty of the order of 5 %.⁷³

PSDs are often assessed only very partially, for example by establishing a mean, or modal or median value. Most of the publications addressing measurement uncertainty of PSDs focus on the uncertainty of these average values. For example, Kuchenbecker et al. recently published the results of an interlaboratory study of the volume-based median particle diameter of commercially manufactured ceramic powders as measured by laser diffraction, which, as opposed to the methods used by Yoshida et al., is an example of an ensemble method.⁷⁴ The between-lab reproducibility of the measurement of the median value of the equivalent diameter of a polydisperse SiC powder of about 1 micrometre was about 15 %. This reproducibility is only one contribution to the measurement uncertainty of results produced with this technique, but it is an important (large) one: other uncertainty contributions are often smaller. The corresponding expanded uncertainty value of 30 % (= 2 x 15 %) can be retained as a realistic uncertainty estimate for median (d_{50}) values of PSDs of a polydisperse but otherwise homogeneous ceramic powder with an average size of about 1 micrometre, measured with laser diffraction.

5.2.3.2 *Measuring particle size distributions in the nanorange*

The term *measurement uncertainty* is mentioned in several of the Chapters of JRC Report 1 (EUR 26567).² Most often the term was used in a qualitative way, as it is difficult to find quantitative evaluations or estimates of measurement uncertainty in reports on the size analysis of nanoparticles.

There are a few publications which employ a so-called 'bottom-up approach' to assess the uncertainty of measurements of the PSD of nanoparticles. In such 'bottom-up approach', individual, technique-specific contributions to the total measurement uncertainty are individually assessed and then combined to obtain an estimate of the total measurement uncertainty. The alternative 'top-down approach' to estimate measurement uncertainty relies on a more holistic attitude, combining the experimentally observed variation between different measurement results (within or between laboratories) with an assessment of the trueness of the measurement result by comparing the obtained average value with the certified value of a reference material (see e.g. ref. 75), or the outcome of an interlaboratory study. These 'top-down' uncertainty estimates are reported in or can be deduced from a few recently published results of method validation studies, and from recent RM certification studies. In the following paragraphs a number of recent publications are briefly mentioned, highlighting their main findings and the type of uncertainty contributions taken into account by the respective authors.

5.2.3.3 *Measurement of average nanoparticle sizes*

For a number of highly monodisperse colloidal materials, it is possible to determine an average, method-defined particle size with great accuracy. For example, NIST has produced certified reference materials consisting of polystyrene spheres with average sizes around 60 nm and 100 nm, with expanded uncertainty values of about 1%.⁷⁶ When the particles become smaller, the typical (relative) measurement uncertainties increase. Nevertheless, a low measurement uncertainty and between-method reproducibility can be maintained (Meli et al.⁷⁷), at least for a number of methods that produce number-based PSDs, such as TEM (3 % for silica particles between 20 nm and 40 nm, De Temmerman et al.⁷⁸).

When measuring less monodisperse materials with ensemble methods, or even with fractionation methods, the measurement uncertainties increase. Results of full validations of DLS and CLS methods, following the top-down approach, were reported by Braun et al.⁷⁹ Expanded uncertainties of the measured average particle diameters varied between 5 % (DLS) and 16 % (CLS). Later, these method validation studies were repeated, using newly available reference materials to better assess the trueness of the method. At the same time, the measurement uncertainties of the DLS method were reported in an original and more specific way, relating measurement uncertainty both with average size and with the polydispersity index of the tested material. The measurement uncertainty was reported to vary from only 2 % for highly monodisperse materials to 4 % for slightly less monodisperse materials ($PI < 0.15$).⁸⁰

The lower measurement uncertainty values of the DLS method compared with the CLS method shall not be mistaken as an argument to prefer DLS over CLS for the implementation of the EC definition. It is indeed known that the DLS results are rather reproducible, but they do not provide reliable PSDs in many cases. The CLS method has a higher baseline uncertainty, as it is highly dependent on the accurate knowledge of the density and size of the calibrants,⁸¹ but it is more robust for work on more polydisperse industrial materials.

An increasing number of relevant interlaboratory studies have been performed. Hackley et al. have compared the interlaboratory reproducibility of the mean size of nominally 30 nm Au nanoparticle (spherical, monodisperse) reference materials and estimated expanded measurement uncertainties from 10 % (AFM and TEM) to 25 % or 30 % (SEM and DLS).⁶⁶ In the collaborative work of Motzkus et al., performed under VAMAS, slightly larger but comparable values were established, for the measurement of silica nanoparticles deposited from an aerosol.⁸² From the same VAMAS study, Hodoroba et al. report the measurement uncertainty of particle size values measured by transmission-mode SEM.⁸³ The authors combine uncertainty contributions from the image magnification calibration, from the choice of threshold levels in the image analysis, and from the coating applied to the nanoparticle, to obtain an expanded uncertainty of about 10 nm, for the individual modes of a particle-number based PSD of spherical silica nanoparticles with average values between 35 nm and 100 nm.

Rice et al. report the results of an interlaboratory study that evaluated a protocol for measuring and analysing the PSD of discrete (non-agglomerated), metallic (Au), spheroidal nanoparticles using TEM.⁸⁴ For these particles, the authors observe an acceptable reproducibility of the average diameter if at least 500 particles are counted (expanded uncertainty of about 5 %), but the uncertainty of the parameters describing the width of the PSD were considerably larger. Hole et al. report results from a series of interlaboratory studies performed in order to gradually improve the reproducibility of the modal diameter of monomodal suspensions of spherical reference materials as measured with particle tracking analysis (PTA).⁸⁵ For these idealised samples, and after several rounds of preliminary comparisons, the reproducibility of the PTA results corresponds with an expanded uncertainty of about 10 % of the measured modal diameters.

Interlaboratory studies have also been used to characterise candidate reference materials, and to obtain their certified size values. Lamberty et al. organised a preliminary interlaboratory study, in order to identify expert laboratories for later use in certification studies.⁸⁶ The expanded between-laboratory reproducibilities of the measured average diameters for the monomodal silica material, were around 10 %, for DLS, CLS, as well as EM methods. The preliminary evaluation allowed the CRM producer (IRMM) to educate the laboratories on a number of analytical quality assurance aspects, resulting in slightly improved but generally comparable between-laboratory reproducibility in the following certification studies.^{87,88}

5.2.3.4 Measurement of particle number concentrations in number-based particle size distributions

Laborda et al. estimated the measurement uncertainty of particle size and number concentrations by single particle ICP-MS.⁸⁹ Extrapolating the values they report to the 95 % confidence level, the uncertainty on the measured size varies from 5 % to 20 % in the range of 100 nm to 40 nm, while the

expanded uncertainty of the number concentrations is about 10 %. These values are comparable to the results of a more profound sp-ICP-MS uncertainty investigation reported by Olesik and Gray, who performed their experiments on silica particles with sizes between 100 nm and 2000 nm,⁹⁰ but much lower than the reproducibility-dominated uncertainties deduced from the sp-ICP-MS interlaboratory study reported by Linsinger et al., obtained on Ag nanoparticle suspensions, which vary around 15 nm for particles ranging from 20 nm to 100 nm.⁹¹

The BAM-N001 certified reference material, consisting of a colloidal suspension of Ag particles, comes with values for d_{10} ,¹ d_{50} and d_{90} .⁹² The certified particle number-weighted d_{50} value, as determined with SAXS, is about 13 nm and its expanded uncertainty 2 nm (or about 17 %). The relative uncertainty of the d_{10} value is larger (about 40 %).

Wohlleben has reported results for centrifugation methods for the determination of number-based PSDs.⁹³ He observed a difference of less than 5 % between the measured number based PSD and the theoretical values deduced from the mixing ratio of the monomodal polystyrene model materials (with sizes between 50 nm and 1000 nm), at least for relative fractions larger than about 15 %.

5.2.4 Specific uncertainty contributions from elements of the nanomaterial definition

The previous section has shown some estimates of uncertainties for the measurement of particle sizes in relatively 'simple' particle populations. The EC definition presents a more specific and bigger measurement challenge. With a number of these specificities one can associate additional uncertainty contributions, which will inevitably lead to larger total measurement uncertainty values than the values reported in the previous paragraph. In this section, the possible significance of a number of these elements from the EC definition is discussed.

5.2.4.1 Minimum external dimension

The expression 'minimum external dimension' can be interpreted in different ways. Two straightforward interpretations are that of the 'minimum Feret diameter' (see EUR 25404⁵³) or of the 'maximum inscribed circle diameter' (the diameter of the largest circle that can be drawn inside the contour of the 2D-image of the particle). Both concepts stem from the theory of image analysis, and they can only be applied for methods that produce images. Report EUR 25404⁵³ also explains that the majority of particle size analysis results are reported as equivalent spherical diameters: the raw signals captured by the instruments are interpreted as if they were produced by perfect spheres. The obtained values are necessarily overestimations of the minimum external dimensions of the measured particles. Using equivalent diameter data to estimate the minimum external dimensions of particles inherently creates an important, but difficult to quantify, additional contribution to measurement uncertainty.

The only way to deal with this additional measurement uncertainty is to interpret the equivalent diameter results with the aid of additional information obtained on the shape of the investigated particles, as obtained with imaging methods. However, also for the usual 2D-imaging methods, there is one major uncertainty contribution related to the assessment of the minimum external dimension. This is the uncertainty associated with the external dimensions of the particle in the 3rd dimension, perpendicular to the plane of the image. This uncertainty component does not interfere too much with particles having an 'equiaxial' shape (approximately spherical), or even for fibre-like particles. However, for plate-like particles, the minimum dimension is typically the dimension perpendicular to the grid or substrate onto which the particles are deposited to enable image acquisition. Of all PSA

¹ d_x is the particle size value for which x % of the particles in the material are smaller than d_x

methods, AFM (or SPM) techniques are most suited to tackle this issue: AFM/SPM techniques perform best in this third, out-of-plane direction, and most easily measure the height of particles with respect to the surrounding substrate surface. Of relevance here is the possible use of VSSA for average platelet thickness determination, as discussed in Section 5.4.1.

5.2.4.2 *Agglomerates and aggregates*

In this report a separate section (5.3) is devoted to the measurement of the size of constituent particles inside aggregates and agglomerates. That section concludes that for this type of materials, the fundamental question is whether the measurement is possible at all. The assessment of the uncertainty of these measurements is only possible when this fundamental question is answered.

One way to deal with the issue is to require that samples are fully dispersed into their constituent particles prior to performing the PSA. Under this assumption, the corresponding additional specific uncertainty contribution is determined by the reproducibility of the (to-be-agreed) dispersion method. This reproducibility will certainly depend strongly on the type of nanomaterial. For a number of materials, such as the carbon black materials relevant for the tyre industry, a standard dispersion method has already been agreed.⁹⁴ Other protocols have been developed and used, giving within-laboratory precision of the results which is acceptable to good, also for materials that are clear mixtures of agglomerates and aggregates.⁹⁵ It should be noted that these protocols do not usually achieve full dispersion into constituent particles, since aggregates, and in some cases strongly bound agglomerates, do not break up into their constituent particles when using known dispersion methods based on ultrasonic energy input and chemical dispersants. Nickel et al. studied the role of dispersion protocols on the between-laboratory reproducibility of the average hydrodynamic diameter of P25 titanium dioxide powders, as measured by DLS.⁹⁶ Their interlaboratory study indicates that aggregated and agglomerated powders can be brought into a reproducible dispersion state, if a well-defined dispersion protocol and similar dispersion tools are imposed and respected, resulting in an expanded uncertainty between 10 % and 20 % of the average diameter (around 200 nm) measured by DLS. The constituent particle size of P25 varies around 19 nm (for the anatase particles) and 37 nm (for the rutile particles),⁹⁷ illustrating that full dispersion was not achieved for this material. In the absence of a rigorous protocol, dispersion procedures are a source of additional measurement uncertainty.⁹⁸

5.2.4.3 *Width of the size range*

In its purest form, the EC definition requires that for each particle in a representative sample one decides whether its minimum external dimension is bigger than 100 nm or its maximum external dimension is smaller than 1 nm. If this is the case, then the particle is binned as 'non-nano', the other particles are counted as 'nano'.

In practice, the lower threshold of 1 nm is less important, and the focus of the development of new dedicated measurement methods is on the 100 nm threshold: one assumes that all particles detected with a physical dimension smaller than 100 nm have at least one dimension between 1 nm and 100 nm. Whether this assumption is always justified or not, it is of practical use since the detection limit of most methods is larger than 1 nm.

Also of concern is the counting of the particles that are much bigger than 100 nm. They often do not contribute significantly to the particle-number based PSD, but they do interfere significantly with the measurement, as they literally can hide smaller particles (in imaging methods) or less directly (as they produce strong signals that make the signals from the smaller particles undetectable).

There are few methods that can make this assessment for individual particles from the 1 nm level to well above the 100 nm threshold in a single measurement step. Either their upper measurement limit is too low, or their lower measurement limit is too high, or their dynamic range (the ratio of upper and lower measurement limit in a single measurement) is too small. As a consequence, to assess whether a material is a nanomaterial or not, necessitates the combination of multiple measurement results, obtained in different size ranges, coming from the same method or even from

different methods. This so-called *stitching* of partial PSDs is a well-known PSA challenge, not only for the nanoparticle size range but also for larger particle sizes.

It has been pointed out in previous JRC reports,^{2,53} and also shown in many publications, that the comparison and direct combination of the results of different PSA methods is limited by the method-defined nature of the values they produce. Anderson et al. show that the difference between methods can be up to 10 % even for the more straightforward measurements of the average diameters of monomodal materials.⁹⁹ Nevertheless, there is a lot of knowledge to gain from the combination of results of different methods,^{68,100,101,102} and the number of 'tiered approaches' for the assessment of the EC definition is increasing. It may be an open question as to how this combined wisdom can be expressed in the format of a measurement uncertainty, but in theory the combination of the information from the different methods should result in a reduced uncertainty in comparison with the measurement uncertainties of the results of the individual methods.

5.2.5 Volume-specific surface area

The proposal to use VSSA as a complementary definition of nanomaterial was made by Kreyling et al.¹⁰³ and has been retained, with some caveats, in the EC definition. For a full discussion on the theoretical use of VSSA as a proxy technique for implementation of the EC definition the reader is referred to Section 5.4.1. This section focuses on the associated measurement uncertainty.

Surface area is an industrially very relevant measurand for fine powders, and values are measured for many materials. Usually, the Brunauer-Emmet-Teller (BET) specific surface area is determined, and this can be done with different adsorption gasses following different experimental procedures and data analysis methods. For a particular method, the accuracy of surface area measurements is typically much better than the accuracy of particle size measurements, especially for agglomerated and aggregated materials. NIST, for example, reports a measurement uncertainty of about 1.2 % on the specific surface area of the reference material SRM 1898, a commercial and heavily aggregated/agglomerated titanium dioxide powder with a specific surface area of about 55 m²/g.⁹⁷ This material was also used in an interlaboratory comparison to reveal that the between-laboratory reproducibility (95 % confidence level) is about 2.5 %.

To use a BET value in the implementation of the EC definition, it first has to be transformed from a mass-specific surface area to a volume-specific surface area. This is done by multiplying the BET value with the density of the particles. This density value is not always known exactly, and therefore the calculation will result in an increase of the measurement uncertainty, e.g. by about 5 % as mentioned also earlier, when comparing DLS and CLS uncertainty budgets.⁸¹

Other methods to measure surface area exist, and are based on e.g. diffusion charging or mobility measurements, or on electron tomography.^{95,104} However, there is to the authors' knowledge no reliable information available about the measurement uncertainty of these methods in the nanoscale regime. And it must be noted that the differences between surface areas measured via different methods are considerable.¹⁰⁵

5.2.6 Outlook

5.2.6.1 Acceptable measurement uncertainty

Method validation studies need to be designed for a purpose. The main aim of a method is to produce values with a measurement uncertainty that is compatible with the intended use of the measurement result. This judgement, and therefore the design of a method validation study, cannot be made unless the acceptable measurement uncertainty is defined. It is possible that acceptable measurement uncertainties will be defined for specific regulatory applications of the EC nanomaterial definition. If not, then the customer needing PSA data will have to decide on which measurement uncertainty he is willing to accept. If he has to prove that a material is a nanomaterial (or that it is not a nanomaterial) the measured value shall be higher (or lower) than the 50 % criterion by an amount equal to the measurement uncertainty. De facto, measurement uncertainty

makes the 50 % threshold higher (or lower) if you need to prove that a material is a nanomaterial (or the opposite).

5.2.6.2 CRM production

The assessment of measurement uncertainty via the top-down method validation approach relies ultimately on the availability of suitable certified reference materials, to determine the trueness component of the total measurement uncertainty. On the other hand, the production of certified reference materials critically depends on the availability of validated methods and qualified laboratories.¹⁰⁶ CRM producers are used to struggling with this hen-and-egg dilemma, which can only be solved in a stepwise, sustained effort, gradually increasing the complexity of the measurement challenge. This has already resulted in the development of a series of CRMs of increasing relevance for the EC definition, and will ultimately result in CRMs that are representative also for polydisperse industrial materials. In this process, collaboration between different expert laboratories is shown to be the only viable and reliable route.¹⁰⁷ Collaborative projects, e.g., the work in FP7 projects such as NanoDefine, will certainly contribute to this development.

5.2.6.3 Interlaboratory comparison studies

In the absence of specific and suitable certified reference materials, the most prominent source of realistic estimates of the measurement uncertainty associated with specific methods to assess a material against the EC definition, are interlaboratory comparison studies. It is noted that ILCs provide information about the method reproducibility, but do not automatically produce information about the trueness of the method. To achieve this information, the ILCs should be carried out with materials that have at least been appropriately characterised with TEM, or that have been specifically created from mixtures of well characterised particulate samples.

Several ILCs have already taken place, but often on simpler (monomodal, well-dispersed, equiaxial) particles, and none with a method specifically designed for the implementation of the EC definition. It is foreseen, e.g. in the FP7 project NanoDefine, to organise such ILCs, and to compare across laboratories the results of particle size measurements on industrially relevant particulate materials.

5.2.6.4 Material-specific in-house method validation studies

As explained above, measurement uncertainty has a method-component, but also a laboratory- or operator-specific component, as well as a (test) material specific component. The in-house method validation studies will have to assess aspects such as linearity, working range, limit of detection, limit of quantification, trueness, selectivity, precision (repeatability, intermediate), and robustness. The generic method validation concepts are not specific for the methods that are needed to implement the EC definition. But it is likely that more specific guidance on which uncertainty contributions to take into account and how to combine them will be given in specific contexts, such as that of collaborative research projects. This could lead to more formalised guidance documents such as, for example, ISO 11352, which provides a set of procedures to enable laboratories working on the assessment of water quality to estimate the measurement uncertainty of their results.¹⁰⁸

5.3 Measuring the size of constituent particles in aggregates

The current EC definition requires measurement of the size of individual, constituent particles, also if they are aggregated or agglomerated into bigger particles. This section assesses the availability of analytical means to realize these measurements, today and in the foreseeable future, and addresses the measurement uncertainty associated with them.

5.3.1 Aggregates, agglomerates and their dispersion into constituent particles

Most PSA methods measure sizes correlated with external dimensions of 'bits of material', that are 'moving' as one entity or that appear on an image as one entity. These may be single particles, but it may also be a group of constituent particles that are aggregated or agglomerated. To measure the

size of the aggregated or agglomerated particles with these methods, one needs to disperse (here used in the sense of 'break up') the aggregated or agglomerated particles before the size measurement.

Aggregates and agglomerates are differentiated by the strength of the forces keeping their constituent particles together, with the aggregates consisting of strongly bonded particles and agglomerates of weakly bonded particles. In this context, agglomerates do not pose particular problems: as they are bound weakly, they can be dispersed relatively easily into their constituent particles using an appropriate dispersion protocol. It may be necessary to try several dispersion protocols to confirm that the results are robust against different conditions, and one should be aware that re-agglomeration of the dispersed agglomerates can occur; otherwise their dispersion is usually rather straightforward.

The latter conclusion does not hold for the stronger bound assemblies ("aggregates"), which will therefore be the main focus of this section. (Also ISO 26824 recognises this by stating that the constituent particles of aggregates and agglomerates are often aggregates.⁶²) Therefore, it could be useful to develop new test approaches for the sizing of the constituent particles specifically for aggregated materials. The definition of "aggregate" as "a particle comprising of strongly bound or fused particles" is less than helpful in this context for two reasons:

a) There is no commonly accepted definition of "strong". Breaking up bound particles requires energy input, where higher energies will break up stronger bound agglomerates, but not (all) aggregates. Increasing the energy input will eventually break the constituent particles themselves. It is therefore possible to create nanoparticles from aggregates consisting of constituent particles larger than 100 nm, thus potentially creating nanoparticles, leading to a wrong classification.

b) Fusion of metallic or ionic particles (one example of an aggregation process leading to strong bonds) creates chemical bonds that differ in no way from the bonds within a particle. The same is true for covalent bonds, or for the coalescence of particles via Ostwald ripening. This means that the internal bonds inside aggregated particles do not differ from those inside the original primary particles. The main distinction between the aggregates and the primary particles is the history of the new entity. If the fusion or coalescence is not complete, this history may sometimes still be inferred from the shape of the particle, but strong fusion can eventually erase any hint of the original particles, leading eventually to a single, dense body. This means that there is a continuous variety of binding strengths, with easily dispersible agglomerates on the one side, leading via physically entangled particles, hardly dispersible agglomerates, sintered particles with still an intermediate phase between the original particles to dense bodies with no indication of any individual particle. The strength that sintering can achieve is demonstrated by the fact that many industries rely on sintering of metal powders to produce sturdy, load-bearing products.

5.3.2 Constituent particles and primary particles

In the responses to the JRC survey² and in the discussions around the review of the definition, it is noted that the term "constituent particle" is sometimes incorrectly used as a synonym for "primary particle". As explained in section 4.3.4, a "primary particle" is an original source particle, which, despite having undergone a growth process, fusion, covalent binding or coalescence with other particles, sometimes still can be inferred from the shape and structure of a larger particle. However, these inferred primary particles have often lost their individual existence and the larger particle cannot be disintegrated into the original, primary particles. The understanding, and the definition currently proposed in ISO, for the term "constituent particle" is that of an 'identifiable, integral

component of a larger particle'. This suggests that an ensemble can still be disintegrated into the various constituents, and shows that primary particles are often not constituent particles.^J

For facilitating this discussion, the shorthand definition of aggregate as "smallest dispersible unit" (of course using the dispersion protocol that results in the best dispersion) can be used.⁹⁴ Using this definition means that this section can be limited to aggregates, because we assume that the dispersion of particles sticking together, where possible (as in agglomerates), will be common practice in the preparation of samples for the analytical assessment of the EC definition.

5.3.3 Measurement of constituent particle size inside smallest dispersible units

Distinguishing constituent particles inside aggregates means probing the bound or fused ensemble in order to identify the boundaries between the constituent particles. This endeavour is hampered by two facts:

a) If particles are severely fused, the former boundaries between primary particles may be invisible or may have vanished completely, meaning that an aggregate may be interpreted as a single particle. Only previous knowledge can decide whether the material is an aggregate of fused or covalently bound particles or a single particle.

b) Many materials, also particulate materials, are 'polycrystalline': their microstructure is built up of small grains each having a crystalline structure. The boundaries between these crystals (usually called grain boundaries) inside a single, polycrystalline particle can incorrectly be interpreted as boundaries between constituent particles.

Any possibility to identify constituent particles in aggregates must avoid both sources of bias. They must probe the interior of aggregates to elucidate the size of constituent particles. Currently, there are two possibilities to do this, namely transmission electron microscopy (TEM) and x-ray diffraction (XRD), which were both reviewed in previous reports^{2,53} and compared in a recent paper.¹⁰⁹

5.3.3.1 Transmission electron microscopy

Transmission electron microscopy (TEM) can, to a limited extent, reveal the internal structure of non-dispersable aggregates.¹¹⁰ Judging whether an apparent boundary corresponds to a real boundary between two constituent particles is a tedious and delicate task that requires heavy involvement of highly qualified personnel. Even if both time and manpower are available, it is often impossible to judge whether the boundary seen is the remains of a grain boundary within a single polycrystalline particle or a still existing boundary between particles. In addition, the intrinsic image quality can make sizing of constituent particles impossible. Even TEM is therefore not able to decide in all cases whether a particle is a single particle or an aggregate of multiple constituent particles.

5.3.3.2 X-ray diffraction

X-ray diffraction (XRD), in its simplest form, only yields the average crystallite size of crystalline materials, but gives no signals that relate to particle size of amorphous materials.

While a constituent particle can consist of several crystallites, any single crystallite can only be part of one constituent particle. The crystallite size therefore sets the minimum possible size for constituent particles. XRD therefore can, for monodisperse crystalline constituent particles of known

^J Lego[®] can be used to illustrate this distinction: In a building made of Lego bricks, the individual bricks would be the constituent particles (as the building can be disintegrated into the various bricks), but the polymerisation nuclei of the plastic material (which in this case are not visible any longer) would be the primary particles.

shape, decide that a material is not a nanomaterial (if the crystallite size is above 100 nm). However, XRD does not allow a positive identification of nanomaterials (unless all particles can be shown to be monocrystalline). For polydisperse constituent particles XRD is not generally reliable for classification as non-nanomaterial.

5.3.4 Conclusion and outlook

The above paragraphs lead to the conclusion that it is currently not possible to reliably measure the size of constituent particles inside aggregates for many materials. Furthermore, giving an uncertainty of a typical measurement result for those materials for which it is possible, is meaningless, as the possible outcomes range from relatively accurate to completely unreliable.

A number of existing methods to (semi-)automatically distinguish and count constituent particles inside aggregates are currently being developed further for potential application on nanomaterials. One such method is based on the fractal properties of aggregates as captured with TEM images.¹¹¹ However, this has led to only few published papers with data obtained on nanomaterials. In addition, these approaches give the size of the original particles inferred from the image, which may have morphed into a single particle. The obtained results therefore give a minimum size of constituent particles.

While it is of course impossible to make reliable predictions about the future, one must bear in mind that hardly anything distinguishes single polycrystalline particles from fused or covalently bound aggregates, especially those for which fusion or sintering has progressed strongly: chemical bonds have no "memory", which means that there is no difference between the bonds within and between covalently bound or fused constituent particles. As there are therefore hardly any properties that can be exploited to make such a distinction, it is likely that the possibilities to distinguish aggregates of nanoparticles (which are nanomaterials according to the current EC nanomaterial definition) from nanostructured single particles (which are not nanomaterials according to the EC definition) will also be very limited in the future, as long as no clear and unambiguous definition of "strong" (in "*strongly* bound particles") exists that allows such a distinction.

This means that also in future it will be virtually impossible for a number of materials to decide whether it consists of single particles or aggregated particles. This situation will continue to exist unless a maximum binding strength (either as energy or via a certain protocol) can be defined which distinguishes aggregates and agglomerates from single particles. It is clear that adopting this practice would require further clarification and guidance, possibly in a revised EC Recommendation.

5.4 Alternative analytical routes to implement the EC nanomaterial definition

5.4.1 Use of specific surface area measurements

5.4.1.1 The basics of specific surface area analysis

Specific surface area (SSA) analysis of powder and/or porous materials is widely used in industry since SSA is related to particle and pore sizes which can fundamentally alter the characteristics and performance of materials. Volume specific surface area (VSSA, expressed in m^2/cm^3) is the SSA in m^2/g multiplied by the particle density (in g/cm^3).

The method most often employed to measure SSA is based on the measurement of nitrogen adsorption according to the theory of Brunauer, Emmett and Teller (BET), and several manufacturers offer instruments for SSA analysis. Standards exist for the application of the BET method, including the general description in ISO 9277:2010¹¹² and several application-specific standards. Certified reference materials are available for testing BET instrumentation. Sample preparation is important in order to avoid erroneous results, since all surface moisture and adsorbed gases must be removed by an appropriate procedure prior to measurement. If carried out according to correct procedures, the reproducibility of the technique may be better than 10 %.

5.4.1.2 The relation between specific surface area and particle size

It is important to note that BET analysis is not a size measurement, and conversion of the VSSA to size parameters requires knowledge or assumptions regarding particle density, shape and size distribution. Without at least a preliminary EM examination of the material being analysed, derived size parameters are likely to be unreliable. The EC definition requires the determination of the size of particles either unbound or contained within aggregates or agglomerates. While loose agglomeration affects BET results in a relatively limited way, any strong particle aggregation will have a significant effect and in that case will render BET-derived size parameters irrelevant with respect to constituent particles within aggregates.

In the following subsections, the relation between SSA and particle size will be investigated for different particle shapes.

Spherical, non-porous particles

A sample of perfectly monodisperse, solid, non-porous, non-aggregated spherical particles with a diameter of 100 nm will have a theoretical VSSA of $60 \text{ m}^2/\text{cm}^3$. Despite the fact that VSSA of a single particle increases more rapidly to the low side of 100 nm than it decreases to the high side of this threshold (due to the $1/D$ dependence of VSSA, with D the sphere diameter), symmetric broadening of the number-based PSD, while maintaining a median size of 100 nm, will reduce the measured VSSA. This is shown in Figure 1 for the example of a Gaussian (normal) distribution function. Most asymmetric broadening functions (e.g. lognormal) would also lead to reduced overall VSSA values for a median size of 100 nm. This is because the contribution of the smaller particles to the overall surface area and to the overall volume diminishes very rapidly with decreasing particle size.

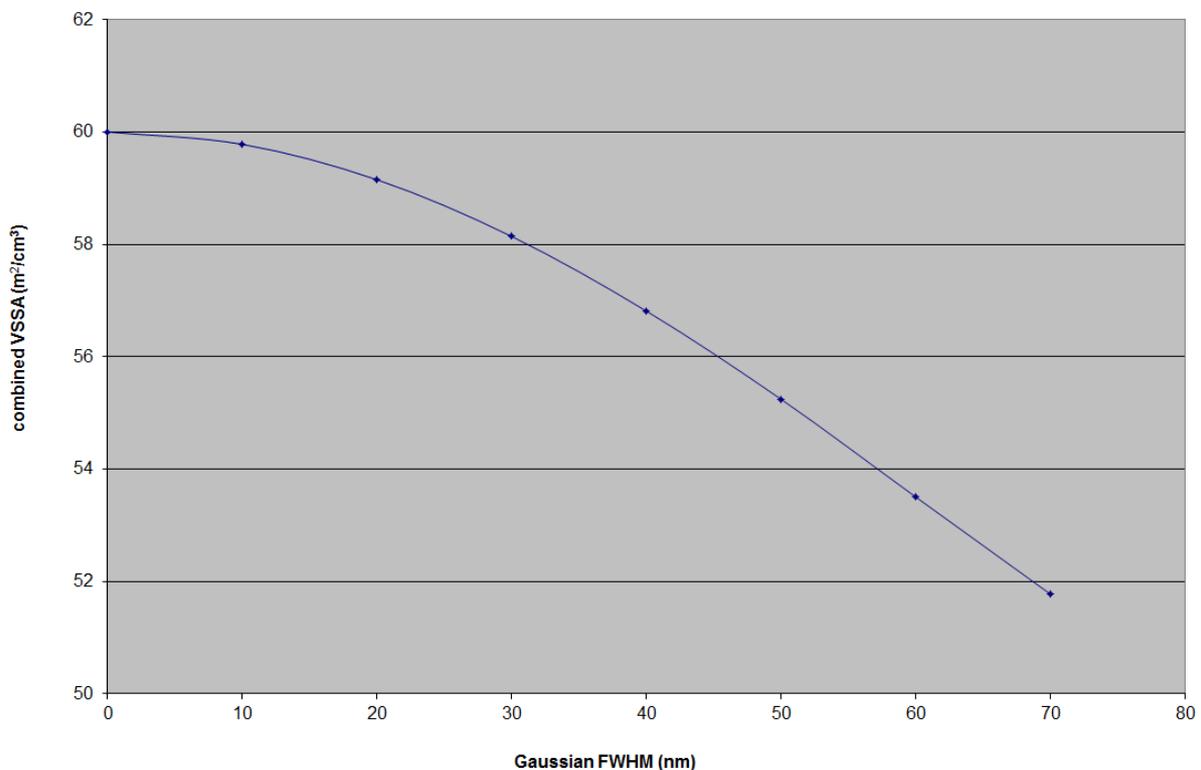


Figure 1: Variation of total VSSA with width of particle size distribution, assuming a median (and mean) particle size of 100 nm and a Gaussian (normal) PSD.

It is possible to construct theoretical PSDs for spherical particles with a VSSA higher than $60 \text{ m}^2/\text{cm}^3$ and more than 50 % of the particles of diameter above 100 nm, but such distributions require most of the particles above 100 nm in diameter to be only just above this value, and the particles below 100 nm to be well below the threshold – in fact between 60 nm and 70 nm is the most “efficient” sub-threshold size-range for increasing sample VSSA as illustrated in Figure 2, which shows the overall VSSA (z axis) calculated for pairs of particles (sizes on the x and y axes). The light blue area shows the region of $\text{VSSA} > 60 \text{ m}^2/\text{cm}^3$ for the particle pairs. It can be deduced from this diagram that the only PSDs for spherical particles that would produce “false positives” for nanomaterial classification would be highly asymmetric and/or bimodal PSDs, where the majority of the particles that are above 100 nm diameter are only just above this value. This is very unlikely to be found in real situations. For example a bimodal distribution with 60 % of particles close to 105 nm diameter and 40 % close to 65 nm diameter would have a VSSA of about $62 \text{ m}^2/\text{cm}^3$.

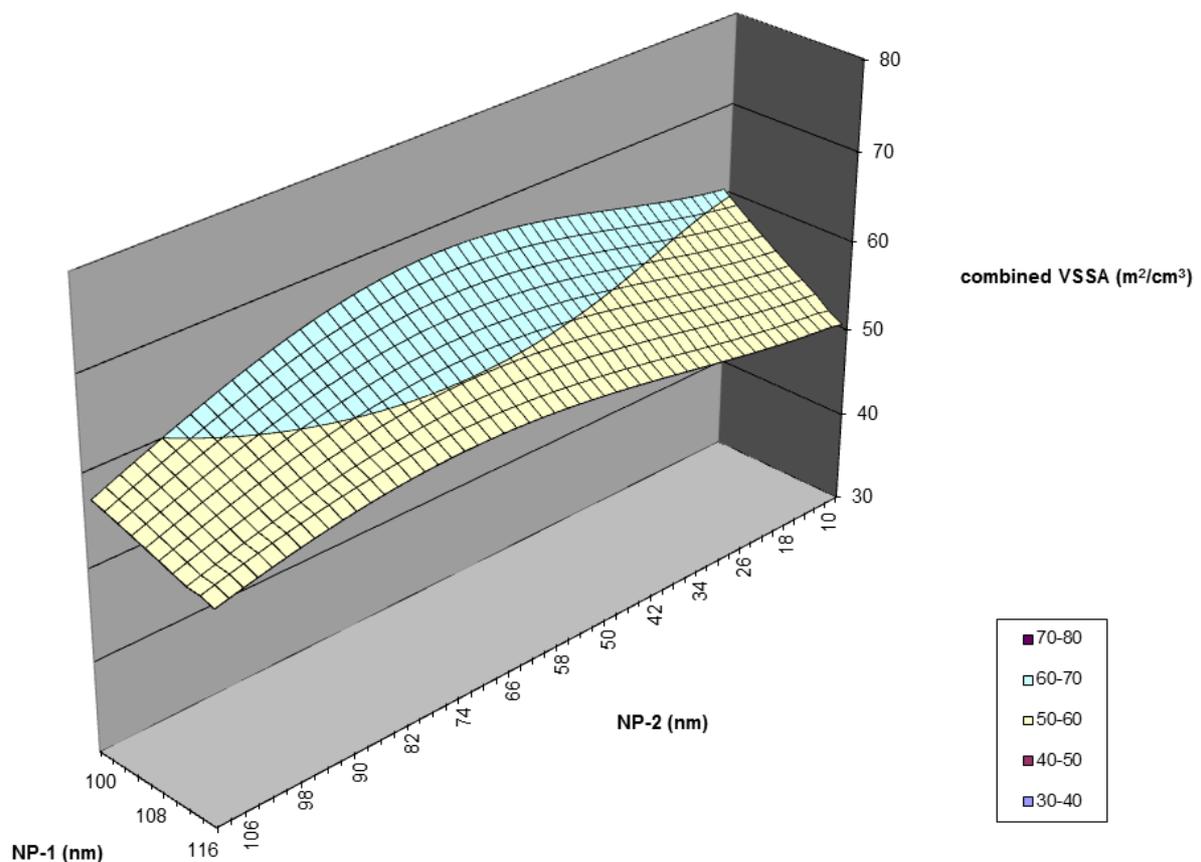


Figure 2: Calculated variation of combined VSSA of pairs of particles (NP1 and NP2) as a function of particle sizes.

It can also be noted that the measured VSSA (assuming that it can be measured with high accuracy) will always be slightly less than the theoretical VSSA, even for samples with no strongly aggregated particle ensembles, due to the fact that in the dry state agglomeration will restrict the available surface area for gas adsorption to some extent. This further reduces the possibility for false positive VSSA results for spherical particles. In fact, ignoring measurement uncertainty will lead to a much higher chance of false positive results than the unlikely event of specific and unusual PSD effects.

In contrast to the “unlikely” chances of false positives for polydisperse samples of spherical particles, the chances of false negatives are clearly much higher. Simple Gaussian broadening of the PSD with a median size just under 100 nm will lead to false negative classification (as can be deduced from Figure 1 above), and in the cases of lognormal (or otherwise asymmetric), bimodal or multimodal

PSDs, there are an infinite number of possibilities for false negatives. As an illustration, we could imagine a bimodal PSD with two narrow size ranges centred around 50 nm and 150 nm. If 80 % of the particles were of diameter 50 nm and 20 % were of diameter 150 nm then the combined VSSA would be about $50 \text{ m}^2/\text{cm}^3$ or a clear “false negative” with respect to the definition.

An even more extreme case of a false negative might be the mixture of 99 % of particles with diameter 10 nm and just 1 % of diameter 150 nm, which has a theoretical VSSA of about $56 \text{ m}^2/\text{cm}^3$. But also for the theoretical case of 1 cm^3 of very fine brittle sand (grain size about $100 \mu\text{m}$, number of particles about 1 million) with 10 million nanoparticles (size 50 nm, representing just 1 billionth of the mass) caused by mechanical grinding or wear of the sand grains, the VSSA would be just $0.06 \text{ m}^2/\text{cm}^3$. Such sand, that would usually not be considered a nanomaterial, meets the size specification of the EC definition, but would not be recognized as one based on VSSA measurement.

It is clear from the above considerations that, for polydisperse spherical particles the chances of false negative classification (i.e. classification as non-nanomaterial when in reality the material should fall under the definition) are high, so VSSA cannot be used reliably for classification of such materials as non-nanomaterials. Conversely, the chances of false positives are low, even for polydisperse samples, so VSSA would be suitable for positive identification – but only for spherical, non-porous particles. On the other hand, if spherical particles can be shown to be non-porous, non-aggregated, and reasonably monodisperse, then VSSA may be a fairly reliable indicator of nanomaterial status, both as nanomaterial and as non-nanomaterial. However this is not representative of the majority of real situations. In real life, particles will have a range of shapes, PSDs, and states of aggregation/agglomeration.

Non-spherical particles

Deviation of the shape from approximately spherical towards most other basic shapes such as platelets or rods/needles, *while maintaining the median minimum external dimension at 100 nm*, will usually, but not always, reduce VSSA. There are some scenarios whereby a $\text{VSSA} > 60 \text{ m}^2/\text{cm}^3$ could be measured for a sample with median minimum dimension $\geq 100 \text{ nm}$:

Porous particles: this is one scenario that may be relatively common depending on the particle type and synthesis route. However, porous particles that are aggregates of smaller constituent particles (that can clearly be distinguished by microscopy) would not qualify themselves as the constituent particles with respect to the current EC definition.

Particles with complex shapes: this may result from a synthesis route whereby smaller particles coalesce (e.g. at high temperature) to the point where they cannot be distinguished as individual particles, but not to the point where the resulting object minimises its surface area by assuming a more spherical shape (see figure 11.1 of ref. 2).

Special particle shapes: monodisperse tetrahedra with a minimum dimension of 100 nm have a VSSA value of $120 \text{ m}^2/\text{cm}^3$, twice that of the current definition's VSSA threshold of $60 \text{ m}^2/\text{cm}^3$. In fact the minimum dimension has to be increased to 200 nm to reduce the VSSA to the threshold. While this is not likely to be representative of most particle types, especially close to or higher than the 100 nm threshold, tetrahedral particle shapes have been reported. Similarly, monodisperse octohedra with minimum dimension = 100 nm have a VSSA of $73.5 \text{ m}^2/\text{cm}^3$, and flat triangular particles with thickness equal to the “height” of the triangular top and bottom surface, have a VSSA of $80 \text{ m}^2/\text{cm}^3$ for a minimum dimension of 100 nm. In these cases to reach the threshold the minimum dimension needs to be increased by a smaller amount than for tetrahedra.

Thus a $\text{VSSA} > 60 \text{ m}^2/\text{cm}^3$ is not necessarily a reliable indicator that a material would be classified as a nanomaterial according to the dimensional criteria of the current definition. While the third case of special particle shapes is likely to be rare for particle sizes close to and above the 100 nm threshold, porous particles and complex particle shapes may be somewhat more common. The chance of “false positive” nanomaterial classification based solely on VSSA measurement is therefore not negligible. The case of approximately spherical “convex” particles may also be quite common and more suitable

for reliable positive classification – but this would need to be studied on a case-by-case basis, and complemented by EM analysis.

In contrast to the “special particle shapes” described above that can lead to “false positives”, there are many non-spherical particle shapes for which the VSSA threshold at which a material would fall under the definition would be lower than $60 \text{ m}^2/\text{cm}^3$; these would lead to “false negative” classification even for relatively monodisperse samples with no aggregation/agglomeration. It is interesting to examine this in some more detail for two specific shapes – needle shaped particles and platelets. These shapes are not so uncommon, and two such materials have been examined in a joint study undertaken by the JRC and Eurocolour.¹¹³

Needle-shaped particles

It is a very straightforward matter to model a polydisperse sample of needle shaped particles if a few simplifying assumptions are made. Modelling very complex samples of (for example) faceted needle shaped particles with different size distributions for each dimension is also quite feasible, but would be of limited use if the model does not match reality. Here we present theoretical VSSA calculations for a few simple cases of needle shaped particles: needles with circular cross sections, and faceted needles with square, rectangular and triangular cross sections.

The VSSA of *circular-cross-section needles*, of diameter $d=2r$ and length l , is given by:

$$\text{VSSA (m}^2/\text{cm}^3) = 1000 \times (2\pi r l + 2\pi r^2) / \pi r^2 l = 1000 (2/r + 2/l)$$

where d and l are in nanometers. This equation, for $l \gg d$ and $r = 50 \text{ nm}$ ($d = 100 \text{ nm}$) approaches the value of $40 \text{ m}^2/\text{cm}^3$ for very long needles.

Square cross-section needles of length l and cross section $a \times a$ will also have a theoretical VSSA, for $l \gg a$ and $a = 100 \text{ nm}$, that approaches $40 \text{ m}^2/\text{cm}^3$ for long needles. For *triangular needles* the situation is different, in an analogous way to tetrahedral particles being a rather special 3D case. In fact needle-shaped particles with a triangular cross section and length much greater than the base, with a minimum dimension of 100 nm have a VSSA that approaches $60 \text{ m}^2/\text{cm}^3$. It can be argued that this particular situation is not likely to be common, since hexagonal crystal symmetry would probably tend to create needle shaped crystallites with a hexagonal cross section, which, like circular and square cross section needles, have a VSSA of $40 \text{ m}^2/\text{cm}^3$ for long crystallites with a minimum dimension of 100 nm . Needle shaped particles with a rectangular cross section and minimum dimension of 100 nm have a VSSA less than $40 \text{ m}^2/\text{cm}^3$. The general equation for the VSSA of a rectangular parallelepiped of sides a , b and c is

$$\text{VSSA (m}^2/\text{cm}^3) = 2000/a + 2000/b + 2000/c$$

where a , b and c are in nanometers. The higher the aspect ratio of the cross section, the more the particle resembles a platelet, with a VSSA ranging between $40 \text{ m}^2/\text{cm}^3$ and that of platelets with minimum dimension of 100 nm , as discussed below. In the JRC/Eurocolour study,¹¹³ the VSSA of “Pigment Yellow 42” was measured by 8 laboratories. This material consists of needle-shaped crystallites, as shown in the electron micrograph supplied by the manufacturer (Figure 3).

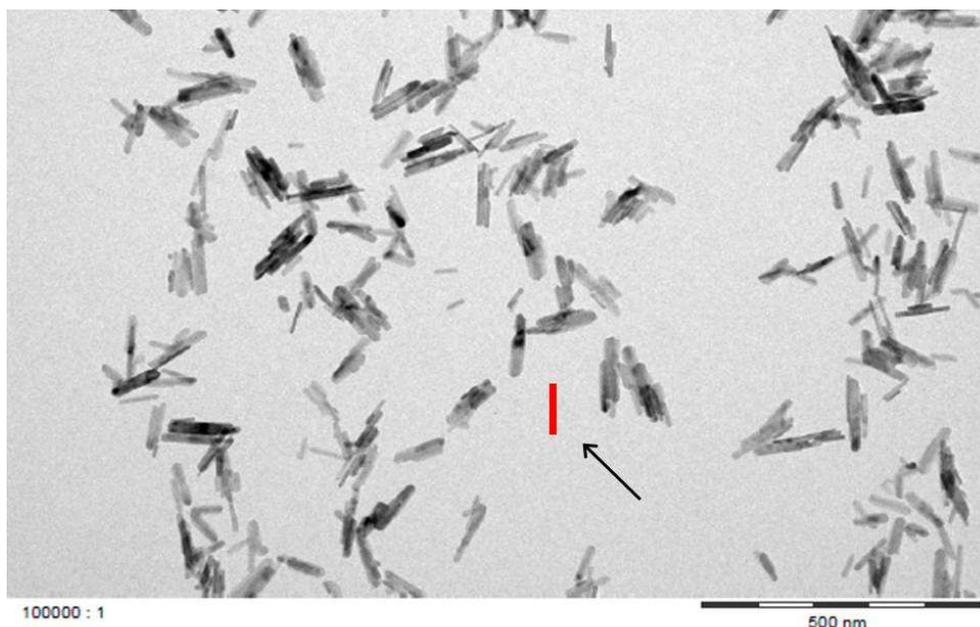


Figure 3: EM micrograph of Pigment Yellow 42 (Image courtesy BASF SE); the indicated red rectangle is representative of a needle shaped particle of circular cross section with a theoretical VSSA of $326 \text{ m}^2/\text{cm}^3$.

The mean VSSA was determined to be $326 \text{ m}^2/\text{cm}^3$ with a standard deviation of $32 \text{ m}^2/\text{cm}^3$. The minimum and maximum values measured were $275 \text{ m}^2/\text{cm}^3$ and $375 \text{ m}^2/\text{cm}^3$ respectively. For comparison, superimposed on the micrograph, in red colour, is a “simulated” image of a particle with a VSSA of $326 \text{ m}^2/\text{cm}^3$, of circular cross section (diameter $d = 13.1 \text{ nm}$) and length $l = 7d$ (the approximate average aspect ratio of the particles shown in the micrograph). It seems that the measured VSSA in this case is a rather good indicator of average particle size. In the dry state, it can be expected that agglomeration, seen also in the micrograph, will reduce the VSSA by a significant amount, so that the real average size of the particles would be slightly less than that used for the simulation.

It should be noted that changes in the aspect ratio inserted in the simulation, affect the calculated VSSA less than changes to the cross-sectional diameter, so for long needle shaped particles VSSA is in fact rather sensitive to the appropriate particle measurement (minimum dimension) with respect to the current definition of nanomaterial. This is an important observation, since if a “safety factor” can be included for particle agglomeration and/or measurement uncertainty, then in the case of nearly-monodisperse needle-shaped particles, VSSA might be accurate enough for both positive and negative classification with respect to the EC definition if a VSSA threshold of $40 \text{ m}^2/\text{cm}^3$ is used instead of $60 \text{ m}^2/\text{cm}^3$. Of course baseline EM studies would have to be performed in order to determine particle aspect ratios, and to ensure that particles are not aggregated and only loosely agglomerated. In any “borderline case” VSSA would have to be abandoned and replaced with careful EM analysis for a reliable classification. From these basic considerations and the example illustrated above, it can be concluded that a more extensive evaluation of VSSA for both positive and negative classification of needle-shaped particulates should be undertaken, including consideration of the effects of polydispersity on the measured VSSA, and whether agglomeration of faceted needles would be likely to render results unreliable.

Platelet or flake shaped particles

For platelets with thickness d much less than the other two dimensions (a and b), it is a simple matter to show that the theoretical VSSA (m^2/cm^3) = $2000/d$ where d is in nm, thus for $d = 100$ nm, the VSSA should be close to $20 \text{ m}^2/\text{cm}^3$.

In the JRC/Eurocolour study,¹¹³ the VSSA of a gold-bronze pigment was measured by 8 laboratories. This material consists of thin metallic flakes of irregular shape, with varying sizes with areas up to several hundred square micrometers. The mean VSSA was determined to be $35.5 \text{ m}^2/\text{cm}^3$ with a standard deviation of $10.5 \text{ m}^2/\text{cm}^3$. The minimum and maximum values measured were $23.7 \text{ m}^2/\text{cm}^3$ and $48.2 \text{ m}^2/\text{cm}^3$ respectively.

Whether the median minimum thickness of these flakes is below or above 100 nm or not (this information not provided by the manufacturer) this example is an illustration of a case where the VSSA lies between the theoretical value of $\sim 20 \text{ m}^2/\text{cm}^3$ for large-area platelet/flake shaped particles with uniform thickness of 100 nm and the threshold VSSA value of $60 \text{ m}^2/\text{cm}^3$ for positive nanomaterial classification specified in the current recommended nanomaterial definition.

The theoretical sensitivity of VSSA to platelet/flake thickness alone also raises the question of which dimension should be used for classification against the EC definition. The most obvious would be the thickness – but for platelets/flakes that are not perfectly flat this might not correspond to specific candidate particle shape parameters such as the Minimum Feret Diameter. It is entirely possible for such materials that a median Minimum Feret Diameter of greater than 100 nm could be determined even if the flakes were all much less than 100 nm in thickness, illustrating that guidance is required regarding choice of size parameter to be used for different particle shapes.

Assuming for now that the appropriate dimension for the EC definition is the platelet or flake thickness, independent of curvature, then it is clear that for such materials (with uniform flake thickness) the VSSA is actually quite a good measurement technique for material assessment with respect to the definition, since for $d \ll a$ or b , the VSSA is only sensitive to the thickness. However, the appropriate VSSA threshold would be $20 \text{ m}^2/\text{cm}^3$ and not $60 \text{ m}^2/\text{cm}^3$ as in the current definition. As in the case of needle-shaped particles, a “safety factor” would need to be included for particle agglomeration and/or instrumental uncertainty, and baseline EM studies would have to be performed in order to ensure that particles are not aggregated and only loosely agglomerated. From these basic considerations and this example, it can be concluded that a more extensive evaluation of VSSA for both positive and negative classification of platelet or flake shaped particulates should be undertaken.

5.4.1.3 Concluding remarks about VSSA determination as a proxy measurement method

From the above sections it can be concluded that VSSA determination is not a reliable particle classification method for “unknown” samples – i.e. where state of aggregation/agglomeration, shape and/or porosity are unknown. Simple comparison of a VSSA measurement on a powder against a threshold value of $60 \text{ m}^2/\text{cm}^3$ would often lead to incorrect classification (false negatives are most likely) if the method were to be used as a proxy “alternative to straightforward implementation”. Nevertheless, there are specific situations where VSSA, with appropriate baseline EM studies, might actually be useful for this purpose.

A first step in a tiered process would be to establish, using a suitable method, presumably EM (possibly in combination with other methods and/or sample dispersion protocols), whether aggregates of constituent particles are present. Aggregation will reduce measured VSSA to some extent and may render VSSA determination useless for classification of a sample as “non-nanomaterial”. If aggregates are not present, and it can be clearly shown that particles are only loosely agglomerated, then the next step would be to establish what particle shape dominates, and whether the material is polydisperse. For platelet/flake shaped particles of approximately uniform thickness VSSA determination against a threshold value of $20 \text{ m}^2/\text{cm}^3$ could be a reliable method of classification both as nanomaterial and non-nanomaterial. This however should be verified by a more

in-depth study, including development of appropriate sample preparation and VSSA measurement protocols and an analysis of what level of “safety margin” would be necessary to avoid false classification. For needle-shaped particles, with two dimensions approximately equal and much less than the length, it is possible that classification against a threshold value of $40 \text{ m}^2/\text{cm}^3$ might be reliable, but in this case verification by an in-depth study, including development of appropriate sample preparation, VSSA measurement protocols and safety margins, would need to be complemented by an analysis of the effects of polydispersity (more likely for needle shaped particles than for platelets) on VSSA.

Particles with all 3 dimensions in or close to the nano-range represent probably the most difficult case since there are many scenarios that can either increase or decrease VSSA, including porosity, polydispersity, aggregation, agglomeration (even if to a limited extent), and shape. For non-porous particles with non-complex shapes (in particular non-concave) a VSSA greater than $60 \text{ m}^2/\text{cm}^3$ is likely to be a reliable indicator for “positive classification” (i.e. as nanomaterial). However, even if samples can be clearly shown to be non-aggregated, for negative classification (i.e. as non-nanomaterial) a large “safety margin” would have to be used to account for the many factors that can lead to a VSSA value less than $60 \text{ m}^2/\text{cm}^3$ for samples with median minimum dimensions less than 100 nm. However, this also merits further study in order to establish what baseline EM information and safety margin might be used in order to contribute to the development of a VSSA-based sample screening approach for non-needle-shaped and non-platelet-shaped particles.

5.4.2 Conversion of other size distributions to particle number based size distributions

The EC definition classifies a material as nanomaterial if a sufficient number of the particles have their minimum external dimension in a defined size range. Therefore, the definition relies on PSA methods that can count how many particles are within certain particle size ranges. Many of the commonly used methods are 'ensemble methods', which produce PSDs based on other metrics, for example the cumulative mass of the samples present in the defined particle size ranges. Ensemble methods can include both non-fractionation (e.g. DLS or LD) and fractionation methods (e.g. CLS or FFF/DLS). To use the results of these methods for assessment against the EC definition therefore requires a conversion of the data from their original metric to an equivalent particle-number based PSD. It is also a condition for nearly all ensemble methods that full dispersion into constituent particles is achieved as remaining aggregates/agglomerates are regarded as single particles.

As discussed in the first report² reliable conversion between PSDs based on different size metrics requires in practice that all particles have the same (regular) shape and approximately the same size, except for fractionation methods which can, at least in principle, deal with polydisperse samples. These conditions may be fulfilled for some materials which are manufactured with a very specific purpose. For those materials, the question whether they are nanomaterial or not is most likely trivial, as the material specifications require them to be either a nanomaterial or not.

The majority of materials, which only need to have a sufficiently "small" particle size that can be achieved either by synthesis ('bottom-up') or grinding ('top-down'), will not have the level of monodispersity (for non-fractionation methods) and regular shape required for reliable data conversion, and will often contain aggregates and/or agglomerates that are difficult or impossible to disperse into their constituent particles. This can limit the usefulness of converted data, depending on the intended use or application of the converted data.

Several potential applications of conversion between different types of size distribution can be envisaged; the most relevant applications, from the point of view of the implementation of the EC definition, are the following:

- the definitive decision as to whether a material fulfills the definition or not,
- the screening (i.e. only positive identification) of potential nanomaterials, and
- the quality control of new production batches.

5.4.2.1 *Decision whether a material fulfils the EC nanomaterial definition*

The inherent uncertainties connected with the conversion of data mean that number-based PSDs obtained via data conversion are most often not sufficiently reliable for unambiguous, definitive classification of a material as nanomaterial.

An additional problem is the fact that the conversion of data from one metric to another often involves various equivalent diameters, which neither correspond directly to any external dimension of the particles, nor to the minimum external dimension used in the EC definition.

Therefore, converted data from non-fractionation methods are usually not suitable for a confident decision as to whether a material falls under the EC definition or not, except for materials consisting of near-monodisperse populations of spherical particles. For fractionation methods that cover the entire size range of interest, polydisperse populations of spherical non-agglomerated/non-aggregated particles can in principle be classified, though establishment and validation of sample preparation and measurement protocols would be necessary.

5.4.2.2 *Positive identification of nanomaterials using unconverted data*

The fact that many ensemble methods give results that are (compared to number based PSDs) biased towards higher diameters, can be exploited to allow positive identification of certain nanomaterials. The rationale is that if the intensity-weighted (or volume-weighted, or mass-weighted) average particle size is below 100 nm, the number-weighted average is certainly below 100 nm. The higher representation of larger particles in the intensity (e.g for DLS) or volume/mass weighted distribution will most likely also outweigh any differences between the effectively determined (e.g. hydrodynamic or Stokes') diameter and the required minimum external dimension. Therefore, unconverted data can usually be considered reliable to positively identify a material as nanomaterial. They can, however, not prove that a material is *not* covered by the definition.

5.4.2.3 *Quality control of new production batches*

In the case that a lot of information on shape, PSD, densities etc. is available from the original classification of the material as nanomaterial or non-nanomaterial, the initial investigations offer the opportunity of establishing the potential links between converted data from ensemble methods and the number-based PSD (e.g. as determined using advanced microscopic techniques). (Converted) data from ensemble methods can be used for new batches to demonstrate that the production process is under control and that the material has the same property as the initially released batch.^K This quality control can in principle also be performed on unconverted data, but conversion may help to highlight critical size regions.

5.4.2.4 *Conclusion*

In summary, conversion between different types of PSD has its merits, but is for the majority of particulate materials not suitable for definitive decisions as to nanomaterial status. Only in the case of fractionation methods used on spherical particles could converted data be considered reliable 'in principle', and then it would need to be complemented by microscopic assessment of constituent particle dispersion and shape, and verification that all particle sizes are within the operating range of the instrumentation. Unconverted data could be used for positive nanomaterial screening, and converted data might have an application in batch quality control.

^K The same approach is widely used in other fields of industry. Fast and easily measurable parameters like colour and density are used to check whether a constant production process is under control.

5.4.3 Simplified binary binning methods

5.4.3.1 *A (deceptively) simple concept*

A priori, implementation of the EC definition does not require the measurement of a very detailed particle size distribution (PSD): it is sufficient to classify (or 'bin') the particles in one of three size classes: smaller than 1 nm, larger than 100 nm, or in between. In most practical situations, the lower size limit is of lesser relevance, reducing the analytical question further to assessing how many particles are smaller (undersize fraction) and how many are larger (oversize fraction) than 100 nm.

In other words, it is sufficient to construct a method that separates particles with a minimum external dimension larger than 100 nm from particles with a minimum external dimension smaller than 100 nm, and to count and compare the number of particles in both fractions. With a good separation method, there would even be no need for size measurements at all. This concept is simple, and therefore deserves a minimum of attention. In the following section, we describe the sieving and filtration methods that in theory could be used, and check their practical value.

5.4.3.2 *Separation by sieving or filtration*

Sieving

A well known method for determining undersize and oversize particle fractions in a polydisperse powder is *sieving*. Sieves separate the particles larger and smaller than the mesh (the 'holes' in the sieve bed), and the weight of the two fractions is measured to determine the relative amount of undersize or oversize particles. The term sieve is also used for more advanced tools, for example in *molecular sieves*, which are materials with very small holes of a uniform size, in which molecules can be absorbed and trapped that are smaller than the material's pore size. Whereas traditional mesh sieves operate at too large particle sizes to be relevant for the EC definition (the smallest have openings of about 5 micrometres), molecular sieves are working only for too small particles, typically around the 1 nm range. Therefore, simple sieving techniques do not play a role in the implementation of the current EC definition.

Filtration

Sieving is a kind of filtration, but the term filtration covers also other techniques, which separate particles in suspensions or aerosols at a certain cut-off value, and some of them do operate in the nanoscale range.

A first and major problem for filtration is the clogging of filters, especially in 'dead-end filtration' methods, where the input ('feed') material is passing through the filter membrane and where the solids/particles that are bigger than the cut-off value are trapped in or on top of the filter. Especially when the PSD is broad and contains a large volume fraction of larger particles, this leads to clogging of the filter. This is less of a problem for an analytical technique, where small sample volumes are treated, then for processing applications, where the filtrate volume and its sustainability are crucial.

For analytical tests one could also use one of the alternative filtration techniques, such as tangential flow or cross flow filtration, in which the feed is not passed through the filter, but repeatedly passes along the surface of the filter. This type of filtration avoids rapid clogging, and, importantly, it results in two suspensions: one containing the particles that have passed the filter, and that are smaller than the filter's cut-off value, and one containing the particles larger than the cut-off value. This is also an advantage, compared to the dead-end filtration systems, where the particles in the larger fraction are packed in the filter or filter cake, and have to be resuspended for the next analytical step.

Two of the EC definition basic elements affect the potential success of filtration. First of all, filters will not consider aggregated and agglomerated particles as separate particles, unless the aggregates and agglomerates are broken up before the filtration process. Also shape plays a role, as the EC definition is based on the minimum external dimension. For elongated, but even more for platelet shaped particles, the filtration efficiency and the cut-off value of a given filter, will be shape dependent: two

particles with the same minimum external dimension but different shape may not pass through the same filter.

5.4.3.3 *Counting the particles after separation*

An important difference with traditional sieve analysis, which produces mass-based particle concentrations, stems from the fact that the EC definition is based on particle numbers. Therefore, instead of weighing the permeate (fraction of the feed material having passed the filter) and retentate (fraction of the feed material not having passed the filter), a particle counting technique will have to be applied to measure the particle concentration in both permeate (undersize) and retentate (oversize) fraction.

For the analytical purposes of the current report, the relevant performance parameter of a filtration system is its separation quality: the filter should not allow particles larger than the cut-off value to pass, and should not stop particles that are smaller than the cut-off value. If this is respected, and if the cut-off value can be chosen to be at 100 nm, then the technique chosen to count the particles in the two fractions can be relatively simple: a simple particle detection, without any sizing capacity, will suffice to determine whether more or less than 50 % of the particles in the original material are in the nanoscale range. One can unfortunately not use the methods that estimate particle number or concentration from an ensemble signal such as light absorption, as this does not take into account that there is likely a distribution of particle sizes within undersize and oversize fractions (see section 5.4.2 on conversion of PSDs).

5.4.3.4 *Conclusion*

If one assumes that the critical size limit in the EC definition is the upper one (100 nm), and if one can find a suitable filtration method with a sharp cut-off at this 100 nm value, then the analytical challenge posed by the EC definition is significantly simplified. This simplifying approach has not yet been studied or used extensively, but it has the potential to become an alternative implementation method for a limited number of materials, if at least the issues of minimum external dimension and aggregation can be solved. If the latter issues are not solved, then a 'simple' counting of particles in undersize and oversize fractions will be insufficient to really assess the EC definition.

This brings us back to considering other, filtration-related techniques, based on a continuous fractionation of the particles in an infinite number of 'size classes', such as field flow filtration, as already mentioned in ref. 2. These techniques have developed into true analytical tools and have already been indicated as very valuable for the implementation of the EC definition in ref. 53, in particular in combination with size measurement methods that are accurate, but depend on the particle population to be monodisperse.

5.4.4 **Considerations of manufacturing information**

The first few pages of this sub-section present an overview of some particle production methods, focusing in particular on the relationship between production process parameters and size characteristics. Then some general conclusions are presented.

5.4.4.1 *Flame aerosol particle production methods*

The number of processes that are in industrial use for the production of nanoparticles in the range of kilotons per year is very limited. In 2002 more than 90 % of the world wide produced volume of nanomaterials was produced in flame aerosol reactors, and this is still the case, 10 years later (carbon black, titania, zinc oxide, fumed silica).^{114,54} With properly adjusted process parameters the large scale production of non-agglomerated nanoparticles of spherical shape can be achieved. In combination with an electrical field applied to the flame by plate or pin electrodes the particle size can be reduced and a narrower PSD can be obtained.¹¹⁵

Contrary to flame aerosol processes, inert gas condensation, physical or chemical vapour synthesis and laser ablation are leading to typically broad and asymmetric PSDs.¹¹⁵ Flame spray nanoparticle

reactors have usually been designed and scaled up for the production of one specific product which they can produce with the desired quality after having optimised the reactor design and the process parameters.^{114,115,116}

While the flame aerosol process is a versatile approach to produce a large variety of different nanoparticle species, and in spite of a reasonable scientific understanding of the effect of process parameters on the quality of the products, a systematic understanding of reactor design correlations on the production process, which would be helpful for the scaling up of production capacity and the extension on the production of other nanoparticle species, has so far not been reached.^{114,115,117} Since the realisation of a nanoparticle reactor based on a specific production process is very variable and optimised for specific products, it is usually not possible to draw quantitative conclusions from the simple knowledge of the production process on the PSD of the produced nanoparticles. However, as the whole effort has been made to reliably produce nanomaterials to a well-defined size in the range of some 10 nm and with a PSD as narrow as possible, the processes mentioned in this section can implicitly be considered as methods to produce materials with an overwhelming fraction of particles below a size of 100 nm.

Knowledge of process parameters such as composition and concentrations of precursors, their feeding rate and others will not be meaningful without detailed knowledge of the reactor design. Moreover, such information on process control parameters will be considered by most producers as confidential since it is the result of a long and cumbersome optimisation process which frequently makes the difference to products of competitors. Additionally, in many cases after the synthesis process an additional processing by mechanical grinding is applied in order to break up agglomerates that are inherently formed in vapour and liquid phase methods.^{118,119}

Usually processes are limited in the smallest particle size they allow to obtain. On the other hand, the production or co-production of larger particles with typical sizes above 100 nm appears to be always possible if the process parameters exhibit intentional or non-intentional deviations from their optimised values. Typically in such cases the precursor is converted fast at high temperatures, leading to a high concentration of the nanoparticle forming substance, which results in high nucleation and growth rates, high collision rates among the small nanoparticles, and to growth by coalescence. The growth limit will be reached when coalescence is stopped as surface energy cannot be further reduced, and when particle growth has decreased their concentration and hence their collision rate to sufficiently low levels to stop also further growth by coagulation.¹¹⁵

The scale-up of a nanoparticle production process producing batches of mg to g of certain nanoparticles per day in the laboratory into continuously operating processes that can be run 24/7, producing nanomaterials at a rate of 250 g/h to 2500 g/h, is a challenge since it is usually accompanied by an increase in nanoparticle size.^{114,115,116} In continuous operation such production rates are equivalent to production capacities of about 2-20 tons per year and reactor. However, plants with production capacities of up to 25 t/h are in operation that are described by Stark and Pratsinis as resembling the "best rockets of the space shuttles departing from Cape Kennedy".¹²⁰

Since the aerosol characteristics are important parameters in process control the droplet formation technique is important. Good control can be achieved by ultrasound where with increasing ultrasound frequency the droplet size is decreasing. Also electro spray methods are used by applying a high voltage to a liquid passing in a capillary.^{115,121}

Teoh et al.¹²² consider FSP as especially convenient for nanoparticle synthesis as it allows the use of less volatile precursors for the liquid feed and exhibits higher flame temperatures (up to 2600 K or even 2880 K) and higher temperature gradients, which facilitates upscaling production while essentially preserving the product properties such as size and PSD. The high gas velocities used in FSP yield short residence times of the growing nanoparticles in the high-temperature field and high temperature gradients along the flame axis. Both effects promote the production of homogeneous, highly crystalline, fine nanoparticles, which are spherical with only very few exceptions (CeO₂ rhomboids, BaF₂ cubes, ZnO nanorods and Fe₂O₃ platelets).^{122,123}

The adjustable process parameters that determine the quality of the nanoparticles produced by flame aerosol synthesis are:^{114,117,123,124,125}

- The chemical form of the precursor, its solubility in the solvent and its physical properties
- The viscosity of the solvent and its enthalpies of evaporation and combustion
- The size of the aerosol droplets, their density in the carrier gas and the flow rate into the reactor
- The physical properties of the carrier gas and their effect on temperature quenching
- The water content in the aerosol
- The type of combustion gas (e.g. H₂, CH₄) and its flow rate into the reactor
- The type of oxidant (e.g. air, O₂) and its flow rate into the reactor
- The velocity difference between the combustion gas and the aerosol entering the reactor
- The geometrical arrangement and size of the nozzles feeding aerosol, combustion gas and oxidant into the reactor, as determined by the burner design of a flame spray reactor^{114,115,125}

The combination of these parameters determines the type (e.g. whether laminar or turbulent) and spatial extension of the flame,¹²⁰ the temperature and temperature gradients in the reactor, and the local concentration and mixing of the reactants. This in turn determines key properties of the production process such as the supersaturation of the precursors that can be achieved in the nucleation and cluster formation phase, the reaction times in certain temperature zones, and the collision probability between clusters and nanoparticles and hence their further growth by coalescence and coagulation.¹²³ Therefore, the fine tuning of the listed process parameters determines the mean size, the PSD, composition, crystallite properties and morphology of the nanoparticles.¹²² It is intuitively clear that the scaling up of a laboratory process to an industrial production with increased material turnover on a larger geometrical scale is a difficult project if the properties of the nanoparticles need to be preserved. Since the best description of the quality of the process is given by a comprehensive analysis of the final product, detailed information on the manufacturing process and the process parameters is not very illustrative.

5.4.4.2 *Wet chemistry methods*

Compared to the methods mentioned so far, wet chemistry methods such as chemical precipitation, sol-gel methods, microemulsion techniques, sono-chemistry and hydrothermal processes play a minor role in large-scale industrial manufacturing of nanoparticles.^{115,121} Nevertheless they are important since various nanoparticulate materials cannot be produced in the required quality by flame aerosol processes.¹¹⁸ Silver and gold nanoparticles are mainly produced by liquid-phase precipitation techniques due to the good control of particle size and the low cost of the raw materials.¹¹⁸ The same holds for semiconductor quantum dots, which are among the smallest nanoparticles that are industrially produced, where stoichiometry, crystallinity and shape of the phases can most suitably be controlled in liquid-phase synthesis processes.¹¹⁸ Also the scale-up of chemical reactors, such as confined impinging jet reactors, stirred tank or vortex mixing reactors to increase production capacity is demanding since essential process parameters such as mixing rates, concentration ratios and reaction times realized in a flowing liquid need to be preserved when increasing the dimensions and material throughput.¹²⁶

Recent efforts appear to focus on microemulsion methods, which allow the production of non-agglomerated inorganic nanoparticles with controlled composition, shape and size. With respect to other wet chemistry methods the improved control is achieved by creating two microemulsions of reverse micelles in a continuous oil phase. The reverse micelles present nm to μm sized aqueous domains filled with small amounts of reactants. After mixing the microemulsions, collisions and coalescence of droplets containing the different reactants create a confined reaction space with limited amount of reactants thereby achieving excellent control over the nanoparticle properties.^{121,127} In this way inorganic nanoparticles below 50 nm size with narrow PSD can be obtained, and metallic nanoparticles in a size range of 2 nm to 5 nm can be produced.¹²⁷

5.4.4.3 *Microwave plasma process*

Very small nanoparticles with a size below 10 nm and a very narrow PSD can reliably be produced by the microwave plasma process,^{115,128} where the nanoparticles are electrically charged. By addition of water vapour to the carrier gas the charge of the nanoparticles can be modified and even neutralized due to collisions with OH⁻ ions which allows a tuning of particles size and PSD.¹²⁸ There are various design options for creating a microwave plasma reactor. Therefore the design and the choice of the key process parameters, mainly gas pressure, frequency and field strength of the electrical field can be adjusted to different needs¹²⁸ and many different nanoparticle species such as GeO, Fe₂O₃, Co₃O₄, TiO₂, ZrO₂, FePt, Ni, WS₂ and microwave coated nanoparticles can be produced with tuneable size and PSD.^{115,128} It is probable that the microwave plasma process can be scaled up for large scale production of nanoparticles.^{115,128}

5.4.4.4 *Mechanochemical processing*

Some nanoparticle species can also be produced in a bottom-up process from the solid phase by high energy dry ball milling at low temperatures.¹¹⁸ Nanoparticles are formed from precursor powders that are mixed with a salt powder which is used as a matrix that prevents aggregation and agglomeration of the desired nanoparticles.^{118,129} Mechanochemical processing leads to the formation of nanosized composite structures from precursor powders by repeated deformation, fracture and welding of the nanometer-sized grains.¹¹⁸ On the freshly created surfaces chemical reactions occur at temperatures that are much lower than those required in alternative production methods such as plasma spray methods.^{118,129} By careful selection of the chemical reaction paths, the stoichiometry of the precursor materials, and the ball milling conditions, nanoparticles can be synthesised which are dispersed in a salt matrix, in which they can even be subjected to additional heat treatments without risk of agglomeration or aggregation before they get separated from the matrix.¹²⁹ In this way nanoparticles of nearly spherical shape, narrow PSD and low level of aggregation can be obtained¹¹⁸ and mean nanoparticle sizes as low as 5 nm have been reported.¹²⁹ The disadvantage may be that additional processes are required to separate the nanoparticles from by-products and matrix material, which creates additional cost and the risk of contamination.¹¹⁸

5.4.4.5 *Relating manufacturing process to the EC nanomaterial definition*

There are only a few systematic investigations concerning the effects of the manufacturing process on the characteristics and quality of nanoparticles. In ref. 118 the case of ZnO nanoparticles is reported, that were produced from the solid, liquid and vapour phase. Their characterization with BET, XRD and DLS agree in particle size for the nanoparticles produced by the solid phase method, which results in small (24 nm) monodisperse particles. The liquid phase method resulted in ZnO nanoparticles of the same size, however with some aggregation or agglomeration detectable by DLS. The vapour phase method yields also aggregated and/or agglomerated particles which exhibit however a two to three times larger size, and it generated nanoparticles with about four times more radicals (normalized to the surface area).^{116,118} In ref. 116 it is therefore emphasized that "the manufacturers should recognize the unique properties of their products compared to those of other manufacturers, based on their production techniques and process conditions". Thus, depending on the desired application of nanoparticles, the user needs information going beyond those related to size since PSD, degree of agglomeration and shape, as well as photocatalytic activity, photoluminescence and UV-Vis transmission may determine whether e.g. ZnO or CeO₂ nanoparticles of a certain manufacturer are suitable for a specific application.

5.4.4.6 *Conclusion*

The above overview gives an idea of the wide range of particle production processes that exist, and also the complexity of these processes and of the relationship between the various production parameters and the final product size characteristics. While modelling of some processes is possible, it is clear that careful characterisation of the products is often the most appropriate method of determining whether the production process is achieving the desired result. It is also clear that a lot of information exists regarding production methods and final product size characteristics, though it is

likely that the reported size parameters are in general not those required for assessment against the EC definition. This said, the overview illustrates that many production processes are in fact optimised to produce powder materials that will almost certainly fall within the recommended EC definition, and that a significant number of products would not need to be re-examined to determine their nanomaterial classification. Additionally, some but not all bottom-up processes are suitable for analysis of constituent (or primary) particles before aggregation/agglomeration takes place post-production, thereby offering an opportunity for more reliable and possibly less costly classification against the EC definition.

It can be argued that only “large” manufacturers of powder materials have the resources necessary to carry out suitable studies to determine whether their products fall under the EC definition, and that such studies may already have been performed as part of their production process development. Small manufacturers with limited resources, using production processes that are not optimised for the production of nanoscale particles (for example top-down milling processes) may face more difficulties, especially if production parameters are varied in order to modify product characteristics. In such cases, costs may be contained by, for example, using the concept of “product families” and “lead products” as outlined in the next section. Where production parameters are not varied, then periodic size analysis combined with process/product consistency control (e.g. VSSA for product consistency) may be used instead.

5.4.5 Possibilities for read-across

There is concern that testing of materials with respect to the current recommended EC definition will place a high economic burden on manufacturers, especially if TEM will be required for a reliable classification of individual materials. This section will take a purely conceptual look at some possible ways to reduce such testing and the associated costs. The section starts by presenting the “read-across” concept as applied in REACH, and then looks at how analogous methods may be applied to nanomaterial testing.

5.4.5.1 What is read-across?

Read-across is a concept applied in REACH that can be used to eliminate unnecessary testing (primarily toxicological and ecotoxicological) of substances and/or to fill data gaps. The following extracts are taken directly from:

http://echa.europa.eu/documents/10162/13628/read_across_introduutory_note_en.pdf :

“Substances that are structurally similar with physicochemical, toxicological, ecotoxicological and/or environmental fate properties that are likely to be similar or to follow a regular pattern may be considered as a *group* of substances. These similarities may be due to a number of factors:

- Common functional group (*i.e.* chemical similarity within the group)
- Common precursors and/or likely common breakdown products *via* physical and/or biological processes which result in structurally-similar degrading chemicals
- A constant pattern in the properties across the group (*i.e.* of physico-chemical and/or biological properties)

For registration of a substance under REACH, the information requirements have to be met. Within a group of substances, a data gap might be filled by *read-across*, as described below...

... The application of the *grouping* concept described above means that REACH information requirements for physicochemical properties, human health effects and/or environmental effects may be predicted from tests conducted on *reference substance(s)* within the group, referred to as *source substance(s)*, by interpolation to other substances in the group, referred to as *target substance(s)*, and this is called *read-across*.

Thus, read-across is regarded as a technique for predicting endpoint information for one substance (*target substance*), by using data from the same endpoint from (an)other substance(s), (*source substance(s)*). Consequently, the read-across approach has to be considered on an endpoint-by-endpoint basis due to the different complexities (e.g. key parameters, biological targets) of each endpoint.

The term *analogue approach* is used when read-across is employed within a group of a very limited number of substances for which trends are not apparent: i.e. the simplest case is read-across from a single source substance to a target substance. Alternatively, with a higher number of substances in a group the term *category approach* is used.

Read-across must be, in all cases, justified scientifically and documented thoroughly. There may be several lines of evidence used to justify the read-across, with the aim of strengthening the case.”

5.4.5.2 What is the equivalent of read-across for “nanomaterial” classification?

With respect to particle size measurements for classification of particulate materials against the currently recommended EC definition, the equivalent of “read-across” would mean that PSD measurement would not be required on a particular material (the “target material” using analogous terminology to REACH) if a measured property of that material which is directly related to the median value of the number-based PSD (with size meaning that particle dimension suitable for assessment with respect to the definition), is sufficiently similar to that of another material (the “source material” using analogous terminology to REACH) for which the median value of the PSD is known, under the premise that all other physicochemical characteristics of the two materials have been shown (as a function of particle size) to have identical influence on the measured property. In such a case the median value of the PSD of the “target material” could be inferred to be sufficiently close to that of the “source material” and if the uncertainty associated with this exercise is low enough, a classification of the material may be made.

This is a highly unlikely scenario for particulate materials, especially for particles that are not chemically identical and created using identical synthesis processes. However, there are a number of cases where it may be possible to avoid extensive testing of many individual materials via an extension of the read-across concept as described in the following sub-section, and/or to classify materials based on groupings and well-characterised “lead products”.

5.4.5.3 Extension of the read-across concept

Classification of particulate materials as being a nanomaterial or not requires a reliable determination as to whether the median value of an appropriate size parameter is above or below 100 nm. The appropriate size parameter is not specified in the definition. One such size parameter is the Minimum Feret Diameter ($x_{F,min}$),⁵³ which can be determined from two-dimensional TEM images. For spherical particles $x_{F,min}$ corresponds to the diameter of the circle that constitutes the 2D image of the particle, but for platelets or rods the smallest physical dimension may not be easy to determine with microscopy techniques, especially for platelets. Guidance is required as to the appropriate parameter to use for a variety of sample shapes (see also section 4.3.5).

The most obvious modifications of read-across as applied to classification of particulate materials might be termed “read-up” and “read-down”:

Read-up would mean that if a measured property of a “target material” that is directly related to particle size (only) can be shown to be characteristic of a material with a median size (size referring to the appropriate particle dimension regarding the EC definition) above that of a “source material”, the median size of which is known, then the median size of the target material can be taken to be above that value (minus possibly a correctly determined uncertainty).

Read-down would mean that if a measured property of a “target material” that is directly related to particle size (only) can be shown to be characteristic of a material with a median size (size referring to the appropriate particle dimension regarding the EC definition) below that of a “source material”,

the median size of which is known, then the median size of the target material can be taken to be below that value (plus possibly a correctly determined uncertainty).

If the theory establishing the relationship of the measured property with particle size is reliable, then the use of a reference (i.e. the “source material”) may be unnecessary in some cases.

There are hypothetical (and possibly some practical) cases where read-up or read-down according to the above definitions might be quite accurate even for small differences in particle size. For example, nearly monodisperse samples of approximately spherical quantum dots would show shifts in optical properties that can be reliably related to increases or decreases in particle size, and monodisperse metallic nanoparticles would show similar shifts in the position of surface plasmon resonance peaks as a function of particle size. However both of these cases are of relevance to the lower end of the nano-range (1 nm to 100 nm) and are not of use where particles are closer to the 100 nm threshold of the EC definition.

VSSA is in fact a good example where “read-down”, without a reference “source material”, may be quite reliable for a large number of dry powder materials. A sample of perfectly monodisperse, solid, non-porous, non-aggregated spherical particles with a diameter of 100 nm will have a VSSA of $60 \text{ m}^2/\text{cm}^3$. Broadening the number-based PSD, while maintaining a median size of 100 nm, will reduce the measured VSSA, as will aggregation or agglomeration of the constituent particles. Deviation of the shape from approximately spherical towards basic shapes such as platelets or rods/needles, *while maintaining the median minimum dimension at 100 nm*, will also reduce VSSA (though there are unlikely exceptions to this such as tetrahedrons or octohedrons). The only obvious scenario whereby a VSSA greater than $60 \text{ m}^2/\text{cm}^3$ would be measured for a sample with median minimum dimension greater than 100 nm would be for porous samples or for samples with constituent particles of rather complex shape. Thus a VSSA $> 60 \text{ m}^2/\text{cm}^3$ is often a reliable indication that the PSD of a material would also classify the material as a nanomaterial. However, a VSSA $< 60 \text{ m}^2/\text{cm}^3$ is clearly NOT a reliable indicator of “non-nanomaterial” status (see also section 5.4.1).

“Read-down” may also be reliable for DLS, given the known issues in dealing with polydisperse or aggregated/agglomerated samples, and the fact that the hydrodynamic diameter of a platelet or rod-shaped particle will usually be higher than the minimum external dimension. If a DLS determined light-intensity-based PSD indicates particles with a mean hydrodynamic diameter of approximately 100 nm or less then it is highly likely that the sample should be classified as a nanomaterial. This is due to the fact that the presence of any significant number of particles with a diameter of greater than 100 nm will certainly shift the DLS peak to higher size values due to the much more intense light scattering from the larger particles. Using the light-intensity-based PSD eliminates the very major artefacts and possible errors that may be introduced in conversion to number based PSD. Conversely, a hydrodynamic diameter of $> 100 \text{ nm}$ is NOT a reliable indicator, except in very specific cases, of non-nanomaterial status.

It is not inconceivable that “read-up” or “read-down” with the use of a reference “source-material” may offer an opportunity for both positive and negative classification with respect to the EC definition. A purely hypothetical example could be where particles are grown (bottom-up) in a liquid phase process using a specific synthesis technique, and immediately stabilised as monodisperse non-agglomerated particles. Even for particles with non-spherical shapes, if a “source material” representing a mean minimum dimension of 100 nm can be used to establish a reference DLS size distribution, then “target materials” synthesized to different sizes but using a more or less identical process might be reliably classified against the source material. The same concept might be applicable to certain gas-phase synthesized particulates and VSSA determination, though aggregation/agglomeration in the dry state may prevent this from being reliable.

Another example could be the use of XRD, where peak-widths are sensitive to constituent particle size, irrespective of the state of agglomeration/aggregation. Comparison of XRD peak widths of samples grown to different constituent particle sizes may offer an opportunity for rapid assessment of “nanomaterial-status”, even for non-spherical particles. In this case it would have to be verified

that the constituent particles are monocrystalline and reasonably monodisperse (especially for positive classification).

It should be noted that due to the various issues associated with most size measurement techniques, “read-up” is unlikely to be reliable for many industrially produced particulates, and the extensive experimental/theoretical justification required for read-up classification in specific cases may be more time-consuming and expensive than straightforward PSD measurements by TEM.

5.4.5.4 *Product families and “lead products”*

A very similar idea to read-up and read-down is that of product families where logical size relationships can be deduced simply from the relative particulate material synthesis parameters. In this case, a group of products may all be classified as “nanomaterial” or “non-nanomaterial” by careful analysis of a “lead product”. This idea is proposed in a joint study by the JRC and Eurocolour.¹¹³ The “lead product” would represent the material with either the highest or lowest median particle size, determined with an appropriate method such as TEM, taking into account the correct size parameters for assessment with respect to the EC definition. Products that logically have a higher or lower median size than the “lead product” – e.g. because of shorter/longer grinding times or shorter/longer particle growth times or lower/higher precursor concentrations at synthesis – would not necessarily have to be characterised using time-consuming and expensive TEM analysis.

5.4.5.5 *Conclusion*

The “read-across” concept as used in REACH is not likely to be generally applicable to the problem of classifying particulate materials with respect to the EC definition. However, in well-justified cases, read-down and product families may represent effective methods for avoiding time-consuming and expensive characterisation of large numbers of materials (see e.g. Section 5.4.1 on VSSA). However, given the known issues with most PSD measurement methods, only in very specific and well justified situations would read-up be acceptable for classification as non-nanomaterial. Also, it is likely that full TEM analysis would be a faster and more economic solution than an extensive read-up justification study, unless large numbers of materials would be covered by the study.

5.5 **The financial implications of the analytical challenges**

5.5.1 **Estimates from JRC report EUR 26567**

The conclusions of JRC report EUR 26567 (ref. 2) on the resources needed for the implementation of the EC definition pointed to the development of an effective and reproducible dispersion protocol as the main driver for very high cost (see Section 5 of ref. 2). Nevertheless, the estimate of the median time per measured sample is below what is needed for the development and execution of many chemical analyses. In terms of cost, and momentarily ignoring the wide range of reported measurement times, an average cost was estimated for the development of a dispersion protocol and instrument set-up (EUR 1.300) as well as for the marginal cost (EUR 350) per additional test sample. It has also to be considered that applying several different measurement methods will increase the incurred cost per material.

5.5.2 **Feedback on the estimates from JRC report EUR 26567**

Despite the caveats mentioned by JRC, several stakeholders have challenged the conclusions of the costs section of the JRC report. For example, there seems to be a discrepancy between the cost of developing a dispersion protocol and instrument set-up, and the marginal cost per additional test sample. The small difference between both estimates suggests that the number of measurements needed during the protocol development is unrealistically low.

More generally, industry claims that the costs associated with experimentally assessing whether a material is a nanomaterial or not, are much higher.¹³⁰ It was stated that the JRC report correctly identified the person-time required for sample preparation but underestimated the time for TEM

image evaluation given the diversity of particulate materials. For a particular large chemicals producer, considering the amount of materials to be assessed (~ 10000), estimating the time for TEM analysis at about 1.85 days per material, and counting 220 working days per year, the effort would correspond to more than 200 person-years of TEM. However, such high numbers are obtained by multiplication of the time needed for one material with a high number of materials – which, regardless of the ease or difficulty for each individual assessment will yield high numbers. It seems reasonable to assume that the companies that have so many different materials in their portfolio are also the very ones who are able to bear the burden of assessment.

In order to decrease the costs, (at least part of) the industrial stakeholders promote the use of VSSA as a broad market screening method, since it achieves for many materials the same classification (nanomaterial or not) as the more expensive TEM method, but at only 2 % of the TEM-analysis cost. However, preference of some for cheaper screening methods should not influence the definition: it is up to the user to decide whether the risk taken by using a screening method is acceptable or not.

5.5.3 Conclusion

It is clear that the exact financial implications are difficult to report as a single, generic number. The variety of materials categorised under the name 'nanomaterial' is one obstacle, but also the perceived and/or real ambiguities in the current EC definition (see Chapter 4) render a robust assessment problematic. With these caveats in mind, and without being quantitative, it is important to remember from this and previous sections that, in general:

- the cost of correctly measuring (within a given measurement uncertainty) the fraction of particles within the 1 nm to 100 nm range, increases when this fraction decreases;
- the cost of reliably categorising a material as being a nanomaterial, or not, increases as the actual fraction of nanoparticles in the material approaches the chosen threshold value because the measurement uncertainty required to make a reliable decision decreases (see also Section 5.2.6.1).

6 MATERIALS EXPLICITLY INCLUDED IN THE DEFINITION

In the 2011 European Commission definition of nanomaterial (2011/696/EU),¹ three materials were explicitly included, on top of the materials meeting the generic size-based criterion:

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

Fullerenes are closed-cage structures consisting of an even number of three-coordinate carbon atoms devoid of hydrogen atoms. ISO defines a fullerene as "molecule composed solely of an even number of carbon atoms, which form a closed cage-like fused-ring polycyclic system with 12 five-membered rings and the rest six-membered rings" (ISO/TS 80004-3:2010).¹³¹ The diameter of the fullerene C₆₀ is around 1 nm.¹³²

Single Walled Carbon Nanotubes (SWCNT) are an allotrope of carbon which take the form of cylindrical carbon molecules.¹³³ According to ISO/TS 80004-3:2010¹³¹ a SWCNT is a hollow nanofibre composed of carbon and consisting of a single cylindrical graphene layer. The diameter of a SWCNT can be around 1 nm and larger and their length can vary from few nms to more than 1 µm.¹³⁴

Graphene is defined by IUPAC (International Union of Pure and Applied Chemistry) as "*a single carbon layer of the graphite structure, describing its nature by analogy to a polycyclic aromatic hydrocarbon of quasi infinite size*".¹³⁵ ISO/TS 80004-3:2010¹³¹ defines graphene as single layer of carbon atoms with each atom bound to three neighbours in a honeycomb structure.

Fullerenes, single wall carbon nanotubes and graphene flakes are usually (e.g. in the scientific literature) regarded as nanomaterials. However, their smallest external dimension can be smaller than 1 nm meaning that they do not fall into the size range (1 nm – 100 nm) specified in the definition. Nevertheless they are considered as nanomaterials due to the derogation.

6.1 Methods to detect and identify fullerenes, single-wall carbon nanotubes and graphene

6.1.1 Fullerenes, graphene flakes and single wall carbon nanotubes in the EC definition

According to the EC definition, fullerenes, graphene flakes and SWCNTs are considered as nanomaterials, even if their minimum external dimension is below 1 nm. It seems to be reasonable to assume that this includes also functionalized SWCNTs and fullerenes. Hence, for these materials the definition replaces the size criterion by a criterion of chemical identity. However, as no material is 100 % pure, criteria need to be specified from when on a material is regarded as a "fullerene", CNT or graphene.

The straightforward approach is to use the same criterion as for other materials: the 50 % threshold value in the particle number based PSD. However, this approach is not clearly imposed in the current Recommendation. If applied, then similar issues would need to be considered, such as the measurement and counting of aggregated SWCNTs, fullerenes or graphene flakes.

An alternative approach is the one outlined in the ECHA document on the "Identification and Naming of Substances under REACH and CLP"¹³⁶, according to which materials consisting for > 80 % by mass of fullerenes, CNTs or graphene should be considered nanomaterials. (Note: this 80 % does not consider solvents or additives.) Substances of a purity of < 80 % are to be considered multi-component systems. Therefore it seems reasonable to use the 80 % as a purity cut-off below which materials are no longer considered for application of the derogation criterion. Since the 80 % criterion is used only as a rule of the thumb for the purposes of REACH, it might be helpful to clarify the applicability of it for the derogation of certain substances in the EC nanomaterial definition.

6.1.2 Standards for the identification of fullerenes, graphene flakes and single wall carbon nanotubes

Currently, there are no published standards that are explicitly designed for the *identification* of fullerenes, CNTs and graphene, neither from ISO, ASTM nor IEC. However, several documentary standards for the characterization of SWCNTs and measurements of their key properties have been published by ISO, ASTM and IEC (see e.g. section 6.5 in ref. 2). Most of these standards aim to characterize the purity of a CNT material, where the identity of the main component (i.e. whether it is a CNT or not) is already beyond doubt from the production process. Similarly, standards are in preparation for the characterization of graphene. Again, the main aim is the characterization of the graphene properties rather than identification of graphene.

For fullerenes, the absence of standard measurement methods to identify them is not a problem: unlike CNTs and graphene, fullerenes are clearly defined molecules that are not different to any other molecule. One might therefore argue that fullerenes are not particles at all, as they do not fulfill one of the key properties of particles, namely continuity in size (whereas e.g. silver nanoparticles can have any size from sub-nm Ag clusters to several hundred nanometres, a fullerene molecule can only have one size specific to this molecule). Therefore, the methods of classical analytical chemistry are sufficient to identify fullerenes. This has been proven by several research papers that quantify the concentrations of fullerenes in various matrices.^{137,138,139}

Compared to fullerenes, the situation is different for graphene, which can come in various sizes. ISO/TC 229 'Nanotechnologies' recently accepted a new work item proposal that should lead to the development of a Technical Report containing a matrix of relevant graphene properties and the methods with the potential to assess these properties. This matrix could serve as an initial guide for developing the necessary international standards in graphene characterization in future. On the other hand, there are currently no industrial applications of graphene, which largely eliminates the need for a standard for the detection of graphene. However, such a method might be needed when products containing graphene are brought to the market.

CNTs are currently the only group of the three explicitly mentioned additions in the definition that already has technical uses, which may make identification more urgent. However, the properties of CNTs differ so markedly from other forms of carbon, that they are clearly traded as such. There is therefore no uncertainty whether a container of raw material labeled as "CNTs" contains indeed CNTs. As the inclusion does not depend on any size, the labeling from the supplier provides sufficient evidence that one is dealing with a nanomaterial. Therefore, there is currently no need for development of standards for the *identification* of CNTs beyond the already existing standard *characterization* methods such as TEM¹⁴⁰ and SEM (in combination with EDX),¹⁴¹ near infrared photoluminescence spectroscopy,¹⁴² ultraviolet-visible-near infrared spectroscopy,¹⁴³ evolved gas chromatography-mass spectrometry,¹⁴⁴ and thermogravimetry.¹⁴⁵

6.1.3 Other measurement methods to identify fullerenes, CNTs and graphene

As fullerenes, CNTs and graphene are subject of intense research, a number of papers have been published that also include their identification. The situation is straightforward for fullerenes: as they are not particles in the common sense of the word but clearly defined molecules, they can be identified for example using their mass spectra. Identification of CNTs and graphene flakes is possible using e.g. microscopic techniques or probing their electrical properties. Raman spectroscopy has been proposed to be used for the identification of graphene flakes. Although a literature review on this subject is beyond the scope of this report, it is clear that methods exist that allow identification.

6.1.4 Conclusion

While there are currently no standardised methods available that are specifically developed for the identification of fullerenes, CNTs and graphene, a number of relevant methods that characterize CNTs and fullerenes are described in the scientific literature. There is no urgent need of

standardisation for identification of graphene, also because it might well be that such method would not yet meet the requirement of market relevance.

6.2 Expansion of the list of explicitly included materials

In this Section, we will discuss the possible extension of the current 'positive' list (of fullerenes, SWCNTs and graphene flakes) to other materials. This will be done mainly by an evaluation, whether other particles exist which have dimensions around the lower size limit of the definition, and are usually regarded as nanomaterials. Particles around/above the upper size limit will not be discussed here, as they are covered under nanostructured materials (Chapter 7). The following materials will be considered in this chapter: different shapes of graphene, nanotubes with composition other than carbon atoms (e.g. titania, silicon and peptides), quantum dots and nanoclay. Thereafter, some general considerations will follow.

6.2.1 Graphene

Besides nanoflakes, graphene can occur in other shapes such as nanoribbons,¹⁴⁶ nanocones or nanodots,¹⁴⁷ and can be found in different chemical forms such as graphene oxide.¹⁴⁸ The most common graphene types are the nanoribbons and the graphene oxides. Nanoribbons are thin strips of graphene and are morphologically different from other forms of graphene. Graphene oxide is a graphene layer which was oxidised. Figure 4 shows three basic types of graphene.

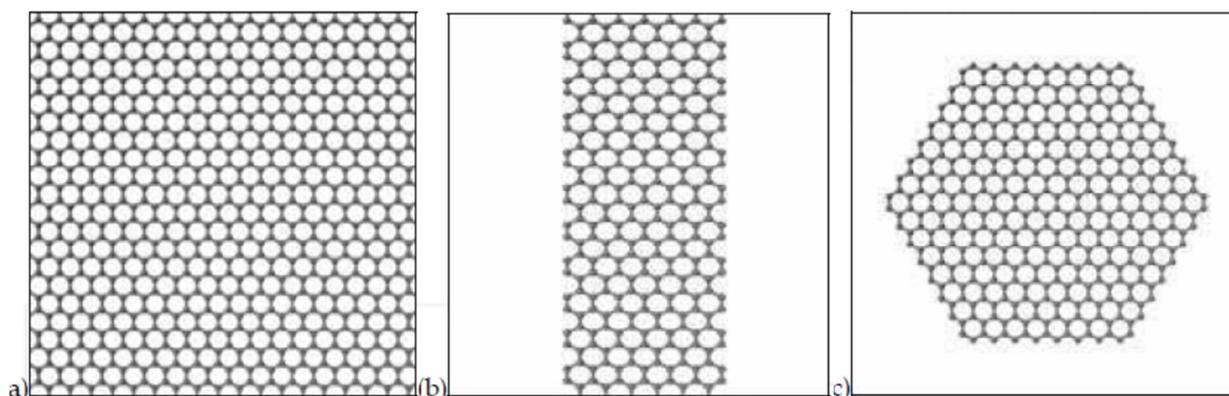


Figure 4: The three basic type of graphene, (a) an infinite graphene sheet or membrane, 2D graphene (b) a graphene nanoribbon, 1D graphene, (c) a graphene nanoflake or graphene nanodot, 0D graphene (© 2011 Ian Snook and Amanda Barnard. Originally published in I. Snook, A. Barnard, in *Physics and Applications of Graphene - Theory*, Dr. Sergey Mikhailov (Ed.), ISBN: 978-953-307-152-7, InTech, DOI: 10.5772/15541, 2011 under CC BY-NC-SA 3.0 license. Available from: <http://dx.doi.org/10.5772/15541>)¹⁴⁷

The question arises, whether it was the intention to include all different graphene shapes by the wording "graphene flakes", and whether the derogation should be modified to provide clarity concerning different graphene shapes. An explicit extension to all particulate graphene forms, including possibly also multi-layer graphene, could simplify the characterisation, as it would not be necessary to determine the shape of the graphene particles, or even to know if it is oxidised or not in order to decide whether it is covered by the EC definition. Non-free-standing graphene could similarly be included, or explicitly excluded.

6.2.2 Nanotubes

The occurrence of carbon in the form of nanotubes is well known, but nanotubes of other chemical elements are also synthesized and manufactured, e.g. titanium dioxide nanotubes, silicon nanotubes or organic nanotubes. The diameters of these different nanotubes are not as small as those of

SWCNTs. Nevertheless, it could be considered to extend the definition to all sorts of nanotubes. This would provide clarity in case the diameter (the "outer" one since only the smallest external dimension is important for the EC Recommendation) is smaller than 1 nm.

There are different definitions of the term "nanotubes". The one given by ISO is a 'hollow nanofibre'.⁵⁷ But this can be ambiguous since nanotubes can also be filled. For example, some nanoparticles which were used during synthesis are present in carbon nanotubes.^{149, 150} Furthermore, there is also some research concerning nanotubes to be filled intentionally with molecules,¹⁵¹ or nanoparticles.^{152, 153} It is not clear from which level of filling nanotubes are still considered as hollow. Therefore, nanotubes are also defined as a cylindrical nanostructured material.¹⁵⁴

ISO also defines "nanorod" as a 'solid nanofiber'.⁵⁷ However, various companies and also academic researchers do often not refer to this definition.^{155,156,157, 158} The definition given by the company READE,¹⁵⁸ for example, is also used: 'nanostructures shaped like long sticks or dowels with a diameter in the nanoscale but having a length that is very much longer; each of their dimensions ranges from 1 nm to 100 nm; standard aspect ratios (length divided by width) are 3-5.'

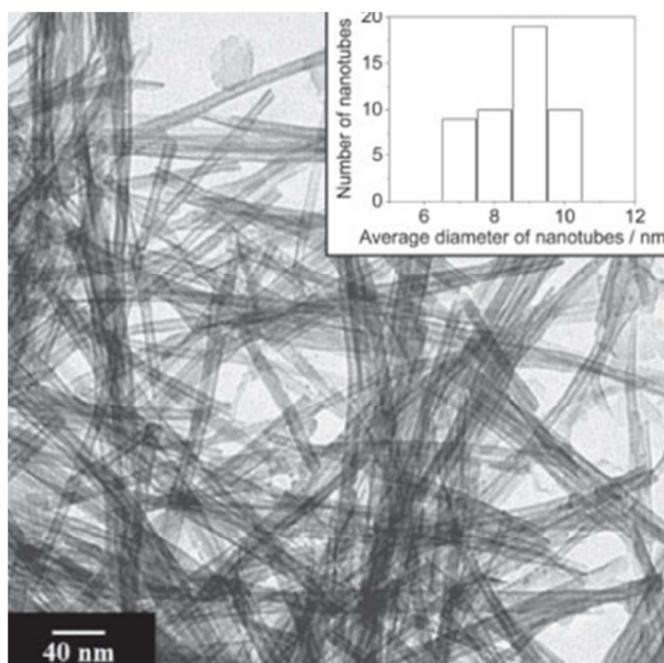


Figure 5: TEM image of the as-prepared titanium oxide nanotubes produced by alkali hydrothermal treatment of anatase TiO₂ powder. Reprinted with permission from O. P. Ferreira, et al., J. Braz. Chem. Soc., Vol. 17, p. 393, 2006.¹⁵⁹

Titanium dioxide nanotubes (TNTs) with diameters ranging between 6 nm and 100 nm are studied because of their photocatalytic properties.¹⁶⁰ TNTs can also be used in dye-sensitized solar cells, electrochromic devices,¹⁶¹ or in biomedical applications. The 3D structure is also optimal for embedding precursors to hydroxyapatite formation.¹⁶² Figure 5 shows a picture of TNTs.¹⁵⁹

Scientists and engineers have already begun to consider the possible uses of silica and silicon nanotubes.^{163, 164, 165} Some researchers are studying their use for a new lithium-ion battery electrode^{166, 167} or explosives sensors due to their porous surface.¹⁶⁸ Their diameters range between 2 nm and 100 nm.^{153,165, 169} Figure 6 shows a picture of silicon nanotube.¹⁷⁰

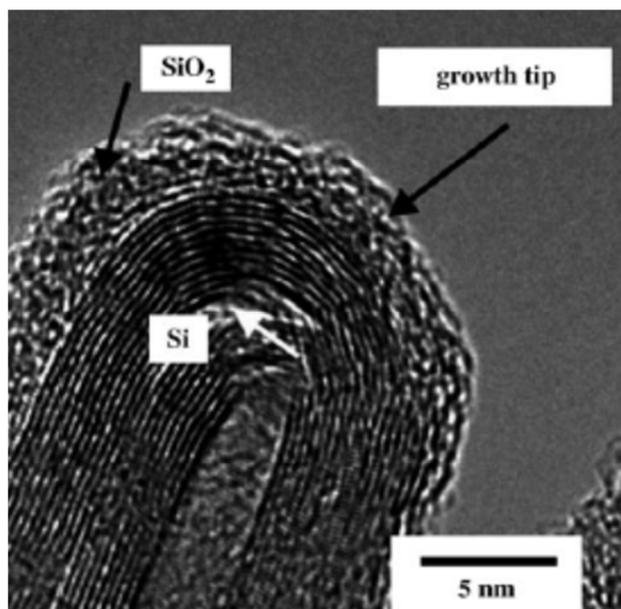


Figure 6: TEM image of a silicon nanotube grown from silicon monoxide under supercritically hydrothermal conditions (Reprinted figure with permission from Y. H. Tang et al., Phys. Rev. Lett. 95, 116102, 2005, Copyright (2005) by the American Physical Society, <http://journals.aps.org/prl/abstract/10.1103/PhysRevLett.95.116102>.¹⁷⁰

Not only inorganic material could be manufactured in tube form. Self-assemblies of peptide nanotubes (PNTs) have appeared as another interesting nanostructure to be explored in the field of nanotechnology.^{171, 172} Patents have already been registered.^{173, 174, 175} The applications of PNTs are various such as tissue engineering, drug release, novel antibacterial agents, biosensors or nanoelectronics.^{161, 176} Figure 7 displays a typical chemical structure for a cyclic peptide and illustrates schematically the self-assembly of such peptides into nanotubes and nanotube arrays.¹⁷⁷

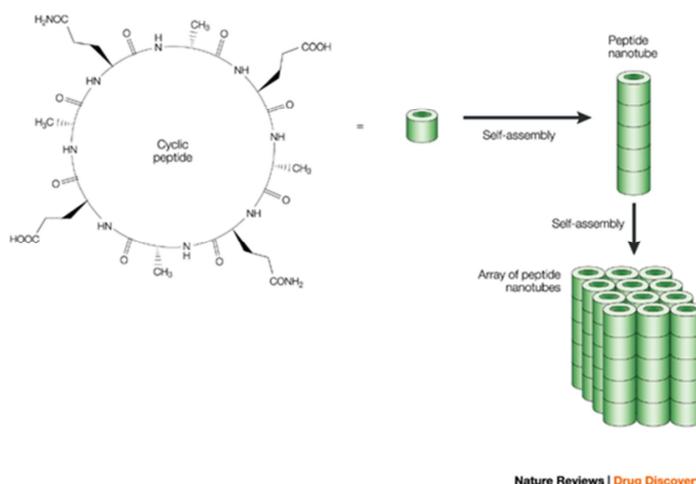


Figure 7: Structure of a cyclic peptide and their schematic self-assembly of peptides into nanotubes (Reprinted by permission from Macmillan Publishers Ltd: C. R. Martin, P. Kohli, Nat. Rev. Drug. Discov., vol. 2, p. 29, 2003.¹⁷⁷

6.2.3 Quantum Dots

Quantum dots are nanocrystals, typically with a size in the range of 1 nm to 10 nm and made out of a hundred to a thousand atoms. These semiconductor materials can be composed of one kind of element, such as silicon or germanium, or a compound, such as CdS or CdSe. According to CEN ISO/TS 27687,⁵⁷ a quantum dot is defined as a 'crystalline nanoparticle that exhibits size dependent properties due to quantum confinement effects on the electronic states'. Due to their optical and semiconductor properties, quantum dots can be used in many sectors: solar cells, bio imaging, LEDs, painting, medical devices.^{178,179,180,181,182,183,184}

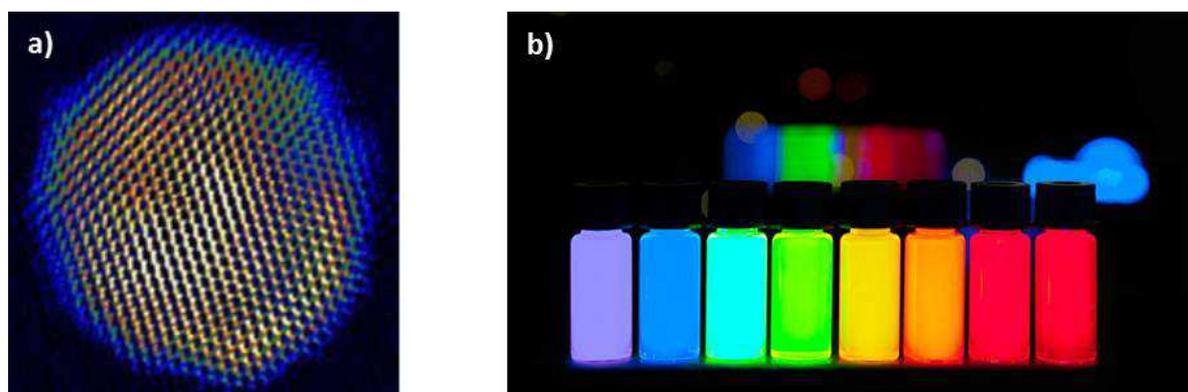


Figure 8: (a) Quantum dots; Image courtesy of Jian-Min Zuo and University of Illinois at Urbana-Champaign, (b) Vials of quantum dots which emit light with a wavelength depending on their size (from ref. 185, used under GNU Free Documentation License, Courtesy Dr. Antipoff)

The synthesis of these nanocrystals is easily controllable but, due to their tiny size, it could be difficult to determine their size without using electronic microscopy.¹⁶⁷ Their external size, though usually in the range between 1 nm and 10 nm, can be close to the lower size limit of the definition. Therefore it might be considered whether they should be explicitly mentioned in the definition along with fullerenes, SWCNT and graphene.

6.2.4 Nanoclay

Nanoclay is defined as clay from the smectite family having a unique layered morphology with layer spacing in the nanometre range.⁵⁵ The most common nanoclay material is montmorillonite, which consists of ~ 1 nm thick aluminosilicate layers as a principal component substituted with a variety of other metal cations like magnesium, calcium or potassium and a varying amount of water molecules.¹⁸⁶

Nanoclay figures on the list of the OECD sponsorship programme for the testing of manufactured nanomaterials,¹⁸⁷ as it is often considered as nanomaterial. However, the relevant external dimension can be close to the lower size limit of the definition. Therefore, it could be considered to mention nanoclays explicitly in the definition.

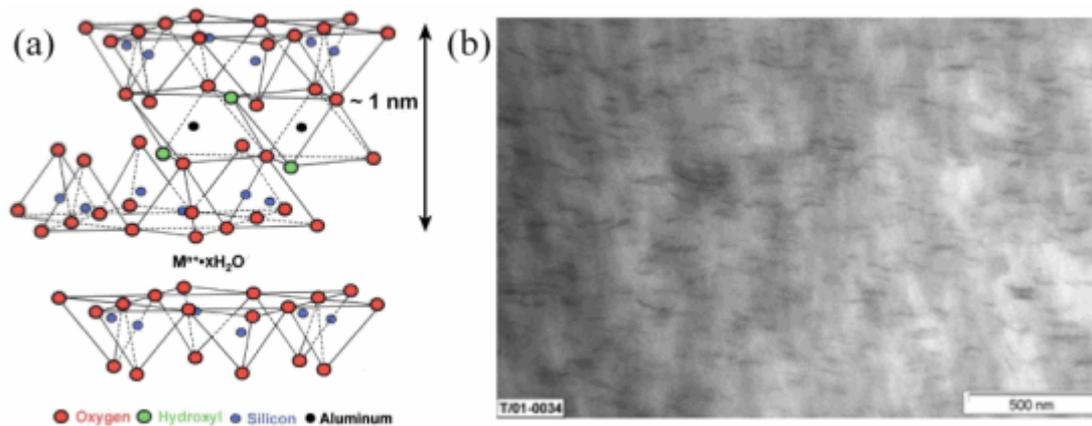


Figure 9: (a) Scheme of nm-thick montmorillonite clay with aluminosilicate layers. (b) TEM micrograph of 2% nanoclay, Nanomer® I.34TCN — Nylon 6 nanocomposite showing complete dispersion of clay layers into distinct plate-like nanoparticles, appearing as dark stripes on the brighter matrix background (from ref. 188, reprinted with permission, image courtesy Nanocor).

6.2.5 General considerations

Ideally, the definition would cover all materials which should be regarded as nanomaterials without the need of any derogation. Any list with explicit inclusions or exclusions questions the rationale of the definition by including materials which do not fulfil the definition. Furthermore, a regular review of such lists might be needed in light of technological developments. However, inclusion (or exclusion) lists can be a pragmatic solution to a policy problem.

As previously said, the smallest external dimension of graphene ribbons and graphene dots, or nanoclay can be smaller than 1 nanometer. To cover these materials, which are widely considered as nanomaterials, univocally in the definition, one has two options: either one broadens the scope of the definition by changing the lower limit of the considered size range (now 1 nm), or one extends the already created list of materials explicitly included in the definition (now consisting of fullerenes, graphene flakes and SWCNTs). Both options have advantages and disadvantages.

It is difficult to choose appropriate quantitative criteria, i.e. size limits, to set the scope of the nanomaterial definition, as there is no unambiguous natural borderline between nanomaterials and non-nanomaterials. Every chosen set of criteria therefore will be chosen by compromising between materials that should have been covered by the general definition, but are not, and materials that are covered by the general definition, but should not. An extension of the scope of the basic definition by changing the lower size limit might result in a considerable increase in the number of unintentionally included materials that fit the definition.

The other option should therefore be considered, which is to extend the inclusion list, e.g. for graphene ribbons and graphene dots, and nanoclay which can be smaller than 1 nm. Currently quantum dots and titania, silica or silicon or peptide nanotubes are usually above 1 nm. However, in light of the quick development in this area one could also consider to include these materials in the list. An extension of the inclusion list could have several advantages. Classifying borderline substances straight away as nanomaterials would save time and costs for the analysis for industry, would make the decision, whether or not a material is a nanomaterial according to the definition, simpler, and would provide legal clarity for industry as well as for the legislator.

7 NANOSTRUCTURED MATERIALS

7.1 Regulatory relevance of nanostructured materials

7.1.1 Introduction

The term "nanostructured material" is frequently used in the scientific literature and in many other publications in the field of nanotechnology. Currently there is no specific definition recommended by the EC for this term. For the purposes of this report the definition by ISO^L is used, i.e., a 'material having internal or surface structure in the nanoscale'. ISO in addition proposes a scheme for a non-exhaustive further classification of nanostructured materials,³ which is also referred to in Report 1:²

- nanostructured powder
- nanocomposite
- solid nanofoam
- nanoporous material
- fluid nanodispersion

In addition to these classes, nanostructured materials that do not fall under that sub-classification scheme such as objects with surface nanostructures and materials with layered nanostructures (layer thickness in the nanoscale) will also be considered here.

The EC definition explicitly covers only particulate materials, i.e., materials consisting of unbound particles and their agglomerates and aggregates (Recitals 4 and 7 and Definition sentence (2)).¹ This is in line with a previous JRC Reference Report⁵³ suggesting that a definition of nanomaterial *for regulatory purposes* should address particulate materials. The EC definition uses the number based PSD of the constituent particles to assess whether a material is a nanomaterial.¹ Aggregates and agglomerates of constituent particles that fall under the EC definition are nanostructured materials according to ISO terminology. Therefore the EC definition already covers certain types of nanostructured materials, including several types of nanostructured powders which consist of nanostructured agglomerates and nanostructured aggregates in the ISO sense.

Consequently, if a legal provision addresses particulate materials and also refers to the EC definition, then particulate nanostructured materials are covered by that particular legal provision. In this context it also remains to be clarified whether the ISO definition of "particle" and the European Commission's position on the interpretation of this term are the same or whether there are certain differences. A discussion on that subject can be found in section 4.3.1 of this report.

7.1.2 Regulatory relevance of nanostructured materials

Nanostructured materials are relevant from a regulatory point of view if they are covered or addressed by regulatory provisions. This relevance can be independent from the fact that the material is nanostructured, for example if a specific regulatory provision covers certain materials, including, but not limited to, nanomaterials.

7.1.2.1 Nanostructured powder

According to ISO/TS 80004-4¹⁸⁹ a nanostructured powder is a powder comprising nanostructured agglomerates, or nanostructured aggregates, or other particles of nanostructured material.

^L ISO terms reproduced from the ISO Online Browsing Platform (<https://www.iso.org/obp/ui/>). Copyright remains with ISO.

Nanostructured powders are relevant from a regulatory perspective in a variety of sectors, e.g., chemicals, cosmetic products, biocides, food additives and food contact materials, but this relevance does not depend on whether they are classified as nanomaterials or not. In the definition by ISO, the nanostructured aggregates and agglomerates are collections of individual nano-objects, and therefore they match the EC definition and are covered by it. Consequently, regulatory provisions which address nanomaterials and use the EC definition also cover nanostructured powders, unless not explicitly excluded. If a nanostructured powder consists of particles which have internal structures at the nanoscale but external dimensions larger than 100 nm it is not covered by the EC definition, but it may fall into one of the subclasses of nanostructured material discussed below.

7.1.2.2 *Materials with surface structures at the nanoscale*

Almost all solid materials have surface structures with nanoscale dimensions. Consequently, extending the definition to materials with surface structures at the nanoscale would make practically all solid materials and objects nanomaterials. Avoiding this and limiting the discussion to certain materials of potential regulatory relevance (e.g., materials with specific surface reactivities) would require defining additional qualifiers for such materials. Such qualifiers could address the purpose, the function or the composition of the surface structure. However, doing this would imply that the term nanomaterial is no longer based only on the size of a material.

In line with these arguments, ISO TS 80004-4 specifies that *"almost all materials always have surfaces with morphological and chemical heterogeneities in the nanoscale. Only surfaces that have been intentionally modified or textured to have morphological or chemical heterogeneities in the nanoscale identify materials as "nanostructured"*¹⁸⁹.

Materials with surface structures at the nanoscale with potential regulatory relevance are for example larger (non-nano-)particles with specific nanoscale features at the surface, such as supported catalysts (e.g. nanoscale metal particles attached to micrometre-sized oxide particles), or novel biocides, where also metal nanoscale particles are attached to larger oxide particles. Since such materials often are the result of particular synthesis routes they may already be covered by the EC definition, if the metal and oxide particles are considered as constituent particles of the material. One could indeed argue that if the nanostructured surface features are made of different materials than the supporting material, then those nanoscale features might be considered as constituent particles. Such a position would avoid the need to include these materials specifically in an extended definition. However, clarification might be needed, e.g. in a recital or a guidance document.

Specific consideration in the context of the EC definition is required for materials consisting of particles which have deliberately engineered nanostructures at their surface. If one or more external dimensions of these particles are within the nanoscale, they are covered by the EC definition. However, if the external dimensions are above the nanoscale they are not covered. There is a discussion among regulators and scientists whether such materials, i.e., particles with external dimensions larger than the nanoscale but with deliberately engineered surface structures in the nanoscale, should be considered as nanomaterials. On the one hand, this would include a group of materials considered as true products of nanotechnology, but on the other hand it would widen the scope of the definition. If such materials were to be included in the definition it would also be necessary to define a specific limit (larger than 100 nm) for their external dimensions, up to which they would be covered by the definition of nanomaterial. Such materials could for example be called "nanostructured particulate materials".

7.1.2.3 *Nanocomposite*

A nanocomposite is a 'solid comprising a mixture of two or more phase-separated materials, one or more being a nanophase'. The term "nanocomposite" refers to an internal structure, and hence nanocomposites are not covered by the EC definition unless they consist of particles with external dimensions in the nanoscale. Examples of nanocomposites are matrix materials reinforced with carbon nanotubes, e.g., polymer matrix composites with finely dispersed nanotubes for improved electrical conductivity, or lightweight, high performance materials for improved strength. An

example for the latter is ultrahigh performance concrete, where silica nanoparticles are embedded in a concrete matrix for reinforcement. Other high performance nanocomposite materials are used for high performance machines, such as turbofans or modern jets. Those nanocomposite materials are usually regulated according to the purposes they are used for (construction materials, aviation) and under the General Product Safety Directive in case of consumer products.¹⁹⁰ Usually the release of particulate nanomaterials from such nanocomposites is not part of their design but may occur during normal wear. The released material most likely consists of particles, and, if their size is in the nanorange, those would be covered by the EC definition, as the definition also covers incidental materials.

A specific form of nanocomposites is layered materials with layer thicknesses in the nanoscale. This class also includes core-shell particles which may have external dimensions above the nanoscale but a core or a shell with a diameter or thickness, respectively, at the nanoscale. Some of these nanocomposites have already been used for decades, for example in microelectronics or in packaging. Epitaxial layers used in micro- and nanoelectronics are designed for specific electronic properties and can have thicknesses down to a few atomic layers. In packaging technology the layers are designed for special barrier properties. Layered materials for electronics – as any other electronic equipment - are relevant from a regulatory perspective regarding the end of their life cycle.¹⁹¹ Layered materials used for food packaging are also relevant from a regulatory perspective.¹⁵ The regulatory relevance of these nanostructured materials is however not dependent on their inclusion in the EC definition.

7.1.2.4 Nanoporous material

Nanoporous materials are solid materials containing a fraction of nanoscale pores. The definitions of solid nanofoam (where most of the volume is occupied by pores) and nanoporous material (also materials with a small fraction of pores) are overlapping. As such, nanoporous materials are not covered by the EC definition (e.g. zeolites), but if the nanoporous materials consist of particles or aggregates/agglomerates of particles they are covered by the EC definition (e.g. silica gels). Nanoporous materials can be irritant and may release nanoparticulate materials when mechanically agitated, but this is true for any other powder as well.

7.1.2.5 Solid nanofoam

Solid nanofoam is a non-particulate material and consists of nanoscale gas bubbles surrounded by solid struts. The defining property can be either the size of the nanopores or the scale of the strut material. Also, the material can contain two continuous phases, if the pore volumes are interconnected, in which case it is the cross-section or thickness of the solid struts that has to be in the nanoscale. Solid nanofoams are not covered by the EC definition unless the material consists of nanofoam particles with external dimensions at the nanoscale. In that case it would be a specific type of nanostructured powder.

Aerogels (a gel in which the liquid component has been replaced with a gas) are a form of a solid nanofoam. Such a material can have extremely low densities and a very high surface area. Solid nanofoams can be very good thermal insulators. Carbon nanofoams are used for example in electronics to make very high capacity elements due to their extremely large surface area. Nanofoams can also be used as chemical absorbers and are being explored for use as drug delivery systems. Metal nanofoams have also been used as very efficient catalysts.

Solid nanofoams are relevant from a regulatory perspective specifically for professional use and regarding the protection of workers handling these materials. The regulatory relevance of solid nanofoams does not depend on whether they are included in the definition of nanomaterial.

7.1.2.6 Fluid nanodispersion

A subset of fluid nanodispersions is the nanosuspensions. There the dispersed phase is a solid. Nanosuspensions are covered by the EC definition in the sense that the dispersed phase is a nanomaterial according to the EC definition. Depending on the language version of the current EC

definition, nanosuspensions are also covered as a whole because they are materials *containing* nanoparticles, or they are not because they do not only *consist* of particles. But in any case, a suspension of nanoparticles could be considered as nanomaterial as the liquid in which the particles are suspended provides only a stable storage or transport or processing means for the particles, similar to the air between the particles of a powder. If the liquid 'matrix' itself has a function, the combination of particles and matrix is a material in itself, and not just the suspension of a particulate nanomaterial. Whether such a material would be considered as nanomaterial, requires clarification, taking also into account the Commissions Q&A guidance documentation on the scope of the definition (Q&A No 13).⁵⁶

Nano-aerosols are materials which consist of a gaseous phase containing freely moving nano-objects. If those nano-objects have external dimensions at the nanoscale, the nano-aerosol is covered by the EC definition. If the nano-objects in a nano-aerosol are non-solid, then, according to the EC position published in the SWD,⁵⁴ it is not covered by the EC definition. Both nanosuspensions and nanoaerosols are relevant from a regulatory perspective, but not necessarily within the scope of the definition of nanomaterial.

7.1.2.7 Nanoemulsions – droplets, micelles, liposomes

A nanoemulsion contains at least one liquid nanophase. The nanophase might consist of droplets, micelles, liposomes or natural vesicles. Micelles and liposomes themselves are nano-objects if they have external dimensions at the nanoscale. At the same time they are nanostructured materials, or more specifically nanoscale capsules, because their shells have a thickness at the nanoscale and they can enclose, fix, transport or release substances. Being nanostructured materials, they can have external dimensions well above the nanoscale. The current position of the EC, as expressed in the SWD,⁵⁴ is that such objects are not covered by the EC definition. However, these materials are relevant from a regulatory perspective, because they are used, or their use is being envisaged in applications for cosmetics, food (e.g., carriers, supplements), or for drug delivery.

Micelles used as carriers for drug delivery or for food supplements are products of nanotechnology and considered as innovative nanotechnology applications. As such, their use in food and other consumer products is widely discussed. Furthermore, they are relevant from a regulatory perspective. In view of the discussion on the nature of particles in section 4.3 and their regulatory relevance it is worthwhile to consider them for inclusion in the definition, if their external dimensions are within the nanoscale, even if they are "soft" materials. If deemed necessary, exclusion of such materials would be possible by sector specific provisions in relevant regulations.

7.2 Methods to characterise nanostructured materials

A discussion on the inclusion of nanostructured materials in the scope of the nanomaterial definition must also include the possible consequences on its practical implementation. This section relates the defining features of nanostructured materials with possible ways to enforce a nanomaterial definition which includes nanostructured materials, through measurements.

7.2.1 Extension of the particle size distribution-based nanomaterial definition

Nanostructured materials are not necessarily particulate materials. They do not distinguish themselves from non-nanomaterials by their external dimensions, but by internal or surface features with dimensions in the nanorange. If one would want to consider including nanostructured materials in the EC definition, then this would necessitate complementing the PSD threshold fraction of 50 %, currently used in the EC definition, with a criterion suitable for materials not consisting of particles.

For example, one could resort to mass-based concentrations, the parameter which is most often used in regulatory texts. However, since the density of nanostructured materials is often not well known, and since it is often impossible to separate the nanoscale features from the rest of the nanomaterial, it is difficult to use a mass-based concentration as defining parameter.

Instead, and as shown in section 15.3 of JRC Report EUR 26567,² nanostructures are usually identified and characterised by imaging methods. From images one can, at least in theory, judge at the same time the size of the nanoscale features, and their volume fraction. One could therefore imagine defining a nanostructured material as a material for which X % of the volume is taken up by (microstructural) phases with a nanoscale dimensional feature (thickness, diameter, ...). For each of these phases one would have to estimate both the minimal dimension (to check whether they are in the nanorange or not) and their volume. A practical example would be a nanocomposite consisting of a continuous matrix (not 'nano') reinforced by X vol-% of small nanoplates (particles with a thickness smaller than 100 nm). These measurements would typically be taken from 2D-images, implying that there are a number of assumptions to be made about the size of the phases in the 3rd dimension. This problem is also mentioned in the discussions on the measurement of particle size from 2D images. Similarly, one could define a certain value for the fraction of the materials surface that has to be covered with nanofilm or nano-topography, for it to be a nanomaterial.

7.2.2 Implementation of an extended nanomaterial definition

Section 15.3 of JRC Report EUR 26567² provides an overview of existing methods for the characterisation of nanostructured materials. It distinguishes decomposable and non-decomposable nanostructured materials.

For decomposable materials, the nanoscale phase(s) can be separated from the non-nanoscale matrix or continuous phase. In this case, the characterization of the nanoscale fraction of the material can be done with the same particle characterization methods as the ones described in section 4 of ref. 2.

For the non-decomposable materials, a number of imaging methods and surface characterisation techniques were identified in ref. 2. Some of these methods are not so new, yet very performant, for example in terms of spatial resolution, but usually they are far from being routinely applied (e.g 3D atom probe tomography¹⁹²). And while the number of relevant techniques, and the number of specific methods to improve the usefulness of existing techniques, increases with every new issue of the relevant journals,^{193,194,195,196,197} the main issues identified,² remain to be solved: preparation of representative and undistorted samples and calibration of the size scales. In terms of the associated costs, the preparation of samples for an investigation of their internal structure is more expensive than an assessment of the external dimensions of well-dispersed particles.

7.3 Conclusions

As a consequence of their regulatory relevance, the following principal types of nanostructured materials can be considered when deciding on an expansion of the scope of the definition:

- Nanoporous materials/solid nanofoams, if they can easily release nanoparticles or disintegrate into nanoparticles;
- "soft" materials, such as micelles, with external dimensions within the nanoscale, if they are products of nanotechnology;
- deliberately engineered *nanostructured particulate* materials; these would include particles with surface nanostructures and soft nanostructured materials (if the term "particle" is used according to the definition by ISO).

If other, non-particulate materials also need to be considered for inclusion in the EC definition, then the PSD-based threshold currently used in the EC definition, will have to be complemented with a separate criterion suitable for materials not consisting of particles.

REFERENCES

- 1 European Commission, *Commission Recommendation of 18 October 2011 on the definition of nanomaterial*, Official Journal of the European Union. 2011/696/EU: 38-40, 2011.
- 2 H. Rauscher, G. Roebben, V. Amenta, A. Boix Sanfeliu, L. Calzolari, H. Emons, C. Gaillard, N. Gibson, T. Linsinger, A. Mech, L. Quiros Pesudo, K. Rasmussen, J. Riego Sintes, B. Sokull-Klüttgen, H. Stamm, *Towards a review of the EC Recommendation for a definition of the term "nanomaterial" Part 1: Compilation of information concerning the experience with the definition*, JRC Scientific and Policy Report, EUR 26567 EN, Eds H. Rauscher, G. Roebben, 2014.
- 3 CEN ISO/TS 80004-1, *Nanotechnologies - Vocabulary - Part 1: Core terms*, ISO, Geneva, 2010.
- 4 <http://nanotechnology.americanchemistry.com/Nanotechnology/Panel-Activities/Nanotechnology-Definitions/Nanotechnology-Panel-Presents-at-Society-of-Toxicology.pdf> Accessed 24/06/2014.
- 5 ICCA, *Core Elements of a Regulatory Definition of Manufactured Nanomaterials*, October 2010.
- 6 German Chemical Industry Association, *VCI position on the definition of the term nanomaterial for use in regulations laying down provisions on substances*, February 2010.
- 7 Scientific Committee on Emerging and Newly Identified Health Risks, *Scientific Basis for the Definition of the Term "nanomaterial"*, http://ec.europa.eu/health/scientific_committees/emerging/docs/scenih_r_o_032.pdf, 2010; Accessed 24/06/2014.
- 8 Report of the ICCR Joint Ad Hoc Working Group on Nanotechnology in Cosmetic Products: *Criteria and Methods of Detection*, Toronto, Canada, July 14, 2010.
- 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*, Official Journal of the European Union L342: 59-209, 2009.
- 10 European Parliament and Council, *Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004*, Official Journal of the European Union. L 304: 18-63, 2011.
- 11 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*, Official Journal of the European Union L 167/1(1): 1-123, 2012.
- 12 European Parliament and Council, *Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use*, 2001.
- 13 European Parliament and Council, *Proposal for a Regulation of the European Parliament and of the Council on medical devices, and amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009*, COM(2012) 542 final, 2012.
- 14 Regulation EU 1183/2012 - *Plastic materials and articles intended for contact with food amending Regulation (EU) No 10/2011*
- 15 Regulation EU 202/2014 - *Plastic materials and articles intended for contact with food amending Regulation (EU) No 10/2011*
- 16 Decree no. 2012-232 of 17 February 2012 on the *Annual declaration on substances at nanoscale in application of article R. 523-4 of the Environment code*.
- 17 Draft Royal Decree on the *market placement of manufactured nanoparticle substances*, Kingdom of Belgium, Federal Public Service for Health, Food Chain Safety and Environment, 2014.
- 18 Danish Environmental Protection Agency, *Information requirements for nanomaterials*, IRNANO, Miljøprojekt nr. 1469, 2013.
- 19 <http://www.nanotechia.org/news/news-articles/danish-epa-starts-public-consultation-national-nanomaterial-product-register>; Accessed 24/06/2014.
- 20 Safety data sheets (SDS): *Guidelines for synthetic nanomaterials*, SECO, 2012.
- 21 FDA Draft Guidance for Industry: *Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology*, June 2011.
- 22 http://www.egg2012.de/tl_files/pdf/Paper/EGG2012_C6_1_Park_presentation_slides.pdf; Accessed 24/06/2014.
- 23 GB/T 19619-2004: *Terminology for nanomaterials*, China, 2004.

24 <http://www.nicnas.gov.au/regulation-and-compliance/nicnas-handbook/handbook-appendixes/guidance-and-requirements-for-notification-of-new-chemicals-that-are-industrial-nanomaterials/nicnas-working-definition-of-industrial-nanomaterial>; Accessed 24/06/2014.

25 <http://www.hc-sc.gc.ca/sr-sr/pubs/nano/pol-eng.php>; Accessed 24/06/2014.

26 ETUC, *ETUC concept of a regulatory definition of a substance in the nanoform*, http://www.etuc.org/sites/www.etuc.org/files/REACH_nanosubstance_definition_ETUC_concept_1.pdf; Accessed 24/06/2014.

27 H. Krop, Technical Workshop on 'Aspects to consider in the technical and scientific review of the European Commission Recommendation of 18 October 2011 on the Definition of Nanomaterial', Brussels, 19th March 2014.

28 *Assessing nanoparticles risks to human health*; Elsevier, 2011.

29 Ramachandran, G.; Paulsen, D.; Watts, W.; Kittelson, D., *Mass, surface area and number metrics in diesel occupational exposure assessment*, J Environ Monit, Vol. 7, p. 728-35, 2005.

30 Pauluhn, J., *Poorly soluble particulates: Searching for a unifying denominator of nanoparticles and fine particles for DNEL estimation*, Toxicology, Vol. 279, p. 176-188, 2011.

31 Pauluhn, J., *Subchronic 13-Week Inhalation Exposure of Rats to Multiwalled Carbon Nanotubes: Toxic Effects Are Determined by Density of Agglomerate Structures, Not Fibrillar Structures*, Toxicological Sciences, Vol. 113, p. 226-242, 2010.

32 Gallagher, J.; Sams, R., 2nd; Inmon, J.; Gelein, R.; Elder, A.; Oberdorster, G.; Prahalad, A. K., *Formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine in rat lung DNA following subchronic inhalation of carbon black*, Toxicol Appl Pharmacol, Vol. 190, p. 224-31, 2003.

33 Tran, C. L.; Buchanan, D.; Cullen, R. T.; Searl, A.; Jones, A. D.; Donaldson, K., *Inhalation of poorly soluble particles. II. Influence Of particle surface area on inflammation and clearance*, Inhalation Toxicology, Vol. 12, p. 1113-26, 2000.

34 Oberdörster, G., *Safety assessment for nanotechnology and nanomedicine: concepts of nanotoxicology*, Journal of Internal Medicine, Vol., 267, 89-105, 2010.

35 Oberdörster, G.; Oberdörster, E.; Oberdörster, J., *Nanotoxicology: An Emerging Discipline Evolving from Studies of Ultrafine Particles*. Environ Health Perspect., Vol.113, p. 823-839, 2005.

36 Teeguarden, J. G.; Hinderliter, P. M.; Orr, G.; Thrall, B. D.; Pounds, J. G., *Particokinetics in vitro: dosimetry considerations for in vitro nanoparticle toxicity assessments*, Toxicological sciences : an official journal of the Society of Toxicology, Vol. 95, p. 300-12, 2007.

37 OECD, *Important issues on risk assessment of manufactured nanomaterials*, 2012.

38 Handy, R. D.; Owen, R.; Valsami-Jones, E., *The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs*, Ecotoxicology, Vol. 17, p. 315-25, 2008.

39 ECHA, *Guidance on information requirements and chemical safety assessment. Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7a – Endpoint specific guidance*, ECHA-12-G-03-EN, April 2012.

40 SCENIHR, *Risk Assessment of Products of Nanotechnologies*, Scientific Committee on Emerging and Newly Identified Health Risks, 2009.

41 OECD, *Guidance on sample preparation and dosimetry for the safety testing of manufactured nanomaterials*, 2012.

42 SCCS, *Guidance on the safety assessment of nanomaterials in cosmetics*, Scientific Committee on Consumer Safety, 2012.

43 EFSA Scientific Committee, *Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain*, EFSA Journal, Vol. 9, p. 1-36, 2011.

44 Bleeker, E. A. J.; Cassee, F. R.; Geertsma, R. E.; De Jong, W. H.; Heugens, E. H. W.; Koers-Jacquemijns, M.; van De Meent, D.; Oomen, A. G.; Popma, J.; Rietveld, A. G.; Wijnhoven, S. W. P., *Interpretation and implications of the European Commission Recommendation on the definition of nanomaterial*, National Institute for Public Health and the Environment - Dutch Ministry of Health, Welfare and Sport, 2012.

45 German Federal Authorities, *Nanomaterials and REACH – Background Paper on the German Federal Authorities' Position*, p. 13, 2012.

46 Heitbrink, W. A.; Evans, D. E.; Ku, B. K.; Maynard, A. D.; Slavin, T. J.; Peters, T. M., *Relationships among particle number, surface area, and respirable mass concentrations in automotive engine manufacturing*. Journal of Occupational and Environmental Hygiene, Vol. 6, 19-31, 2009.

47 Wittmaack, K., *In search of the most relevant parameter for quantifying lung inflammatory response to nanoparticle exposure: particle number, surface area, or what?* Environmental Health Perspectives, Vol. 115, p. 187-94, 2007.

48 Nel, A. E.; Nasser, E.; Godwin, H.; Avery, D.; Bahadori, T.; Bergeson, L.; Beryt, E.; Bonner, J. C.; Boverhof, D.; Carter, J.;
 Castranova, V.; DeShazo, J. R.; Hussain, S. M.; Kane, A. B.; Klaessig, F.; Kuempel, E.; Lafranconi, M.; Landsiedel, R.;
 Malloy, T.; Miller, M. B.; Morris, J.; Moss, K.; Oberdorster, G.; Pinkerton, K.; Pleus, R. C.; Shatkin, J. A.; Thomas, R.;
 Tolaymat, T.; Wang, A.; Wong, J., *A Multi-Stakeholder Perspective on the Use of Alternative Test Strategies for*
Nanomaterial Safety Assessment. ACS Nano, Vol. 7, p. 6422-6433, 2013.

49 Oberdorster, G., *Significance of particle parameters in the evaluation of exposure-dose-response relationships of*
inhaled particles, Inhal Toxicol, Vol. 8, p. 73–89, 1996.

50 Oberdorster, G.; Maynard, A.; Donaldson, K.; Castranova, V.; Fitzpatrick, J.; Ausman, K.; Carter, J.; Karn, B.; Kreyling,
 W.; Lai, D.; Olin, S.; Monteiro-Riviere, N.; Warheit, D.; Yang, H., *Principles for characterizing the potential human*
health effects from exposure to nanomaterials: elements of a screening strategy, Particle and Fibre Toxicology, Vol. 2,
 p. 8, 2005.

51 Jarabek, A. M.; Asgharian, B.; Miller, F. J., *Dosimetric adjustments for interspecies extrapolation of inhaled poorly*
soluble particles (PSP), Inhalation Toxicology, Vol. 17, p. 317-34, 2005.

52 Ministerial Order of 6 August 2012 on the *Content and the conditions for the presentation of the annual declaration*
on substances at nanoscale, in application of articles R. 523-12 and R. 523-13 of the Environment code, 2012.

53 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", JRC Reference Report EUR 25404,
 Joint Research Centre of the European Commission, 2012.

54 European Commission, *Types and uses of nanomaterials, including safety aspects*, Staff Working Paper SWD(2012)
 288 final, 2012.

55 G. Lövestam, H. Rauscher, G. Roebben, B. Sokull-Klüttgen, N. Gibson, J.-P. Putaud, H. Stamm, *Considerations on a*
Definition of Nanomaterial for Regulatory Purposes, Joint Research Centre of the European Commission, EUR
 24403EN, 2010.

56 http://ec.europa.eu/environment/chemicals/nanotech/faq/questions_answers_en.htm. Accessed 28/04/2014.

57 CEN ISO TS 27687, *Terminology and definitions for nano-objects – Nanoparticle, nanofibre and nanoplate*, ISO,
 Geneva, 2008.

58 ISO 14644-6, *Cleanrooms and associated controlled environments -- Part 6: Vocabulary*, ISO, Geneva, 2007.

59 ISO 21501-1, *Determination of particle size distribution -- Single particle light interaction methods -- Part 1: Light*
scattering aerosol spectrometer, ISO, Geneva, 2009.

60 ISO 29464, *Cleaning equipment for air and other gases -- Terminology*, ISO, Geneva, 2011.

61 European Commission, *EC's Second Regulatory Review on Nanomaterials*, COM(2012) 572, 2012.

62 ISO 26824, *Particle characterisation of particulate systems – Vocabulary*, ISO, Geneva, 2013.

63 ISO DTS 80004-2, *Nanotechnologies -- Vocabulary -- Part 2: Nano-objects: Nanoparticle, nanofibre and nanoplate*, ISO,
 Geneva, 2014.

64 Scientific Committee on Consumer Safety, *Guidance on the safety assessment of nanomaterials in cosmetics*,
 SCCS/1484/12, 2011.

65 OECD, *Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials*.
 ENV/JM/MONO(2009)21. OECD Environment, Health and Safety Publications Series on the Safety of Manufactured
 Nanomaterials No. 15. Available online:
<http://search.oecd.org/officialdocuments/displaydocumentpdf/?doclanguage=en&cote=env/jm/mono>, 2009.

66 V. A. Hackley, M. Fritts, J. F. Kelly, A. K. Patri, A. F. Rawle, *Enabling standards for nanomaterial characterization*,
 InfoSIM, p. 24-29, August 2009.

67 ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*, ISO, Geneva, 2005.

68 S. C. Brown, V. Boyko, G. Meyers, M. Voetz, W. Wohlleben, *Toward Advancing Nano-Object Count Metrology: A Best*
Practice Framework, Environ Health Perspect, Vol. 121, p. 1282-1291, 2013.

69 ISO/IEC Guide 98-1, *Uncertainty of measurement -- Part 1: Introduction to the expression of uncertainty in*
measurement, ISO, Geneva, 2009.

70 *Report on the relationship between analytical results, measurement uncertainty, recovery factors and the provisions of*
EU food and feed legislation, with particular reference to community legislation concerning contaminants in food
(Council Regulation (EEC), No 315/93 of 8 February 1993 laying down Community procedures for contaminants in
food) and undesirable substances in feed (Directive 2002/32/EC of the European Parliament and of the Council of 7
May 2002 on undesirable substances in animal feed),

http://ec.europa.eu/food/food/chemicalsafety/contaminants/report-sampling_analysis_2004_en.pdf, Accessed 31/7/14.

- 71 ISO 14488, *Particulate materials – Sampling and sample splitting for the determination of particulate properties*, ISO, Geneva, 2007.
- 72 H. Yoshida et al., *Theoretical calculation of uncertainty region for spherical particles based on a picket fence, quasi-monodisperse particles*, *Advanced Powder Technology*, Vol. 25, p. 524-529, 2014.
- 73 H. Yoshida et al., *Particle size measurement of standard reference particle candidates with improved size measurement devices*, *Advanced Powder Technology*, Vol. 14, p. 17-31, 2003.
- 74 P. Kuchenbecker et al., *Inter-laboratory study of particle size distribution measurements by laser diffraction*, *Part. Part. Syst. Charact.*, Vol. 29, p. 304-310, 2012.
- 75 T. P. J. Linsinger et al., *Validation of methods for the detection and quantification of engineered nanoparticles in food*, *Food Chemistry*, Vol. 138, p. 1959-1966, 2013.
- 76 G. W. Mulholland et al., *Measurement of 100 nm and 60 nm particle standards by differential mobility analysis*, *J. Res. Natl. Inst. Stand. Technol.*, Vol. 111, p. 257-312, 2006.
- 77 F. Meli et al., *Traceable size determination of nanoparticles, a comparison among European metrology institutes*, *Meas. Sci. Technol.*, Vol. 23, doi: 10.1088/0957-0233/23/12/125005, 2012.
- 78 P.-J. De Temmerman et al., *Measurement uncertainties of size, shape, and surface measurements using transmission electron microscopy of near-monodisperse, near-spherical nanoparticles*, *J. Nanopart. Res.*, Vol. 16, doi:10.1007/s11051-013-2177-1, 2014.
- 79 A. Braun et al., *Validation of dynamic light scattering and centrifugal liquid sedimentation methods for nanoparticle characterisation*, *Advanced Powder Technology*, Vol. 22, p. 766-770, 2011.
- 80 V. Kestens et al., *Comparison between correlation analysis and frequency analysis dynamic light scattering for nanoparticle size analysis*, poster at Partec 2013, International Congress on Particle Technology, Nuremberg (DE), April 23-25, 2013.
- 81 H. Woehlecke et al., *In-situ determination of the effective particle density of suspended colloidal silica particles by means of analytical centrifugation*, poster at Partec 2013, International Congress on Particle Technology, Nuremberg (DE), April 23-25, 2013.
- 82 C. Motzkus et al., *Size characterization of airborne SiO₂ nanoparticles with on-line and off-line measurement techniques: an interlaboratory comparison study*, *J. Nanopart. Res.*, Vol. 15, doi 10.1007/s11051-013-1919-4, 2013.
- 83 V.-D. Hodoroaba et al., *Performance of high-resolution SEM/EDX systems equipped with transmission mode (TSEM) for imaging and measurement of size and size distribution of spherical nanoparticles*, *Microsc. Microanal.*, doi:10.1017/S1431927614000014, 2014.
- 84 Rice et al., *Particle size distributions by transmission electron microscopy: an interlaboratory comparison study*, *Metrologia*, Vol. 50, p. 663-678 (2013).
- 85 P. Hole et al., *Interlaboratory comparison of size measurements on nanoparticles using nanoparticle tracking analysis (NTA)*, *J. Nanopart. Res.*, Vol. 15, doi 10.1007/s11051-013-2101-8, 2013.
- 86 A. Lamberty et al., *Interlaboratory comparison of methods for the measurement of particle size, effective particle density and zeta potential of silica nanoparticles in an aqueous solution*, JRC Scientific and Technical Report, RM-10-003, 2010.
- 87 A. Braun et al., *Certification of equivalent spherical diameters of silica nanoparticles in water*, EUR 24620, European Union, 2011.
- 88 K. Franks et al., *Certification of the equivalent spherical diameters of silica nanoparticles in aqueous solution*, EUR 25018, European Union, 2012.
- 89 F. Laborda et al., *Critical considerations for the determination of nanoparticle number concentrations, size and number size distributions by single particle ICP-MS*, *J. Anal. At. Spectrom.*, Vol. 28, p. 1220-1232, 2013.
- 90 J. W. Olesik, P. J. Gray, *Considerations for measurement of individual nanoparticles or microparticles by ICP-MS: determination of the number of particles and the analyte mass in each particle*, *J. Anal. At. Spectrom.*, Vol. 27, p. 1143-1155, 2012.
- 91 T. P. J. Linsinger et al., *International interlaboratory study for sizing and quantification of Ag nanoparticles in food simulants by single-particle ICP-MS*, *Anal. Bioanal. Chem.*, doi:10.1007/s00216-013-7559-9, 2013.
- 92 M. Menzel et al., *Certification report Certified Reference Material BAM-N001, particle size parameters of Nano Silver*, BAM, Berlin, April 2013.

- 93 W. Wohlleben, *Validity range of centrifuges for the regulation of nanomaterials: from classification to as-tested coronas*, J. Nanopart. Res., Vol. 14, doi 10.1007/s11051-012-1300-z, 2012.
- 94 ISO/TS 16176, *Rubber compounding ingredients — Carbon black — Determination of the aggregate-size distribution at ultimate dispersion*, ISO, Geneva, 2011.
- 95 P.-J. De Temmerman et al., *Quantitative characterization of agglomerates and aggregates of pyrogenic and precipitated amorphous silica nanomaterials by transmission electron microscopy*, J. Nanobiotechnology, Vol. 10:24, 2012.
- 96 C. Nickel et al., *Dynamic light-scattering measurement comparability of nanomaterial suspensions*, J. Nanopart. Res., Vol. 16, doi 10.1007/s11051-014-2260-2, 2014.
- 97 National Institute of Standards and Technology, *Certificate of Analysis SRM 1898, titanium dioxide nanomaterial*, June 2012.
- 98 G. Roebben et al., *Interlaboratory comparison of size and surface charge measurements on nanoparticles prior to biological impact assessment*, J. Nanopart. Res., Vol. 13, p.2675-2687, 2011.
- 99 W. Anderson et al., *A comparative study of submicron particle sizing platforms: accuracy, precision and resolution analysis of polydisperse particle size distributions*, J. Colloid and Interface Sc., Vol. 405, p.322-330, 2013.
- 100 N. C. Bell et al., *Emerging techniques for submicrometer particle sizing applied to Stöber silica*, Langmuir, Vol. 28, p. 10860-10872, 2012.
- 101 R. I. MacCuspie et al., *Challenges for physical characterization of silver nanoparticles under pristine and environmentally relevant conditions*, J. Environ. Monit., Vol. 13, p. 1212-1226, 2011.
- 102 M. Hassellöv et al., *Nanoparticle analysis and characterization methodologies in environmental risk assessment of engineered nanoparticles*, Ecotoxicology, Vol. 17, p. 344-361, 2008.
- 103 W. G. Kreyling et al., *A complementary definition of nanomaterial*, Nano Today, Vol. 5, p. 165-168, 2010.
- 104 E. A. F. Van Doren et al., *Determinatino of the volume-specific surface area by using transmission electron tomography for characterization and definition of nanomaterials*, J. Nanobiotechnology, Vol. 9:17, 2011.
- 105 LeBouf et al., *Measuring surface area of airborne titanium dioxide powder agglomerates: relationships between gas adsorption, diffusion and mobility-based methods*, J. Nanopart. Res., Vol. 12, p.7029-7039, 2011.
- 106 T. P. J. Linsinger et al., *Reference materials for measuring the size of nanoparticles*, Trends in Analytical Chemistry, Vol. 30, p. 18-27, 2011.
- 107 T. P. J. Linsinger, H. Emons, *Characterization of reference materials: proposal for a simplification of the options listed in ISO Guide 34*, Accred Qaul Assur, Vol. 18, p. 149-152, 2013.
- 108 ISO 11352, *Water quality – estimation of measurement uncertainty based on validation and quality control data*, ISO, Geneva, 2012.
- 109 V. Uvarov, I. Popov, *Metrological characterisation of X-ray diffraction methods at different acquisition geometries for determination of crystallite size in nano-scale materials*, Materials Characterization, Vol. 85, p. 111-123, 2013.
- 110 P.-J. De Temmerman et al., *Semi-automatic size measurement of primary particles in aggregated nanomaterials by transmission electron microscopy*, Powder Technology, Vol. 261, p. 191-200, 2014.
- 111 A. M. Brasil, T. L. Farias, M. G. Carvalho, *A recipe for image characterization of fractal-like aggregates*, J. of Aerosol Science, Vol. 30, p. 1379-1389, 1999.
- 112 ISO 9277, *Determination of the specific surface area of solids by gas adsorption -- BET method*, ISO, Geneva, 2010.
- 113 JRC-Eurocolour study (to be released)
- 114 K. Wegner and S.E. Pratsinis, *Scale-up of nanoparticle synthesis in diffusion flame reactors*, Chem. Engng. Sci., Vol. 58, p. 4581-4589, 2003.
- 115 D. Vollath, *Nanomaterials*, Wiley-VCH, Weinheim, 2008.
- 116 T. Tsuzuki, *Commercial-scale production of nanoparticles*, in: *Nanotechnology Commercialization*, T. Tsuzuki (ed.), CRC Press, Taylor & Francis Group, Boca Raton, ISBN-13: 978-981-4303-28-6, pp. 139-169, 2013.
- 117 K. Wegner, B. Schimmoeller, B. Thiebaut, C. Fernandez, T.N. Rao, *Pilot plants for industrial nanoparticle production by flame spray pyrolysis*, KONA Powder and Particle Journal, Vol. 29, p. 251-265, 2011.
- 118 T. Tsuzuki, *Commercial scale production of inorganic nanoparticles*, Int. J. Nanotechnol. Vol. 6, p.567-578, 2009.
- 119 N. Osterwalder, C. Capello, K. Hungerbühler, W.J. Stark, *Energy consumption during nanoparticle production: How economic is dry synthesis?* J. Nanopart. Res., Vol. 8, p. 1-9, 2006.

- 120 W.J. Stark and S.E. Pratsinis, *Aerosol flame reactors for manufacture of nanoparticles*, Powder Technology, Vol. 126, p. 103-108, 2002.
- 121 M. Eslamian and M. Shekarriz, *Recent advances in nanoparticle preparation by spray and micro-emulsion methods*, Recent Patents on Nanotechnology, Vol. 3, p. 99-115, 2009.
- 122 W.Y. Teoh, R. Amal, L. Mädler, *Flame spray pyrolysis: An enabling technology for nanoparticles design and fabrication*, Nanoscale, Vol. 2, p. 1324-1347, 2010.
- 123 R. Strobel and S.E. Pratsinis, *Flame aerosol synthesis of smart nanostructured materials*, J. Mater. Chem., Vol. 17, p. 4743-4756, 2007.
- 124 G.L. Messing G.L., S.-C. Zhang, G.V. Jaynathi, *Ceramic powder synthesis by spray pyrolysis*, J. Am. Ceram. Soc., Vol. 76, p. 2707-2726, 1993.
- 125 S.E. Pratsinis, *Flame aerosol synthesis of ceramic powders*, Prog. Energy Combust. Sci., Vol. 24, p. 197-219, 1998.
- 126 D.L. Marchisio, L. Rivautella, A.A. Barresi, *Design and scale-up of chemical reactors for nanoparticle precipitation*, AIChE Journal, Vol. 52, p. 1877-1887, 2006.
- 127 M. Boutonnet, S. Lögdberg, E.E. Svensson, *Recent developments in the application of nanoparticles prepared from w/o microemulsions in heterogeneous catalysis*, Current Opinion in Colloid and Interface Science, Vol. 13, p. 270-286, 2008.
- 128 D. Vollath, *Plasma synthesis of nanopowders*, J. Nanopart. Res., Vol. 10, p.39-57, 2008.
- 129 T. Tsuzuki and P.G. McCormick, *Mechanochemical synthesis of nanoparticles*, J. Mater. Sci., Vol. 39, p. 5143-5146, 2004.
- 130 W. Wohlleben, at Technical Workshop on 'Aspects to consider in the technical and scientific review of the European Commission Recommendation of 18 October 2011 on the Definition of Nanomaterial', Brussels, 19th March 2014.
- 131 ISO TS 80004-3, *Nanotechnologies -- Vocabulary -- Part 3: Carbon nano-objects*, ISO, Geneva, 2010.
- 132 https://www.beckmancoulter.com/wsrportal/bibliography?docname=AN-12744A_BCPCS005PE_PCS%20Sizing%20Fullerene.pdf, Accessed 04/04/2014.
- 133 J. Prasek, J. Drbohlavova, J. Chomoucka, J. Hubalek, O. Jasek, V. Adam, R. Kizek, *Methods for carbon nanotubes synthesis—review*, J.Mater. Chem, Vol. 21, p. 15872, 2011.
- 134 http://www.stanford.edu/group/cpima/education/nanotube_lesson.pdf, Accessed 04/04/2014.
- 135 <http://goldbook.iupac.org/G02683.html>, Accessed 04/04/2014.
- 136 European Chemicals Agency, *Guidance for identification and naming of substances under REACH and CLP*, ECHA-11-G-10.2-EN, 2014.
- 137 Chae, S-R., Hotze, E. M., Xiao, Y., Rose, J., Wiesner, M.R., *Comparison of methods for fullerene detection and measurements of reactive oxygen production in cosmetic products*, Environmental Engineering Science, Vol. 27, p. 797–804, 2010.
- 138 Wang, C.A., Shang, C.A., Westerhoff, P.B., *Quantification of fullerene aggregate nC₆₀ in wastewater by high-performance liquid chromatography with UV-vis spectroscopic and mass spectrometric detection*, Chemosphere, Vol. 80, p. 334-9, 2010.
- 139 Anacleto, J.F., Perreault, H., Boyd, R.K., Pleasance, S., Quilliam, M.A., Sim, P.G., Howard, J.B., Makarovskiy Y., Lafleur, A.L., *C₆₀ and C₇₀ fullerene isomers generated in flames. Detection and verification by liquid chromatography/mass spectrometry analyses*, Rapid Communications in Mass Spectrometry, Vol. 6, p. 214-220, 1992.
- 140 ISO/TS 10797, *Nanotechnologies -- Characterization of single-wall carbon nanotubes using transmission electron microscopy*, ISO, Geneva, 2012.
- 141 ISO/TS 10798, *Nanotechnologies -- Characterization of single-wall carbon nanotubes using scanning electron microscopy and energy dispersive X-ray spectrometry analysis*, ISO, Geneva, 2011.
- 142 ISO/TS 10867, *Nanotechnologies -- Characterization of single-wall carbon nanotubes using near infrared photoluminescence spectroscopy*, ISO, Geneva, 2010.
- 143 ISO/TS 10868, *Nanotechnologies -- Characterization of single-wall carbon nanotubes using ultraviolet-visible-near infrared (UV-Vis-NIR) absorption spectroscopy*, ISO, Geneva, 2011.
- 144 ISO/TS 11251 *Nanotechnologies -- Characterization of volatile components in single-wall carbon nanotube samples using evolved gas analysis/gas chromatograph-mass spectrometry*, ISO, Geneva, 2010.
- 145 ISO/TS 11308 *Nanotechnologies -- Characterization of single-wall carbon nanotubes using thermogravimetric analysis*, ISO, Geneva, 2011.

- 146 E. Kan, Z. Li, J. Yang, *Graphene Nanoribbons: Geometric, Electronic, and Magnetic Properties*, in *Physics and Applications of Graphene - Theory*, Dr. Sergey Mikhailov (Ed.), ISBN: 978-953-307-152-7, InTech, DOI: 10.5772/14112, 2011.
- 147 I. Snook, A. Barnard, *Graphene Nano-Flakes and Nano-Dots: Theory, Experiment and Applications*, in *Physics and Applications of Graphene - Theory*, Dr. Sergey Mikhailov (Ed.), ISBN: 978-953-307-152-7, InTech, DOI: 10.5772/15541, 2011.
- 148 D.R. Dreyer, S. Park, C.W. Bielawski, R.S. Ruoff, *The chemistry of graphene oxide*, Chem. Soc. Rev., Vol. 39, p. 228, 2010.
- 149 C. Bussy, E. Paineau, J. Cambedouzou, N. Brun, C. Mory, B. Fayard, M. Salomé, M. Pinault, M. Huard, E. Belade, L. Armand, J. Boczkowski, P. Launois, S. Lanone, *Intracellular fate of carbon nanotubes inside murine macrophages: pH-dependent detachment of iron catalyst nanoparticles*, Part Fibre Toxicol, Vol. 10, p. 24, 2013.
- 150 C. Laurent, E. Flahaut, A. Peigney, A. Rousset, *Metal nanoparticles for the catalytic synthesis of carbon nanotubes*, New J. Chem, Vol. 22, p. 1229, 1998.
- 151 T. W. Chamberlain, A. M. Popov, A. A. Knizhnik, G. E. Samoilov, A. N. Khlobystov, *The Role of Molecular Clusters in the Filling of Carbon Nanotubes*, ACS Nano, Vol. 4, p. 5203, 2010.
- 152 G. Korneva, H. Ye, Y. Gogotsi, D. Halverson, G. Friedman, J. C. Bradley, K. G. Kornev, *Carbon Nanotubes Loaded with Magnetic Particles*, Nano Lett., Vol. 5, p. 879, 2005.
- 153 T. Wang, Y. Fu, L. Chai, L. Chao, L. Bu, Y. Meng, C. Chen, M. Ma, Q. Xie, S. Yao, *Filling Carbon Nanotubes with Prussian Blue Nanoparticles of High Peroxidase-Like Catalytic Activity for Colorimetric Chemo- and Biosensing*, Chem-Eur J, Vol. 20, p. 2623, 2014.
- 154 http://www.princeton.edu/~achaney/tmve/wiki100k/docs/Carbon_nanotube.html, Accessed 22/05/2014.
- 155 Y. W. Jun, S. M. Lee, N.J. Kang, J. Cheon, *Controlled Synthesis of Multi-armed CdS Nanorod Architectures Using Monosurfactant System*, J. Am. Chem. Soc., Vol. 123, p. 5150, 2001.
- 156 C. Shi, S. Soltani, A. M. Armani, *Gold Nanorod Plasmonic Upconversion Microlaser*, Nano Lett., Vol. 13, p. 5827, 2013.
- 157 http://www.nanopartz.com/gold_nanorods.asp, Accessed 22/05/2014.
- 158 <http://www.reade.com/products/113-nanorod-materials-nanowire-nanopillar-/606-nanorods-nanorod-arrays-quantum-rods->, Accessed 22/05/2014.
- 159 O. P. Ferreira, A. G. Souza Filho, J. Mendes Filho, O. L. Alves, *Unveiling the Structure and Composition of Titanium Oxide Nanotubes through Ion Exchange Chemical Reactions and Thermal Decomposition Processes*, J. Braz. Chem. Soc., Vol. 17, p. 393, 2006.
- 160 T. Sekino, *Synthesis and Applications of Titanium Oxide Nanotubes*, in *Inorganic and Metallic Nanotubular Materials-Recent Technologies and Applications*, T. Kijima (Ed.), ISBN 978-3-642-03620-0, Springer, DOI: 10.1007/978-3-642-03622-4, 2010.
- 161 P. Roy, S. Berger, P. Schmuki, *TiO₂ Nanotubes: Synthesis and Applications*, Angew. Chem. Int. Ed., Vol. 50, p. 2904, 2011.
- 162 Y. C. Nah, I. Paramasivam, P. Schmuki, *Doped TiO₂ and TiO₂ Nanotubes: Synthesis and Applications*, ChemPhysChem, Vol. 11, p. 2698, 2011.
- 163 R.Q. Zhang, S.T. Lee, C. K. Law, W. K. Li, B. K. Teo, *Silicon nanotubes: Why not?*, Chemical Physics Letters, Vol. 364, p. 251, 2002.
- 164 J.A. García-Calzón, M.E. Díaz-García, *Synthesis and analytical potential of silica nanotubes*, Trend. Anal. Chem., Vol. 35, p. 27, 2012.
- 165 D. F. Perepichka, F. Rosei, *Silicon Nanotubes*, Small, Vol. 2, p. 22, 2006.
- 166 Z. Wen, G. Lu, S. Mao, H. Kim, S. Cui, K. Yu, X. Huang, P. T. Hurley, O. Mao, J. Chen, *Silicon nanotube anode for lithium-ion batteries*, Electrochemistry Communications, Vol. 29, p. 67, 2013.
- 167 T. Song, J. Xia, J. H. Lee, D. H. Lee, M. S. Kwon, J. M. Choi, J. Wu, S. K. Doo, H. Chang, W. I. Park, D. S. Zang, H. Kim, Y. Huang, K. C. Hwang, J. A. Rogers, U. Paik, *Arrays of Sealed Silicon Nanotubes As Anodes for Lithium Ion Batteries*, Nano Lett., Vol. 10, p. 1710, 2010.
- 168 A. Yildirim, H. Acar, T. S. Erkal, M. Bayindir, M. O. Guler, *Template-Directed Synthesis of Silica Nanotubes for Explosive Detection*, ACS Appl. Mater. Interfaces, Vol. 3, p. 4159, 2011.
- 169 C. Gao, Z. Lu, Y. Yin, *Gram-Scale Synthesis of Silica Nanotubes with Controlled Aspect Ratios by Templating of Nickel-Hydrazine Complex Nanorods*, Langmuir, Vol. 27, p. 12201, 2011.

- 170 Y. H. Tang et al., *Self-Assembled Silicon Nanotubes under Supercritically Hydrothermal Conditions*, Phys. Rev. Lett., Vol. 95, 116102, 2005.
- 171 S. Scanlon, A. Aggeli, *Self-assembling peptide nanotubes*, Nanotoday, Vol. 3, p. 22, 2008.
- 172 E. Gazit, *Self-assembled peptide nanostructures: the design of molecular building blocks and their technological utilization*, Chem. Soc. Rev., Vol. 36, p. 1263, 2007.
- 173 <http://www.google.com.mx/patents/US20050124535>, Accessed 07/04/2014.
- 174 <http://www.google.st/patents/WO2013122642A2?cl=en>, Accessed 07/04/2014.
- 175 <http://www.freepatentsonline.com/y2014/0072801.html>, Accessed 07/04/2014.
- 176 A. B. Seabra, N. Durán, *Biological applications of peptides nanotubes: An overview*, Peptides, Vol. 39, p. 47, 2013.
- 177 C. R. Martin, P. Kohli, *The emerging field of nanotube biotechnology*, Nat. Rev. Drug. Discov., Vol. 2, p. 29, 2003.
- 178 <http://ion.chem.usu.edu/~tapaskar/Britt-Quantum%20Dots.pdf>, Accessed 07/04/2014.
- 179 T. Jamiesona, R. Bakhshia, D. Petrovaa, R. Pockocka, M. Imanib, A. M. Seifaliana, *Biological applications of quantum dots*, Biomaterials, Vol. 28, p. 4717, 2007.
- 180 http://www.pv-magazine.com/archive/articles/beitrag/quantum-dots--the-pros-and-cons-in-pv-_100010173/86/?tx_ttnews%5BbackCat%5D=214&cHash=b70bb78d3041688a35dbb83efeb87b34#axzz2yClVDMvH, Accessed 07/04/2014.
- 181 <http://www.lanl.gov/newsroom/news-releases/2013/December/12.10-nontoxic-quantum-dots-improves-solar-cells.php>; Accessed 07/04/2014.
- 182 <http://www.reportsnreports.com/reports/231562-quantum-dot-and-quantum-dot-display-qled-market-shares-strategies-and-forecasts-worldwide-nanotechnology-2013-to-2019.html>; Accessed 07/04/2014.
- 183 A. J. Nozik, M. C. Beard, J. M. Luther, M. Law, R. J. Ellingson, J. C. Johnson, *Semiconductor Quantum Dots and Quantum Dot Arrays and Applications of Multiple Exciton Generation to Third-Generation Photovoltaic Solar Cells*, Chem. Rev., Vol. 110, p. 6873, 2010.
- 184 http://www.coatingsys.com/yahoo_site_admin/assets/docs/What_are_%E2%80%98Quantum_Dots%E2%80%99_and_their_applications_in_coatings%E2%80%A6.24175532.pdf; Accessed 07/04/2014.
- 185 http://commons.wikimedia.org/wiki/File:Quantum_Dots_with_emission_maxima_in_a_10-nm_step_are_being_produced_at_PlasmaChem_in_a_kg_scale.jpg, Accessed 31/07/2014.
- 186 http://www.coatingsys.com/yahoo_site_admin/assets/docs/What_is_intercalation_and_exfoliation_of_clay.30231020.Pdf; Accessed 07/04/2014.
- 187 http://www.oecd.org/env/ehs/nanosafety/List_of_representative_MN_for_testing_Nov_2012.pdf; Accessed 07/04/2014.
- 188 <http://www.sigmaaldrich.com/materials-science/nanomaterials/nanoclay-building/nanoclays-montmorillonites.html>; Accessed 07/04/2014.
- 189 ISO TS 80004-4 Nanotechnologies – Vocabulary – Part 4: Nanostructured Materials, ISO, Geneva, 2011.
- 190 Directive 2001/95/EC of the European Parliament and of the Council of 3 December 2001 on General Product Safety, OJ No L 11 of 15 January 2002.
- 191 Directive 2012/19/EU of the European Parliament and of the Council of 4 July 2012 on waste electrical and electronic equipment (recast), 2012.
- 192 D. J. Larson et al., *Information storage materials: nanoscale characterisation by three-dimensional atom probe analysis*, Acta Mat., Vol. 52, p. 2847-2862, 2004.
- 193 K. Kimura et al., *Imaging of Au nanoparticles deeply buried in polymer matrix by various atomic force microscopy techniques*, Ultramicroscopy, Vol. 133, p. 41-49, 2013.
- 194 X. Y. Wang et al., *Reconstruction and visualization of nanoparticle composites by transmission electron tomography*, Ultramicroscopy, Vol. 113, p. 96-105, 2012.
- 195 A. K. Kambham et al., *3D site specific sample preparation and analysis of 3D devices (FinFETs) by atom probe tomography*, Ultramicroscopy, Vol. 132, p. 65-69, 2013.
- 196 A. Grenier et al., *3D analysis of advanced nano-devices using electron and atom probe tomography*, Ultramicroscopy, Vol. 136, p. 185-192, 2014.
- 197 K. Babinsky et al., *A novel approach for site-specific atom probe specimen preparation by focused ion beam and transmission electron backscatter diffraction*, Ultramicroscopy, Vol. 144, p. 9-18, 2014.

Europe Direct is a service to help you find answers to your questions about the European Union
Freephone number (*): 00 800 6 7 8 9 10 11

(*): Certain mobile telephone operators do not allow access to 00 800 numbers or these calls may be billed.

A great deal of additional information on the European Union is available on the Internet.
It can be accessed through the Europa server <http://europa.eu/>.

How to obtain EU publications

Our priced publications are available from EU Bookshop (<http://bookshop.europa.eu/>),
where you can place an order with the sales agent of your choice.

The Publications Office has a worldwide network of sales agents.
You can obtain their contact details by sending a fax to (352) 29 29-42758.

European Commission
EUR 26744 EN – Joint Research Centre – Institute for Reference Materials and Measurements

Title: 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

Author(s): Gert Roebben, Hubert Rauscher, Valeria Amenta, Karin Aschberger, Ana Boix Sanfeliu, Luigi Calzolari, Hendrik Emons, Claire Gaillard, Neil Gibson, Uwe Holzwarth, Robert Koeber, Thomas Linsinger, Kirsten Rasmussen, Birgit Sokull-Klüttgen, Hermann Stamm

Luxembourg: Publications Office of the European Union

2014 – 83 pp. – 21.0 x 29.7 cm

EUR – Scientific and Technical Research series – ISSN 1831-9424 (online)

ISBN 978-92-79-39427-0

doi: 10.2787/97286

JRC Mission

As the Commission's in-house science service, the Joint Research Centre's mission is to provide EU policies with independent, evidence-based scientific and technical support throughout the whole policy cycle.

Working in close cooperation with policy Directorates-General, the JRC addresses key societal challenges while stimulating innovation through developing new methods, tools and standards, and sharing its know-how with the Member States, the scientific community and international partners.

Serving society
Stimulating innovation
Supporting legislation

