JRC SCIENTIFIC AND POLICY REPORTS

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

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2014

Report EUR 26567 EN

Joint
Research
Centre

European Commission
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This publication is a Science and Policy Report by the Joint Research Centre of the European Commission.

JRC89369

EUR 26567 EN

ISBN 978-92-79-36601-7 (print)
ISBN 978-92-79-36600-0 (pdf)

ISSN 1018-5593 (print)
ISSN 1831-9424 (online)

doi:10.2788/36237 (online)

Luxembourg: Publications Office of the European Union, 2014

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March 2014

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EXECUTIVE SUMMARY

The Recommendation (2011/696/EU) of the European Commission (EC) on the definition of the term 'nanomaterial' includes an article on its review in the light of experience and of scientific and technological developments by December 2014. This report collects information on scientific-technical issues that should be considered when reviewing the current EC nanomaterial definition.

Section 2 presents an overview of nanomaterial definitions, to identify where the EC definition diverges from other definitions. To better understand reasons for such differences, Section 3 shows how and in which context the EC nanomaterial definition is currently used.

An important element in the development of this report was the collection of input from a broad range of stakeholders directly contacted by JRC in the frame of a dedicated online survey about their experience with the implementation of the definition (Section 7). Parts of the outcome of this survey are also presented in more specific sections of the report, such as Section 9 which deals with elements of the current definition for which (further) clarification is requested by the survey respondents.

Section 4 provides an update to the JRC Reference Report on 'Requirements on measurements for the implementation of the European Commission definition of the term nanomaterial' published in 2012. This section is complemented by an estimation of the resources needed to measure the particle size distributions of materials (Section 5), and by a list of documents that can provide guidance for the identification of nanomaterials and the quantification of their constituents (Section 6). Examples of recently measured particle size distributions are provided in Section 10 as reality check.

Section 11 describes the possibilities and limitations of alternative measurement approaches to complement or replace the direct measurement of *particle number based distributions of the particles' minimum size*, i.e. of the parameter on which the nanomaterial definition is based. The aspect of converting measurement data from other metrics to the metric relevant for the EC nanomaterial definition is discussed in Section 14. This conversion is often affected by the shape of particles, as discussed in Section 13. Section 12 describes main manufacturing techniques for nanomaterials, as knowing how a nanomaterial is manufactured can help to better understand measurement results.

Section 8 lists those activities that have provided or are likely to provide in the near future a further scientific basis for regulations that require a nanomaterial definition.

Section 15 tackles the issue of nanostructured materials, which currently are not covered by the EC nanomaterial definition. An overview is given of different classes of nanostructured materials, and on how they can be manufactured and characterised.

Section 16 provides information on natural and incidental nanomaterials, and on the challenges to distinguish them from manufactured nanomaterials. This distinction may also become a major issue, if measurements inside matrices of regulatory relevance (food, cosmetics, environmental compartments) would be needed, as discussed in Section 17.

The JRC welcomes all feedback to the compiled issues. Based on this report and the feedback received, JRC will write a second report. In that follow-up report the JRC will provide an assessment of the EC nanomaterial definition and the issues compiled in this first report, in relation to the objective of reviewing the current EC definition. In a third report JRC will provide recommendations to improve content and the implementation of the EC Definition as well as related communication aspects.

Acronyms

3D-APT	Three-dimensional Atom Probe Tomography
AES	Auger Electron Spectrometry
aFl-FFF	Asymmetrical Flow Field-flow Fractionation
AFM	Atomic Force Microscopy
ASTM	American Society for Testing and Materials
AUC	Analytical Ultracentrifuge
BCR	Community Bureau of Reference
BET	Brunauer-Emmett-Teller
BF TEM	Bright Field Transmission Electron Microscopy
CEN	European Committee for Standardization
CLS	Centrifugal Liquid Sedimentation
CRM	Certified Reference Material
DLS	Dynamic Light Scattering
DMA	Differential Mobility Analyser
DCS	Differential Centrifugal Sedimentation
DG ENV	Environment Directorate-General
EC	European Commission
ECD	Equivalent Circle Diameter
ECHA	European Chemicals Agency
EDX	Energy-dispersive X-ray Spectroscopy
EELS	Electron Energy Loss Spectroscopy
EFTEM	Energy Filtered Transmission Electron Microscopy
EHS	Environment, Health and Safety
EM	Electron Microscopy
ERM	European Reference Material
ET	Electron Tomography
FIB	Focussed Ion Beam
FP7	Seventh framework programme of the European Community for research and technological development including demonstration activities
HAADF	High-Angle Annular Dark Field
HR-TEM	High Resolution Transmission Electron Microscopy
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
ITS	Integrated Testing Strategy
JRC	Joint Research Centre
LD	Laser Diffraction
NGO	Non-governmental Organisation
NM	Nanomaterial
OECD	Organisation for Economic Co-operation and Development
PSD	Particle size distribution
PTA	Particle Tracking Analysis

RSD	Relative Standard Deviation
SAXS	Small-Angle X-ray Scattering
SEM	Scanning Electron Microscopy
SMPS	Scanning Mobility Particle Sizer™
sp-ICPMS	Single Particle Inductively Coupled Plasma Mass Spectrometry
SPM	Scanning Probe Microscopy
STEM	Scanning Transmission Electron Microscopy
TC	Technical Committee
TEM	Transmission Electron Microscopy
TRPS	Tunable Resistive Pulse Sensing
TSEM	Transmission Scanning Electron Microscopy
VAMAS	Versailles Project on Advanced Materials and Standards
VSSA	Volume Specific Surface Area
WAB	Warren-Averbach-Bertaut
WH	Williamson-Hall
WPMN	Working Party on Manufactured Nanomaterials
XRD	X-ray Diffraction

Table of content

1 INTRODUCTION

In October 2011 the European Commission (EC) published a Recommendation on the definition of nanomaterial (2011/696/EU),[1] here subsequently referred to as the *EC Definition*. The purpose of this definition is to enable determination when a material should be considered a nanomaterial (NM) for regulatory purposes in the European Union. The definition covers natural, incidental and manufactured materials and is based solely on the size of the constituent particles of a material, without regard to specific functional or hazard properties or risks.

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

> *'Nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm.*
>
> *In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %.*

The Recommendation 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 m^2/cm^3. However, a material which, based on its number size distribution, is a nanomaterial should be considered as complying with the definition […] even if the material has a specific surface area lower than 60 m^2/cm^3.*

Detailed and technical information about this definition is available in the related "questions and answers" section of the EC's website.[2]

The current EC Definition was the result of a process of more than 2 years of discussion among Commission services and EU agencies and included an open public consultation on a preliminary version of the current definition. This process also involved a thorough discussion on all elements of such a definition, including the defining properties and the types of material that should be covered by the EC Definition.

The EC Recommendation includes a commitment to review its' definitionit by December 2014 in the light of experience and of scientific and technological developments. The review will encompass all aspects, but should particularly also assess whether the number size distribution threshold of 50 % should be increased or decreased, and whether to include materials with internal structure or surface structure in the nanoscale.

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

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

In view of the upcoming review of the current EC Definition of the term 'nanomaterial' and noting the need expressed by DG ENV and other Commission services for a set of scientifically sound reports as the basis for this review, JRC prepares three consecutive reports. This is the first of these reports (Report 1), which has the objective to compile information concerning the experience with the definition. In the second report (Report 2) the JRC will provide an assessment of the EC Definition and the issues compiled in this first report, in relation to the objective of reviewing the current EC Definition. On the basis of the compilation in Report 1 and the assessment in Report 2, JRC will provide recommendations to improve content and the implementation of the EC Definition as well as related communication aspects.

2 ANNOTATED LIST OF NANOMATERIAL DEFINITIONS USED IN INTERNATIONAL OR NATIONAL FORA, INCLUDING A BRIEF PERCEPTION ANALYSIS

2.1 Introduction

In 2010 the Joint Research Centre (JRC) of the European Commission published a report entitled *"Considerations on a Definition of Nanomaterial for Regulatory Purposes"*.[3] Chapter 3 of that report provides an overview of proposed nanomaterial definitions. One year later, in October 2011, the EC Recommendation on the definition of nanomaterial was published.[1] This chapter gives an overview on changes on nanomaterial definitions and new definitions that have been published after the editorial deadline of Ref. 3 in 2010.

In the EU, legally binding nanomaterial definitions were adopted as part of sector-specific legislation (e.g. food, biocides). Adaptations to the EC Definition are foreseen for pieces of legislation already including a nanomaterial definition and published before 2011, e.g. the Cosmetic Products Regulation and the Regulation on the Provision of Food Information to Consumers (see Tables 1 and 2 of the Annex to Section 2). While the EC Definition contains a broad description of the term nanomaterial, including also naturally occurring nanoparticles, definitions adopted in product specific legislation often restrict their field of applicability to *intentionally manufactured* or *engineered nanomaterials*. Differences in the above mentioned definitions are mainly due to the necessity to limit the definition to the type of compounds the specific legislation is addressing.[4]

National Registries or mandatory Reporting Schemes for NMs are also available or foreseen in some countries (e.g. France, Belgium, Denmark and Switzerland) and NM definitions are therefore adopted in this context to distinguish or identify which materials shall be registered or reported. Several international committees and organisations as well as certain non-European countries provided definitions, working definitions, guidance and other documents addressing the issue of nanomaterial definition and nanotechnology terminologies. Many of these documents are opinions or recommendations on a definition of nanomaterial and are therefore non-normative documents.

Although many of the documents referred to below use the same term 'nanomaterial' it should be kept in mind when comparing those documents that these definitions and the criteria used to describe certain materials were developed for different purposes.

Table 3 of the Annex to Section 2 provides a general overview of the main elements of the nanomaterial definitions used in national and international flora.

2.2 Definitions by international organisations and committees

2.2.1 International Organization for Standardization (ISO) and European Committee for Standardization (CEN)

Since 2010 ISO has published several new documents related to nanotechnology terminology and nomenclature. Definitions relevant to this report are found in ISO/TS 80004-4:2011, *Nanotechnologies - Vocabulary* - Part 4: *Nanostructured materials.*[5] Other recent documents (ISO/TS 80004-5:2011, *Nanotechnologies -Vocabulary* – Part 5: *Nano/bio interface*, [6] and ISO/TS 80004-7:2011, *Nanotechnologies -Vocabulary* - Part 7: *Diagnostics and therapeutics for healthcare*.[7]) are less focused on nanomaterials.

The earlier published ISO documents most relevant for this report are ISO/TS 27687:2008 (Terminology and definitions for nano-objects – Nanoparticle, nanofibre and nanoplate)[8] and ISO/TS 80004-1:2010 (Vocabulary – Part 1: Core terms).[9] In the currently ongoing systematic revision process of these two documents, the nano-specific terminology will be aligned with the more general particle characterisation terminology, recently published as ISO 26824:2013 (Particle characterization of particulate systems – Vocabulary),[10] containing the following definitions for terms as 'particle', 'agglomerate', 'aggregate' and 'primary particle':[A]

Particle	Minute piece of matter with defined physical boundaries
Note 1	A physical boundary can also be described as an interface.
Note 2	A particle can move as a unit.
Note 3	This general particle definition applies to nano-objects
Agglomerate	Collection of weakly or medium strongly bound particles where the resulting external surface area is similar to the sum of the surface areas of the individual components.
Note 1	The forces holding an agglomerate together are weak forces, for example van der Waals forces or simple physical entanglement.
Note 2	Agglomerates are also termed secondary particles and the original source particles are termed primary particles.
Aggregate	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.
Note 1	The forces holding an aggregate together are strong forces, for example covalent bonds, or those resulting from sintering or complex physical entanglement, or otherwise combined former primary particles.
Note 2	Aggregates are also termed secondary particles and the original source particles are termed primary particles.
Primary particle	Original source particle of agglomerates or aggregates or mixtures of the two
Note 1	Constituent particles of agglomerates or aggregates at a certain actual state may be primary particles, but often the constituents are aggregates.
Note 2	Agglomerates and aggregates are also termed secondary particles.

[A] Terms from the ISO Online Browsing Platform (https://www.iso.org/obp/ui/). Copyright remains with ISO.

It is important to note that the European Committee for Standardization (CEN), and in particular CEN/TC 352, the CEN Technical Committee on Nanotechnologies, is not developing a terminology different from that of ISO. Instead, CEN/TC 352 follows closely and contributes to the terminology work in ISO/TC 229, the ISO Technical Committee on Nanotechnologies. The terminology documents published by ISO are systematically submitted to the CEN members for the approval of their adoption as CEN/ISO documents.

This policy is in line with the EC Mandate M/461, which states: '… in various policy documents, the Commission, the European Parliament and the European Economic and Social Committee have highlighted the need for definitions and a common terminology at the global level. The Commission therefore invites CEN and its members to actively take part in international work on definitions'. Nevertheless, in the implementing agreements and contracts following from the same mandate M/461, CEN is also requested to take into account the existence of the EC nanomaterial definition, and to develop standards (e.g. measurement standards) that can help implement the EC nanomaterial definition.

2.2.2 Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR)

In December 2010 SCENHIR published a scientific opinion on the current status of issues on the "*Scientific Basis for the Definition of the Term 'Nanomaterial'*".[11] The Opinion did not provide a new nanomaterial definition, however, the Committee was requested to identify the most suitable metrics to identify nanomaterials. According to SCENIHR the definition of nanomaterial should use the number based particle size distribution and not the mass fraction, as a minimal fraction of the mass could contain large numbers in the low size range, while a low number of large sized particles would represent most of the mass. Also for dose-response effects, the hazard is associated more with the number of particles or the total surface area rather than the mass.

As there is no scientific evidence to use 100 nm as upper limit for nanomaterial identification, the Committee concluded that it is important to consider the 'whole nanoscale metric (1 nm to 999 nm)' when deciding for an approach for the risk assessment of nanomaterials. The application of a tiered approach using an intermediate threshold (e.g. 500 nm upper threshold and 100 nm lower threshold) was proposed.[11] Based on this approach materials would fall into three separate categories:

Category 1 - median size above 500 nm.

These materials are considered to have a lower probability to show nano-specific properties, therefore a classical risk assessment is suggested by SCHENIR.

Category 2 - 500 nm > size > 100 nm:

These materials are qualified as nanomaterials if, based on an extrapolation of an assumed size distribution shape, measurements of the average particle size and its standard deviation, it is predicted that more than 0.15% (or any specified percentage) of the particle size distribution is below 100 nm (based on total particle number), and therefore a nano-specific risk assessment should be performed. If these criteria are not met a classical risk assessment should be performed.

Category 3 - 100 nm > size > 1 nm:

These materials are qualified as nanomaterials and a nano-specific risk assessment is required.

2.2.3 American Chemistry Council (ACC)

The Nanotechnology Panel of the American Chemistry Council presented on March 2013 a poster entitled "*Comparative assessment of nanomaterial definitions and considerations for implementation*" at the 52nd annual meeting of the Society of Toxicology.[12] The Panel steered a comparative assessment of regulatory definitions of nanomaterials and made recommendations on the key aspects that should be included in all regulatory definitions. The Panel concludes that regulatory definitions of nanomaterials should be consistent in the following core elements:

1.	**Solid, particulate substances**
2.	**Distributional threshold of 10% by weight**
3.	**Continued use of 1-100 nm to define nanoscale**
4.	**Exclude naturally occurring and incidentally produced nanomaterials**
5.	**Describe the characteristics of solubility that make it biologically relevant**
6.	**Focus on materials with novel properties not present in non-nano forms**
7.	**Differentiate between aggregates and agglomerates, and consider the potential for these structures to break down into nanoscale particles**

2.2.4 International Cooperation on Cosmetics Regulation (ICCR)

The International Cooperation on Cosmetics Regulation (ICCR) initiative is a group of cosmetic regulatory authorities from the United States, Japan, the European Union and Canada. As stated in the report of the ICCR Joint Ad Hoc Working Group on Nanotechnology in Cosmetic Products: Criteria and Methods of Detection,[13] for purposes of the International Cooperation on Cosmetics Regulation, *"a substance used in a cosmetic is considered a nanomaterial if it is an insoluble ingredient, intentionally manufactured, with one or more dimensions in the realm of 1 to 100 nanometers in the final formulation and is sufficiently stable and persistent in biological media to allow for the potential of interaction with biological systems."*

2.2.5 International Council of Chemical Associations (ICCA)

The International Council of Chemical Associations (ICCA) has released in 2010 a document entitled *"ICCA Core Elements of a Regulatory Definition of Manufactured Nanomaterials".*[14] In that document the following five internationally harmonized core elements for a definition of manufactured nanomaterials are reported:

1.	**Solid, particulate substances**
2.	**Intentionally manufactured at the nano-scale**
3.	**Consisting of nano-objects with at least one dimension between 1 and 100 nm on the basis of ISO**
4.	**And their aggregates and agglomerates**
5.	**With a weight based cut-off either** • **10 wt % or more of nano-objects as defined by ISO or** • **50 wt% or more of aggregates/ agglomerates consisting of nano-objects**

2.2.6 German Chemical Industry Association (VCI)

The German Chemical Industry Association released in February 2010 a document entitled "*VCI position on the definition of the term nanomaterial for use in regulations laying down provisions on substances*".[15] The following definitions of *nanomaterials* and *nano-objects* are included in the document:

Nanomaterials	Intentionally manufactured, solid, particulate substances, either in powder form or as dispersions or as aerosols, consisting of nano-objects and their aggregates and agglomerates, (i) which contain, when measured by standardized and recognized methods, at least 10 wt.-% of nano-objects (ii) or which have, when measured by appropriate methods, a volume specific surface area larger than 60 m^2/cm^3.
Nano-objects	Discrete particles with one, two or three external dimensions between approximately 1 nm and 100 nm.

2.3 Definitions and nanomaterial specifications from EU sector specific legislation

The European Union sector specific legislations already including a nanomaterial definition are the Cosmetic Products Regulation No 1223/2009,[16] the Food Information to Consumers Regulation No 1169/2011,[17] the Biocidal Products Regulation No 528/2012[18] and the Recast of the Novel Foods Regulation (amending Regulation EC No 258/97).[19] The latter was already included in the 2010 JRC Reference Report and will not be discussed here. Nanomaterial specifications are also included in the EU Regulation No 10/2011 on Plastic Materials[20] and are foreseen in the European Union Medical Devices Regulation.[21]

2.3.1 Cosmetic Products Regulation

The nanomaterial definition included in the Cosmetic Product Regulation No 1223/2009,[16] differently from the EC definition, limits the term nanomaterial to *insoluble* or *biopersistent* and *intentionally manufactured* materials, therefore excluding all soluble and/or naturally occurring materials with dimensions at the nanoscale. An adaptation to the EC Recommendation definition is however foreseen (particle size distribution, particle, agglomerate and aggregate definitions integrated). The term *intentionally manufactured,* used in the actual Cosmetic Products Regulation definition is proposed to be substituted with *manufactured to perform/fulfil a specific function or purpose*. The proposed new definition, together with the definition at the moment in use can be found in Table 1 of the Annex to Section 2.

2.3.2 Food Information to Consumer Regulation

Article 3, Chapter 1 of the Food Information to Consumer Regulation No 1169/2011[17] provides a definition of engineered nanomaterial. An adaptation of this definition to that provided in the Recommendation 2011/696/EU is however foreseen (draft DG SANCO).[22] Both actual and proposed definitions are reported in Table 2 of the Annex to Section 2.

2.3.3 Biocides Regulation

Article 3 of the new Biocidal Products Regulation (No 528/2012)[18] provides a definition of nanomaterial which was adapted from the EC definition:

Nanomaterial	A natural or manufactured active substance or non-active substance 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 1-100 nm. Fullerenes, graphene flakes and single-wall carbon nanotubes with one or more external dimensions below 1 nm shall be considered as nanomaterials.
Particle	A minute piece of matter with defined physical boundaries
Agglomerate	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
Aggregate	A particle comprising strongly bound or fused particles

2.3.4 Medicinal Product Regulation

No specific provisions for nanomaterial are included in the medicinal product legislation (Directive 2001/83/EC).[23] The European Medicinal Agency published in 2006 a Reflection Paper (EMEA, 2006)[24] which states that the *nanometre scale ranges from the atomic level at around 0.2 nm (2 Å) up to around 100 nm*. This definition differs in the lower limit (0.2 nm instead of 1 nm) from the EC definition. On the

Agency website,[25] nanotechnology is instead defined as follow: nanotechnology is the use of tiny structures - less than 1,000 nanometres across - that are designed to have specific properties. The upper limit in this definition differs from the ones specified in the EC recommended definition, some medicinal products considered by the Agency as nanomedicines, i.e. liposomes, can have in fact dimensions larger than 100 nm.

2.3.5 Medical Devices Regulation

A *"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"*,[21] is under scrutiny of the European Parliament. A nanodefinition adapted from the EC definition is included in Art 2.1 (15) of the draft Regulation:

Nanomaterial	'nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1-100 nm.
Particle	A minute piece of matter with defined physical boundaries
Agglomerate	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
Aggregate	A particle comprising strongly bound or fused particles

2.3.6 Regulation on plastic materials and articles intended to come into contact with food

The *Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food*[20] provides some specifications for engineered nanomaterials without explicit definition of the term:

(23)"New technologies engineer substances in particle size that exhibit chemical and physical properties that significantly differ from those at a larger scale, for example, nanoparticles. These different properties may lead to different toxicological properties and therefore these substances should be assessed on a case-by-case basis by the Authority as regards their risk until more information is known about such new technology. Therefore it should be made clear that authorisations which are based on the risk assessment of the conventional particle size of a substance do not cover engineered nanoparticles".

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

2.4 National definitions of nanomaterials

Some small changes in nanomaterial definitions already mentioned in the JRC 2010 report[3] (e.g. Canada, Australia) were found. New definitions were also identified for countries such as France, Switzerland, Taiwan, China, etc.

2.4.1 France

The French Ministry of Ecology, Sustainable Development, Transport and Housing, has published on February 2012 the Decree no. 2012-232 [26] on the mandatory reporting of nanomaterials. The Decree applies to "*substances at the nanoscale",* using a definition mainly based on the EC recommended definition, but which, differently from the EU recommendation, restricts the "*substances with nanoscale status*" only to substances intentionally manufactured at the nanoscale.

Substance at nanoscale	Substance as defined in article 3 of EC Regulation no. 1907/2006, intentionally produced at nanometric scale, containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for a minimum proportion of particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm.

The minimum proportion of the number size distribution is specified to be 50% in a joint Order (Ministerial Order of 6 August 2012)[27] issued by the Ministers of environment, agriculture, health labour and industry. In the Decree 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".*

2.4.2 Belgium

Belgium is planning to adopt a legislation that will establish a national registry for nanomaterials. Within the TRIS notification (2013/369/B) of 4 July 2013[28] the scope of the draft legislation is stated: nanomaterials as defined in the EC Recommendation 2011/696/EU definition, but excluding naturally occurring and incidental nanomaterials, as well as pigments. Also excluded are nanomaterials which fall under other EU legislation (e.g. biocides, food products).

2.4.3 Denmark

The Danish Environmental Protection Agency (Miljøstyrelsen) launched on July 2013 a public consultation related to a draft executive order [29,30] for a register for mixtures and products that contain or release nanomaterials. The definition of nanomaterials included in the executive order follows the European Commission definition. Certain activities or products are excluded from the registration requirement: nanoproducts sold between businesses and products that fall under specific regulations (e.g. food, feed, pharmaceuticals, medical devices, cosmetics, pesticides and waste). The following specific products in which nanomaterials are used are also excluded:

- Nanosized products of substances in REACH Annex V
- Products where the material is not consciously produced in nanosize
- Products where the nanomaterial is in a fixed matrix
- Products where the nanomaterial is used as printing ink directly on the product or on labels on the product
- Textiles where the nanomaterial is used as printing ink or to colouring of the textile
- Paints and wood protection products containing titanium dioxide where the sole purpose for the titanium dioxide is to colour the product
- Products of rubber or rubber parts that contain the nanomaterials carbon black or silicon dioxide.
- Products imported for private use
- Products used for research and development

2.4.4 Switzerland

The Swiss Secretariat for Economic Affairs (SECO) has recently published a guideline document for the compilation of safety data sheet for synthetic nanomaterials (SECO et al. 2012).[31] In the chapter on "*Applicability of the guidelines and their individual definition*" the following nanomaterial definition is given:

Nanomaterial	A material whose particle size distribution includes over 1% nanoparticles (1-100 nm) in an unbound state, either as an aggregate or as an agglomerate. Fullerenes, graphene flakes and single-wall carbon nanotubes are classed as nanomaterials even if they have dimensions of less than 1 nm. Should the particle size distribution not be known, then any material with an average grain size less than 500 nm will be classed as a nanomaterial.

The definition adopted, as explained in the guideline, is mainly based on the definitions of nanomaterial of the 2011/696/EU Recommendation[1] and on the ISO definition CEN ISO/TS 27687.[8] The differentiation between nanoparticles, nanofibers and nanoplates and the definition of agglomerates and aggregates (not cited here) are adopted from the ISO definition.

Two important aspects of this definition shall be highlighted: the percentage used for the particle size distribution (1% versus 50% of the EC recommendation) and the 500 nm upper size limit to be used when the PSD is unknown. The adoption of 500 nm as upper size limit is justified by the following considerations: a) in size distributions of manufactured nanomaterials (MNM) with a maximum at 500 nm, a large fraction of the MNM can still be in the low nm range; b) potential nanospecific interaction with cells can occur for sizes < 300 nm.[32]

2.4.5 Norway

In Norway, chemical products must be registered in the Norwegian Product Register of Chemicals at the Norwegian Environment Agency, which includes chemicals that are classified as dangerous, and whose quantity produced in/imported to Norway and/or placed on the market each year is 100 kg or more. If the chemical contains nanomaterials this must be declared in the registration, and information on any substance in nano form must be given for all mandatory declared chemicals, including the identity of the constituent that is in nano form. Only intentionally added nanomaterials need to be registered in the Norwegian Product Register of Chemicals, and the definition of nanomaterials follows the EC Recommendation 2011/696/EU.

2.4.6 The United States of America

In the USA a legally binding definition of nanomaterial does not exist. The American Food and Drug Administration (FDA) released in 2011 a draft guidance to industry entitled *"Considering Whether an FDA-Regulated Product Involves the Application of nanotechnology"*.[33] As reported in the guidance, when considering whether and FDA-regulated product contains nanomaterials or otherwise involves the application of nanotechnology, FDA will ask:

1. *Whether an engineered material or end product has at least one dimension in the nanoscale range (approximately 1 nm to 100 nm); or*
2. *Whether an engineered material or end product exhibits properties or phenomena, including physical or chemical properties or biological effects, that are attributable to its dimension(s), even if these dimensions fall outside the nanoscale range, up to one micrometer.*

Once the guidance will be finalised, the agency means to apply these considerations to all FDA-regulated products. Once more scientific information and on-field experience will be available, FDA's comprehensive approach may become less broad and eventually more specific and well-tailored to each FDA-regulated product (e.g. cosmetics, food). [34]

The FDA Center for Drug Evaluation and Research (CDER) has also published in June 2010 a Manual of Policies and Procedures (MAPP 5015.9)[35] for chemistry, manufacturing, and controls (CMC) reviewers within the Office of Pharmaceutical Science (OPS). The aim of the document is to identify nanotechnology-based products and to report the information related to those products in a nanotechnology database. The nanomaterial definition included in the document is the following: *"Nanomaterial/Nanoscale Material: Any materials with at least one dimension smaller than 1,000 nm"*. The list of the information that a CMC reviewer should document (if available) in the appropriate CMC review is also reported:

- *Whether the application contains nanomaterials.*

- *What type of nanomaterial is included in the product.*
- *Whether the nanomaterial is a reformulation of a previously approved product.*
- *Whether the nanomaterial is part of the drug substance (active pharmaceutical ingredient (API)) or the drug product (carrier, excipient, or packaging).*
- *Whether the particle size was described in the application and what the reported particle size (average primary particle size, size range distribution, aggregation status, agglomeration status) is.*
- *Whether the techniques used to assess particle size are thoroughly described with respect to their adequacy.*
- *Whether the nanomaterial is soluble or insoluble in an aqueous environment (e.g., gold nanoparticle (insoluble) versus nanocrystal (soluble)).*
- *What other properties of the nanomaterial (e.g., surface charge, surface properties) were measured and reported in the application and how those properties were measured (e.g., surface probe microscopy, laser Doppler, electrophoresis).*

Furthermore, the U.S. Environmental Protection Agency (EPA) proposed in 2011 significant new use rules under the Toxic Substances Control Act for two rutile-based chemical substances (CAS No. 389623–01–2 and CAS No. 389623–07–8).[36] Based on toxicity concerns EPA restricts companies from manufacturing these substances with a d10 particle size less than 100 nanometers, where d10 particle size presents the particle size, as determined by laser light scattering, at which 10 percent by weight of the substance measured is smaller. This way EPA effectively introduced specific criteria for those two materials at the nanoscale.

2.4.7 Taiwan

The Council of Labor Affairs, within the context of Chemical Substance Nomination & Notification (2012), provides the following definition of nanomaterial:[12]

Nanomaterial	A nanomaterial is one which is intentionally manufactured or designed and meets any of the following conditions: A) Material with one or more external dimensions or an internal or surface structure on the scale from 1-100 nm; B) It is smaller or larger than the nanoscale above in all spatial dimensions and exhibits one or more nanoscale phenomena/property (for example, increased intensity and chemical reactivity).

2.4.8 Korea

The Republic of South Korea has established the "National Nano-safety Strategic Plan (2012/2016)". The Ministry of Knowledge and Economy and the Korean Agency for Technology and Standards, published in 2011 a *"Guidance on safety Management of Nano-based products", (Korean Agency for Technology and Science Public Notice No.2011-0108 of 12 May 2011 (나노제품의 안전관리에 관한지침)),*[37] where nanomaterials are defined as follows:

Nanomaterial	Means nano-objects and nano-structured materials (including materials having an internal nano-sized structure or materials with condensed nanoparticles), in a solid form, that are smaller than 100 nm in any dimensions.

2.4.9 China

China has released several standards related to nanotechnology.[38] A definition of nanomaterial can be found in "GB/T 19619-2004: 纳米材料术语 (Terminology for nanomaterials)" and it is effective since April 2005.[39]

Nanomaterial	Is the material which has a structure in the three-dimensional space in at least one dimension in the nanometer scale (from 1 nm to 100 nm range of geometric dimensions), or constituted by the nano-structure unit and a material with special properties.

2.4.10 Australia

The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) introduced the first regulatory program for “industrial nanomaterials” as of January 1, 2011. The working definition[40] adopted in this context is the following:

Industrial nanomaterials	Materials intentionally produced, manufactured or engineered to have unique properties or specific composition at the nanoscale, that is a size range typically between 1 nm and 100 nm, and is either a nano-object (i.e., that is confined in one, two, or three dimensions at the nanoscale) or is nanostructured (i.e., having an internal or surface structure at the nanoscale). Notes to the working definition: • intentionally produced, manufactured or engineered materials are distinct from accidentally produced materials • 'unique properties' refers to chemical and/or physical properties that are different because of a material's nanoscale features when compared with the same material without nanoscale features, and result in unique phenomena (e.g. increased strength, chemical reactivity or conductivity) that enable novel applications • aggregates and agglomerates are considered to be nanostructured substances

- where a material includes 10% or more number of particles that meet the above definition (size, unique properties, intentionally produced) NICNAS will consider this to be a nanomaterial.

2.4.11 Canada

In October 2011 Health Canada published a "Policy Statement on Health Canada's Working Definition for Nanomaterials".[41]

Health Canada considers any manufactured substance or product and any component material, ingredient, device, or structure to be nanomaterial if:

a)	*It is at or within the nanoscale in at least one external dimension, or has internal or surface structure at the nanoscale, or*
b)	*It is smaller or larger than the nanoscale in all dimensions and exhibits one or more nanoscale properties/phenomena.*

The definitions of the terms "*nanoscale*", "*nanoscale phenomena*", "*manufactured*" used for the purpose of this definition are the same of those already stated in the 2010 JRC report.[3]

2.4.12 Other Asian Countries

Additional relevant information on activities in other Asian Countries such as India, Thailand, Indonesia, Japan and Malaysia can be found in the External Liaison report of the Asia Nano Forum to ISO/TC 229 'Nanotechnologies'.[38]

3 INVENTORY OF WHERE THE EC DEFINITION IS CURRENTLY USED IN THE EU

As of November 2013 the Definition is two years old and not implemented fully in any legislation. There is a number of instances in the EU, where a definition of the term "nanomaterial" is provided which was adapted from the EC Recommendation. Those cases are listed in the following inventory. A discussion on which elements are identical and which are different from the EC Recommendation can be found in Section 2.

3.1 EU Legislation

Biocides Regulation No 528/2012

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

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

A "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", was adopted by the Commission and is under scrutiny of the European Parliament. A definition of the term "nanomaterial", adapted from the EC Recommendation, is included in Art 2.1 (15) of that draft Regulation.

European Union Cosmetic Products Regulation No 1223/2009 *and*
Regulation on the Provision of Food Information to Consumers No 1169/2011

Adaptations of the currently legally binding definitions in the latter two Regulations to that provided in EC Recommendation 2011/696/EU are foreseen, with sector specific provisions. The definition used in the Cosmetic Products Regulation is also relevant for the notification of nanomaterials as cosmetic ingredients in the Commission's Cosmetic Products Notification Portal (CPNP).

Guidance on REACH

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

3.2 National Legislation and Registries

France

The French Ministry of Ecology, Sustainable Development, Transport and Housing, has published on February 2012 the Decree no. 2012-232 on the mandatory reporting of nanomaterials. The Decree uses a definition on *"substances at the nanoscale"* mainly based on the EC Recommendation.

Belgium

Belgium plans to establish a national registry for nanomaterials, and uses an adaption of the EC Recommendation to define the scope of that registry (TRIS notification (2013/369/B) of 4 July 2013).

Denmark

The Danish Environmental Protection Agency has released a draft executive order for a register for mixtures and products that contain or releases nanomaterials. The definition of nanomaterials included in the executive order follows the European Commission definition.

Norway

The Norwegian Product Register of Chemicals at the Norwegian Environment Agency uses the EC Recommendation 2011/696/EU. Only intentionally added nanomaterials need to be registered there.

Switzerland

The Swiss Secretariat for Economic Affairs (SECO) has recently published a guideline document for the compilation of safety data sheets for synthetic nanomaterials. This definition is mainly based on the definitions of nanomaterial of the 2011/696/EU Recommendation and on the ISO definition CEN ISO/TS 27687.

3.3 Research projects

NanoDefine

This FP7 project (2013-2017) is developing a decision manual and practical guidelines on the implementation of the EC Recommendation (www.nanodefine.eu).

3.4 Research papers explicitly addressing the EC definition of nanomaterial

The following list includes selected technical papers which explicitly refer to the EC Definition of nanomaterial and discuss its elements and/or its application.

- F. Laborda, J. Jiménez-Lamana, E. Bolea and J. R. Castillo , Critical considerations for the determination of nanoparticle number concentrations, size and number size distributions by single particle ICP-MS, J. Anal. At. Spectrom.: 28, 1220-1232, 2013
- L. Calzolai, D. Gilliland, F. Rossi, Measuring nanoparticles size distribution in food and consumer products: a review , Food Addit. Conta.: Part A, 29, 1183 – 1193, 2012
- W. Wohlleben, L. Ma-Hock, V. Boyko, G. Cox, H. Egenolf, H. Freiberger, B. Hinrichsen, S. Hirth, R. Landsiedel, Nanospecific Guidance in REACH: A Comparative Physical-Chemical Characterization of 15 Materials with Methodical Correlations, Journal of Ceramic Science and Technology, Vol.4, No.2, Pages 93-104, 2013
- P. Staniland, Nanotechnology and Sun Care - A Risk Review , Household and Personal Care, vol. 8(2), 18-23, 2013
- E. A. J. Bleeker, et al., Considerations on the EU definition of a nanomaterial: science to support policy making, Regulatory Toxicology and Pharmacology 65, 119-125A, 2013
- A. D. Maynard, Don't define nanomaterials, Nature, 475, 31, 2011.

- S. Greßler, A. Gazso, Definition of the term "nanomaterial", Nano Trust-Dossier No. 039en, May 2013, Institute of Technology Assessment of the Austrian Academy of Science.
- W. Wohlleben, Validity range of centrifuges for the regulation of nanomaterials: from classification to as-tested coronas, J. Nanopart. Res., Vol. 14, 1300, 2012
- J. Warren, The EU definition of nanomaterials – An American perspective, International Labmate, July 2012
- P.-J. De Temmerman, J. Lammertyn, B. De Ketelaere, V. Kestens, G. Roebben, E. Verleysen and J. Mast, Measurement uncertainties of size, shape and surface measurements using transmission electron microscopy of near-monodisperse, near-spherical nanoparticles, J. Nanopart. Res., Vol. 16, citation ID 2177, 2014
- T.P.J. Linsinger, Q. Chaudhry, V. Dehalu, P. Delahaut, A. Dudkiewicz, R. Grombe, F. von der Kammer, E.H. Larsen, S. Legros, K. Loeschner, R. Peters, R. Ramsch, G. Roebben, K. Tiede, S. Weigel, Validation of methods for the detection and quantification of engineered nanoparticles in food, Food Chemistry, Vol. 138, p. 1959-1966, 2013
- D. Gilliland, U. Hempelmann, eds., Interlaboratory comparison of particle size distribution measurements applied to industrial pigments and fillers, in publication, 2014
- O. Geiss, C. Cascio, D. Gilliland, F. Franchini, J. Barrero-Moreno, Size and mass determination of silver nanoparticles in an aqueous matrix using asymmetric flow field flow fractionation coupled to inductively coupled plasma mass spectrometer and ultraviolet–visible detectors, J. Chromat. A, Vol. 1321, p. 100-108, 2013

Many more technical papers do not explicitly refer to the EC definition of nanomaterial, but they are of direct relevance and may well have been inspired by the EC Definition of nanomaterial, for example:[B]

- C. Motzkus et al., Size characterisation of airborne SiO_2 nanoparticles with on-line and off-line measurement techniques: an interlaboratory comparison study, J. Nanopart. Res., Vol. 15, 1919, 2013
- S. B. Rice, C. Chan, S. C. Brown, P. Eschbach, Li Han, D. S. Ensor, A. B. Stefaniak, J. Bonevich, A. E. Vladar, A. R. Hight-Walker, Jiwen Zheng, C. Starnes, A. Stromberg, Jia Ye and E. A. Grulke, Particle size distributions by transmission electron microscopy: an interlaboratory comparison case study, Metrologia, Vol. 50, p. 663-678, 2013
- E. A. F. Van Doren, P.-J. R. H. De Temmerman, M. A. D. Francisco, J. Mast, Determination of the volume-specific surface area by using transmission electron tomography for characterisation and definition of nanomaterials, J. Nanobiotechnology, Vol. 9:17, 2011
- K. Loeschner, J. Navratilova, S. Legros, S. Wagner, R. Grombe, J. Snell, F. von der Kammer, E. H. Larsen, Optimisation and evaluation of asymmetric flow field-flow fractionation of silver nanoparticles, J. Chromatography A, Vol. 1272, p. 116-125, 2013
- W. Anderson, D. Kozak, V. A. Coleman, A. K. Jamting, M. Trau, A comparative study of submicron particle sizing platforms: Accuracy, precision and resolution analysis of polydisperse particle size distributions, J. Colloid and Interface Science, Vol. 405, p. 322-330 (2013)

[B] These publications were mentioned in the replies to question C3 of the survey on experiences made during the implementation of the EC recommendation of a definition of nanomaterial (Which recent scientific publications are particularly relevant for the implementation and review of the EC nanomaterial definition?). See also Section 7.

4 UPDATE ON RELEVANT MEASUREMENT METHODS

4.1 Background

The recent JRC Reference Report EUR 25404[42] summarises its findings about individual measurement methods in a Table, which is reproduced here as Table 4.1. The listed methods were investigated for their capability to characterize 'raw' nanomaterials, as required for the implementation of the nanomaterial definition. The ability of methods to measure or detect nanomaterial ingredients inside products or complex matrices, e.g. for labeling purposes[43] is an equally important issue.[44],[45] However – and this is often not well-understood – this measurement problem is less relevant for the nanomaterial definition itself, and is therefore not discussed here, in this section. The issue of relevant matrices in which eventually to detect or quantify nanomaterials is introduced at the end of this report (Section 17).

The following paragraphs provide an update of the JRC Reference Report[42] with new or additional information on relevant measurement methods. Information was acquired through the survey that is further described in Section 7. In the survey, the respondents were asked to list the methods which they use for size distribution measurements of particulate materials, as well as the measurement methods that have recently been developed or improved in a way that makes them a likely candidate method to help implement the EC definition of nanomaterial in the near future. Additional information was obtained from a review of relevant literature that was newly released or not yet consulted for the redaction of the EUR 25404 report.

In line with the 2012 JRC Reference Report,[42] this section first discusses a number of generic issues, relevant across measurement methods. It also summarises the efforts made to match or combine the results obtained with different methods. Finally, it presents the main developments for each of the methods discussed in the 2012 JRC Reference Report, and presents all additional methods that were mentioned more than once in the survey, for which a commercially available instrument exists.

4.2 Generic measurement issues

4.2.1 Particle size analysis

Several of the difficulties associated with the measurement of number based particle size distributions, as required for the implementation of the EC Definition (see the 2012 JRC Reference Report[42]), are not specific for the field of nanomaterials. Particle size analysis in general, also for larger particles, is a challenging measurement area. Most techniques provide 'apparent' or 'equivalent' particle size values, and these values are strongly affected by the sample preparation (in particular the powder or particle dispersion step) that is an essential part of every particle size analysis method. The following paragraphs indicate which of these generic particle size analysis issues are aggravated or especially relevant for measurements on nanomaterials.

Table 4.1: Main characteristics of particle size methods relevant to the nanomaterial definition

Method name (abbreviation)	**Measurement range and medium (limiting factors)**	**Type of size distribution of raw data**	**Can deal with challenges of particular types of nanomaterials?** (scale: ++, +, 0, -, --)*				**Standards for use of method for size analysis available?**
			poly-dispersity	**non-spherical particles**	**low-density materials**	**aggregates**	
Electron microscopy (EM)	1 nm and higher; dry (dynamic range)	number-based	+	long: + flat: -	-	-	yes
Dynamic light scattering (DLS)	5 nm to 500 nm; suspension (sedimentation, scattering intensity)	(no distribution, or scattering-intensity–based)	--	--	+	--	yes
Centrifugal liquid sedimentation (CLS)	20 nm and higher; suspension (particle density)	extinction-intensity–based	+	--	-	--	yes
Small-angle X-ray scattering (SAXS)	5 nm and higher; suspension (dynamic range)	scattering-intensity–based	0	-	0	--	yes
Field flow fractionation (FFF)	1 nm to 200 nm; suspension (dynamic range)	(depends on detector)	+	-	+	--	no
Particle tracking analysis (PTA)	25 nm and higher; suspension (scattering intensity)	number-based	+	--	0	--	no
Atomic force microscopy (AFM)	1 nm and higher; dry (dynamic range)	number-based	+	long: + flat: +	0	-	yes
X-ray diffraction (XRD)	1 nm and higher; dry (only for crystalline materials)	(no distribution measured)	--	--	-	+	yes

* scale: ++ = very well, + = well, 0 = moderately, - = not well, -- = not at all.

4.2.2 Sample preparation

The dynamic nature of the morphology and agglomeration/aggregation state of nanoparticle materials has been present in the background of many of the discussions surrounding the characterisation of nanomaterials. It was also mentioned and addressed in the JRC Reference Report EUR 25404. A new and very explicit call to better recognise this issue was made recently by Baer et al. [46]: '… it is desirable (to) … recognise the … challenges associated with reproducible synthesis and characterisation of nanomaterials, including the difficulties of maintaining desired material properties during handling and processing due to their dynamic nature…'.

The point-of-view of Baer et al. is that of surface characterisation. One may argue that the particle morphology (i.e. shape and size) of commercial nanomaterials is more stable than the chemical composition of their surface and of the layers attached to their surface. Indeed, the particle morphology should be sufficiently stable to preserve the functional, commercially relevant, surface properties over longer periods of time. Nevertheless, the dynamic nature of nanomaterials is an issue for most particle size characterisation techniques, if the measurements disturb the stable format of the commercial material. In particular, for the investigation of materials with techniques requiring the separation of nanoparticles from solution (e.g. electron microscopy), Nurmi et al.[47] distinguish several critical factors, including the removal of residual solutes and solvents, elimination of non-structural water, erosion of original surface coatings and the reactions that may occur upon exposure to oxygen or other potentially reactive species.

But also the measurements made in liquid dispersions of nanoparticle powders may be influenced by (time-dependent) sample preparation effects. Tourbin et al.[48] point to the effects of even a simple dilution on the size distribution and stability of colloidal (nanoparticle) suspensions. Also, for different dispersion methods (stirring vs. sonication, with vs. without surfactant) and different materials (SiC and TiC), Mejia et al. report different time-dependencies of agglomeration and hence particle size distribution, measured with CLS.[49] This however seems not to have affected the primary SiC and TiC particle size as assessed with high-resolution TEM. On the other hand, Mejia et al. also show that the length of multi-wall carbon nanotubes (MWCNTs) does change with probe sonication time.[50] Similar observations of increased variability in measurement results upon ultrasonication were reported by Meissner et al.[51] and Roebben et al.[52]

The paragraphs above call for a discussion not only about 'with which technique' to measure, but also about 'when (or in which state)' to measure. Adhering to agreed, common and sufficiently detailed sample preparation procedures is a necessary condition for the reproducibility of measurement results. The awareness of this issue is clearly increasing as more and more interlaboratory comparisons and collaborative research projects are performed and completed.[53,54]

4.2.3 Method validation and standardisation

Many of the methods listed in this document are not very new to the academic or research community. However, that does not imply that they are sufficiently developed for use in the industrial or control laboratory settings that are relevant for regulation. This is one of the main concerns raised by industry in their responses to the survey. Routine measurements in industry are mainly based on volume-, mass- or intensity based particle size distributions. Several industry representatives point out that, standard methods for particle number-based size distributions have only been tested on an investigative level. For transparency reasons, industry requests that implementation of a definition like the EC Definition would only be done with methods that are already validated and established for measurements of a great number of samples. In essence, this is a call for (more) method validation.

4.2.3.1 Method validation and measurement uncertainty

Method validation is a process that is often neglected, underestimated or not well understood. Method validation first of all requires a clear definition of the intended measurement goal. This means that a specific measurement technique may well require multiple method validations, if the technique is to be used for different purposes (e.g. for different kinds of (nano-)materials). The assessment of whether a material is a nanomaterial according to the EC nanomaterial definition is such a specific purpose, and obviously no measurement methods had been specifically validated for use with the nanomaterial definition, until the definition was agreed.

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 25404 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. The outcome of a validation study for a method to implement the EC nanomaterial definition therefore cannot be summarised in a simple 'yes' or a 'no'; the validated method shall be declared valid for a defined scope of materials and property values.

In addition, an important outcome of a method validation is information on the measurement uncertainty of the property values obtained when applying the method. At least the main contributions to the method's uncertainty budget should be identified during the validation study, and it should be shown that the combined uncertainty can meet a performance criterium that was set before the start of the method validation. Very little evidence is found in literature on this subject, also because there is no indication of an acceptable measurement uncertainty in the current guidance documents related to the nanomaterial definition. Nevertheless, some of the most advanced efforts will be mentioned in the sections on specific measurement methods (see 4.3 and 4.4).

Recently, Linsinger et al.[55] presented a possible design for a method validation study for the detection and quantification of engineered nanoparticles in food. At present, such a design has not yet been developed for the EC nanomaterial definition.

4.2.3.2 Standardisation

Method validation necessarily has to precede standardisation: a method that was never validated should not be given the status of a normative standard. This is why the Study Group on Metrology of ISO/TC 229 developed a Metrology check-list,[56] which encourages candidate proposers and evaluators of a new measurement method for standardisation to consider the classical components of a method validation (working range, limits of quantification and of detection, selectivity, sensitivity, repeatability, intermediate precision, reproducibility, trueness (or absence of bias), robustness). The initial responses to the ISO/TC 229 metrology check-list clearly indicate that most members of the nanotechnology community are not yet familiar with the details of the method validation concept. In scientific or industrial areas other than nanotechnology, awareness for method validation has often been pushed by the need for measurements in a regulatory context. An example is validation of methods according to 2002/657/EC, which is concerned with the performance of analytical methods to detect e.g. residues in animal products. The EC nanomaterial definition may well be this trigger for the nanotechnology area.

4.2.4 Reference materials and representative test materials

In 2011 an overview of different available nanoscale reference materials (RMs) was published.[57] However, in the last two years several relevant new certified reference materials (CRMs) were released. More recently, Stefaniak et al.[58] made an overview of the reference materials needs and intentions of different organisations active in the area of nanotechnology and in related EHS issues. The paper illustrates how the issue of reliable nanoparticle size analysis, as relevant for the EC nanomaterial definition implementation, is only exemplary to the broader discussion about other properties also relevant for EHS studies of nanomaterials.

4.2.4.1 Reference materials with certified mean particle diameter

In 2000 Mulholland et al.[59] provided an overview of the available reference materials for use in the calibration of nanoparticle size analysis instruments. All listed materials are polystyrene latex materials. For these highly spherical and monodisperse materials, very low certified uncertainties can be obtained (e.g. NIST SRM 1963, 100 nm nominal diameter, expanded relative uncertainty of 1 %; characterisation via Differential Mobility Analysis (DMA)).

More recently, and in view of the upcoming nanomaterial definitions, JRC-IRMM produced CRMs from industry-sourced colloidal silica materials. A critical step in this process was the search for a sufficient number of qualified laboratories to participate in the CRM characterisation studies. To allow candidate laboratories to demonstrate their proficiency, JRC-IRMM had to organise a preliminary ILC.[60] This effort was successful for a few measurement methods, as it appears from the ERM-FD100 and ERM-FD304 certification studies.[61, 62] However, for several measurement methods, it was not possible to find the required statistically relevant number of qualified laboratories to certify the corresponding method-defined size value. This lack of qualified laboratories remains a concern for JRC, for reference material producers in general, but also for all organisations and authorities relying on the availability of reliable, commercially offered analytical services.

4.2.4.2 Reference materials with certified particle size distribution

Sieving and sedimentation based techniques have traditionally been used to certify the particle size distribution of powders consisting of particles with (equivalent) diameters of 1 μm and larger (e.g. CRMs produced under the EU Community Bureau of Reference (BCR) programme). Pioneering work in the 100 nm to 1 μm region, using laser diffraction, has also been done for a series of Japanese RMs,[63] including detailed analysis of the uncertainty associated with the certified values.[64]

However, still largely absent are the CRMs with a certified, particle number based size distribution stretching into the nanorange. An exception is the BAM-N001 CRM, a monodisperse colloidal silver, which carries certified values for d_{10}, d_{50} and d_{90} of its number based size distribution (certified value d_{50} = 12.6 nm). BAM-N001 is characterised with SAXS and Transmission Scanning Electron Microscopy (TSEM).[65]

An overview of available reference nanomaterials can be found on the BAM website.[66]

4.2.4.3 Representative test materials

In addition to the above discussed (certified) RMs, there are a number of initiatives or projects in which common or shared test materials are used. In particular, in the frame of the OECD WPMN Sponsorship programme the JRC-IHCP is hosting a repository of nanomaterials (the NM-xxx series, including TiO_2, ZnO, SiO_2, Ag and MWCNT materials) from which samples are sent across the world to participants in

this OECD test programme. These materials are typically less monodisperse than the reference materials mentioned above, but thereby also closer to the industrially relevant materials. Often these samples are used to develop or check test methods for properties for which the homogeneity and stability of the test materials has not been explicitly tested. It was proposed recently to call these materials 'representative test materials' (RTMs).[67] It is expected that the increasing set of data and information about these more polydisperse materials will eventually lead to or help with the certification of new reference materials that can be used by routine laboratories to validate their in-house methods and to demonstrate their proficiency.

4.2.5 Combination of results obtained with different methods

Motzkus et al.[68] report the results of a VAMAS (Versailles Project on Advanced Materials and Standardisation) interlaboratory comparison (ILC) on techniques for characterising the size distribution of airborne nanoparticles. Of relevance for this report is the comparison of the 'off-line' imaging methods transmission electron microscopy (TEM), atomic force microscopy (AFM) and scanning electron microscopy (SEM) for spherical particulate nanomaterials with monomodal but also with bimodal size distributions. These methods characterised particles on substrates or grids produced following several aerosolisation and deposition protocols. Satisfactory between-laboratory and between-method reproducibility was achieved for the mean values of the individual modes in the bimodal size distribution of these spherical and well-dispersed materials (first population near 40 nm, second population near 85 nm), but significant differences were observed in the width of and the area underneath both peaks in the size distribution. Specific recommendations were made for the use of SEM and TEM instruments.

Meli et al.[69] present the results of an iMERA-Plus project which demonstrate that the average particle diameters of spherical (reference) materials measured with AFM, small-angle X-ray scattering (SAXS), SEM and transmission (mode) scanning electron microscopy (TSEM) perfectly match (within the measurement uncertainty). DLS is singled out as a technique for which this conclusion does not hold.

The study by Anderson et al.[70] compared DLS, CLS and PTA with Tunable Resistive Pulse Sensing (TRPS), a newer technique (see also Section 4.4.6) and with TEM, which was presented as a reference technique. The results demonstrate that the techniques have a good precision (repeatability of results of the same technique on the same material), but that the comparability of the measured average size values in the range from 200 nm to 500 nm is acceptable only in the case of monomodal spherical particles (polystyrene in the study). Even for this simple type of monomodal material, the broadness of the size distribution, required to assess e.g. the EC nanomaterial definition, varies significantly, with values up to 6 times that of the peak width as measured by TEM.

A detailed comparison and combination of TEM, analytical ultracentrifugation (AUC), DLS, asymmetrical flow – field flow fractionation (aFl-FFF) and XRD was presented by Dieckmann et al.,[71] who investigated the sub 10 nm-region for quantum dot materials. The results of the different methods agree with each other, but the conclusions cannot be directly extended to other less dense particles, which are too light to sediment during AUC, or lack the electron density to provide sufficient contrast for TEM and the scattering intensity for DLS.

Summarising, most papers conclude with at least one or several of the following, mutually compatible, conclusions:

- Particle size values obtained with different methods or in different laboratories match within each other's measurement uncertainty, if the test material is 'simple' enough (spherical, monodisperse, dense).
- For polydisperse and aggregated/agglomerated materials particle size values and particle size distributions obtained with different methods do not agree, as they are based on different physical principles which lead to different measurement results for these materials.
- For polydisperse and aggregated/agglomerated materials particle size values and particle size distributions obtained in different laboratories do not agree well, as they are very dependent on even small differences in sample preparation.

One of the conclusions of the JRC Reference Report EUR 25404 was that no single measurement method was available to assess, across all possible nanomaterials, the compliance with the EC nanomaterial definition. Instead, the combined use of several size analysis techniques could provide a way out. This suggestion has been made by many authors as a conclusion of their respective studies to compare results obtained with different particle size analysis techniques (examples are listed in the reference section[72, 73, 74, 75, 76, 77] but also in the ECHA Guidance document ECHA-12-G-03-EN [78]).

Several documents discuss in a more systematic way the possibilities of combining the results of different methods:

ECHA has proposed an integrated testing strategy (ITS) for granulometry.[78] The ECHA testing strategy distinguishes materials with or without the potential to release particles or fibres of an inhalable size. For nanomaterials having such potential, a more detailed test route is recommended. In the same guidance document ECHA also recommends a specific ITS for shape analysis. These testing strategies are developed for the purposes of REACH, but not with the intent to be used specifically for the implementation of the EC nanomaterial definition.

Recently, ISO/TC 229 has created a study group to investigate the possibility of a tiered approach for the identification of a material as a nanomaterial. The current discussion document uses a combination of an initial screening (based on circumstantial information) for the 'obvious' cases, with specific surface

area (SSA) (step 2), and further use of CLS, DLS, laser diffraction (LD), EM or other methods, in a 3rd step, reserved only for borderline materials.

An interlaboratory comparison of particle size distribution measurements applied to industrial pigments and fillers was organised by JRC and Eurocolour.[79] The study collects results obtained with DLS, LD, CLS and SSA instruments, and compares the outcome of these studies with results from EM measurements. The study proposes a tiered approach to classifying particular pigments according to the EC nanomaterial definition.

4.3 Developments in previously assessed measurement methods

In this section the methods discussed in JRC Reference Report EUR 25404[42] are revisited and relevant changes as compared to the findings in Table 4.1 are indicated.

4.3.1 Electron microscopy (EM)

4.3.1.1 Traditional electron microscopy

EM is considered by many as a reference method for particle size analysis, and EM images having great value to better understand the results obtained with other measurement methods.

In terms of achievable spatial resolution, progress is steadily being made, both for SEM and for TEM. Given the growing availability of high-end (field-emission) SEMs, their value for the implementation of the EC nanomaterial definition is increasing. Nevertheless, TEM remains the superior technique in terms of resolution. An excellent illustration of the different capabilities of SEM and TEM in the imaging of nanoparticles or nanostructures is presented by Jackman et al.[80] The paper proposes a method that reduces the effect of electron beam broadening inside the SEM test sample (which is the main reason for a better spatial resolution of TEM) for a particular case (sizing of nanotubes).

Motzkus et al.[81] have shown that TEM and SEM produce reliable and mutually comparable values of the mean values of both peaks in a bimodal colloidal silica material. Satisfactory between-laboratory reproducibility was achieved for the mean values of the individual modes in the bimodal size distribution of these spherical well-dispersed materials (first population near 40 nm, second population near 85 nm), but significant differences were observed in the width of and the area underneath both peaks in the size distribution. Specific recommendations were made especially for the use of SEM and TEM instruments.

For more polydisperse materials the limited 'dynamic range' of image analysis methods remains a challenge: it is not possible to capture at the same time a sufficient number of small particles and a sufficient number of larger particles on the same image or on series of images obtained at the same magnification. This implies that a posteriori combination is needed of partial size distributions into a total size distribution. This challenge is not new, and may also be required when more tests will be performed on pre-fractionated particle samples, also with techniques other than EM.

4.3.1.2 Automation and validation of electron microscopy for measurement of nanoparticle size distributions

Electron microscopy essentially consists of three consecutive steps: sample preparation, image acquisition and image analysis.

- Automation of the sample preparation step is most difficult. Systematic monitoring of the reproducibility of sample preparation protocols will remain necessary.

- Automation of the image acquisition step is to some extent possible, and, for example, the selection of image positions should be done in an as automated and randomised manner as possible, to avoid bias introduced by operator decisions on what is a representative location on the sample.[82]

- The most significant progress is made in the automation of image analysis, which is important, because the manual analysis of hundreds of particle images (the number depending on the width of the size distribution) is an important cost factor. A first step in the image analysis is the detection of the particles on a background of uneven contrast; adaptive thresholding algorithms are investigated specifically for the nanoparticle case.[83] Once the particles are detected on the images, their dimensions have to be determined. De Temmerman et al.[82] and Rice et al.[84] both describe verifications of automated TEM image analysis procedures for the measurement of size distributions of discrete, spheroidal nanoparticles.

Similar procedures have also been applied to assess the external dimensions of aggregated and agglomerated materials, e.g. pyrogenic and precipitated amorphous silica materials.[85] The absence of certified reference materials for aggregated and agglomerated materials makes it difficult to fully validate the method for use with these materials, but the results of the method show to be at least internally consistent. A major question, at this point, is whether the automated image analysis methods can also be used to assess the size of particles that are aggregated together.

Based on recent publications, ISO/TC 229/JWG2 and VAMAS are setting up an international interlaboratory study to validate protocols, including automated image analysis, for the measurement of the particle size distribution of several particulate nanomaterials. The first discussions among the participating experts have tempered the initial ambition to fix a method that would assess the primary particle size. Instead, the size of well-dispersed particles of different shape will be investigated, as well as the aggregate size of an aggregated material. Input will also be obtained from the carbon black community, for which ASTM recently released a new version of its ASTM D3849 – 13.[86]

Images shown in Section 10 indicate some of the problems with which one is confronted when assessing the size of particles that remain aggregated also after sample preparation. Experts are working on this issue, with the aim of developing digital image analysis software to correctly separate or cut aggregated particles, e.g. based on watershed algorithms. These algorithms are not necessarily new, but to the authors' knowledge there are no publications yet describing their use with aggregated nanoparticles.

4.3.1.3 Electron microscopy on delicate samples

A remaining concern for all EM investigations is that of electron beam damage, i.e. the effect of the electron beam on the delicate structure of small amounts of material, especially organic materials, but also inorganic materials. Examples are the crystallization of regions in amorphous materials and the transformation of phases, both of which can change the morphology of certain particles. A brief, but recent overview of the different kinds of damage and the threshold doses is given by Jiang et al. in the introduction of their paper.[87]

Another obstacle for the use of traditional electron microscopy for samples containing water or other volatile fractions, is the high-vacuum chamber in which the samples are placed for analysis. 'Environmental' forms of electron microscopy (i.e. in chambers with a controlled low pressure instead of a high vacuum) provide a way out, at the expense however of spatial resolution and instrument cost.[88] Efforts have been made to develop other sample preparation methods, e.g. plunge freezing for Cryo-TEM.[89] Solutions such as chemical treatments, fixation, encapsulation or cryogenic methods require indeed more specific sample preparation, as described in a review paper by Dudkiewicz et al.,[90] who studied the case of food samples.

4.3.1.4 Electron tomography (ET)

ET was mentioned in the JRC Reference Report EUR 25404 as a potential approach to solve in the future the problem of judging 3D particles based on 2D images. A few additional publications with ET results relevant for this report have been released. The more spectacular images show atomic resolution 3D-pictures of an individual nanoparticle. These images are known both from popular magazines,[91] and from high-end peer-reviewed journals.[92] However, the cost and limited availability of the instrument (aberration-corrected scanning transmission electron microscope) used to make these atomic resolution pictures limit its use on a large scale in a regulatory context.

ET can also be performed with less unique transmission electron microscopes. Van Doren et al.[93] report as a proof of principle the use of (conventional) bright field ET to measure the volume-specific surface area (VSSA) of a limited number of Au and silica particles. While Wang et al.[94] report a number of ET limitations and artefacts, which would obstruct the use of ET to measure the size of primary particles in close-packed aggregates, Van Doren et al. refer to recent developments in ET instrumentation that may in future lead to the possibility to analyse larger numbers of particles, as required for the reliable implementation of the EC nanomaterial definition.

4.3.1.5 Conclusion

Summarising, most authors agree on the use of EM techniques as the de-facto reference method. More diverging are the opinions about the practicability of the method to investigate large numbers of materials, referring to the laborious nature of the 2D-image analysis and the cost of the equipment (see

also, e.g.,[95]). Most progress is made in the automation of the image analysis. The available literature on the validity of the automated EM image analysis methods for sizing of particles within aggregated/agglomerated nanoparticles, and on the effect of sample preparation on the outcome of the image analysis routines, is yet limited. Also the combination of EM results obtained at different magnifications, to cover the full size range of a polydisperse material, remains an issue.

Overall, the results newly available on the performance and abilities of the EM method confirm the results of the JRC Reference Report 25404.

4.3.2 Dynamic light scattering (DLS)

Recent studies (e.g. [96, 97, 98, 99]) confirm also for the DLS method the results of the JRC Reference Report 25404.

Particular conclusions are drawn from the following studies:

Khlebtsov et al.[100] report the appearance of false peaks in the lower part of the particle size distribution in the case of non-spherical strongly scattering particles. A solution proposed by those authors relies on the measurement of the scattered light intensities at widely varying scattering angles, which is not possible for standard DLS instruments. The authors also established that the widths of the DLS-based size distributions are overestimations.

Jamting et al.[101] report some progress in the use of DLS, but the progress remains limited to bimodal particle suspensions with well-separated peaks in the size distribution.

4.3.3 Centrifugal liquid sedimentation (CLS)

CLS is considered by many as one of the more promising and industrially available methods to measure the particle size distributions required for the implementation of the EC nanomaterial definition. The method, however, does not give access to the primary particle size. This is recognised, e.g., in the title of the standard ISO 15825:2004 'Rubber compounding ingredients -- Carbon black -- Determination of aggregate size distribution by disc centrifuge photosedimentometry', for which ISO/TC 45/SC 3 (Raw materials (including latex) for use in the rubber industry) has recently opened a periodic revision.

Wohlleben[102] studied the reliability of CLS results obtained with AUC and demonstrated the successful measurement of number based size distributions but only for those materials that could be well dispersed.

One of the issues to be solved for CLS is that of the effective particle density, which is often unknown, especially for particles that are not homogeneous in composition or porous (e.g. agglomerates and aggregates). This issue is recognised in e.g. the reports on the certification of the certified reference materials ERM-FD100 and ERM-FD304. Possible approaches to the measurement of the effective particle density have been presented[103, 104, 105, 106] and a proposal to start a new project group to develop a standard method is being discussed in ISO/TC 24/SC4 (Particle characterisation).

Overall, the results newly available on the performance and abilities of the CLS method confirm the results of the JRC Reference Report 25404.

4.3.4 Small-angle X-ray scattering (SAXS)

SAXS results are reported regularly as outcome of research projects, but these are mainly related to monodisperse nanoparticle suspensions.[107] It is noted that the technique will deduce the particle size from the scattering of X-rays at sharp transitions in electron density. That means SAXS is less affected by adsorption of low-density surface layers on the surface of dense metallic or inorganic particles. As a consequence, SAXS will likely not include the thickness of a layer of polymer functional groups on the surface of such particles, which is sometimes added onto a nanoparticle to improve suspension stability by steric hindrance.

No major developments are reported since the publication of the JRC Reference Report 25404.

4.3.5 Field flow fractionation (FFF)

FFF, a separation technique that can be coupled to different size measurement instruments, is increasingly used in the characterisation of nanoparticles,[108,109] but it is mainly used in academic and research settings.[102] It appears that the development of dedicated, material-specific test protocols will be required in order to obtain reliable particle size distributions (e.g. [110]). Carrier liquid composition, membrane material, cross flow rate and spacer height are named by Loeschner et al. as having significant influence on the nanoparticle separation/fractionation process, and '…each new type of nanoparticle requires a careful study of all the mentioned parameters'. The authors recommend combining the size information deduced from FFF-based sizing methods with the results from TEM studies.

The newly available information on the performance and abilities of the FFF method do not alter the related conclusions presented in the JRC Reference Report 25404.

4.3.6 Particle tracking analysis (PTA)

Several papers report about the improved results obtained with PTA as compared to DLS.[111] The abilities of PTA are improving as new data analysis software is becoming available, increasing e.g. the power of the method to resolve peaks in the particle size distribution.[112] Nevertheless, Bell et al.[113] report PTA size distribution results and compare them with results of other tests, showing that the measured size distributions are wider than those of TEM measurements. But, in comparison with DLS, the authors stress the advantage of directly measuring the particle number based size distribution, without needing a data transformation requiring knowledge of the test material's refractive indices.

In the framework of the QualityNano Research Infrastructure, a number of consecutive ILCs were organised to test the comparability of size distribution measurements obtained with PTA on spherical

particles.[114] The study has shown that for spherical particles and under the guidance of a well written protocol, reproducible and accurate modal particle size results can be obtained down to a particle size that depends on the material (30 nm for Au particles, 100 nm for polystyrene and silica particles).

Overall, the results newly available on the performance and abilities of the PTA method confirm the results of the JRC Reference Report 25404. But it can be noted that in 2012 ASTM published a guidance document that explains the basics of the technique and provides good practice for the use of a particular type of PTA instruments. Recently, the drafting of a standard test method has been initiated in ISO/TC 24/SC 4.

4.3.7 Atomic force microscopy (AFM)

As mentioned earlier, the study by Motzkus et al.[115] showed reliable AFM results when characterising the mean values of the peaks in a bimodal size distribution of silica nanoparticles. Several other studies were made to optimise the sample preparation for size measurements with AFM. AFM requires a good, non-agglomerated deposition of the nanoparticles on a flat substrate.[116] New deposition methods (spin coating, fluid cell deposition, gradual immersion[117]) improve the efficiency of the size measurements, but they still rely on having a well-dispersed suspension of the particles from which to start deposition.

Overall, the conclusions of the JRC Reference Report 25404 (see Table 4.1) on AFM remain valid.

4.3.8 X-ray diffraction (XRD)

Crystalline nanomaterial powders show broadened x-ray (but also electron) diffraction peaks due to the limited size of the diffracting crystallites. Three main approaches appear as standardized methods to exploit peak broadening of XRD peaks in terms of crystallite sizes.

The very basic use of Scherrer's formula to interpret XRD spectra remains a popular technique for the estimation of crystallite particle size analysis of crystalline powders, both in academia[118, 119, 120] and in industry.[121, 122] Other methods exist and are worth mentioning, because differences in peak shapes coming from instrumental and sample contributions can create non-correctable artefacts when using the Scherrer equation.

An example is the Williamson-Hall (WH) analysis, which is more suited to discriminate between crystallite size effects and microstrain effects. The size-parameter extracted from WH analysis is also always larger than real crystallite sizes, but smaller than the ones obtained from Scherrer's equation.

A more reliable approach is the Warren-Averbach-Bertaut (WAB) analysis, which consists of a Fourier analysis of peak profiles providing a measure of the crystallite sizes, shapes, microstrains and distributions. Real crystallite sizes are obtained from the Fourier decomposition coefficients, together with microstrains and their anisotropies. Finite crystallite sizes act as Fourier truncation effects, i.e. the truncations reveal the number of diffracting planes in a given direction. Distributions of sizes and

microstrains are measured by the second-order derivatives of the Fourier coefficients. Such sizes are then always equal to or smaller than the sizes of domains or grains visible in microscopy images. WAB analysis does not suffer the previous geometry limitations, because it incorporates a clean intrinsic calibration. However, it was originally practiced on individual peak profiles, which is not easy for nanocrystalline powder diffraction diagrams, because of strong peak overlaps, furthermore enhanced in polyphasic samples.

Also Rietveld analysis can be used, which is a more advanced approach to the fitting of the powder diffraction spectra. This analysis combines the contributions of crystal structure, volume fractions, crystallographic defects (from 0D, like atomic vacancies, to 3D, like limited sizes), and other material and instrumental parameters to simulate the measured diffraction spectra. Dealing with sizes, and in contrast to the WAB analysis, the Rietveld analysis does not need peak deconvolution steps, since it processes by comparison between experimental and simulated diagrams, reinforced by refinement strategies (e.g. least-squares). It is then a strict convolution of all effects visible by diffraction, a procedure much less subjected to artefacts than deconvolution.

Nonetheless, powder diffraction diagrams, even Rietveld-analysed, can suffer from preferred crystallite orientations, layering, residual stresses ..., i.e. size determination depends on these factors. The most advanced methodology to treat also these aspects and work also on as-manufactured samples is the so-called Combined Analysis method. Most of the corresponding softwares are freely available on the internet and widely used world-wide. All commercial instruments provide data that can be analysed using such softwares, which also provide batch-type analyses for routine treatments.

With XRD a number of major limitations remain, as for example the inability to size non-crystalline samples and the inability to distinguish between crystallites or intracrystalline substructures[123] from primary or constituent particles.

4.3.9 Determination of specific surface area by BET

In the NanoGenoTox project, BET Brunauer-Emmett-Teller (BET) specific surface area measurements were compared with results obtained with SAXS and electron tomography. The booklet summarising the outcome of the project indicates that further work is needed to assess the comparability between BET and SAXS measurements and that electron tomography is not yet ready for high-throughput analysis.[124]

Overall, the results newly available on the performance and abilities of the BET method confirm the results of the JRC Reference Report 25404. More information on the use of BET in the context of the EC Definition of nanomaterial can be found in Section 11.

4.4 Additional, new or emerging measurement methods

In addition to the methods discussed in the previous sections, there are quite a number of other particle size measurement methods which have been used to solve specific particle size measurement problems in the nanoscale. In this section some of these methods are listed and briefly discussed.

Most of the selected methods were taken from the answers to the survey question 'Are you aware of measurement methods that have recently been developed or improved in a way that makes them a likely candidate method to help you implement the EC definition of nanomaterial in the near future?' If the respondents selected "yes" (about 30 %) they were asked to list the sources of their information (publications, reports, etc.) for each proposed method. Additional methods were identified by JRC in open literature.

4.4.1 Laser diffraction (LD) and static light scattering (SLS)

In Section 4.3.2, DLS is described, a popular technique based on light scattering and the variation of scattered light intensity in time due to the (Brownian) motion of nanoparticles. The laser diffraction and the static light scattering (SLS) or multi-angle light scattering (MALS) methods also rely on light scattering, more precisely on the angle-dependence of the scattered light.

Laser diffraction is a very common particle sizing technique in the industrial practice. The method is based on the Mie theory of light scattering that links the angle of a scattered light beam to the size of the particle that scattered the incident beam. ISO 13320:2009 is the international standard that provides general guidance on the technique.[125] The technique is applicable to particle sizes ranging from approximately 0.1 µm to 3 mm, but with special instrumentation and under certain conditions, the applicable size range can be extended below 0.1 µm. For non-spherical particles, an equivalent diameter is reported, which is related to the size of spherical particles that match the measured scattering pattern.

Static light scattering (SLS) is based on the angular dependence of the intensity of light scattered by particles which are smaller, but not much smaller than the wavelength of the incident light. In this Rayleigh scattering regime, there are variations in the phase of the light scattered from different parts on the particle. This can lead to angle-dependent constructive and destructive interference. When measuring the intensity of scattered light over multiple angles (MALS), one can derive the root mean square radius of the scattering particle, which under certain assumptions can be transformed into a geometric size. MALS measurements are possible in a size range between about 10 nm to about 500 nm. MALS is often used in combination with FFF instruments.

4.4.2 Acoustic spectroscopy

One of the methods not discussed in the JRC Reference Report EUR 25404 is acoustic spectroscopy, which is based on the measurement of the (excess) acoustic (or ultrasound) attenuation spectrum of

particle dispersions (as compared to the dispersion medium without the particles). Its main advantage is the possibility to determine particle size distribution in (relatively) high-concentration colloidal suspensions, avoiding the need to severely dilute and thereby potentially change the original sample, as is the case for most other methods mentioned in this report.[126] Dukhin et al.[127] confirmed the reliability of the method to measure particle mass based size distributions for a 50 g/kg monomodal silica colloid of about 20 nm (average size).

4.4.3 Aerosol based measurement techniques

Many particle size measurements are done on aerosolised particles, in particular in investigations of environmental or occupational settings.[128, 129] Those methods are not discussed in JRC Reference Report 25404. One reason is the fact that the measurement of aerosolised nanoparticles does not provide access to the primary or constituent particles, but to the size of agglomerates and aggregates. One may however argue that this first reason is also an issue for many of the non-aerosol-based techniques. A second reason is the increased dynamics in an aerosolised nanomaterial, with an increasing tendency to agglomerate with decreasing particle size. These agglomeration effects are at least reduced when measurements are done on a substrate or in a liquid suspension. In the future the aerosol based measurement techniques may be considered more closely.

One interesting example is the differential electrical mobility analysis, which is a mature and standardised technique used also in the certification of the size of spherical particle reference materials. Bell et al.[130] demonstrate the precision and resolution of the method as compared with other particle size analysis (PSA) techniques. However, the method requires larger amounts of samples compared to many other PSA techniques and suffers from the inherent disadvantages of aerosol-based methods mentioned earlier.

4.4.4 Single-particle inductively coupled plasma-mass spectrometry (sp-ICP-MS)

The method has been used to identify spherical Au nanoparticles as a nanomaterial according to the EC definition.[131] Also, in the frame of the FP7 project NanoLyse, an interlaboratory study on the reliability of this method was held.[132] Based partly on the outcome of this study, a new work item proposal for the preparation of a technical specification is being considered in ISO/TC 229. A related but less developed technique is the 'microdroplet generator (or MDG)-ICP-TOF-MS'.

4.4.5 Charged particle beam microscopy

The number of installed microscopes using charged particle beams (instead of electron beams), e.g. Helium Ion Microscopy, is increasing. These instruments can provide complementary information (e.g. about light elements in the sample) as compared to the traditional electron microscopes.[133]

Nevertheless, their availability remains limited and standardisation or validation of their use as PSA methods has not been attempted yet.

4.4.6 Coulter counter or (Tunable) resistive pulse sensor ((T)RPS) or scanning ion occlusion sensing (SIOS)

Coulter counters deduce the number and size of particles based on the change in resistance across a channel through which the particles are sent. The principle is not new and used in e.g. blood cell counting. Improvement in the manufacturing of small (and tunable) microchannels or holes has enabled the reduction of the lower particle size limit of this measurement method. The method's principle (measurement of a change in resistance) implies that the measured size must be interpreted as the diameter of a sphere with the same volume as the actually measured particle.

Anderson et al.[134] demonstrated that, in the region between 200 nm and 500 nm, this technique has a better resolution (in terms of separation of multiple modes in a size distribution) than PTA and DLS. However, no validation test results are known for particle size measurements in the nanorange, and a standardised method applicable to the nanoscale does not exist. Also, Anderson et al. report that the method is not well suited for polydisperse materials, as the larger particles would easily block the channel or pore/hole where the measurement takes place. Bell et al.[135] report SIOS size distribution results consistent with TEM, but point to a minimum measurable particle size of 50 nm. An advantage is the direct measurement of a particle number based size distribution with signals directly proportional to the particle volume.

4.4.7 Chromatography techniques

The basic principles of hydrodynamic chromatography and size-exclusion chromatography have been mentioned in the JRC Reference Report EUR 25404.

A related family of methods is called 'capillary electromigration separation techniques' and is described by Pyell.[136] For free-solution capillary electrophoresis (CE), the author points to the broadening of peaks in the elution times that is due to heterogeneity (variability in shape and zeta-potential) of the migrating particles. Only if the latter electrophoretic heterogeneity is limited (i.e. in the case of the 'model' spherical particles of uniform composition), the elution times can be converted into equivalent spherical diameters, also in bimodal or multimodal size distributions. For gel electrophoresis (GE) the analysis of the data relating electrophoretic mobilities with (apparent) size values becomes more complex.

Overall, the use of chromatographic techniques for measuring nanoparticles according to the EC nanomaterial definition remains limited.

4.5 Conclusions

This section reviewed significant developments and improvements in the measurement methods relevant for the implementation of the nanomaterial definition, since the writing of the JRC Reference Report EUR 25404 (published in 2012).

Within the period of less than 2 years, over which the update was made, progress has been moderate. It is acknowledged that the EC definition of nanomaterial is not the only driver for developing instruments with the ability to characterise the morphology of small particles. It has always been an academic and industrial challenge to offer new measurement instruments with improved spatial resolution.

The main elements of progress are found in the dedicated method validation exercises that address whether an existing instrument can be used to reliably, as required in the regulatory context, assess number-based particle size distributions. It is important to describe in full the outcome of such a validation study, which cannot be summarised in a general, simple 'yes, valid' or a 'not, not valid'; instead, a validated method is valid for a defined scope of materials and property values. This caveat is not specific to the field of PSA of nanomaterials; it is in line with the outcome of other method validation campaigns in other measurement areas as well where a broad diversity of materials is studied.

Currently more measurement methods are being validated for determining the number based particle size distribution for specific types or kinds of (particulate) nanomaterials.

5 ESTIMATION OF RESOURCES NEEDED TO MEASURE THE SIZE OF DIFFERENT NANOMATERIALS BY AVAILABLE METHODS

5.1 Results of the survey on experiences from relevant actors in the implementation of the definition

The survey presented in Section 7 also included a question on the level of resources the respondents used for the implementation of the EC definition of nanomaterial (e.g., manpower, instrumentation, consultancy, etc.). Respondents were asked to add a 'quantitative estimate of the most significant costs (person-hours, instrument time, consumables etc.) for the type of material(s) that is (are) relevant for your organisation' and to specify the materials in question.

Although the question was addressed to organisations actually performing measurements, most replies did not provide quantitative information but rather expressed a feeling or a fear of how high these costs would be, or what investments would be necessary for an implementation of the definition. Several respondents declared that the necessary resources would be 'significant', 'very high', 'tremendous' or 'extreme', either referring to TEM measurements only or without giving further quantitative details. A general statement that it would involve several measurement techniques was also given in some cases. The need for establishing new techniques in the laboratory was mentioned. A number of respondents declared that such an estimate could not be provided or is not possible. In some cases costs were declared as intangible and dispersed throughout the organisation.

Some companies mentioned their involvement in internal nanomaterials working groups, without any quantification of the resources. One large company provided estimates for their entire nanomaterial programme, not detailing implementation cost.

The survey therefore did not allow a conclusive quantitative assessment of the resources needed to decide whether a material was a nanomaterial or not.

5.2 Additional information obtained from targeted enquiries

As the survey was not successful in providing a quantitative assessment of costs, an even more targeted approach was chosen. 24 instrument manufacturers and laboratories were asked about their best estimation of the resources needed for a given measurement method to decide whether a material is a nanomaterial according to the EC definition or not, of which 10 provided answers. Methods included in this investigation were DLS, CLS, EM, AFM, SAXS, PTA and sp-ICPMS. Laboratories were selected amongst instrument manufacturers and amongst those participating recently in the production of the certified reference materials (e.g. ERM-FD100 and ERM-FD304) and included instrument manufacturers, national metrology institutes, research organisations, control authorities, universities and commercial laboratories. The aim was to get at least two estimates per method. However, only one was received for EM and none at all for PTA and sp-ICPMS. The low number of answers naturally limits the statistical

significance of the investigation, and the nature of the addressees with the bias towards research laboratories could result in an overestimation of the required time compared to highly efficient routine laboratories.

Respondents were asked to give their best estimation of resources for general infrastructure (instrument cost) as well as resources (i) incurred once for a type of a material (method development), (ii) incurred for each measurement series and (iii) incurred for each measurement. In particular respondents were asked to assess

a) the time required for the development of a suitable dispersion protocol (needed once for a certain type of material)
b) the time required for instrument set-up and calibration (needed for each measurement series)
c) the time required for sample preparation (needed for each sample)
d) the time required for the actual measurement (needed for each sample)
e) the time required for evaluation and reporting (needed for each sample)
f) the cost of consumables needed for a measurement (needed for each sample)
g) the list price of the instrument (needed once every 5 years to keep the laboratory running)

The effect of points a), b) and g) can be reduced by a higher sample throughput, i.e. are subject to economies of scale, whereas costs for points c) to f) are incurred for each sample and can only be reduced by technological progress.

Specifying resources in units of time was preferred to a specification in any currency, as salaries for laboratory staff vary significantly over the EU. Naturally, all of these estimates are broad approximations and depend to a large extent on the previous experience of the respondents with the specific nanomaterials with which they deal, but they do allow an assessment of the economic burden that may be generated by the definition and derived legislation for nanomaterials.

It was expected that these enquiries would result in a clear separation between 'expensive' and 'inexpensive' methods. However, this was not the case: time estimates for the various steps in an analysis varied widely even for one and the same method and overlapping time estimates were received for e.g. DLS and EM. While it has to be borne in mind that with only 1-3 time estimates per method the investigation is not statistically significant, the data do not support a separation into 'inexpensive' and 'expensive' methods and rather indicate that resources required depend more on the efficiency and organisation of the laboratories than on the methods themselves. Listing the resource estimates per method would not lead to smaller ranges, therefore the required resources are discussed together for all methods. It should be pointed out that one method alone will often be insufficient to decide whether a material is a nanomaterial or not. Therefore the discussion below focuses on one material. If several methods are applied, the resources would have to be scaled up accordingly.

5.2.1 Development of a dispersion protocol

For many particulate materials, in order to correctly determine the size of the constituent particles, materials must first be fully disagglomerated. Disagglomeration may sometimes be simple, but may also

be highly challenging for certain particle types and it is difficult to estimate a priori whether disagglomeration will be easy or not. Disaggregation generally cannot be achieved without changing the character of the material and destroying the sample, which would make conclusions about the original sample invalid. In some cases, analysis by electron microscopy may be necessary to assess whether sufficient disagglomeration has occurred.

This step was cited by all respondents as the most difficult and critical step. Time estimates ranged from 1 person-hour to three person-weeks (120 h), with most laboratories estimating about 8 person-hours. One laboratory stated that, when faced with that request, it would spend one person-day on the development of a dispersion protocol. If that effort was not successful in one person-day, it would report back to the customer to decide on the further steps. However, full disagglomeration may not be required in many cases. If a material is positively identified as nanomaterial, further disagglomeration will not change this assessment.

The main problem in the development of dispersion protocols is that for the current definition, most sizing techniques require dispersion of agglomerates or aggregates into their individual constituent parts. This generates uncertainty (as to whether complete dispersion has been achieved) and legal insecurity, as there is always the possibility that another laboratory (from a customer, enforcement authority, lobby group etc.) applies a stricter dis-agglomeration regime which may change the material classification. Sufficient energy to break up aggregates might also break up constituent particles into smaller entities, thus creating nanoparticles from larger particles that originally did not fall under the definition. The development of a suitable dispersion protocol clearly is challenging.

5.2.2 Sample preparation

Estimates for the time required for sample preparation, i.e. application of the dispersion protocol and bringing the sample into a state susceptible to measurement ranged from 15 minutes to 2 person-hours, with a median time of 45 minutes. Imaging techniques (AFM, EM) tend to lie on the upper range as the sample needs to be deposited on a support.

5.2.3 Instrument set-up and calibration

Time required for instrument set-up and calibration was estimated to lie between 30 minutes and 1.5 person-hours. AFM seems to require more calibration time: depending on the desired accuracy, 2 to 24 person-hours were thought to be necessary. The median time required over all instruments was estimated as 1 person-hour. It should be noted that even nominally calibration free methods like DLS and SAXS generally require verification of correct working of the instrument before use.

5.2.4 Measurement time

5 minutes to 6 person-hours were regarded as necessary to perform the actual measurement, with a median value across all methods of 40 minutes. Again, even for a method like DLS, which is perceived as "simple", estimates ranged from 5 minutes to 2-4 person-hours, depending on the material.

5.2.5 Evaluation and reporting

Estimates for the time required for data analysis and reporting ranged from 10 minutes to 80 person-hours. The 80 person-hours are an extreme value, quoted in one case as worst-case scenario for AFM measurements. Excluding that extreme value, all estimates were shorter than 8 person-hours. It should be noted that the time estimated for EM was not significantly larger than for other methods: an estimate of 3 person-hours for manual picture evaluation plus one person-hour for reporting was given, which is in line with an estimate for DLS where one estimate ranged from 1 person-hour (simple report) to 1 person-day for a fully detailed analysis. 1 person-hour seems to be a realistic median estimate.

5.2.6 Consumables

Consumables were in all cases quoted as minor expense, ranging from "a few Euro" to up to EUR 200 for AFM.

5.2.7 Instrument cost

Instrument costs vary widely, with SAXS, EM and AFM being the most expensive with list prices quoted from 165,000 to 300,000 EUR. EM instruments currently used are often research instruments and hence in fact too sophisticated for sizing alone (the same is true for AFM). With other instruments ranging from EUR 30,000 to 100,000, instrument costs, assuming depreciation over 10 years, are EUR 3,000 to EUR 30,000 per year. To this the cost of service and maintenance contracts needs to be added.

5.3 Summary and conclusion

Based on the estimates above, the median time for the development of a dispersion protocol is about 8 person-hours (range 1-120 person-hours), median time needed per measurement series is about 1 person-hour (range 30 min to 1.5 person-hours) and marginal time required per measured sample is about 2.5 person-hours (range 30 min to 16 person-hours). Fixed instrument costs per year, based on depreciation over 10 years range from EUR 3,000 to EUR 30,000. Applying an hourly rate of EUR 140[C], this amounts to costs of EUR 1,300 for development of a dispersion protocol and instrument set-up plus marginal costs of EUR 350 per sample. These estimates are supported by discussion with a representative of a commercial laboratory estimating the cost of one high-end analysis as EUR 1,500 to 2,000 per sample (which corresponds to 11-14 person-hours at an hourly rate of EUR 140).

[C] Hourly rate of the testing laboratory of the Germanischer Lloyd Prüflabor GmbH, 2011: http://www.gl-group.com/pdf/GLP_Preisliste_01-2011_Stand_05_01_2011.pdf

As the wide ranges show, the median values are only rough estimates and real costs may differ widely. The time (and hence cost) needed for some particular materials may be significantly longer or shorter and for some materials it may be highly challenging to perform a reliable and affordable analysis. Furthermore, applying several different measurement methods will add to the incurred cost per material. More time (e.g. for the development of a dispersion protocol) will be needed for unknown samples than for familiar samples in a routine process control setting.

The number of samples that have to be tested per material depends on the broadness of the particle size distribution, the amount of material produced (higher volumes require more samples to obtain a representative sample selection), the homogeneity of the material (variation of size distribution within and between production batches) and the required accuracy. Therefore a general statement on the number of samples to be measured for the assessment of a material cannot be made at this point.

Despite these necessary caveats, the expectation that the costs of analysis are extraordinary high, as raised by the participants of the survey described in Section 5.1, is not supported by the data from the targeted enquiry described in Section 5.2. Estimated median times are below of what is needed for development and execution of many chemical analyses, where often several person-days of method validation and several person-hours per measurement are required. The main driver for very high cost seems to be the development of a suitable dispersion protocol.

Nevertheless, it should be taken into consideration that these cost are per materials. For companies producing many materials, the total cost will increase with the number of materials. However, experience from similar materials should also reduce the time needed for the development of dispersion protocols and measurement approaches.

6 LIST OF RELEVANT GUIDANCE DOCUMENTS REGARDING THE DETECTION, IDENTIFICATION AND QUANTIFICATION OF NANOMATERIALS, DEVELOPED AT EU AND EUROPEAN NATIONAL GOVERNMENT LEVEL AND BY STANDARDISATION BODIES, INCLUDING SECTOR GUIDANCE DOCUMENTS AT THE INTERNATIONAL LEVEL

6.1 Introduction

In relation to the implementation of the Commission Recommendation for a definition of Nanomaterials, some international harmonisation and standardisation bodies have developed standards or guidance that are either directly addressing the determination of the particle number based size distribution (or an average size) of nanomaterials or certain groups of nanomaterials, or that may be relevant for creating a knowledge basis to develop appropriate standards and/or guidance. Standards or guidance on the detection, identification and quantification of nanomaterials are crucial for the effective implementation of the Recommendation.

The compilation presented in this section addresses documents developed at supranational and national EU level as well as by the main harmonisation and standardisation bodies. This collection includes as well reference to some documents developed by some U.S. bodies, having regard to the strategic role that this country plays in the nanotechnological field. Some selected scientific publications are as well included in a final section.

The authorities in all EU Member States were contacted for providing documents already existing at national level within the EU, and response was received from Sweden, Ireland and France. The response from Sweden and Ireland both stated that those member states do not have a national definition of nanomaterial and thus neither any associated documents. Ireland made reference to a series of documents which are listed in Table 6.1. France responded providing documents relevant for the cosmetics legislation and using that definition.

The following bodies or organisations were identified in this review as possible sources of relevant documents and their documents were examined:

- EC (European Commission)
- ECHA (European Chemicals Agency) Guidance
- EFSA (European Food Safety Authority)
- EMA (European Medicinal Agency)
- OECD (Organisation for Economic Cooperation and Development) Test Guidelines Programme (TGP)
- OECD Working Party on Manufactured Nanomaterials (WPMN)
- CEN (Comité Européen de Normalisation)
- ISO (International Organization for Standardization)
- NIST (US National Institute of Standards and Technology)
- European national government level (as identified on 03 Sep. 2013)

In addition, some suggested guidance can be found in the scientific literature.

Table 6.1: Information received from Ireland and France

Information from Ireland	
Authority/area of responsibility	**Information provided**
Health and Safety Authority (HSA) and the Science Foundation Ireland (SFI)	We can confirm that there is no existing national legislation, definition(s), guidelines and guidance documents specifically concerning nanomaterials in Ireland.
Irish Environmental Protection Agency (EPA).	Nanotechnology: public engagement with health, environmental and social issues. STRIVE Report 61 - Pádraig Murphy (2010) http://www.epa.ie/pubs/reports/research/health/STRIVE_61_Nano_Murphy_web.pdf Nanotechnology: Environmental and Human Health Impacts STRIVE Report 79 - Michelle Nic Raghnaill, Meredith Brown, Dong Ye, Mattia Bramini, Kenneth Dawson and Iseult Lynch (2011) http://www.epa.ie/pubs/reports/research/health/STRIVE_79_web.pdf 2011 SKEP Report – Co-funded by EPA. "Nanomaterial in REACH - evaluation of applicability of existing procedures for chemical safety assessment to nanomeaterials". Katarzyna Malkiewicz, Michala Pettitt, Kenneth A. Dawson, Arho Toikka, Sven Ove Hansson, Janne Hukkinen, Iseult Lynch, Jamie Lead http://www.skep-network.eu/Libraries/Network_documents/SKEP_Nanomaterials_in_REACH_Report.sflb.ashx 2011 Abstract of PhD Thesis Development of a risk assessment methodology for evaluating ecotoxicological dispersion and human risks from nanoparticles through the environmental pathways Niall O'Brien, University College Dublin(2011) http://www.epa.ie/researchandeducation/research/striveprogramme/postgraduateprogrammes/doctoral/abstractniallobrien/

Irish Medicines Board (IMB)	There is no definition of 'nanomaterials' or 'nanomedicines' in our national legislation for medicinal products. The IMB refer to the EMA work in this area which the Commission are probably aware of - i.e. the EMA reflection paper on nanotechnology-based medicinal products for human use (EMEA/CHMP/79769/2006) The IMB are not aware of anything in good manufacturing practice (GMP) which defines what a nanoparticle is specifically.
Information from France	
Authority/area of responsibility	**Information provided**
Direction des dispositifs médicaux thérapeutiques et des produits cosmétiques	Report: Etat des connaissances relatif aux nanoparticules de dioxyde de titane et d'oxyde de zinc dans les produits cosmétiques en termes de pénétration cutanée, de génotoxicité et de cancérogenèse. Saisine 2008 BCT0001. Afssaps : Rapport relatif aux nanomatériaux dans les produits cosmétiques

It is noted that the U.S. standardisation organisation body (ANSI) has recently launched a public database containing references to published standards and guidance documents relevant to the nanotechnology area. The database is accessible at http://nanostandards.ansi.org. In addition to published documents, the database also contains information on documents currently under preparation.

6.2 EU guidance documents

At the EU level, the following guidance documents from the different Directorates General from the Commission or the EU Agencies were identified:

EC

The European Commission has provided guidance on general and specific questions related to the EC Definition of nanomaterial:

- http://ec.europa.eu/environment/chemicals/nanotech/faq/questions_answers_en.htm

The following two documents are directly related to the definition and its implementation:

- Lövestam, G., Rauscher, H., Roebben, G., Sokull-Klüttgen, B., Gibson, N., Putaud, J.P., Stamm, H. (2010). Considerations on a definition of nanomaterial for regulatory purposes. EUR 24403. Luxembourg, European Commission Joint Research Centre. ISBN 978-92-79-16014-1, ISSN 1018-5593, doi 10.2788/98686. Available online: http://ihcp.jrc.ec.europa.eu/our_activities/nanotechnology/report-definition-nanomaterial
- Linsinger, T. P. J., Roebben, G., D. Gilliland, L. Calzolai, F. Rossi, P. Gibson, C. Klein (2012). Requirements on measurements for the implementation of the European Commission definition of the term "nanomaterial". EUR 25404. Luxembourg, European Commission Joint Research Centre. ISBN 978-92-79-25602-8 (pdf), 978-92-79-25603-5 (print). doi: 10.2787/63490 Available online: http://publications.jrc.ec.europa.eu/repository/bitstream/111111111/26399/2/irmm_nanomaterials%20(online).pdf

The next document is related to sizing of particulate materials (including fibres), it might be possible to expand it for the use for a range of nanoparticles:

- Riego Sintes J. (ed.) (2002) “Guidance Document on the Determination of Particle Size Distribution, Fibre Length and Diameter Distribution of Chemical Substances”. EUR 20268 EN Luxembourg, European Commission Joint Research Centre. ISBN 92-894-3704-9. Available online: http://publications.jrc.ec.europa.eu/repository/bitstream/111111111/5555/1/EUR%2020268%20EN.pdf

The following document contains suggested guidance on how to determine several parameters relevant for the EC Definition of nanomaterial:

- Hankin S.M., Peters S.A.K., Poland C.A., Foss Hansen S., Holmqvist J., Ross B.L., Varet J. and Aitken R.J. (2011) Specific Advice on Fulfilling Information Requirements for Nanomaterials under REACH (RIP-oN 2). - Final Project Report Available online: http://ec.europa.eu/environment/chemicals/nanotech/pdf/report_ripon2.pdf

Further guidance documents:

- Scientific Committee on Consumers Safety (SCCS) (2012). Guidance on the Safety Assessment of Nanomaterials in Cosmetics. Available online: http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_s_005.pdf

- Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) (2010). Scientific Basis for the Definition of the Term “nanomaterial”. ISSN 1831-4783. ISBN 978-92-79-12757-1. doi:10.2772/. Available online: http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_032.pdf
- Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) (2008). Opinion on the Scientific Aspects of the Existing and Proposed Definitions Relating to Products of Nanoscience and Nanotechnologies. Available online: http://ec.europa.eu/health/archive/ph_risk/committees/04_scenihr/docs/scenihr_o_012.pdf
- Scientific Committee on Consumer Products (SCCP) (2007) Opinion on Safety of Nanomaterials in Cosmetic Products. Available online: http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_123.pdf

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. Available online: echa.europa.eu/documents/10162/13632/appendix_r7a_nanomaterials_en.pdf.

EFSA

- EFSA Scientific Committee; Scientific Opinion on Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain. EFSA Journal 2011;9(5):2140. doi:10.2903/j.efsa.2011.2140. Available online: www.efsa.europa.eu/en/efsajournal/doc/2140.pdf

EMA

- EMA/538503/20101st International Workshop on Nanomedicines 2010 Summary Report. EMA, Human Medicines Development and Evaluation. London. Available online: **www.ema.europa.eu/docs/en_GB/document_library/Report/2010/10/WC500098380.pdf**

6.3 CEN Standards and guidance documents

As well at EU level, several CEN Technical Specifications are currently under development, which are relevant to the implementation to nanomaterial definitions. Although their final titles may change and it is anticipated that the final documents will only be available within three to five years, they are mentioned here for the sake of completeness:

- CEN Technical Specification (TS) “Guide to the identification and definition of measurands required for characterizing, evaluating functional properties and performance of materials at the nanoscale” (Project Leader: Charles Clifford (National Physical Laboratory), UK)
- CEN Technical Specification (TS) "Protocols for whole life cycle assessment of nanoscale materials, devices and products" (Project Leader: Jeremy Hunt (National Physical Laboratory), UK)
- CEN Technical Specification (TS) "Detection and identification of specific nano-objects" (Project Leader: Michael Stintz (Technische Universität Dresden), Germany)

6.4 OECD Test Guidelines

At a global level the OECD is the main harmonisation body regarding safety testing of chemicals in general through the Test Guidelines Programme (TGP), and nanomaterials in particular via the WPMN. There is one Test Guideline related to determination of size of particulate and fibrous chemicals that might be adapted to the size determination of nanomaterials. It was developed by the **OECD TGP**:

- OECD (1981*),* Test No. 110: Particle Size Distribution/ Fibre Length and Diameter Distributions*,* OECD Guidelines for the Testing of Chemicals, Section 1, OECD Publishing. doi: 10.1787/9789264069688-en

The OECD WPMN reviewed the available Test Guidelines in the

- OECD (2009) 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)21

and provided guidance relevant for determining some properties related to the EC Definition of nanomaterial:

- OECD (2009). Guidance Manual for the Testing of Manufactured Nanomaterials: OECD Sponsorship Programme. ENV/JM/MONO(2009)20/REV. Environment, Health and Safety Publications. Series on the Safety of Manufactured Nanomaterials. No. 14. Available online: http://search.oecd.org/officialdocuments/displaydocumentpdf/?doclanguage=en&cote=env/jm/mono(2009)20

A document was published that includes some guidance on how to prepare the nanomaterials whose size, size distribution and other characteristics need to be determined and on some suitable techniques:

- OECD (2012), Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials. ENV/JM/MONO(2012)40. OECD Environment, Health and Safety Publications. Series on the Safety of Manufactured Nanomaterials No. 36. Available online: http.//search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono(2012)40&doclanguage=en

Several proposals for new or updated Test Guidelines related to nanomaterials testing are currently in preparation within a collaboration of these two OECD groups. None of them deals with general detection, identification or quantification of nanomaterials, but with (eco)toxicological effects and environmental behaviour. Nevertheless a

- Report on an OECD Workshop on Physical-Chemical Properties of Manufactured Nanomaterials and Test Guidelines in collaboration with ISO/TC 229: Nanotechnologies

is currently in the last phases of preparation. It can be anticipated that proposals regarding development of test guidelines on physico-chemical characterisation of nanomaterials, including size and size distribution, can stem from the recommendations in this report.

6.5 ISO Standards and guidance documents

At global level ISO is the main source of standards for industrial applications. ISO has adopted its own set of definitions related to the products of Nanotechnologies:

- ISO/TS 27687:2008 Nanotechnologies – Terminology and definitions for nano-objects – Nanoparticle, nanofibre and nanoplate

that is currently under revision. This actually does not provide guidance on the detection, identification or quantification of nanomaterials, it constitutes the background for the ISO related activities.

Among others, ISO has developed a number of Technical Specifications (TS) and Technical Reports (TR) to characterise certain nanomaterials, none of these documents deal with general detection, identification or quantification of nanomaterials; they are material specific. Although most of them do not directly address the size determination, they may provide additional information supporting the characterisation of nanomaterials.

For example, for carbon nanotubes:

- ISO/TS 13278:2011. Nanotechnologies -- Determination of elemental impurities in samples of carbon nanotubes using inductively coupled plasma mass spectrometry

Single-wall carbon nanotubes:

- ISO/TS 10797:2012 Nanotechnologies -- Characterization of single-wall carbon nanotubes using transmission electron microscopy
- ISO/TS 10798:2011. Nanotechnologies -- Characterization of single-wall carbon nanotubes using scanning electron microscopy and energy dispersive X-ray spectrometry analysis
- ISO/TS 10867:2010. Nanotechnologies -- Characterization of single-wall carbon nanotubes using near infrared photoluminescence spectroscopy
- ISO/TS 10868:2011. Nanotechnologies -- Characterization of single-wall carbon nanotubes using ultraviolet-visible-near infrared (UV-Vis-NIR) absorption spectroscopy
- ISO/TS 11251:2010. Nanotechnologies -- Characterization of volatile components in single-wall carbon nanotube samples using evolved gas analysis/gas chromatograph-mass spectrometry
- ISO/TS 11308:2011.Nanotechnologies -- Characterization of single-wall carbon nanotubes using thermogravimetric analysis

Multiwall carbon nanotubes:

- ISO/TR 10929:2012. Nanotechnologies -- Characterization of multiwall carbon nanotube (MWCNT) samples
- ISO/TS 11888:2011. Nanotechnologies -- Characterization of multiwall carbon nanotubes -- Mesoscopic shape factors

Calcium carbonate:

- ISO/TS 11931:2012. Nanotechnologies -- Nanoscale calcium carbonate in powder form -- Characteristics and measurement

Titanium dioxide:

- ISO/TS 11937:2012. Nanotechnologies -- Nanoscale titanium dioxide in powder form -- Characteristics and measurement

Some other TS or TR are more general and address nanoparticles, nanomaterials or nano-objects:

- ISO/TS 10808:2010. Nanotechnologies – Characterisation of nanoparticles in inhalation exposure chambers for inhalation toxicity testing
- ISO/TR 11360:2010. Nanotechnologies -- Methodology for the classification and categorization of nanomaterials
- ISO/TS 12805:2011. Nanotechnologies -- Materials specifications -- Guidance on specifying nano-objects
- ISO/TS 12025:2012. Nanomaterials -- Quantification of nano-object release from powders by generation of aerosols
- ISO/TS 14101:2012. Surface characterization of gold nanoparticles for nanomaterial specific toxicity screening: FT-IR method
- IEC/TS 62622:2012. Artificial gratings used in nanotechnology -- Description and measurement of dimensional quality parameters
- ISO/TS 17200:2013. Nanotechnology -- Nanoparticles in powder form -- Characteristics and measurements
- ISO/TR 13014:2012. Nanotechnologies — Guidance on physicochemical characterization for manufactured nano-objects submitted for toxicological testing

In addition there are a number of ISO Standards and Technical Specifications in relation to particle size and morphology characterisation that may be relevant for nanomaterials, possibly after appropriate adaptations:

- ISO 5725-1:1994. Accuracy (trueness and precision) of measurement methods and results -- Part 1: General principles and definitions, ISO, Geneva.
- ISO 14887:2000. Sample preparation: Dispersion procedures for powders in liquids, ISO, Geneva.
- ISO 13318-1 (2001) Determination of particle size distribution by centrifugal liquid sedimentation methods – Part 1: General principles and guidelines
- ISO 13318-2 (2007) Determination of particle size distribution by centrifugal liquid sedimentation methods – Part 2: Photocentrifuge method
- ISO 13318-3 (2004) Determination of particle size distribution by centrifugal liquid sedimentation methods – Part 3: Centrifugal X-Ray method
- ISO 13320 (2009) Particle size analysis – Laser diffraction methods
- ISO/TS 13762 (2001) Particle size analysis – Small angle X-ray scattering method
- ISO 15900 (2009) Determination of particle size distribution – Differential electrical mobility analysis for aerosol particles
- ISO 21501-1 (2009) Determination of particle size distribution – Single particle light interaction methods – Part1: Light scattering aerosol spectrometer

- ISO 21501-2 (2007) Determination of particle size distribution – Single particle light interaction methods – Part2: Light scattering liquid–borne particle counter
- ISO 21501-3 (2007) Determination of particle size distribution – Single particle light interaction methods – Part3: Light extinction liquid-borne particle counter
- ISO 21501-4 (2007) Determination of particle size distribution – Single particle light interaction methods – Part4: Light scattering airborne particle
- ISO 14488: 2007. Particulate materials -- Sampling and sample splitting for the determination of particulate properties, ISO, Geneva.
- ISO 16700:2004: Microbeam analysis -- Scanning electron microscopy -- Guidelines for the calibration of Electron Microscopy image magnification, ISO, Geneva.
- ISO 13321:1996, Particle size analysis -- Photon correlation spectroscopy, ISO, Geneva.
- ISO 13322-1:2004, Particle size analysis -- Image analysis methods --Part 1: Static image analysis methods, ISO, Geneva.
- ISO 13322-2:2006 -Particle size analysis- Image analysis methods Part 2: Dynamic image analysis methods, ISO, Geneva.
- ISO 22412:2008, Particle size analysis -- Dynamic light scattering (DLS) , ISO, Geneva
- ISO 9276-1:1998, Representation of results of particle size analysis --Part 1: Graphical representation, ISO, Geneva.
- ISO 9276-1:1998/Cor 1:2004, Representation of results of particle size analysis -- Part 1: Graphical representation -- Technical Corrigendum 1, ISO, Geneva.
- ISO 9276-2:2001, Representation of results of particle size analysis --Part 2: Calculation of average particle sizes/diameters and moments from particle size distributions, ISO, Geneva.
- ISO 9276-3:2008, Representation of results of particle size analysis --Part 3: Adjustment of an experimental curve to a reference model, ISO, Geneva.
- ISO 9276-4:2001, Representation of results of particle size analysis --Part 4: Characterization of a classification process, ISO, Geneva.
- ISO 9276-5:2005, Representation of results of particle size analysis --Part 5: Methods of calculation relating to particle size analyses using logarithmic normal probability distribution, ISO, Geneva.
- ISO 9276-6:2008, Representation of results of particle size analysis --Part 6: Descriptive and quantitative representation of particle shape and morphology, ISO, Geneva.
- ISO/TR 13097:2013 —Guide for the characterization of dispersion stability.

ISO has as well developed some standards for shape and surface characterisation of particles that are potentially adaptable to nanomaterials:

- ISO 20998-1 (2006) Measurement and characterisation of particles by acoustic methods – Part 1: Concepts and procedures in ultrasonic attenuation spectroscopy
- ISO 9277 (2010) Determination of the specific surface area of solids by gas absorption – BET method
- ISO 15901–1 (2005) Pore size distribution and porosity of solid materials by mercury porosimetry and gas adsorption – Part 1: Mercury porosimetry
- ISO 15901–2 (2006) Pore size distribution and porosity of solid materials by mercury porosimetry and gas adsorption – Part 2: Analysis of mesopores and macropores by gas adsorption
- ISO 15901–3 (2007) Pore size distribution and porosity of solid materials by mercury porosimetry and gas adsorption – Part 3: Analysis of micropores by gas adsorption

6.6 Guidance from other organisations

6.6.1 NIST

- J. S. Taurozzi, V. A. Hackley, M. R. Wiesner (2012). Preparation of Nanoparticle Dispersions from Powdered Material Using Ultrasonic Disruption. Version 1.1. Natl. Inst. Stand. Technol. Spec. Publ. 1200-2. Available online: dx.doi.org/10.6028/NIST.SP.1200-2

The US National Cancer Institute NCI has a number "Assay Cascade Protocols" developed by a collaboration of the National Cancer Laboratory and NIST:

- http://ncl.cancer.gov/working_assay-cascade.asp

6.6.2 Scientific Literature

- C. Blasco, Y. Picó (2011). "Determining nanomaterials in food." TrAC - Trends in Analytical Chemistry **30**(1): 84-99.

- Calzolai, L., D. Gilliland, et al. (2012). "Measuring nanoparticles size distribution in food and consumer products: a review." Food Addit. Contam. Part A **29**(8): 1183-1193.

- Oomen, A. G., M. Bennink, et al. (2011). Nanomaterials in consumer products - Detection characterization and interpretation. N. I. f. P. h. a. t. Environment. Bilthoven**:** 94.

- Roebben, G., Rasmussen, K., Kestens, V., Linsinger, T. P. J., Rauscher, H., Emons, H., Stamm, H. (2013) Reference materials and representative test materials: the nanotechnology case. Journal of Nanoparticle Research, Vol. 15, pp. 1455-1468.

- von der Kammer, F., P. L. Ferguson, et al. (2012). "Analysis of engineered nanomaterials in complex matrices (environment and biota): General considerations and conceptual case studies." Environmental Toxicology and Chemistry **31**(1): 32-49.

7 SUMMARY OF EXPERIENCES FROM RELEVANT ACTORS IN THE IMPLEMENTATION OF THE DEFINITION INCLUDING BEST PRACTICES AND OPEN CHALLENGES

7.1 Introduction

A survey has been performed by the EC's Joint Research Centre (JRC) in preparation and support of the 2014 review of the EC definition of nanomaterial to collect feedback from key actors on their experience with the implementation of the definition. In addition to respond to the survey, the addressees were asked to provide results of the size distribution measurements of specific particulate materials, if available.

Relevant actors with supposed practical experience in that particular field were identified from industry or trade associations, private companies, EU agencies, international organisations, government authorities, academic/research organisations, non-governmental organisations (NGOs), and other organisations. The invitees to the survey were encouraged to notify it to other relevant organisations. Several organisations not included in the initial list of invitees sent requests on their own initiative to participate in the survey. All those additional requests to participate in the survey were granted after verification that the request came from an organisation with practical experience in nanomaterials. In total, 255 invitations were sent out to stakeholders not being part of the European Commission.

The survey was carried out in a secure way, i.e., each addressee received individual login credentials, with which up to four independent replies could be uploaded to the survey. Most respondents, however, preferred to upload a single reply. The responding organisation had to identify itself, anonymous replies were automatically rejected by the IT system.

The questionnaire for the survey can be found in Annex A to Section 7. In addition, the addressees were asked whether they could provide reliably measured particle size distributions for materials with a large fraction of fine particles that provide a basis to decide whether or not the material should be classified as nanomaterial. The template for providing such distributions with additional information can be found in Annex B to Section 7.

The survey was launched on August, 9, 2013, and it was closed on September, 27, 2013. In total, 63 replies were received by the deadline, 67 % of which came from private companies, industry and trade associations.

The global response statistics for the survey can be found in Annex C to Section 7.

The results of some questions of the survey are included in other sections of this report.

These are:

Question	Section
Is the wording of the EC definition of nanomaterial clear and unambiguous?	9
Is it clear to which materials the EC definition of nanomaterials applies?	9
Are the individual elements (terms, thresholds, etc.) of the EC definition clear?	9
What level of resources do you use for the implementation of the EC definition of nanomaterial (e. g., manpower, instrumentation, consultancy, etc.)? Please add also a quantitative estimate of the most significant costs (person hours, instrument time, consumables etc.) for the type of material(s) that is (are) relevant for your organisation. Please specify the material(s).	5
Survey section D: Provision of measured particle size distributions	10
For which matrices (consumer products, food and feed, cosmetics, biocides, substances, etc.) do you envisage or predict a future need to determine the nanomaterial fraction (i.e. volume or mass percentage of nanomaterial in the matrix, but not the size distribution) by in-situ measurements?	17

There were a number of cases where the reply to a specific question was more within the scope of a different question of the survey. Such replies were also analysed in this report, but in the context of the appropriate questions.

7.2 Cumulative summary of the replies to the questions of the survey

7.2.1 How would you describe your organisation's general experience with the implementation of the EC recommendation of a definition of nanomaterial?

Authorities/Agencies

Most relevant for regulatory purposes are definitions and provisions in EU legislation that exist or are expected in the near future (cosmetics, food, biocides, medical devices). In those fields separate definitions of 'nanomaterial' have already been developed. The alignment of these definitions with the EC Recommendation, including additional sector specific provisions, would be appreciated by the respondents.

The EC definition itself is perceived as clear, even seen as a gold standard for the identification of nanomaterials by one respondent, but (i) specific documentation on how to apply the definition is not yet available and (ii) methods to implement it are lacking: standardised methods and reference materials are urgently needed. One national authority used the EC definition as a reference to derive a national definition for registration purposes. Another national authority uses the EC definition directly for registration purposes and considers it well suited for that. It was also mentioned that implementing the EC definition in CLP (where there is no tonnage threshold) would improve the data available on

existing NMs and lead to better consumer protection. It was also mentioned that if the process of identifying NMs for labelling is concern driven, then including additional characterisers in the definition (e.g., manufactured to perform/fulfil a specific function or purpose) would be counterproductive.
Where relevant, the authorities are aware of typical difficulties faced by industry and, in the absence of standardised methods, accept a variety of other methods, depending on the nanomaterial. In sectors where nanomaterials are relevant, but where no legally binding nanomaterial definition exists, the EC definition is appreciated and used as a pragmatic working definition, though a legally binding definition would be preferred.
A number of the respondents admitted that they have so far little experience with the implementation of the definition and need more time to work with it.

NGOs

NGOs are not directly involved in the implementation of the definition but rather contribute to the discussion on adapting legislation, e. g., replacing existing legally binding NM definitions with the EC definition. Here the lack of a uniform definition across legislation provided by the authorities is criticised. It was also remarked that the EC definition should distinguish between natural, incidental and engineered NM, possibly also including other characterisers than size, but those characterisers were not specified.

Trade/industry associations

Most important for trade and industry associations are legally binding definitions of nanomaterial in Regulations (cosmetics, food, biocidal products,…), because they provide a legal basis for their operations.
The respondents would welcome a better harmonization of the definitions of nanomaterial across legislation and with the EC definition. There are also concerns regarding non-harmonized national regulations based on different definitions.
A specific discrepancy was mentioned, if a nanomaterial is covered by several sector specific Regulations, e. g. by REACH (as a chemical substance) and by the Cosmetic Products Regulation (as ingredient). In that case different definitions would apply which would cause confusion.
Regarding the implementation of the definition, the lack of standardised, validated measurement methods is generally seen as major drawback. Practical aspects of the implementation need to be clarified as soon as possible, given the current major measurement challenges. The respondents doubt if at present the definition can be practically implemented. Given the lack of standardised methods some

respondents started their own initiatives to develop an accepted methodology. The concept of 'constituent' particle was also seen as problematic.

One respondent remarked that the potential impacts for products defined as nanomaterials may be significant. The fact that the definition may be modified in different sectorial applications, combined with the difficulty in measurement would result in significant practical problems for the producers and downstream users. The implementation of the definition is seen as resource intensive and challenging by some associations.

The associations report that for their members it would be very helpful if the following questions were answered: 'Why is there an overarching definition, as some elements of it are difficult to interpret? How do I know if my substance is a nanomaterial? Which measurement methods should be used to identify whether a substance is falling under the scope of the EC Definition of nanomaterial or not? Why are well-known products covered by the definition?'

The majority of the respondents from trade and industry associations report that the definition is not viewed as neutral as it is intended and stated in the definition text. Rather these associations, their members and their customers have the concern that it is linked to a certain perceived hazard that is associated with nanomaterials in general. The associations report that they are faced with many questions on the hazard and the safety of use of nanomaterials and that there is the concern that the attribute 'nanomaterial' has an unwarranted stigma attached to it. As a consequence, identification as a nanomaterial might in the future bring potential legislative restrictions. The respondents have the concern that this could result in well known, long established products suddenly being seen in a negative light or with restrictions on use or being rejected by consumers based on unreliable measurement data.

Most respondents also have the concern that many materials produced for a long time and used safely would now fall under the definition of nanomaterial. It was mentioned that the majority of insoluble particulate materials could become nanomaterials according to the EC Recommendation. One reply claimed that with the 50% threshold the definition encompasses too many substances that should not be considered as NM (e. g., sand, pigments).

One respondent remarked that as a result of this new 'nanomaterial' classification, these materials would be regulated as hazardous substances with repeated risk assessment and evaluation requirements.

Some respondents prefer to give up the hazard-neutrality of the definition and suggest narrowing the scope to nanomaterials where a true nano-specific health risk cannot be excluded.

Private Companies

Most respondents in this category raised the same or similar issues as the trade and industry associations. Salient points were

- Most important for companies are nanomaterial definitions which are relevant for different markets with specific Regulations which include legally binding definitions (cosmetics, food, biocides, French national market, etc.)
- There is some uncertainty as to the relevance of the definition to the companies' products
- Harmonisation of the definition across legislation would be appreciated; there is uncertainty and confusion because of different existing provisions (REACH, cosmetics, food, etc.)
- The lack of appropriate and routine methods including preparation protocols is a big concern; measurement techniques need to be appropriate for the material and comparable across different samples and laboratories. Implementation is generally seen as a challenge, because there is no fast, easy and cheap process to measure the particle size distribution
- The companies would like to know which methods and measurement techniques should be used presently
- Most companies are concerned that products that are well-known, produced for a long time, and with well-established toxicological profiles would fall under the definition. Furthermore, there is a perceived link between the definition and a hazard that might be associated with nanomaterials in general. There is concern that nanomaterials are discussed in a regulatory context as if they posed totally unknown risks and were of 'high concern' which might require a precautionary approach in legislation
- Some companies are facing the situation that customers are looking at the current discussion on nanomaterials very critically and perceive the definition, the nano-product registers and labelling requests (the latter two are currently also either being discussed or in the introduction phase) as stigma for their downstream products. As a consequence, the customer perception towards nanomaterials tends to be negative in general
- There is the feeling that nearly all poorly soluble particles would fall within that definition, they contain a fraction with external dimensions at the nanoscale.
- The impact of the definition on the product portfolios is uncertain
- Some companies have the opinion that the scope of the definition is too broad, they suggest that it should be narrowed, e.g. to new, innovative materials intentionally produced at the nanoscale and the criteria should be made implementable (aggregates/number %)
- For the VSSA criterion the pore structures of some materials is not considered adequately
- The discussion always focusses on the same materials (synthetic amorphous silica, carbon black, titanium dioxide, silver, carbon nanotubes, zinc oxide), leading to different perception of other, similarly structured substances (e.g. aluminium oxide [Al_2O_3], calcium carbonate [$CaCO_3$] etc.)

Research institutions and academia

The replies coming from research/academia indicate that most of those organisations are not yet involved deeply in questions on the implementation of the definition. The need and challenge to distinguish between natural and engineered nanomaterials is mentioned, and also the request of

industry to develop valid and robust methods to decide whether a material falls under the EC Definition. Furthermore, the upper size limit of 100 nm is challenged, and there is the request to make a clearer distinction between nanomaterial and nanoparticles. In some cases however there is a very active involvement to support the implementation of the EC Definition by scientific expertise and the development of suitable measurement methods.

7.2.2 Are you satisfied with the 'Questions and Answers' section provided by the European Commission?

Replies to this question:

Yes	24	38%
No	39	62%

If the respondents selected 'no' they were asked how the 'Questions and Answers' (Q&A) section could be improved.

The main suggestions for improving the 'Questions and Answers' section are listed below.

- Issues raised in the replies to the previous questions may be partly solved in the 'Questions and Answers' section
- The Q&A section should be oriented on the daily business, e.g., statements such as *'A definition will not come at any direct economic costs. It is simply a categorisation of certain materials based on their size'* are not true. (REACH)
- The difference between aggregates and nanostructured materials should be explained.
- The term 'particles' should be better explained. The current explanation is not accepted by all respondents: e. g., micelles and proteins also have defined boundaries. The term 'constituent particles' should be defined and refer to the relevant working item of ISO/TS 80004-2, which is currently in preparation.
- The discussion on nanostructured materials should be improved
- Further attention should be given to address under what circumstances a nanoscale topography becomes a concern.
- The Q&A should provide more guidance and actual responses to analytical issues and not only provide more information on the term(s). Technical aspects should be included in the section (e. g., measurement methods; that a range of measurement methods is required; applicability of the test depends on the material; when can the VSSA be used). For each nanostructured material (aggregate, agglomerate, nano-foam, nanostructured surface etc.) one should work out and communicate an instruction how to analyse and classify it.
- The conclusions of the second regulatory review should be mentioned, specifically regarding potential risks to human health and the environment. Furthermore, a reference to GAARN and ECHA NMWG could be added regarding the management of risk.
- The answer to the question 'Can the definition be used without measurement and standards being available?' could be a clear yes (if the definition would not build on measurements).

- A cross-reference to the JRC work should be included on methods development
- The 'Question and Answers' catalogue arises from deficiencies in the definition. These deficiencies need to be tackled first.
- A discussion on topics such as nanoparticles embedded in matrices and on issues how to distinguish agglomerates / aggregates from bigger particles would be welcomed
- The use of the regulatory wording of REACH also in the nano Q&A is recommended (e. g., multiple interpretations of final product are possible).
- There should be a section which describes the differences in the approach of the EU and ISO with regard to the definition.
- The section on preparations / articles should be clarified.
- There should be a table similar to table 3 in the Commission Staff Working Paper SWD(2012) 288 final, where possible nanostructures are listed
- Links to documents upon which the individual answers are based upon would be helpful

7.2.3 Are you aware of any guidance on the implementation of the definition, other than the 'Questions and Answers' section provided by the European Commission?

If the respondents selected 'yes' they were asked to provide that guidance. The respondents were also asked whether that guidance is clear and if not, to specify the elements of that guidance which should be improved.

Replies on the awareness of guidance other than the Q&A section:

Yes	36	57%
No	27	43%

Replies on the clarity of that specific guidance:

Yes	13	36%
No	23	64%

The following documents are regarded as guidance by the respondents:

- The Cefic interpretation of some elements of the EU Commission recommendation on the definition of nanomaterial (2011/696/EU) of 15 February 2013 (*mentioned by many of the industrial respondents*)

  Note: One respondent stated that the Cefic interpretation of the EC definition is unclear in the sense that some elements of the EC definition are unclear
- The JRC report *'Requirements on measurements for the implementation of the European Commission definition of the term 'nanomaterial' '* (Linsinger et al.; EUR 25404 EN. Luxembourg (Luxembourg): Publications Office of the European Union; 2012. JRC73260) is seen as guidance by many respondents.

  Note: The JRC report is considered as being clear in some cases, other respondents mentioned that the guidance could benefit from improving the methods to detect and characterise nanoparticles, in complex as well as model media. In addition it was mentioned that an upper size limit is required to calculate the 50 % number size distribution. The report also emphasizes the ambiguous elements and the difficulties which the industry has to comply with.
- Cosmetics Europe Nano Guidance Package, Part II: Interpretation of the Definition of the Term 'nanomaterial' according to the EU Cosmetic Products Regulation 1223/2009;

Note: this guidance is considered as being clear by the respondents

- All ECHA guidance documents addressing aspects specific to nanomaterials are a source of complementary information

  Note: It is not regarded as being entirely clear because it does not fully reflect the outcome of on-going discussions in various ECHA, OECD, etc. bodies.
  Recommendations are seen as too general and seem to be based on RIPoN2 only, but they include a good listing of methods. Update should integrate the report from 1st GAARN meeting (Best practices on physicochemical and substance identity information for nanomaterials), the JRC Communication, and recommendations of the OECD WPMN (notably the Guidance on Sample Preparation and Dosimetry for the SafetyTesting of Manufactured Nanomaterials ENV/JM/MONO(2012)40).

- The JRC-Eurocolour interlaboratory study on commercial pigments is also listed as guidance document several times

  Note: his report is not yet published as of March 2014

- Several organisation internal briefings on the EC definition, which were subsequently published
- The article *Considerations on the EU definition of a nanomaterial: Science to support policy making* (Bleeker E.A.J. et al., Regulatory Toxicology and Pharmacology 65 (2013) 119–125)
- Interpretation and implications of the European Commission Recommendation on the definition of nanomaterial. National Institute for Public Health and the Environment (RIVM Letter Report 601358001/2012, The Netherlands)
- Current discussion in ISO/TC 229 on 'the tiered approach identifying nanomaterials and not nanomaterials to the definition'
- Nano support project, scientific technical support on assessment of nanomaterials in REACH registration dossiers and adequacy of available information, final report on analysis and assessment (Task I, step 3 & 4 & 5) and options for adapting REACH (Task II, step 1), 12 March 2012.
- DG-SANCO guidance on nanomaterials (http://ec.europa.eu/health/scientific_committees/opinions_layman/nanomaterials/en/index.htm)
- Information from the German National Helpdesk 'Guide on Characterization of nanomaterials'. The Federal Institute for Occupational Safety and Health (BAuA) August 2012
- ECHA presentation: Characterization of nanomaterials for REACH dossiers - best practice. Abdelqader Sumrein.30 October 2012 - ECHA information http://echa.europa.eu/chemicals-in-our-life/nanomaterials
- ISO/TC 229 approval of different measurement methods
- ISO/TC 256 Sample preparation for Disc Centrifuge Particle sizing
- Smart nano EU Method development project
- COM(2012) 572 final and SWD(2012) 288 final are also listed as guidance by one respondent, including the SRI report referred to in these documents

7.2.4 Has your organisation been facing issues in implementing the definition's specification on size distribution?

Replies to this question:

Yes	44	70%
No	19	30%

If the respondents selected 'yes' they were asked to describe these issues in more detail.

The following issues were indicated, the overwhelming majority of them coming from trade and industry associations and from private companies:

- The absence of standard methods available to measure the PSD in a regulatory context.
- The absence of guidance for measurements
- Measurement of the number based size distribution: companies have little or no experience, equipment is not available in the companies, high cost and time consuming, no validated methods exist for measurement and for sample preparation, but results depend on the method used and on sample preparation. Representativeness of the sampling and of the measurement is an issue. Aggregates (and their constituent particles) are an issue. Measurements are difficult for polydisperse materials or non-spherical particles.
- Sample preparation in itself is a problem, because it can change the size distribution of a material
- Conversion from mass/volume based to number based distribution is problematic and error-prone. However, most of the methods that are readily available to companies produce mass based distributions, which need to be converted to number-based size distributions.
- Specific measurement methods are applicable only for a narrow size range. This creates a problem if the material has a broad size distribution.
- Some minerals with a sheet or needle-like structure (1 or 2 dimensions in the nano range) would be regarded as nanomaterials. They cannot be measured with light diffraction techniques because the evaluation of the data is normally based on the assumptions that the particles are quasi-spherical.
- Uncertainty regarding the nanolabelling of cosmetic products. In the current legally binding definition in that regulation there is no threshold above which an ingredient would be regarded as nanomaterial. In that Regulation the terms intentionally manufactured, soluble and biodegradable have not been defined either.
- If a company uses a material supplied by a third company, the reliability of the given information is uncertain.
- There is little added value to measure the number based size distribution of certain materials which are already registered in REACH and have a well-established toxicological profile.
- For implementation in sectorial Regulations the threshold of 50% appears too high
- The decision whether a material is a nanomaterial may need to rely on the opinion of an expert team.
- By trying to make the definition all-encompassing, many materials are unintentionally included for which no concerns, uncertainty, or even nano-specificity, exists.
- It is not clear to which extent the so called 'nano-tail' must be considered for substances, which are actually not considered as manufactured as nanomaterials by the concerned companies. Within a narrow interpretation many unexpected substances would suddenly be/are nanomaterials.

7.2.5 Does your organisation make use of size distribution measurements of particulate materials?

Replies to this question:

Yes	42	67%
No	21	33%

If the respondents selected 'yes' they were asked to identify the material(s) for which the method is used and which methods are used in-house:

For each method listed, please identify the material(s) for which the method is used.

Which of these methods are used by your organisation in-house?

The respondents were also asked whether there are borderline cases:

Are there borderline cases, i.e., materials for which it was difficult to decide whether they are nanomaterials according to the EC definition?

Replies to this question:

Yes	30	71%
No	12	29%

The respondents were also asked to describe such borderline cases.

Replies to these questions in tabular form as submitted in response to the survey can be found in Annex D to Section 7.

Notes to the replies:

- Size distribution measurements obtained with a variety of methods were indicated by the respondents. These methods include all methods as discussed in the JRC Reference Report (EUR 25404 EN) on measurement methods. Large companies with a portfolio of particulate materials generally are well equipped with a variety of complementary methods. Smaller companies often have a selection of methods available in house and use external laboratories to obtain additional measurement data on their products
- In a number of cases the material for which the method(s) are used is not given by the respondents
- Addressees were asked to provide actual methods used and the materials to which they were applied. General discussions on measurement difficulties were not expected as replies for these questions.
- The addressees were also asked to provide specific borderline cases rather than a theoretical discussion on hypothetical cases. However, although in many cases the respondents mention that there are borderline cases, they did not provide an actual description of such cases. Rather, they provide a more general discussion on why many borderline cases are expected or obtained with specific methods, or in relation to the wording of the EC Recommendation.

7.2.6 Are you aware of measurement methods that have recently been developed or improved in a way that makes them a likely candidate method to help you implement the EC definition of nanomaterial in the near future?

Replies to this question:

Yes	20	32%
No	43	68%

If the respondents selected 'yes' they were asked to list the sources of their information (publications, reports, etc.) for each proposed method.

The responses often included methods as well as documents which give guidance rather than reporting newly developed measurement methods. Both are listed below:

- *Methods:* Field Flow Fractionation, Single Particle Inductively Coupled Plasma Mass Spectrometry, Nanoparticle Tracking Analysis
- automated EM analysis, sp-ICP-MS, (micro droplet generator)-MDG-ICP-TOF-MS
- Expectations for new methods from the NanoDefine project (started in November 2013) were listed several times
- *Guidance documents*: Techniques discussed in the JRC Reference Report EUR 25404 EN, European Union, Luxembourg (2012): Requirements on measurements for the implementation of the European Commission definition of the term 'nanomaterial'.
- Report on the JRC-Eurocolour project (expected to be published soon)
- ASTM, 2012. E2834-12 Standard Guide for Measurement of Particle Size Distribution of Nanomaterials in Suspension by Nanoparticle Tracking Analysis (NTA). http://www.astm.org/Standards/E2834.htm
- REPORT FOR INTERNATIONAL COOPERATION ON COSMETICS REGULATION Joint Regulators - Industry Working Group: Characterization of Nanomaterials III - Solubility, Stability & Persistence and Size Measurement in Complex Media – draft final report May 2013. Section 6.2.2 in the report provides a review of scientific literature where they have been looking at particle size in complex media e.g. final formulations
- ISO/AWI TR 18196 ISO/TS 10797:2012 and ISO/TR 10929:2012 ISO/TS 11931:2012 and ISO/TS 11937:2012 ISO/TS 17200:2013

7.2.7 Would you consider pragmatic solutions such as measurements of other, related material properties (e. g., specific surface area), and/or provision of information about the manufacturing process be acceptable as a substitute for size measurement for specific regulatory purposes?

Replies to this question:

Yes	36	57%
No	16	25%
No opinion	11	18%

If the respondents selected 'yes' they were asked for further information:

If yes, please specify and give reasons

- The replies did not show a clear trend. Several respondents had doubts whether 'pragmatic' solutions were helpful, whereas others would take them into consideration, if the measured alternative properties are unequivocally linked to size distribution. The assumptions upon which a certain solution is based must be transparent. In any case 'pragmatic' solutions should arrive at a consistent decision.
- Information on the manufacturing process was seen as helpful, if it yields consistent size distribution
- *Specific surface area (SSA) measurements* would be acceptable if specific surface area is indeed easier/cheaper to measure and unequivocally linked to size distribution. However, substituting size measurements with information about the manufacturing process may only be appropriate if there is sufficient evidence that the specific manufacturing process is yielding consistent material size distribution.

- SSA can be used in combination with information about the manufacturing process; it could also be used within a tiered approach. Sometimes BET may be sufficient; SSA could be used as a predictor. Volume-specific surface area (VSSA) can be good for substances, but poor for mixtures.
- Other respondents rejected SSA as alternative method. VSSA is of limited value and only applicable to dry matter with spherical uncoated particles
- Surface area measurement with the BET technique enables to calculate particle size. Thus, if the use of an alternative sizing method is justified and applicable for the specific particle type, it should be considered
- A risk based approach should be considered that includes for example exposure – if no exposure is expected there would be no need to measure
- Conditions of use should be taken into account (i.e., is release expected?)
- Dustiness could be used as proxy (for materials that only exist as aggregates)
- Only 'relevant' nano-dimensions should be considered, i.e., those that determine the physicochemical properties of the material, maybe in combination with BET
- Use of 'read across' based on similar chemistry and application could be envisaged
- Knowledge of production conditions or agreed sample preparation techniques for similar materials
- Possible proxies can make use of properties of particular materials (e.g., for pigments the particle size is in direct relation with the colour and other characteristics)
- There might be some solutions to avoid TEM measurements. See, for example: (i) J. Ceram. Sci. Tech., 04[02] 93-104 (2013) DOI: 10.4416/JCST2012-00045, Brown, S. C., Boyko, V., Meyers, G., Voetz, M., and Wohlleben, W. Towards Advancing Nano-object Count Metrology – A Best Practice Framework; (ii) Environ. Health Perspect., Wohlleben, W and Müller P, Classification strategies for regulatory nanodefinitions in "Nanomaterials throughout their lifecycle: Human exposure, hazard, safety". eds. Wohlleben, Kuhlbusch, Lehr, Schnekenburger. Taylor & Francis 2013.
- As far as no reliable method exists, other solutions shall be used; Problem: link of method (e.g. spec. surface area) to nanoscale, which is also not given in all cases
- A tiered or two-step approach is suggested (activities in ISO/TC 229), e.g., SSA-TEM, DLS with specific dispersion instructions – e.g., recommending shear forces to be used prior to DLS as part of the sample preparation

In a number of cases the 'pragmatic' solutions suggested would require a change of the wording or the scope of the definition. These suggestions are included in the replies to the question *'Do you propose any change to the EC definition?'* (see below).

7.2.8 Do you propose any change to the EC definition?

Replies to this question:

Yes	53	84%
No	10	16%

The respondents were also asked to specify their reply to this question:

Please specify and/or give reasons for your answer to the previous question.

The respondents proposed changes to different elements of the current definition. These changes are here grouped according to those elements.

- Size, size range
    - A new open discussion should be conducted on the size range
    - Size should be understood as equivalent spherical diameter
    - There should be an upper and lower limit below and above which particles should not be included when determining the number size distribution threshold of 50%.
    - Is there an actual need for a lower limit of 1 nm? Could the definition instead potentially state that the definition of nanomaterials applies to measurable materials below 100 nm?

- Threshold
    - The threshold (of 50%) should be reconsidered
    - The metrics for the threshold should be mass based
    - The 50% number threshold should be replaced by 10% mass threshold
    - Measurement of mass based particle size distribution should be accepted until there are standardized methods for size distribution (because at present there are no definitive recommendations as for the most suitable dose metric for the risk assessment of nanomaterials)
    - The scientific basis for the specified 50 % threshold should be reviewed and the threshold should be lowered based on the level of knowledge or data uncertainty to a threshold between 1% and 50%.
    - A threshold for 50% or between 1 and 50 % for EHS issues should be more discussed for the regulatory purpose.
    - The threshold '50% particles in the number size distribution' should be significantly higher to include fewer substances.

- Coverage of specific materials
    - Only manufactured/ intentionally manufactured nanomaterials should be covered
    - A clarification that 'manufactured' means 'intentionally manufactured to give new properties' should be included
    - The very broad scope goes beyond a reasonably manageable scale for regulatory agencies and industry. For this reason the focus should be on nanomaterials, which are developed to exhibit novel characteristics, such as improved physical or chemical properties compared to the same material without nanoscale features.
    - Nanotubes in general should be included (also TiO_2, silica nanotubes)
    - The reference to the graphene based materials (fullerenes, single wall nanotubes and flakes) in point 3 of the commission recommendation should be extended to also encompass other shapes/forms of graphene materials, such as cones and ribbons
    - Single wall nanotubes, flakes and spheres made from other materials than carbon should also be considered for the derogations in point 3?
    - The definition should cover only solid materials and this should be explicitly stated in the definition
    - A risk-based approach supported by independent experts should be applied when defining nanomaterials, and in particular it is recommended to exclude nanomaterials with only 1 dimension in the nanoscale (platelets) from the scope of the nanomaterial definition due to negligible risk.
    - Traditional materials such as Carbon Black (for which robust safety data exist) should be excluded from the definition.
    - Porous materials should be discussed

- Additional or fewer properties

    - The definition should also take into account novel functions or nanospecific properties
    - Aggregates should be excluded (they do not release NPs, and it is difficult to distinguish constituent particles), and only agglomerates should be retained. If the phrase on aggregates/agglomerates would be deleted, simpler and less expensive methods could be used (e.g., DLS)
    - The main issue of concern should be the distinction between an agglomerate that might release nanoscale particles and an aggregate that does not
    - Only insoluble materials relevant for regulatory environments should be included – soluble nanomaterials need not be covered by the definition because special considerations for regulatory purposes would not be needed
    - The VSSA proxy should be taken out
    - The aspect ratio could be included as additional property
    - The definition should be based on differences in toxicological behaviour due to the size of the particles, not on the size only
    - Parameters responsible for the most adverse effects should be determined and measured
    - The definition should not cover any material that due to its manufacturing contains a low amount (usually < 1% by weight) of nanoparticles. This could be achieved by specifying in the definition that only materials for which the medium particle size by weight is < 500 nm or for which the amount of nanoparticles in the particle size distribution by weight is above 1%.

- Terminology/wording

    - The definitions of 'constituent particle' and 'unbound' should be clarified
    - Constituent particles could be defined as not breakable and fused from primary particles
    - The term 'nanomaterial' should be replaced by 'nanomaterial mixture'. If there are more than 50% nanoparticles it should be called 'apparent nanomaterial'

- Regulatory relevance

    - An automatic trigger should be introduced to lower the threshold in the Recommendation as soon as new measurement methods become available, to avoid long revision processes. Such an automatic lowering of the threshold should also be applicable to sector specific Regulations (Cosmetics, Food).

- Suggestions which address guidance

    - BET should be used only in certain cases, which need to be specified.
    - VSSA should be allowed as negative criterion (A material should not be considered as a nano-material in the sense of the definition if the specific surface area by volume of the material is lower than 60m^2/cm^3.)
    - An explicit statement that large molecules, even if crystallized, are not nanomaterials should be made.
    - An explicit statement is needed whether or not all crystallized large molecules which are milled to powder, or suspensions of any large molecule, must be analysed if they fall into the scope of the EC Recommendation.
    - The best improvement would be to distinguish materials to airborne and solid/dispersed. For each category it would be much easier to stipulate measurement method and relevant size distribution threshold for distinguishing between nanomaterials/non-nanomaterials.

- The following definitions were suggested in two replies

    - *`Nanomaterial' means an insoluble or biopersistent and intentionally manufactured material which meets the following two criteria: (i) containing particles, in an unbound state or as an agglomerate and where, for 1% (weight/weight) or more of the particles in the size distribution,*

the three external dimensions are in the size range 1 nm-100 nm, and (ii) which exhibits new characteristics in contrast to the same material without nanoscale features.

- First, nanoparticles should be defined and then nanomaterials. Based on this a definition could be: *'Nanoparticles are defined as natural, incidental or manufactured particles with one or more external dimensions is in the size range 1 nm - 100 nm. By derogation from the above, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanoparticles. Materials in which, based on number size distribution, 50 % of their constituent particles are nanoparticles are defined as nanomaterials. Nanoparticles may occur in nanomaterial in unbound state, as aggregates or agglomerates or may be incorporated to a solid or liquid matrix.'*

- Miscellaneous suggestions

  - Definition should be used internationally (including non-EU)
  - For identification of primary particles an 'actual dispersion technique' should be used
  - Quantitative criteria should be removed. This way the implementation of the EC Recommendation would not have to rely on measurements.
  - Harmonization with ISO definition would be beneficial
  - The size-label nano does not represent an intrinsic hazard characteristic. Therefore, size should not trigger regulatory requirements and the respondent recommended not to use a regulatory definition at all. The ISO definition could be used for technical standardisation.
  - The definition is a starting point, which now has to be tested and possibly revised.
  - It should be specified that any nanomaterial has a defined physical boundary. This could be used to distinguish a nanomaterial from a (nano)porous substance.
  - Specify for each element of the definition an analytical method with a reference to an international standard
  - The best way how to improve the definition is to define measurement method according to the category of material

7.2.9 Request for additional comments

The respondents could also any additional comments which they felt would be of particular use in the review process of the EC definition of nanomaterial. Here, some of the replies reiterated answers to previous questions.
The main points indicated in the replies are listed here.

- Criteria must be measurable, and no interpretation should be required

- Guidance for implementation of the definition in each piece of legislation would help clarify what it means in practice when a material fulfils the criteria.

- A tiered approach would be useful

- The definition is less than two years old and not implemented fully in any legislation. Innovation on the development of new methods is driven by necessity and 2 years is short in that context. There is not very much experience in implementing the definition and so changes at this stage may be premature. More experience with the current definition is therefore needed.

- No explanation is given for the derogation for fullerenes, graphene flakes, single wall carbon nanotubes. Other non-carbon substances could show similar properties.

- For more details a round table discussion of analytical experts (including industry) and regulators may help. The economic impact and competitiveness of the European industry has to be considered as well in this respect.

- The use of several definitions within the European regulatory framework is a drawback.
- The added value derived from creating an arbitrary category of matter (nanomaterial) is questionable
- Once a revised definition is developed, a robust review and comment period by stakeholders would be appreciated.
- A regulatory definition of nanomaterial is not necessary.
- The respondents mentioned several times that any definition for regulatory purposes must be accompanied by the analytical methods to be used. These methods and protocols have to be validated according to scientific standards; otherwise the definition could not be used in regulatory context.
- If a threshold is kept in the definition, it should not be lowered below 50%
- If only big companies can afford relevant measurement equipment the free movement of substances, mixtures, articles and products would be no longer ensured.
- The following criteria could be applied for the establishment of a product register which should be used as a basis for risk assessment: (1) Consideration of the Commission Recommendation on the definition of nanomaterial as an initial point, (2) Limitation to specifically manufactured nanomaterials (no natural or biological materials should be included), (3) Exclusion of products where exposure of nanomaterials is most likely irrelevant (e.g. the interior of electronic devices)
- The EC definition is welcome in focusing attention on the reality of existing materials, but it does not answer all concerns because the question is phrased poorly. It will always be size and properties, not size alone.
- Particle coatings will also become an issue and are not addressed at all. There are no naming conventions for particle coatings.

7.2.10 Other than the EC definition of nanomaterial, are there any other relevant 'nanomaterial' definitions in the area (geographical or sectorial) relevant for your organisation?

Replies to this question:

Yes	45	71%
No	16	25%

If the respondents answered 'yes', more information was requested:

If yes, provide a reference to this/these definitions and specify the most significant difference(s) between the EC definition of nanomaterial and the other definition(s).

All relevant definitions are listed in Section 2 of this report and differences to the EC Definition of nanomaterial are discussed in Section 2 as well. The following definitions were mentioned in the replies. The need for a harmonised definition was highlighted by many respondents.

- Definition of nanomaterial in the Regulation on Biocidal Products 528/2012
- Definition of nanomaterial in the Cosmetic Products Regulation 1223/2009
- Definition of nanomaterial in the Regulation in the Provision of Food Information to Consumers 1169/2011

- Definitions summarized by the American Chemistry Council http://nanotechnology.americanchemistry.com/Nanotechnology/Panel-Activities/Nanotechnology-Definitions/Nanotechnology-Panel-Presents-at-Society-of-Toxicology.pdf
- ISO/TC 229; Nanomaterial definitions in the ISO/TS 80004-1,
- OECD definition of nanomaterial
- The definition used in the French Decree of 17 February 2012 (Decret No 2012-232): Art. R. 523-12.
- Definition by US-FDA
- Definition by NICNAS (Australia)
- Definition by Health Canada
- Belgium – Definition in the proposed registration obligation
- ICCA definition

7.2.11 Which recent scientific publications are particularly relevant for the implementation and review of the EC nanomaterial definition? (Max. 10 publications)

The publications listed in the replies to this question are included in Section 8 of this report.

8 LIST OF SIGNIFICANT RELATED ACTIVITIES (E.G. FP7 RESEARCH PROGRAMMES, SCIENTIFIC REVIEWS AND OPINIONS) THAT ADDRESS THE SCIENCE BEHIND THE MAIN ELEMENTS OF THE DEFINITION AND ITS APPLICATION E.G. HAZARD VS. PARTICLE SIZE

8.1 Introduction

On the 18th of October 2011, the European Commission (EC) recommended that the definition of a nanomaterial includes "natural, incidental or manufactured materials containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of particles in the number size distribution, one or more external dimensions is in the size range 1 nm – 100 nm", where particles are defined as minute pieces of material with defined physical boundaries, an aggregate is a body of two or more particles that are strongly bound or fused together, and an agglomerate is a body of two or more particles that are weakly bound together by physical interactions, e.g., van der Waal forces. Additionally the application of volume specific surface area was also acknowledged as a characteriser of potential nanomaterials.

In this chapter lists of significant scientific activities (e.g. FP7 research programmes, scientific reviews and opinions) which address the science behind the main elements of the nanomaterial definition are introduced. For the clarity of the document, the lists are included in Annex 1 (Relevant European Commission Scientific Projects), Annex 2 (Relevant peer reviewed scientific literature) and Annex 3 (Relevant "grey" literature: report, books and opinions) to Section 8. A selection of references, which address issues beyond the elements included in the EC recommendation, such as a possible relation between particle size and hazard, was also identified.

8.2 The choice of relevant references

The EC Definition of nanomaterial considers three basic features of nanomaterials, namely size of particle/aggregate/agglomerate, the number based particle size distribution and the volume specific surface area of the material.

With respect to these parameters the following references were considered to be relevant for the scope of this review:

- References in which the meaning of *particle size distribution in mass, volume and number* is discussed along with the challenges related to the appropriate metrology. Special attention was paid to the references which discuss these issues in view of regulatory requirements and standardisation.

- References which discuss the *influence of the size of the particles/agglomerates/aggregates on the physico-chemical properties as well as on the toxicological profile* of the material. Specifically

references were considered in which additional information on a threshold of the size of the particles/agglomerates/aggregates, at (below or above) which particular changes in the characteristics of the nanomaterial can be observed.

- References in which the *impact of the specific surface area on the physico-chemical properties and toxicological profile* of the material are discussed.

Although the nanomaterial definition does not take into account information on coating or any other surface treatment, additional representative references on the influence of the coating/corona on properties of nanomaterials were also considered valuable for the purpose of this review. Furthermore also references in which the EC Recommendation for the nanomaterial definition was critically discussed were considered relevant as they may bring additional perspective to the discussion. Even though the EC Recommendation for a definition of nanomaterial includes all types of materials, i.e., 'natural, incidental or manufactured', references, in which challenges resulting from identification of the origin of the nanomaterial are discussed, were taken into consideration as well, as they may highlight the difficulties associated with developing measurements techniques for regulatory purposes.

The search for the references was performed in the scientific database SCOPUS, in the Google Web Search, on the websites of the EC research programmes and on the specific websites of regulatory authorities.

The selection of the relevant scientific peer reviewed papers based on the results obtained from the search in the SCOPUS database in which different combinations of specific keywords (*nano**, *size*, **toxic**, *surface area*, *size distribut**, *definition, requirement**, *regulation** *method**, *metrolog**, comparis*, *EC*) were used.

The opinion of SCENIHR[11], which was used as one of the bases to the EC Recommendation for the definition of the 'nanomaterial', was published at the end of 2010 and includes references published up to 2010. Therefore the literature search considered only the period between 2008 and 2013.

The application of above given conditions on the 'title and abstract' of the article in the performed literature search resulted in more than 6000 publications, which potentially could be relevant for the scope of this report. Taking into consideration this high number of scientific sources the search was additionally limited to 'reviews' only which resulted in more than 600 articles out of which 91 were chosen as representative sample of relevant scientific literature. Nevertheless some original research articles were also included in the list, as they were directly associated with the basic elements of the recommended by EC nanomaterial definition.

The same criteria were applied on the Google Web Search and 39 items of grey literature were identified as relevant and included in the list of references.

Furthermore, for the purpose of this review only the research projects founded by European Commission Research programs were taken into consideration.

In the EC research program 2735 projects in which term 'nano' appears, were identified. 55 were considered to be relevant for the purpose of this review. The majority of the included projects belong to the NanoSafety Cluster initiative and their detailed description can be found in the Compendium of Projects in the European NanoSafety Cluster published in 2012 and 2013.

Additional references relevant to the scope of this review are also included in other sections (e. g., 2, 6, 10, 15 and 16) of this report.

9 ELEMENTS IN THE DEFINITION THAT COULD BE FURTHER CLARIFIED

9.1 Background

In the survey mentioned before, the following questions were asked:

- 'Is the wording of the EC definition of nanomaterial clear and unambiguous?'
- 'Is it clear to which materials the EC definition of nanomaterials applies?'
- 'Are the individual elements (terms, thresholds, etc.) of the EC definition clear?'

For each of the above yes/no questions, the majority of answers was negative (about 2/3). With an industry participation of about 2/3 in the survey (combining results of industry or trade associations and private companies) an easy but premature conclusion is that the definition is not 'clear' to industry. However, a more detailed analysis of the results moderates this conclusion: while the majority of industry responses to the above question are negative, indeed, some of the industry respondents find the definition clear. And vice versa, while most of the non-industrial respondents find the definition clear, a significant number of non-industrial respondents find (aspects of) the definition not so clear.

More and useful information is obtained when analysing the answers to the three follow-up questions, corresponding to the three yes/no questions:

- 'Please explain why you do not consider the wording as clear or unambiguous.'
- 'Please explain why it is not clear to which materials the EC definition of nanomaterials applies.'
- 'Please identify the elements that are unclear and give reasons.'

In the following, first a summary is given of the 'raw' responses to the survey. Thereafter, separate sections are devoted to a number of elements or terms in the definition which have been mentioned most. These sections also build on feedback received from stakeholders through routes other than the survey, as well as feedback from within JRC.

9.2 Survey responses

9.2.1 Is the wording of the EC definition of nanomaterial clear and unambiguous?

As stated above, the majority (41 out of 63) of the responses were negative. Authorities and NGOs more often replied 'yes', whereas trade and industry associations and private companies more often replied 'no'.

If the respondents selected 'no' they were asked to explain why they do not consider the wording as clear or unambiguous. The following are the main points that were addressed in the replies (including a number of points that do not exactly answer the question):

- The scope is too broad, it should be narrowed and be more precise.
- The regulatory purpose of the definition should be included in the definition itself.

- Clarification and/or precise definitions should be provided for the following terms: 'natural', 'incidental' and 'manufactured'; 'particle' (specifically in the context of size distribution could refer to 'aggregate' or to 'primary' particles); 'intentionally manufactured'; 'unbound state'; 'constituent particle'; 'aggregate' and 'agglomerate'; 'weakly' and 'strongly' bound

- The definition is ambiguous because there is no standardised or at least validated method how to determine whether a material is covered by it.

- Concerning the proxy function of VSSA, it is not clear when it can be used and when not.

- It is unclear whether non-solid materials are included or not. If they are not covered by the definition, then an explicit statement that, 'nanomaterials are solid state materials' is missing, as well as a statement that, 'large organic materials, even if crystallized, are not nanomaterials'.

- The definition of the EC actually covers an ensemble of 'nanomaterial' and 'non-nanomaterials', which is confusing. Suggestion: It should state, for example, 60% pure nanomaterials or replace 'nanomaterials' by 'nanomaterials mixture'.

9.2.2 Is it clear to which materials the EC definition of nanomaterials applies?

The majority (44 out of 63) of the responses were negative.

If the respondents selected 'no' they were asked to explain why it is not clear to which materials the EC definition of nanomaterials applies. The difficulties described fall into two main groups: (i) measurement-related and (ii) the terminology used in the definition.

The main measurement related points, which were repeatedly brought up, were:

- Because of the difficulties in reliably, or at least reproducibly, measuring particle size distribution by number and the lack of appropriate methods it would be hard for many materials to decide whether they would be covered by the definition.

- There is the problem that each measurement method, and often each instrument and each operator, produces a different result, sometimes differing by more than one order of magnitude. This is a big problem in determining whether a given material falls under the EC definition.

The points relating more to the individual elements and the terminology used in the EC definition were:

- It should be made clearer whether 'soft' and non-solid materials are included in the definition.

- In a strict implementation, the definition embraces almost any material including very coarse materials because of the 50% threshold.

- There is the need for clear interpretation of the term 'material' (e.g. vs. the term 'substance').

- It is not clear how non-pure substances (mixtures, nanoparticles embedded in a matrix, etc.) are covered by the EC Recommendation.

- In certain cases multiple interpretations of a single term are possible (referring to the Questions and Answers documents by DG ENV).

- If in a material nanoparticles are formed temporarily, is this a nanomaterial?

- The possibility to deviate from the 50% threshold creates some uncertainty. Clarification is necessary as to which circumstances might be required to modify the 50 % threshold in the number size distribution to a lower one (as is foreseen in the definition).

- The coverage of the following substances is not clear: amorphous substances like synthetic amorphous silica, carbon black, surface treated pigments, suspensions and emulsions, liquid aerosols.
- Clarification on other shapes of graphene besides flakes is requested.
- Should polycrystalline materials be regarded as aggregates consisting of nanoparticles?

Other points raised by the respondents were:

- The Recommendation has no legal basis yet in sector specific legislation and in REACH. If it were implemented in sector specific legislation then the various sectorial definitions should not deviate too much from each other.
- It is a recommendation and hence it is not legally binding, therefore the scope of application is vague.
- If this definition is used in regulatory frameworks it should be tailored to the materials targeted by these regulatory frameworks.

9.2.3 Are the individual elements (terms, thresholds, etc.) of the EC definition clear?

Again, the majority (40 out of 63) of the responses were negative. The respondents which selected 'no' were asked to identify the elements that are unclear and give reasons.

The main points are:

- The need to clarify or define the terms 'matter', 'material", aggregate', 'agglomerate', 'similar', 'particle', 'constituent particle', 'unbound state', 'weakly/strongly bound' and 'size' was mentioned repeatedly. The replies overlap with the replies to the question on whether the wording of the definition is clear.
- 'Matter' could for example refer to solid or liquid matter.
- For 'agglomerate' the definition in ISO/TS 27687:2008 was recommended.
- A clear definition for fused, non-breakable aggregates is missing.
- 'similar' – what is 'similar' in the definition of agglomerate?
- 'particle' needs a clearer definition (e. g., a particle comprised of other particles is a particle in itself)
- 'size' is not clear because its value depends on the measurement method. What is the 'size' of an irregularly shaped particle?
- The combination of several elements creates a challenge.
- When is a specific concern sufficient to warrant lowering the 50% threshold? Which error for determining the 50% threshold is acceptable?
- An upper size limit is lacking beyond which larger particles would not be taken into account for the size distribution.
- If other forms below 1 nm would become relevant, the definition would not cover them.
- ISO definitions on nanotechnology vocabulary (ISO TS 80004-1:2010) could be used for clarification of some terms.
- In general, confusion between the EC definition and that of ISO should be avoided.
- The proxy function of the VSSA is not clear.

- Are only solid materials covered?
- Measurement methods for the implementation of the EC definition should be given.

9.3 Analysis of the main recurring issues

9.3.1 The terms 'particle' and 'particulate'

The terms 'particle' and 'particulate' are used in several places in the recommendation.

It is argued by some respondents that the definition of the term 'particle' as 'minute piece of matter with defined physical boundary' can be interpreted in different ways. Also it is noted that the definition itself is not clear as to whether it is truly limited to particulate materials. On the latter issue, the 2012 Staff Working Paper on the nanomaterials[137] on the market, and the existing Q&A document[2], are more explicit than the definition itself. These documents also explicitly exclude the categories 'liquid droplets', 'proteins' and 'micelles'. It is suggested by many survey respondents to add the term 'solid' or 'solid state' in the definition. For micelles, it is argued that they should be excluded because their spatial structure is not fixed and can be broken apart by shear forces, whereas they would reappear when the shear forces are removed.

9.3.2 Size / external dimension / shape effect / size range / threshold

9.3.2.1 Size and external dimension

The term *size* is essential for the Recommendation, as well as the term *external dimension,* which is used explicitly in paragraph 2 of the definition. It is noted that, except for hard, dense, spherical particles, the terms *size* and *external dimension* are difficult to define unambiguously. The survey respondents often refer to and generally agree with the JRC Reference Report EUR 25404, of which Section 2.3 concludes as follows: 'Implementation of the definition requires measuring a suitable, commonly agreed characteristic external particle dimension under defined conditions and comparing the result with the limit values set in the definition (1 nm and 100 nm)'.

9.3.2.2 Size range over which to measure

The lower and upper sizes of the nanoscale as used in the EC nanomaterial definition are 1 nm and 100 nm respectively. But the definition de facto also requires measurements outside this size range, in order to evaluate the ratio of particles inside and outside the 1 nm to 100 nm range. In practice, this is an issue mainly for particles larger than 100 nm. Survey respondents indeed noted the need for considering an upper size limit above which particles should not be taken into account when measuring the particle number size distribution.

9.3.2.3 Threshold values

Clarification is also requested about the need to have the varying threshold value in the number size distribution (50 % down to 1 %). In the words of one of the survey respondents '…this may lead to the situation where the threshold is different in different legislative frameworks, leading to a certain material being identified as nanomaterial in one framework, while being identified as non-nanomaterial in another'.

9.3.3 Primary or constituent particle, unbound state, aggregates and agglomerates

Survey respondents point to the ambiguity in the use of the term 'unbound state' and question why on the one hand the definition is limited to unbound particles, but on the other hand includes aggregated particles, in which the particles are bound. Some note that only few (particulate) nanomaterials succeed to remain unbound in different media, whereas most nanomaterials either decompose to ionic form or automatically aggregate or agglomerate. Another question relates to the status of the nanomaterial when bound in matrix (e.g. a paint). As the particles are no longer unbound, does the material cease to be a nanomaterial? Or does the presence of the nanomaterial in the matrix render the composite material also a nanomaterial (e.g. carbon nanotubes reinforced plastic)? The issue of nanostructured materials is further discussed in Section 15.

Further, it is noted that it is often impossible to recognise the primary particles in an aggregate particle (e.g. in (partly) amorphous materials). It is pointed out that the term 'particle' in the context of most particle size analysis methods refers to the aggregate (following the use of adequate methods to disperse agglomerates), and not to the primary particles. A definition of what is understood exactly by 'constituent particles' was suggested as a way out.

Caveats are made about crystalline materials consisting of fused crystallites. In a conservative interpretation of the current nanomaterial definition, almost every crystalline powder would be a nanomaterial. This issue was also raised in Section 2.2.3 of the JRC Reference Report EUR 25404.

A survey respondent questions the use of ‘weakly’ and ‘strongly’ in the definitions of agglomerate and aggregate and warns that, 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.

9.3.4 Measurement methods and method-defined measurement results

Some survey respondents indicate that the definition as such is sufficiently clear, but that it is not clear how to implement it, i.e. to apply it to real materials. They argue that it will not be clear which materials are nanomaterials, as long as the validated and/or standardized methods for evaluating particle number based particle size distributions, taking account of practical constraints, are absent. A particular

challenge comes from the need to measure the minimum external dimensions of the particles, and it is claimed that this limits the number of directly applicable measurement methods. (See also Section 4.)
Respondents also point to the ambiguity that comes from the fact that size measurements are 'method-defined': different size measurement methods produce different size values for the same material, because they measure different size features. In that sense survey respondents claim that 'as long as no method (incl. sample preparation) is defined, discrepancies between the methods will lead to different results and finally to a non-uniform classification of one and the same material'.
The issue of measurement uncertainty is also raised: which measurement uncertainty is acceptable in the implementation of the definition? None of the respondents made any concrete suggestion on a way forward in this respect.

9.3.5 Specific surface area

Currently, the definition lists two independent criteria to define a nanomaterial. The primary definition is based on particle size: a nanomaterial contains particles of which 50% have one or more external dimensions between 1 nm and 100 nm. A second approach is offered as well: a nanomaterial has a volume-specific surface area of > 60 m^2/cm^3. The latter value corresponds to the volume-specific surface area of perfect spheres with a 100 nm diameter. It must be noted that this surface area criterion cannot be used to disqualify a material as a nanomaterial. (Note also that there is no upper value for the surface area value that corresponds with the lower size value of 1 nm.)
However, there are many materials with particle sizes larger than 100 nm which have volume-specific surface areas larger than 60 m^2/cm^3 (e.g. microporous materials). It was suggested to add an explicit statement that a material with a VSSA greater than 60 m^2/cm^3, is not a nanomaterial if its number size distribution shows less than 50 % of the particles with one or more external dimensions in the size range 1 nm to 100 nm. This is of particular relevance to materials coated with a porous or non-dense surface layer, for which the simple relationship between particle size and surface area is lost.

9.3.6 'To contain' or 'to consist of'

The English version of the nanomaterial definition uses the word 'contain': a material is a nanomaterial if it *contains* particles that meet a certain size distribution. In earlier, draft versions of the definition (e.g. as presented in the public consultation in 2010), the word *consist* was used instead of *contain*. A material *consisting* of particles is more defined than a material *containing* particles.
The use of the term *contain* in the released definition could suggest that also a material which only 'contains' a minor particulate fraction is a nanomaterial in itself, if the small particulate fraction meets the definition criteria. A strict interpretation of the definition in this sense renders practically every material and substance a nanomaterial; but it is clear for all involved in writing the definition that this was not the intention.

Interestingly, whereas the German version uses the term 'enthält' (= contains) the Dutch version of the definition uses the term 'bestaat uit', which is the equivalent of 'consist of'. Also the existing Q&A document uses the term 'consist of'.

Several respondents request to call a material containing e.g. 60 % of particles in the nanorange, a 60 % pure nanomaterial, or a 'nanomaterials mixture' (instead of a nanomaterial).

9.3.7 Nanomaterials not covered by the EC definition

Some respondents claim that the scope and purpose of the definition is not clear or sufficiently explained (e.g. should it include all nanomaterials, or only those of (regulatory) concern?). Several (EU and international, non-EU) respondents also mention the confusion arising from the existence of other international nanomaterial definitions, in particular the ISO definition. They call upon qualifying the term nanomaterial in the EC recommendation, e.g. with an adjective referring to its intended use, that is limited to or specific for regulation. Also CEN/TC 352 has recently voiced the concern that the EC recommendation is indeed not sufficiently clear in this respect. CEN/TC 352 therefore suggested that in the next version of the definition it should be mentioned explicitly that the definition is for use in the regulatory context, and therefore would define only nanomaterials that are relevant in the regulatory context (see also next section).

It is a fact that the current EC nanomaterial definition is focused on particles. This approach was inspired by the SCENIHR[11] and JRC[3] reports. SCENIHR and JRC recommended that in the first place be dealt with the particulate nanomaterials for which human and environmental exposure is more likely than for 'embedded' nanomaterials, as in electronic devices, where elements with nanoscale dimensions are strongly bound to larger scale structures. In the recently published Staff Working Paper on the nanomaterials on the market[137], a number of other types of nanomaterials are mentioned (such as the nanostructured materials discussed in Section 15), corresponding with definitions from e.g. ISO (also adopted by CEN).

A survey respondent pointed out that the definition should more clearly describe that a number of materials are excluded (examples given are mixtures, composite materials, non-particulate materials such as thin films and long nanofibers, large organic molecules,…). Others question why traditional materials (such as silica or carbon black) should now, suddenly, become nanomaterials. They suggest that these materials be explicitly mentioned as not being nanomaterials, which can only be solved by placing them in an exclusion list. On the other hand, one has also mentioned the need to extend the number of materials to be explicitly included in the definition (e.g. other forms of graphene materials).

9.3.8 Internationally harmonised terminology

Several respondents suggest a rephrasing of the EC Definition of nanomaterial using internationally accepted terms, e.g. terms defined in ISO, not with the intention to modify the EC nanomaterial definition, nor to approve of it. Instead, they call for rewriting the EC Definition in a clearer way.

CEN/TC 352 has made the following explicit proposal to achieve this (CEN/TC 352/N307, November 20, 2013):

"The fraction of nanomaterials[a] relevant for EU regulation comprises natural, incidental[b] or manufactured[c] material containing particles, fibres*, or plates in an unbound state or as an aggregate[e] or as an agglomerate[f] and where 50 % or more of the particles, fibres, or plates in the number size distribution are nano-objects[d] (i.e. nanoparticles[d], nanofibres[d], or nanoplates[d]).

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 %."

[a], [b], [c]: see ISO/TS 80004-1:2010; [d], [e], [f]: see CEN ISO/TS 27687:2008; *: fibres comprise wires, tubes, and rods similar to the definition of nanofibre in CEN ISO/TS 27687:2008."

Other respondents also refer to the terminology used in REACH, which is in some instances different ('material' vs 'substance', 'final product') from the terminology used in the EC nanomaterial definition, and suggest that some harmonisation could bring clarification.

9.3.9 Manufactured and engineered nanomaterials with unique nanoscale properties

The nanomaterial definition of the EC recommendation covers all materials, whether of natural origin, incidentally produced, or manufactured.

Nevertheless, the term 'manufactured' is not clear for all survey respondents. This is of more significance for the nanomaterial definitions in other EU regulatory documents, which limit the scope of their nanomaterials definition, e.g. by using the term manufactured (or 'intentionally manufactured' or 'engineered'). Survey respondents asked whether this term means 'purposely designed' or whether it can include 'produced incidentally' e.g. by milling of natural minerals.

Other respondents request the definition to be limited to manufactured or engineered nanomaterials. Thereby materials from natural or incidental origin would be excluded, which may be more in line with the regulatory ambition of the EC nanomaterial definition. Alternatively, it is also proposed to keep the current, broad, difficult-to-implement definition, and to define a second, clearer definition, for specific regulatory purpose.

A number of survey respondents question why the EC Commission recommendation does not restrict its field of application only to materials that have unique properties due to their nanoscale structure.

10 INFORMATION ON ACTUALLY MEASURED PARTICLE SIZE DISTRIBUTIONS FOR A REPRESENTATIVE SET OF MATERIALS (IN PARTICULAR BUT NOT EXCLUSIVELY NANOMATERIALS, I.E. ALSO OTHER MATERIALS WITH A HIGH SHARE OF FINE PARTICLES, E.G. REGISTERED OR NOTIFIED PURSUANT TO EU LEGISLATION), INCLUDING DETAILS ON THE QUANTIFICATION METHODS USED

10.1 General considerations

The following data on actually measured particle size distributions for a representative set of materials were selected from different sources and give information on a range of different materials potentially containing nanoparticles. In particular, the focus of the data is on industrial pigments, titanium dioxide and silicon dioxide.

The data presented here come from several different sources:

(i) Work carried out for a basic inter-laboratory comparison of particle size distribution measurements applied to industrial pigments and fillers. A report of that project is currently in preparation for publication.

(ii) The JRC scientific and policy report on 'Synthetic Amorphous Silicon Dioxide (NM-200, NM-201, NM-202, NM-203, NM-204): Characterisation and Physico-Chemical Properties'.[138] A substantial part of the data in that report comes from the NANOGENOTOX Joint Action in which several European laboratories participated. In addition, results and data from the JRC laboratories are included.

(iii) Data on the characterization of TiO_2 materials in the JRC repository of representative materials.

(iv) Data submitted to JRC following an invitation to industrial and public bodies to submit relevant information via a questionnaire on 'Experiences made during the implementation of the EC recommendation of a definition of nanomaterial' organized by JRC during the third quarter of 2013.

The particle size distribution data presented in the following sections have been measured with a variety of different techniques that include Electron Microscopy, Centrifugal Liquid Sedimentation, Dynamic Light Scattering, BET to measure specific surface area. These techniques measure different properties of the materials under study and give particle size distributions based on different metrics that are not easily and immediately converted one into another.[42] Thus, it is not unexpected that the results of PSD measurements could vary quite a lot between different techniques.

10.2 Data from a basic inter-laboratory comparison of particle size distribution measurements applied to industrial pigments and fillers

The study[D] was organized to evaluate a number of instrumental methods of measuring particle size distributions that have been applied to industrially relevant powder pigments. The aim of the study was

[D] A report on the outcome of that work programme, carried out by the JRC and Eurocolour (the association of European Pigments, dyes and fillers industry), is currently in preparation.

to produce and evaluate data which could be considered representative of that obtainable in industrial laboratories using existing instrumental facilities operated by experienced but not specialised operators. Four methods were used and benchmarked against Electron Microscopy (EM): Laser Diffraction, Dynamic Light Scattering, Centrifugal Liquid Sedimentation (CLS) and Volume Specific Surface Area (VSSA).

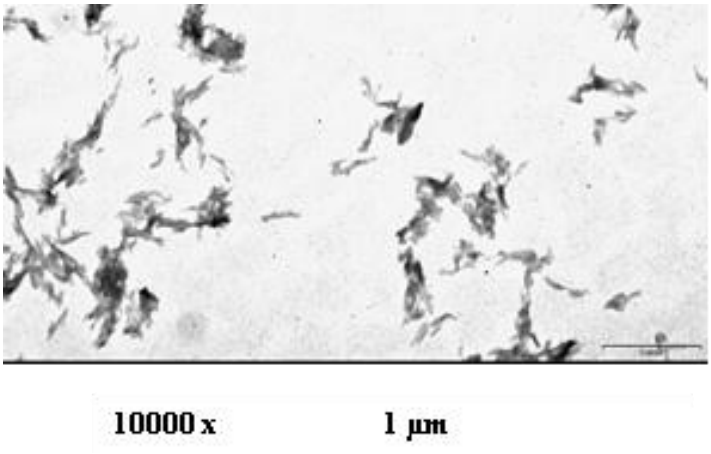
Of the eight analysed pigments we report here two cases: one material that clearly falls in the nanomaterial category and consists of needle-shaped constituent particles, and one material that the manufacturer claims is in the non-nanomaterial category, but which has a median constituent particle size of 130 nm (according to the manufacturer) not far away from 100 nm threshold of the nanomaterial definition.

For each sample we show a representative electron microscopy image taken by the manufacturer and a table summarizing some of the available information on the material and its particle size distribution as reported in the study. The various columns in the tables report the D50 number distribution (in nanometres) provided by the producer (column 1), the resulting classification according to the producer (nanomaterial, not nanomaterial) (column 2), the mean Volume Specific Surface Area (in m^2/cm^3) together with the min-max range measured by the different laboratories (column 3), the mean D50 number size distribution (in nanometres) together with the min-max range measured by the different laboratories by centrifugal liquid sedimentation (column 4).

Material 1. Organic azo-dye

This pigment is a transparent, reddish yellow organic pigment, which is mainly used in printing inks, plastics and industrial coatings. The pigment is strongly agglomerated/aggregated. The dispersibility especially in aqueous media is very poor. It is one of the finest organic pigments available. According to the manufacturer more than 99% of the primary particles have a dimension of less than 100 nm.

The image below shows an electron microscopy micrograph of the sample.



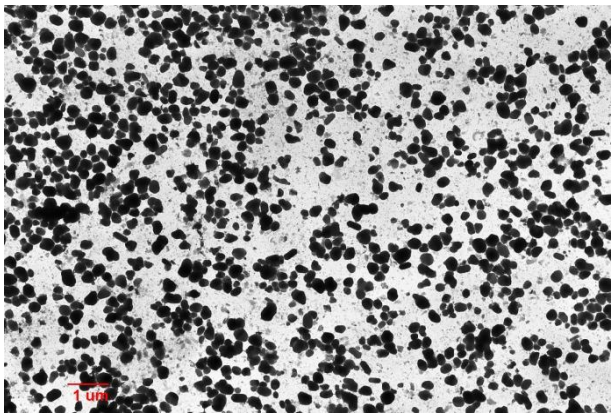
Organic azo-dye		
Size information from the manufacturer/nm*	VSSA (mean, range reported)	D50n (mean, range reported) CLS-based
47 nm*	85.7 (58.0 – 110.0) m^2/cm^3	42 (30 – 45, with one outlier of 29474) nm

(*type of size parameter not specified, manufacturer-supplied value)

Material 2. Titanium dioxide, anatase

This is an uncoated high purity titanium dioxide white pigment product optimised for use in fibres applications. It is of the anatase crystal form manufactured using the sulphate route process. Anatase products typically have a smaller primary particle size than the rutile crystal form.

The image below shows an electron microscopy micrograph of the sample.

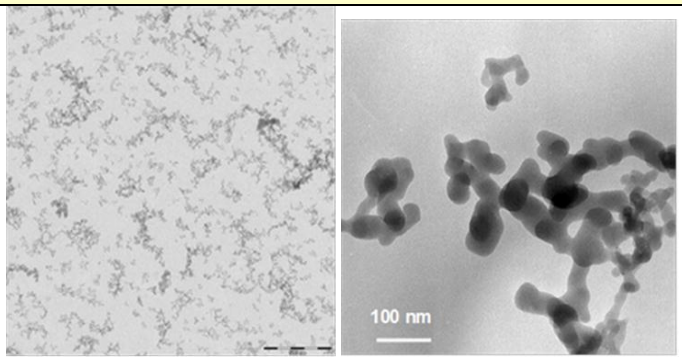


Titanium dioxide (anatase)		
Size information from the manufacturer/nm*	VSSA (mean, range reported)	D50n (mean, range reported) CLS-based
130 nm*	34.84 (33.0 – 37.3) m^2/cm^3	480 (13 – 1850) nm

(*type of size parameter not specified, manufacturer-supplied value)

10.3 Data from the JRC scientific and policy report "Synthetic Amorphous Silicon Dioxide (NM-200, NM-201, NM-202, NM-203, NM-204): Characterisation and Physico-Chemical Properties" including data from NANOGENOTOX

NM-203 is a synthetic amorphous silica produced via a high temperature process (the "thermal route"), hosted in the repository of representative test materials (RTMs) at JRC. RTMs come from a single batch, which is sufficiently homogeneous and stable with respect to one or more specified properties. They are implicitly assumed to be fit for their intended use in the development of toxicity methods which target properties other than those for which homogeneity and stability have been demonstrated.[67]

Method	Institution	Results
TEM	CODA-CERVA, IMC-BAS	High porosity nanostructured material which may be considered aggregates of primary silicon dioxide particles. Mean diameter (nm): 48 ± 4 Feret min: 33.5 nm (median of 4889) Feret max: 53.2 nm (median of 4889). % of aggregates <100nm: 88 ± 2. Morphology of aggregates/agglomerates: Low sphericity, angular.
Representative TEM picture(s)		
TEM	CODA-CERVA, IMC-BAS	 Aggregates with complex open structure.
Particle size distribution		
SAXS	CEA	Parameters could not be fitted.
TEM	CODA-CERVA	Primary particle size: 13 ± 6 nm
	IMC-BAS	Primary particle size: 45 nm
	INRS	Primary particle size: 16 ± 3 nm
TEM	CODA-CERVA, IMC-BAS	Number (expressed in %) of SAS NM particles smaller than 100 nm, 50 nm and 10 nm < 100 nm – 77.5%, < 50 nm – 48.4% < 10 nm – 0.3%
DLS	CEA	The material is polydisperse. The intensity size distribution, which consists of two main peaks is very broad and reveals the presence of large aggregates of few micrometres. Ultra-pure water dispersion (intra vial study) Z-average (nm): 172.9 ± 9.2. PdI: 0.427 ± 0.025, FWHM peak width: 82.5 ± 11.3

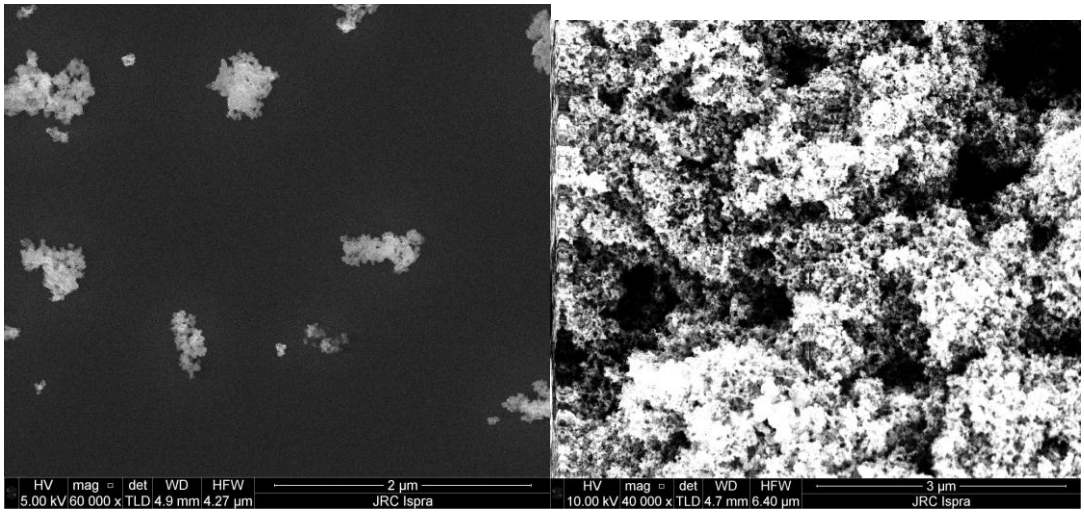
		Ultra-pure water dispersion (inter vial study) Z-average (nm): 176.9, PdI: 0.425, FWHM peak width: 73.15 nm
	JRC	The material is polydisperse. The intensity size distribution, which consists of two main peaks is very broad and reveals the presence of large aggregates of few micrometres. • miliQ water dispersion. Z-average (nm): peak 1: 133, peak 2: 221 • culture media dispersion Z-average (nm): peak : 94.5, PdI: 0.123 • PBS dispersion Z-average (nm): peak: 170.3, PdI: 0.202
	NRCWE	Ultra-pure water dispersion (intra vial study) Z-average (nm): 147.5±4.5. PdI: 0.244±0.017, FWHM: 84.4±10.4 Ultra-pure water dispersion (inter vial study) Z-average (nm): 146.8, PdI: 0.06, FWHM: 83.8±0.6
	INRS	The material is polydisperse. - Ultra-pure water dispersion (intra vial study) Z-average (nm): 245.7±37.2. PdI: 0.299±0.024
CLS	JRC	Peak (nm): 64, half width: 50, CLS PdI: 1.35
Specific Surface Area		
BET	IMC-BAS	203.92 (m^2/g)
SAXS	CEA	167.2±13.4 (m^2/g)
TEM-tomography	CODA-CERVA	219±23 (m^2/cm^3) (Volume specific surface area)
BET	JRC	Sample stored at 40°C: single point: 192.4628 (m^2/g); multi point: 198.0809 (m^2/g). Sample stored at -80°C: single point: 189.8376 (m^2/g); multi point: 195.4241 (m^2/g).

10.4 Data from the characterisation of TiO_2 materials in the JRC repository of representative materials.

Materials: NM100 (TiO_2 Anatase); NM104 (TiO_2 Rutile); NM105 (TiO_2 Anatase/Rutile)

Sample Reference	**Concentration (mg/mL)**	**Sonication (*)**	**CLS (Modal value of the weight distribution nm)**	**BET Surface Area m²/g**
NM100	0,1 mg/ml	15' VT	249	10,03
NM104	0,1 mg/ml	15' VT	64	57,07
NM105	0,1 mg/ml	15' VT	86	52,81

(*)VT = Vial Tweeter



Scanning electron microscopy of NM-104 Scanning electron microscopy of NM-105

The measured BET surface in m^2/g can be converted into volume specific surface area (VSSA) by multiplying it with the density of titanium dioxide (4.23 g/cm^3), thus obtaining VSSA values of around 40 m^2/cm^3, 230 m^2/cm^3 and 210 m^2/cm^3 for NM100, NM104 and NM105, respectively. These values are consistent with EM images that suggest the presence of aggregates formed by primary particles smaller than 40 nm for NM104 and NM105. It is worth noting that the CLS measurements are substantially larger than the values obtained from EM and BET measurements, probably due to the presence of strongly bound aggregates that cannot be dispersed. In any case, even the CLS measurements would lead to the correct classification of NM104 and NM105 as nanomaterials.

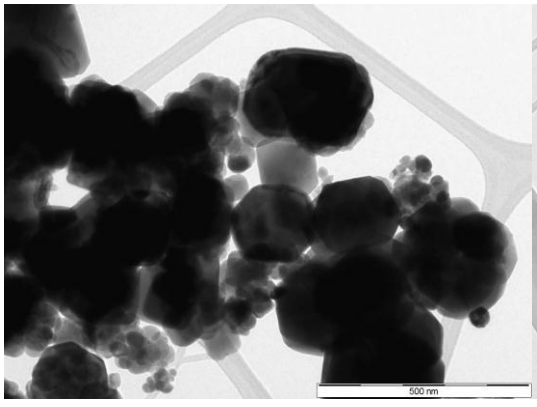
10.5 Data submitted to JRC following an invitation to industrial and public bodies to submit relevant data via the questionnaire on "Experiences made during the implementation of the EC recommendation of a definition of nanomaterial"

Twenty out of the 63 answered submitted (corresponding to 32% of the total) indicated that they have reliably measured particle size distribution for materials with a large fraction of fine particles. The replies provided quite varied levels of details that made in several cases difficult to estimate the quality of the data submitted. In this report the two submissions are included which provide sufficient details to allow an estimation of the data quality. This point highlights the need for the development of a harmonized approach for reporting data on particle size distribution of potential nanomaterials. The submission reported here as Case 3 did not provide particle size distributions or sufficient details to estimate the data quality. It is included here to illustrate some challenges associated with size measurements of constituent particles.

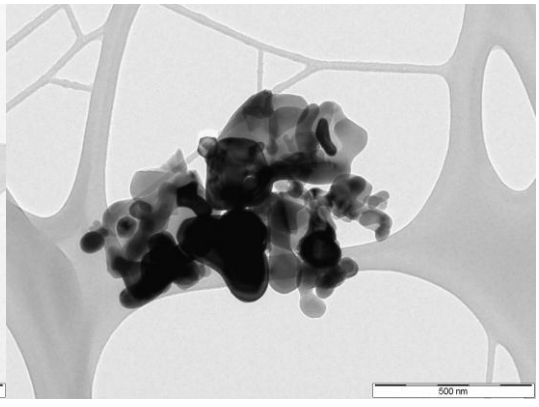
Case 1:

Data submitted from Precheza, Czech Republic (© by Precheza)

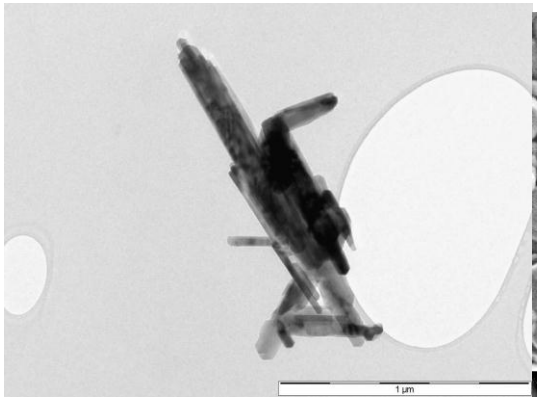
Type	Batch	DLS	VSSA	SEM		TEM	
		D50		minFeret	ECD	minFeret	ECD
		nm	m^2/cm^3	nm	nm	nm	nm
Anatase	143689	148	43	107	118		
Rutile - Surface treated	143255	160	105	166	193		
Rutile untreated	142989	167	33	141	163		
Red iron oxide	215355	222	51	107	124	94	110
Yellow iron oxide	900031	393	51	88	179	108	255
Black iron oxide	215048	104	47	93	107	97	106



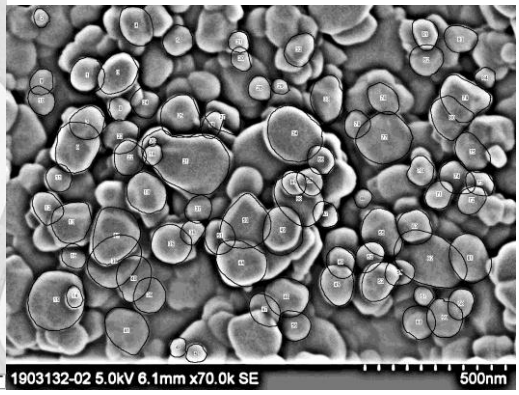
TEM image for Black iron oxide



TEM image for Red iron oxide



TEM image for Yellow iron oxide



TEM image for anatase TiO2

Case 2:

Data on Laponite.

There are four grades of Laponite in common use in cosmetic products.

Laponite® grade name	**IUPAC name**	**INCI name**	**CAS No.**	**EINECS No.**
Laponite® XLG	silicic acid, lithium magnesium sodium salt	lithium magnesium sodium silicate	53320-86-8	258-476-2
Laponite® XLS (see note 1)	silicic acid, lithium magnesium sodium salt	lithium magnesium sodium silicate	53320-86-8	258-476-2
Laponite® D	silicic acid, lithium magnesium sodium salt	lithium magnesium sodium silicate	53320-86-8	258-476-2
Laponite® XL21	lithium magnesium sodium fluoride silicate	sodium magnesium fluorosilicate	85085-18-3	

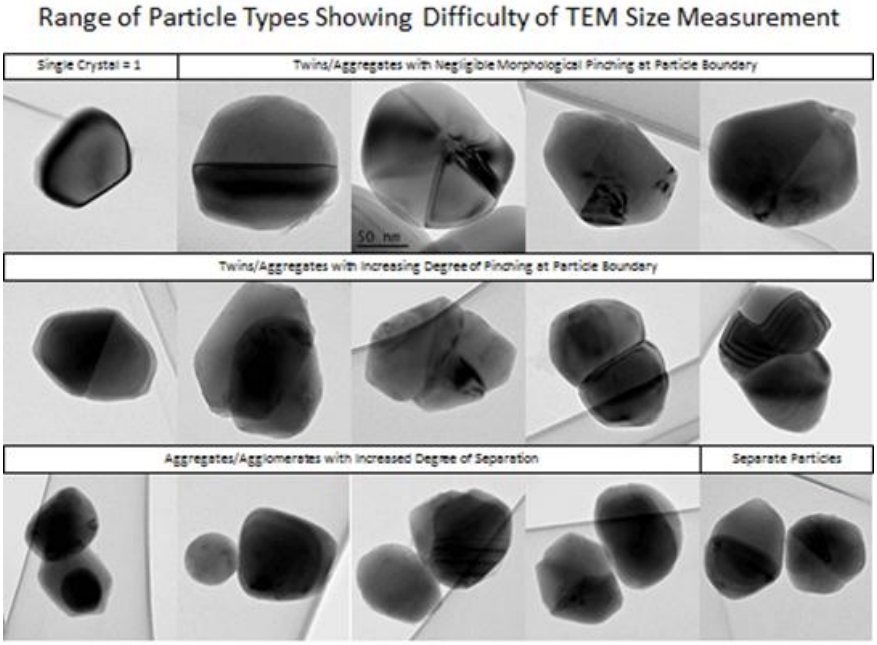
(Note 1: Laponite® XLS is a mixture of 90-95% of lithium magnesium sodium silicate and 5-10% of a dispersing agent which is an inorganic water soluble salt; the full INCI name for Laponite® XLS is lithium magnesium sodium silicate (and) tetrasodium pyrophosphate).

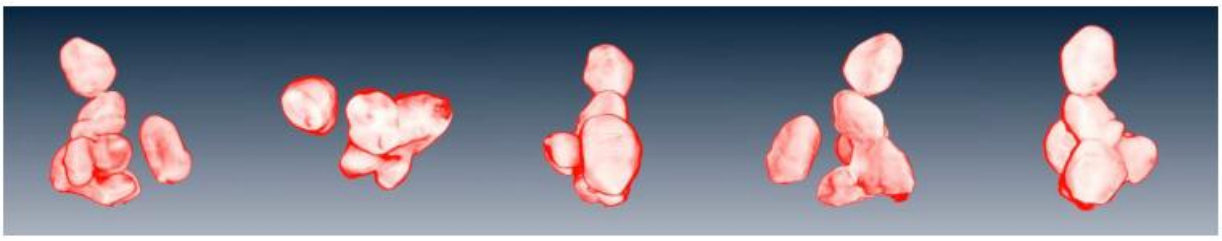
Class of compound: silicate

Laponite® type	**Type 1** **CAS no. 64060-48-6**	**Type 2** **CAS no. 53320-86-8**
Example grades	XL21	XLG, XLS, D
(a) "powder as supplied" average particle size (D50)	30 µm	48 µm
(b) "dispersed colloid" average particle size (Z50)	37 nm	32 nm

<u>Case 3:</u> Data submitted by CEFIC Titanium Dioxide Manufacturers Association (TDMA) on pigment grade Titanium Dioxide.

Sample preparation for TEM measurements is as follows: The TiO_2 is wetted in a suitable high viscosity liquid and then subjected to a shear force using a high-speed off-axis gyratory mill. The resultant dispersion is then diluted before being placed on to a carbon coated copper grid. The solvent is then flashed off before the sample is introduced into the TEM. The sample is examined and representative images captured for sizing by a commercially available image analysis package





Five different viewing directions of an electron tomogram taken of Pigmentary TiO_2, illustrating the challenges

The pictures (© by TDMA) illustrate difficulties firstly in determining the number of particles due to crystal twinning and aggregation and secondly the difficulties in counting the number of particles due to

overlapping of particles seen in the electron micrograph image (use of a 2 dimensional technique to count 3 dimensional particles).

The sample was also measured by BET giving a value of 9.3 m^2/g, which equates to a volume specific surface area (VSSA) of approximately 37 m^2/cm^3.

11 POSSIBILITIES AND LIMITATIONS OF ALTERNATIVES TO 'STRAIGHTFORWARD IMPLEMENTATION' (I.E. BY CONSTITUENT PARTICLE SIZE DISTRIBUTION DETERMINATION), SUCH AS MANUFACTURING INFORMATION, PROXIES OR CONVERSIONS

11.1 Introduction

It is clear from the results of the survey presented in previous sections, and various reports and publications recently[42] that the experimental determination of whether a sample of particulate material falls under the definition of 'nanomaterial' currently recommended by the European Commission poses significant difficulties and challenges.

This section will outline several possible approaches to 'straightforward implementation' (meaning possibly time-consuming sample preparation and direct, particle number based measurement of constituent particle size distributions of final products) that might be used to help to resolve this problem in some cases. These include:

1) Substitution of final product size distribution determination by size distribution determination (or other appropriate characterisation/information) of constituent particles sampled at a suitable stage in the manufacturing process of products or ingredients.
2) Use of proxy measurements such as Volume-Specific Surface Area (VSSA) determination, light scattering methods like DLS possibly combined with FFF, UV-visible spectrometry or similar techniques like colorimetry, sedimentation techniques, X-ray diffraction, etc.
3) The use of simple sizing and other analysis methods (e.g. BET, DLS, FFF/DLS, PTA, CLS, or optical spectrometry) as process control methods, or alternatively the careful monitoring of process parameters, that might be used in combination with less regular and more accurate particle sizing analyses. This pragmatic approach may allow a certain level of confidence regarding the size characteristics of manufactured particulates, while containing costs.
4) Manufacturing information, meaning that certain manufacturing techniques for certain materials will almost certainly result in constituent particle size distributions that fall within the current EC nanomaterial definition, so that without any accurate size distribution measurements these may be labelled as nanomaterials.

The section will discuss the possibilities and limitations of the above-mentioned alternatives in a more general rather than precise way, since some approaches may be applicable to certain products or processes while being unsuitable for others. Some of them may only assist manufacturers in correctly classifying their products and may not be of use to other laboratories attempting to analyse final products. Should an alternative to 'straightforward implementation' for product classification be proposed by either a manufacturer or regulator, it would probably have to be justified on a case-by-case basis.

Very many different methods may be used for particle/nanoparticle manufacturing. The most obvious classification is into bottom-up approaches, whereby the particles are created via chemical processes either in the gas phase or in the liquid phase, and top-down approaches, whereby solid materials, generally in the form of course mesh powders, are ground down into finer and finer particles. Other top-down methods are possible, but are not in general suitable for high volume nanoparticle production. Details of these methods are outlined elsewhere in this report.

11.2 Early sampling following primary/constituent particle synthesis – Sampling during the production process

Most bottom-up methods, whether gas-phase or liquid-phase are suitable for sample extraction and analysis immediately following (or even during) the particle synthesis process and before they are either incorporated into products, or collected and packaged for distribution as a raw material, or ingredient for other products. The samples thus collected will usually be simpler to analyse with respect to their constituent particle size distribution and often methods less expensive than electron microscopy (for example FFF combined with DLS) may sometimes be employed for this. Some knowledge of particle shapes should be available via microscopic analysis of the particles produced via the specific production process, and it is likely that the size measurement method would probably need to be verified by, for example, comparative TEM studies. Such sampling may provide an opportunity for the manufacturer to make a clear decision as to whether a final product falls under the nanomaterial definition or not. This may be a more economic and reliable method than post-production analysis of material that may have strongly agglomerated or changed in other ways, for example due to subsequent particle processing or storage conditions.

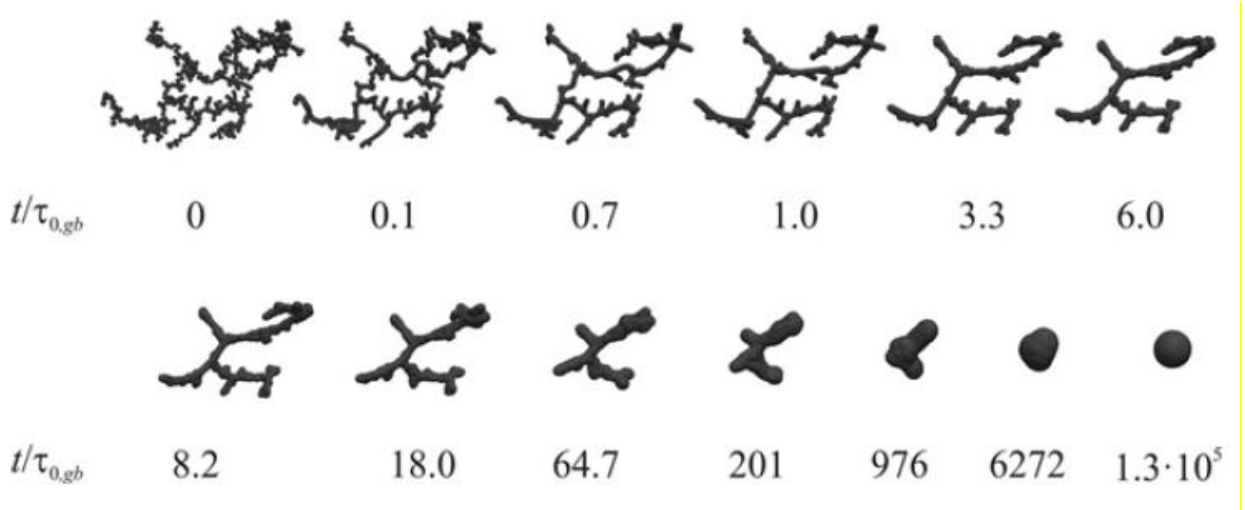


Figure 11.1: Snapshots from a simulation of an aggregate undergoing sintering by grain boundary diffusion that was initially made by diffusion-limited cluster–cluster agglomeration of 512 primary particles (from Ref. 139. Copyright Elsevier Ltd. Reprinted with permission. License number 3293011464905).

While some processes (many liquid-phase processes) may be sampled so that the primary particles can be stabilised and measured, other processes (most high temperature gas-phase processes) may involve some degree of sintering of primary particles and progressive evolution of particle shapes and degree of coalescence during the production[139], as illustrated in Figure 11.1.

In such cases, sampling of the primary particles before irreversible aggregation has occurred may be impossible, and if significant sintering effects (coalescence and merger of small particles through diffusion) occur, the constituent particles in the final aggregates or agglomerates may be much larger than the primary particles from which they were formed. Sampling immediately after the particle production process therefore may collect agglomerates and aggregates which then need to be analysed using microscopic methods in order to determine the size distribution of the constituent particles.

Top-down production processes for very fine particulates are not compatible with such 'early' sampling methods. In some cases sample collection immediately after the grinding/milling process, with immediate dispersion and size stabilisation in suspension, may allow a more relevant determination of the particle size distribution using 'low cost sizing methods' than would be possible after extended storage times, but this is unlikely to be representative of the true constituent particle size distribution since particle size reduction takes place in parallel to aggregation and agglomeration processes. Only careful comparison of the results with TEM analyses (which would anyway be necessary to take particle shape into account) might enable this approach to be acceptable.

11.3 Proxy particle-sizing methods

Few so-called particle/nanoparticle sizing techniques actually directly measure particle sizes. HR-TEM and AFM are two such methods, though both are based on complex technology (especially high-resolution transmission electron microscopy, HR-TEM) and are subject to several error sources when it comes to measuring size distributions of particle ensembles. Even HR-TEM, as it is an imaging technique, can be seen as an 'indirect method' for size measurements, while only AFM relies on direct contact between a measuring tip and the particle being measured. Sizing techniques are described in Section 4, but it can be stated that the majority can be considered as 'proxy methods' for particle size distribution determinations, relying on mathematical models, and usually several assumptions, to convert measured signals to size information. Since HR-TEM is relatively expensive, time consuming and not generally considered appropriate for industrial process monitoring, and AFM while being much cheaper is also time consuming and poses major challenges for size distribution determination, the question then arises as to which proxy methods are suitable, for which products and processes can they be applied, and how accurate can their results be considered.

The EC recommendation for a definition of 'nanomaterial' does not specify which sizing techniques are appropriate for 'direct" or 'straightforward' implementation. It does specify Volume Specific Surface

Area (VSSA) determination (normally achieved by the BET technique[140]) as a suitable proxy method to positively identify a nanomaterial, but not to classify a particulate sample as non-nanomaterial. For implementation of the definition proxy methods that can be used for both positive and negative classification would be desirable, even if these need to be backed up by 'direct' methods.

Details about several different (proxy) particle sizing techniques have been presented in a previous report,[42] together with a comprehensive overview of their strengths and weaknesses with respect to implementation of the EC definition of nanomaterial. It is clear that no single technique is suitable for implementation of the definition for all particulates (with the possible exception of HR-TEM), but the question can be asked if any of the faster or less costly methods might be used reliably for nanomaterial/non-nanomaterial classification in specific cases (e.g. for a particular synthesis process of a certain nanomaterial type). The answer will often be no, but in very specific situations, possibly yes.

For example, in the case of dry powder samples of non-porous particles, where their density can be determined with high accuracy, and all the particles can be shown to be (for example) spherical or close to spherical, not too polydisperse, not aggregated and not agglomerated to any significant extent, then following a suitable comparison/calibration exercise with microscopic techniques BET might be employed successfully for implementation of the EC Definition. For liquid-phase processes that are known (or can be shown) to result in very monodisperse size distributions of stabilised primary particles with known shape, following appropriate validation for specific particle types, DLS might be able to reliably classify samples as nanomaterials or non-nanomaterials. In combination with Field Flow Fractionation, it might also be able to deal with polydisperse samples (of stabilised primary particles). Similarly, other techniques like PTA, CLS, XRD, etc., might also in certain cases be used for material classification, but only after proper validation and careful case-by-case justification.

Another possible proxy method that is not described in reference 42, and that in itself is not a size distribution measurement technique, is optical spectrometry. For example, silver and gold nanoparticles show surface plasmon resonance bands which vary with nanoparticle size, moving to longer wavelengths at increasing particle sizes. For quantum dots, optical characteristics can clearly be used to provide some information regarding particle size. In some specific cases, even top down processes (e.g. for pigment preparation) might be checked for batch consistency using UV-visible spectrometry or colorimetry. This would require extensive calibration of the optical characteristics of a product against particle size distribution as determined by other techniques (specifically EM), but may be sufficient to determine whether the batch is within acceptable limits or not with regard to its nanomaterial or non-nanomaterial classification.

11.4 Production process monitoring

Another approach for particulate material classification with respect to the current recommendation may be to use production process monitoring together with periodic product analysis. If the dependency of the product constituent particle size on different production parameters can be reliably demonstrated, then process parameter monitoring might offer an economic and reliable alternative for a manufacturer to be able to either classify product materials (where the parameters are changed for different batches) or to ensure that a product remains either nanomaterial or non-nanomaterial over time. The closer the average constituent particle size is to the upper limit of the EC definition of 100 nm, the less reliable this approach will be. However for many products with average constituent particle sizes well over or under 100 nm the method should be reliable.

A similar approach, which may be more practical or reliable in some situations, consists of using a 'proxy method' such as DLS, BET, XRD, PTA, CLS, spectrometry, etc., to monitor the consistency of the final product. While these methods suffer from various disadvantages, and may not be reliable for implementation of the EC definition, it may often be the case that any significant modification of constituent particle shapes or size distributions will be reflected in modified results of these analyses (see Section 13). This pragmatic approach, like all others would need to be carefully justified in specific cases, especially if production parameters are changed from batch to batch in order to achieve different product characteristics.

11.5 Special manufacturing cases

Most bottom-up fine particulate manufacturing methods are quite capable of producing a wide range of constituent particle sizes and shapes depending on the process parameters employed. However, for certain particle types, some may be almost incapable of creating constituent particles with all dimensions above 100 nm unless process parameters very far than those used industrially are employed, especially if the production process does not include an effective enough sintering stage. In such cases, according to the EC Definition the product will be a nanomaterial even if the collected powder or suspension is allowed to subsequently agglomerate or aggregate strongly (sintering on the other hand may change the constituent particle size). This is an example of where the knowledge about the manufacturing process could be an element to consider when classifying the product as a nanomaterial.

There is a lot of information available about the influence of synthesis process parameters on particle sizes, for a wide range of synthesis methods and nanoparticle types. However, much of this would need to be validated for specific industrial production processes or individual plants, and although it would require extensive positive collaboration and data-sharing from producers, a broad examination of actual

product characteristics could allow, for specific nanoparticle types/compositions, correlation of production methods and process parameters with nanomaterial or non-nanomaterial classifications.

12 MOST COMMON MANUFACTURING TECHNIQUES USED FOR NANOMATERIAL PRODUCTION

12.1 Introduction

Knowledge about manufacturing techniques may provide useful information about the properties, in particular the size distribution of nanoparticles. Indeed, some techniques allow a better control of shape and size of the particles than other techniques. Moreover, some methods enable a production with fewer impurities than others. Therefore, this section provides an overview about the most common manufacturing techniques for nanomaterials, and gives examples for each technique.

One can classify the manufacturing techniques used for nanomaterial production in 'top down' and 'bottom up' strategies (Figure 12.1).[141,142]

The term 'top down' production applied to nanomaterial synthesis define their fabrication process by bulk material reduction or decomposition. In nanotechnology, milling/attrition processes is typical for top down strategy.[143]

The 'bottom up' strategy refers to the nanoparticle synthesis by assembling atomic or molecular components.[144] This could be achieved by several processes such as inert gas condensation,[145] pulsed laser ablation,[145] precipitation methods,[141] sol gel processes,[141] or hydrothermal techniques.[141,146] These processes are briefly introduced in this chapter.

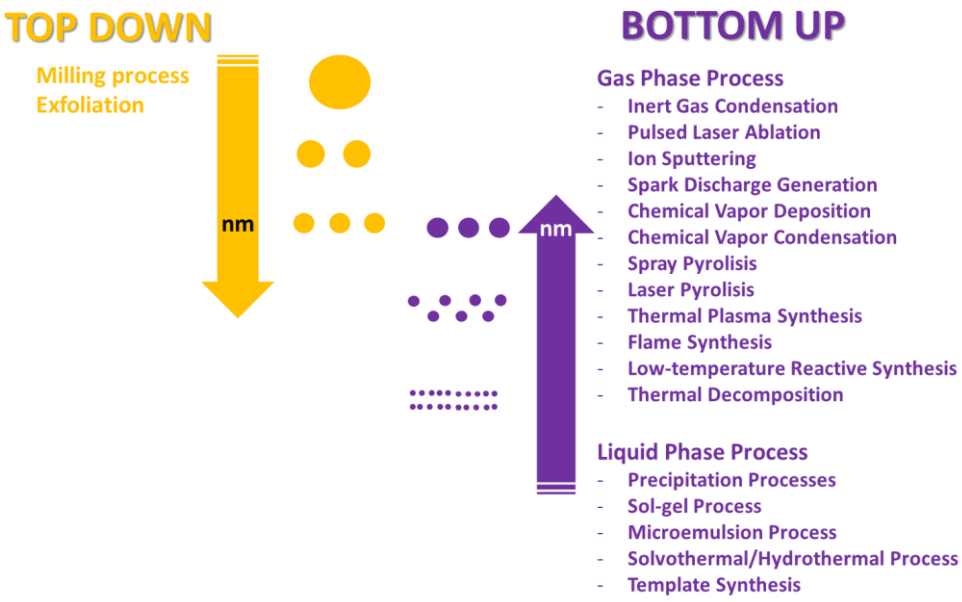


Figure 12.1: Approaches to produce nanoparticles

12.2 Top down processes

The milling process is an attrition approach in which the nanoparticles are formed in mechanical devices.[143] This method is applied for the production of metallic, alloyed, ceramic and polymeric nanoparticles, e.g.:

- *Metals and alloys: NiNb,*[143] *FeCo,*[143] *NiAl*[143]
- *Ceramics: TiC ,*[143] *SiGe*[143] *Li_2O,*[143] *$LiNbO_3$,*[143] *B_2O_3,*[143] *TiO_3,*[143] *TiO_2,*[143] *PZT*[143] *(zirconium titanate)*
- *Polymeric: PMMA (poly(methyl methacrylate)),*[143] *PEP (poly(ethylene-altpropylene)),*[143] *PI (polyisopropene)*[143]

Milling is a simple, inexpensive and widely used technique which can be applied to a broad range of materials. However, this technique does not allow fully controlling the particle shape or size. With this production method, a size range from nanometre to submicrometre can be obtained during the same process and particulate materials obtained this way may contain a considerable fraction of nanoparticles, even if the objective was to obtain submicrometre particles. Moreover, the milled products may contain impurities, which originate from the milling atmosphere, grinding media or control agents added to the powder during the process.

Exfoliation is a common method to produce graphene[147] Different starting materials can be used, such as graphite oxide, pristine graphite, graphite intercalation compounds or expanded graphite.[147] The process can be carried out using different techniques leading to ***mechanical*** (stirring, shaking or ultrasonication), ***thermal*** or ***electrochemical exfoliation***.[147] Thermal techniques are generally faster and can produce graphene in a gaseous environment[147]

- *Carbon: graphene*[147]

12.3 Bottom up processes

Applying bottom up methods,[141,142] nanomaterials can be synthesised from precursors in the solid, liquid or gas[145] phase following physicochemical principles of atomic and molecular organization.

12.3.1 Gas phase techniques[145]

Gas phase processes are very common industrial-scale methods for the production of nanomaterials in powder form or as thin layer. These techniques are simple, inexpensive, and can have high yields production.

In these methods, nanoparticles are synthesized from a vapour phase mixture[146] (see Figure 12.2). A precursor is heated up to form a vapour phase, which is thermodynamically unstable in comparison to a solid material. This process induces a supersaturation state, which favours reactions between molecules. Under certain conditions (e.g. degree of supersaturation, temperature, pressure, residence time) homogenous growth of the nucleus occurs, which induces the formation of primary particles, i.e., particles which have nucleated independently, and subsequently leads to the formation of nanoparticles by coalescence. [145]

The gas phase techniques could be classified according to the precursor phase and the energy source used for the synthesis of nanomaterials.

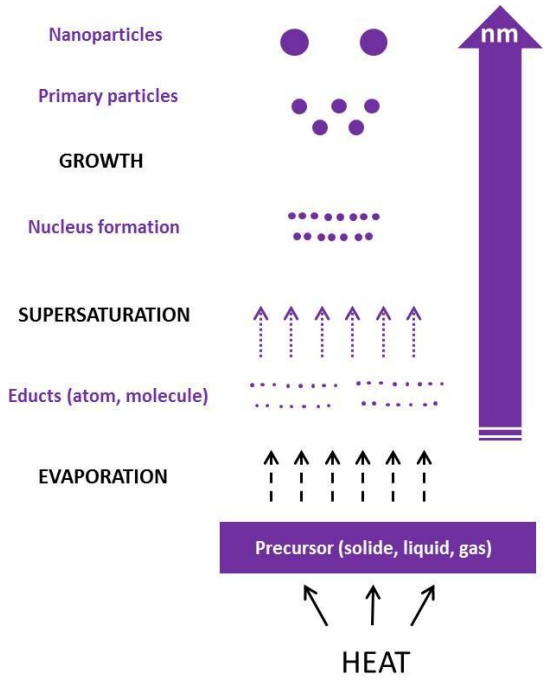


Figure 12.2: Formation of nanoparticle during a gas phase synthesis

12.3.2 Gas phase techniques using solid precursors

Inert Gas Condensation[145,148] is a gas phase process in which the supersaturation phase is achieved by evaporating a solid material in a cold inert gas (usually He or Ar) at low pressure conditions (see Figure 12.3). The most commonly use vaporization methods are resistive evaporation, laser evaporation and sputtering. Particle size can be controlled by changing pressure or the type of the utilised inert gas. This process is applied on industrial scale for a wide range of materials. Addition of reactive species, like O_2, to the inert gas allows synthesizing nanoparticles of various ceramic materials.

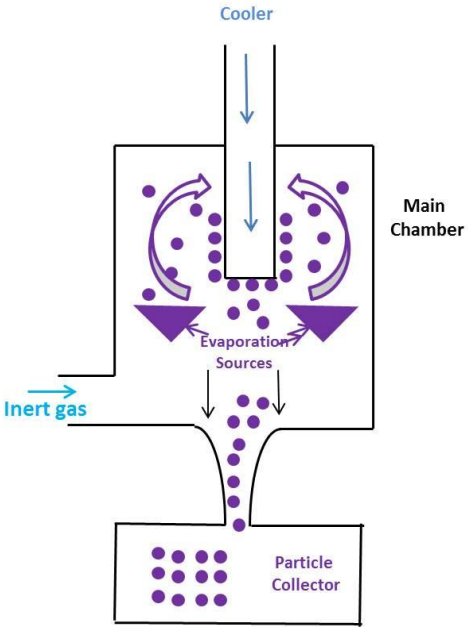


Figure 12.3: Inert Gas Condensation

Examples of nanoparticles which could be synthetized by this method are given below:

- *Metals: Bi,*[145] *Ag,*[145] *Au,*[145] *Fe_2O_3,*[149] *Fe_3O_4*[149]
- *Composites: PbS,*[145] *Si/In,*[145] *Ge/In,*[145] *Al/In,*[145] *Al/Pb*[145]
- *Ceramics: SiO_2,*[145] *Al_2O_3*[145]
- *Carbon: Graphene*[147]

Pulsed Laser Ablation[145, 150] is a gas phase process in which a pulsed laser is used to rapidly heat a thin layer of a substrate solid material (from 1 µm to 0.1 µm), which vaporises without being readily evaporated. This ablation leads to the formation of energetic plasma above the substrate, from which the nucleation process occurs. Modification of the laser pulse durations and energy allows to control the relative amounts of ablated particles or atoms and hence the size and shape of the particles. Nevertheless the formation of the nucleus in this technique cannot be considered as entirely homogenous process and therefore the control of the size and shape is somehow limited. Additionally, in ***Pulsed Laser Ablation*** process only a small amount of nanoparticles can be produced. Examples of nanoparticles which could be synthetized by this method are given below:

- *Metals: Fe_2O_3,*[145] *Fe_3O_4,*[145] *CuO_2,*[151] *Au,*[152]
- *Composites: Sb_2S_3,*[153] *FePt,*[154]
- *Ceramics: TiO_2, SiO_2,*[145] *SiH,*[145] *Al_2O_3*[150]
- *Carbon: nanodiamonds,*[155] *SWNT*[156]

In the ***Spark Discharge Generation*** method, a high current arc (or spark) is applied to evaporate the electrode material in an inert gas and hence to synthetize nanoparticles (see Figure 12.4). This process produces very small amounts of nanoparticles (5 g/kWh) but it is reasonably reproducible. Scale-up for the industrial requirements is possible, and particle size can be controlled via the energy per spark.[157] This method can be applied to the production of any type of conductive material including semi-conductors as well as composite material.

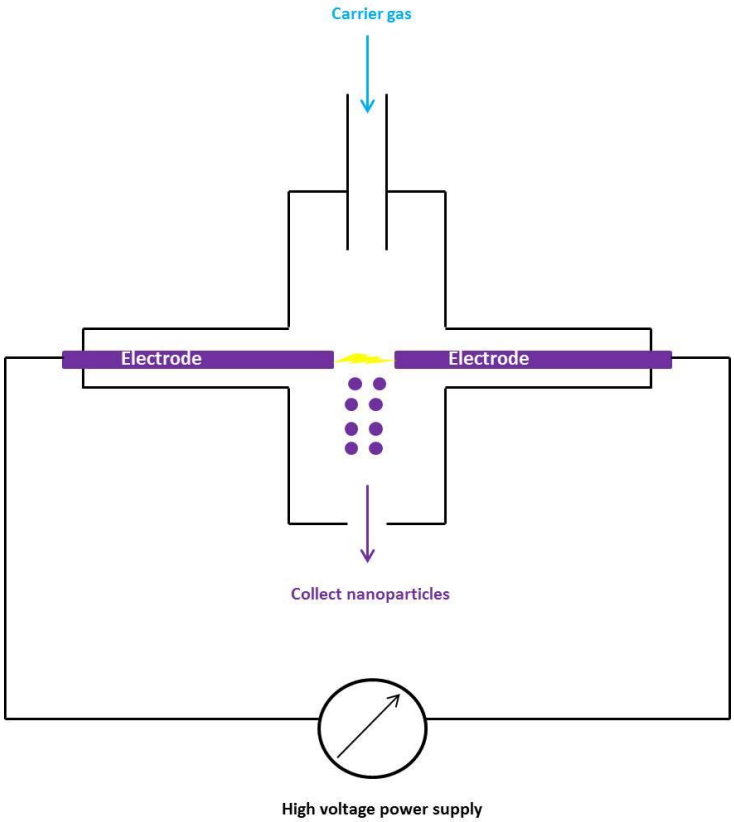


Figure 12.4: Spark Discharge

Examples of nanoparticles which could be synthetized by this method are given below:

- *Metals: Au,*[157] *Ag,*[157] *Cu,*[157,158] *W,* [157] *Sb,* [157] *Ni,*[145] *Pt,*[159] *Fe*[159]*, Fe_2O_3,*[159] *V,*[159] *VO_2*[159]
- *Composites: CuNi,*[160] *SiC,*[150] *CrCo,*[161] *Au-Pd,*[161] *Ag-Pd,*[161]
- *Ceramics: MgO_2* [157]
- *Carbon: nanoparticles and agglomerate with different shape,*[159] *SWNT,* [161] *Fullerene*[161]

The ***Ion sputtering*** process is a method in which material is vaporized from a solid surface by bombardment of inert gas ions, causing an ejection of atoms. This process must be carried out at relatively low temperatures. This technique allows one to control the resulting nanoparticle including size and shape.[143] Examples of nanoparticles which could be synthetized by this method are given below:

- *Metals: Au,*[162] *VO_2* [163]
- *Composites : TiN,*[150] *AlN,* [150] *Al-Cu,*[162] *CdSe,* [162] *CdTe* [162]
- *Ceramics: Al_2O_3,* [150] *SiO_2*[150]

12.3.3 Gas phase processes using liquid or vapour precursors

In this approach, the supersaturation phase required for the homogenous nanoparticles synthesis is achieved by chemical reactions of heated gases

Chemical Vapour Synthesis is a method in which vapour phase precursors are brought into a reactor under conditions which enable nanoparticle formation. The precursors can be solid, liquid or gas at ambient conditions, but have to be delivered to the reactors as a vapour.

Chemical Vapour Deposition (CVD) is a well-known process in which the substrate introduced to the reaction chamber to be exposed to volatile precursor, which reacts or decompose on the substrate surface to form an expected deposit.[143] The CVD reaction needs activation energy which can be provided in several ways. These methods also differ in the means by which chemical reactions are initiated. CVD covers techniques as Atmospheric Pressure Chemical Vapour Deposition (APCVD), Low Pressure Chemical Vapour Deposition (LPCVD), Metal-Organic Chemical Vapour Deposition (MOCVD), Plasma Assisted Chemical Vapour Deposition (PACVD) or Plasma Enhanced Chemical Vapour Deposition (PECVD), Laser Chemical Vapour Deposition (LCVD), Photochemical Vapour Deposition (PCVD), Chemical Vapour Infiltration (CVI), and Chemical Beam Epitaxy (CBE). Examples of nanoparticles which could be synthetized by this method are given below:

- *Metals: NiOx,[164] ZnO [165]*
- *Carbon materials: graphene,[166] SWNT, [167] MWNT, [168] Fullerene,[169] Nanodiamonds[170]*

Another process from this class of techniques is ***chemical vapour condensation (CVC)***, which involves pyrolysis of vapours of organometallic, carbonyls, hydrides, chlorides and other volatile precursors in gaseous, liquid or solid state, in a reduced pressure atmosphere. The reaction allows synthesis of mixtures of nanoparticles. This process allows nanoparticle production with a narrow distribution in size. This method is currently used for industrial synthesis of commercially available nanopowder.[171] Examples of nanoparticles which could be synthetized by this method are given below:

- *Metals : Fe_2O_3,[172] Fe_3O_4,[172] W,[145] Co,[171] Cu,[145] CuO_2[145]*
- *Composite: Fe_3N,[173] WS_2[174]*
- *Ceramics: TiO_2[175]*

In the ***spray pyrolysis*** (also called ***aerosol decomposition synthesis***, or ***droplet-to-particle conversion***) process, a nebulizer is injecting very small droplet of precursor solution directly into a hot reactor where reaction occurs directly in the droplet solution.[145] This process enables adjustable sizes, narrow size distribution and good stoichiometry of the nanoparticles. Spray pyrolysis is a relatively simple, reproducible and low cost technique.[176] Examples of nanoparticles which could be synthetized by this method are given below:

- *Metals: Cu,[145] NiO,[177] Ag[178]*

- *Composites: ZnS*[179]
- *Ceramics: TiO_2,* [145] *ZnO*[176]

In the ***laser pyrolysis*** process, precursors are heated by absorption of laser energy (generally an infrared (CO_2) laser is used). The energy is either absorbed by one of the precursors or by an inert photosensitizer. This technique enables a localized heating and a rapid cooling. This technique is a very flexible and versatile process.[180] These nanoparticles could be applied for structural nanoceramics, wear resistant coatings and functional nanomaterials for optoelectronics, photonics and bio-imaging.[180] Laser pyrolysis method has an application in the production of different types of nanoparticles:

- *Metals : Fe_2O_3,* [180] *FeC,*[181] *Fe_4N*[181]
- *Composites: MoS_2* [145,180]
- *Ceramics: Si,*[145] *SiC,*[145] *Si-C-N,*[180] *SiO_2,* [180]*TiO_2,*[180] *Al_2O_3*[180]

In the ***thermal plasma synthesis***, precursors are injected into thermal plasma, which allows the supersaturation phase to achieve nanoparticles synthesis. It occurs upon cooling while exiting the plasma region. Different types of thermal plasmas could be used as ***dc (direct current) plasma jet, dc arc plasma, rf (radio frequency) induction plasma, microwave-generated plasma.*** The inductively coupled plasma (ICP) used in combination with an aerosol spray called ***spray ICP*** is often used too. Multicomponent oxides as well as simple materials can be obtained by this method, e.g.

- *Metals : CuO,*[182] *NiFe,*[183] *Bi,*[184] *Bi_2O_3* [184]
- *Composites: C_3N_4*[185]
- *Ceramics: ZnO* [186] *TiO_2,*[187] *Al_2O_3* [188]

In the ***flame synthesis***, nanoparticles are produced by using the flame to initiate chemical reactions (Figure 12.5) in order to promote the nanoparticles growth. This technique is inexpensive and most common industrial approach to nanoparticles synthesis. This process is typically use for oxide nanoparticles production because of the oxidizing environment of the flame. Recently the possibility of expanding flame synthesis to a large variety of materials and enabling a control of morphology and particle size was reported in the scientific literature. It is also possible to inject liquid precursor directly into the flame with the ***flame spray pyrolysis*** process.

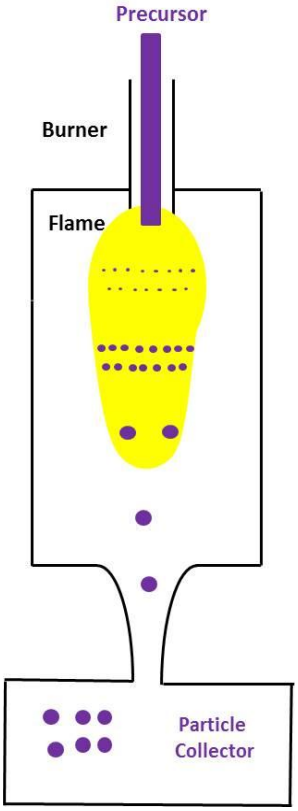


Figure 12.5: Flame synthesis

Examples of nanoparticles which could be synthetized by this method are given below:

- *Metals : Fe_2O_3,* [145] *Fe_3O_4,* [189]
- *Ceramics: ZnO,* [190] *ZrO_2,* [191] *TiO_2,* [191] *Al_2O_3,* [191] *SiO_2,* [191] *$ZnAl_2O_4$* [192]
- *Carbon: CNT,* [191] *fullerenes (C_{60}, C_{70})* [191]

Thermal decomposition can also be used to synthetise nanoparticles, particularly graphene. In this case, the growth of graphene can be realized on insulating silicon carbide (SiC) surfaces by high-temperature annealing in vacuum and allows large-scale production of graphene-based devices.

- *Carbon: graphene*[193]

12.4 Liquid phase processes

The particle formation mechanism is the same as in the gas phase production, but precursors are in solid or liquid phase. In general, liquid phase processes allow a better control of the shape, size, and other parameters of the nanoparticles. The more common liquid phase methods to synthetize nanoparticles are the coprecipitation method, the sol-gel process and the hydrothermal synthesis.

The most common industrial technique for production of nanomaterials is ***coprecipitation method***. During this process, the nucleation, growth and agglomeration occur simultaneously. In this synthesis, soluble precursor(s) are placed in the solvent and precipitate to form nanoparticles. This technique is

suitable for production of a variety of nanomaterials and allows controlling the size and shape of the nanoparticles. This method can be applied to the synthesis that occurs both in aqueous and non-aqueous solvents. In many cases, an organic capping agent is added to the reaction mixture to prevent agglomeration or/and as reducing agent. Metallic nanoparticles can be also synthetized by electrochemical reduction or decomposition of metallorganic precursors. This method enables the production of metal chalcogenide nanoparticles by reactions of molecular precursors. The coprecipitation process can be supported by microwave treatment or sonication, which provide a rapid heating of the reaction mixture. Examples of nanoparticles which could be synthetized by this method are given below:

- *Metals: Cu,*[194] *Ag,*[194] *Au,* [194] *Ni,* [194] *Fe,* [194] *Ru ,* [194] *ZnO,* [194] *SnO_2,* [194] *Sb_2O_3* [194]
- *Composites: CuPt,*[195] *PdPt,*[195] *AuAg,* [195] *CdSe*[194]
- *Ceramics: Al_2O_3,*[196] *TiO_2* [197]

The **Sol-gel process** is a wet chemistry technique which uses either a chemical solution or colloidal particles (sol) to produce an oxide or alcohol-bridge network (gel) for reactions like polycondensation or polyesterification. Metal alkoxyde or metal cations in aqueous media such as Si, Fe, Ti, Zr are typical precursors. Many reaction parameters such as pH, temperature, method of mixing, the nature and concentration of anions have to be controlled in order to provide good reproducibility of the synthesis but this technique gives a possibility for a good control of size and shape of the produced nanoparticles. This process is especially successful in the synthesis of metal oxides (e.g. ceramics, glasses, films and fibres). Highly porous nanomaterials like zeolites or silicates can also be synthetized with this method. Examples of nanoparticles which could be synthetized by this method are given below:

- *Metals : Fe_2O_3,* [194] *Fe_3O_4* [194]
- *Composites: $AgSiO_2$,* [194] *$AuSiO_2$,* [194] *$PbTiO_3$* [194]
- *Ceramics: ZrO_2,* [194] *TiO_2,* [194] *SiO_2* [194]

In the **micro-emulsion** process (see Figure 12.6) two different liquid phases are mixed to create a dispersion of micelles which than serve as a nanoreactors for the synthesis of nanoparticles. In this technique two types of **stable** liquid mixture are used: oil phase and water phase with surfactant and eventually a cosurfactant. The two main branch of this process are direct micro-emulsion technique in which oil is instilling to the water/surfactant solution and reverse micro-emulsion technique where the water is instilling in oil. This process can be applied to metals and metal oxides, alloys, ceramics and polymers. Metal nanoparticles are synthetized by reduction through adding a reducing agent. For metal oxides, the synthesis bases on the precipitation of oxides phenomena in aqueous solution. This method

can be also applied to polymerisation or sol-gel. The size of the nanoparticles can be controlled adjusting parameters like e.g. the type of the surfactant, the molecular ratio of water to surfactant, reagents concentration or rate and type of stirring.

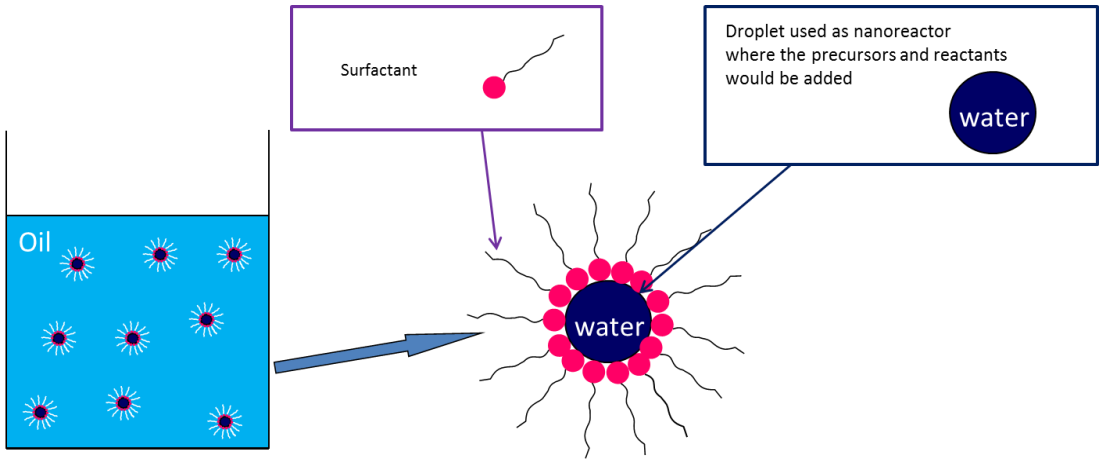


Figure 12.6: Reverse micro-emulsion synthesis

Examples of nanoparticles which could be synthetized by this method are given below:

- *Metals : Co,* [194] *Ag,* [194] *Pd,* [194] *Bi,* [194] *Ni,* [194] *Pt,* [194] Fe_2O_3, [194]
- *Composites :* $FePt_3$ [194]
- *Ceramics:* Al_2O_3, [194] TiO_2, [194] SiO_2 [194]
- *Polymeric: PMMA (poly(methyl methacrylate),*[198] *PPy (Polypyrrole)*[199]

In **hydrothermal** and **solvothermal processes**, solvents are brought to temperatures well above their boiling points in a sealed vessel (e.g. bomb, autoclave etc.) under internal autogeneous pressure. When the solvent used is water, this method is called hydrothermal process; otherwise it is called solvothermal process. These conditions (temperature and pressure) provide milder and friendlier reaction conditions where different reactions types can occur such as complexation or reduction. Hydro- and solvo- thermal techniques can be applied to produce a variety of nanoparticle types such as metals, semiconductors, ceramics or polymers. These techniques enable precise control of particle size, shape, size distribution and crystallinity depending on parameters such as reaction temperature, reaction time, solvent type, surfactant type and precursor. Examples of nanoparticles which could be synthetized by this method are given below:

- *Metals : Ag,*[200] *Cu,*[201] *Fe,*[201] *Ni* [201]
- *Composites: ZnSe,*[202] *SnSe,* [202] $CuInSe_2$ [202]
- *Ceramics:* TiO_2, [194] SiO_2 [201]

- *Carbon: MWNT,* [201] *SWNT* [201]

The **templated syntheses** involves reactions which were previously described (i.e. polymerisation, reduction...). Templates are used for direction of the nanoparticles during their growth which allows to control size. In recent years, synthesis of metal and metal oxide nanoparticles on polymer, protein or peptide templates have gained an increasing amount of attention.

- *Metals:* Fe_3O_4, [194] *Au,* [194] *Pd,* [194] *Pt* [194]
- *Composites:* $CoFe_2O_4$ [194]
- *Ceramics:* TiO_2

12.5 Summary

This chapter provides an overview about the most common manufacturing techniques to produce nanoparticles, including information about the capacity of each technique to control the properties of the nanoparticles, in particular size distribution and shape.

Generally, top down processes allow less control over the nanoparticles production than bottom up processes. Bottom up processes include various techniques, like gas phase techniques such as inert gas condensation, spray pyrolysis or flame synthesis and liquid phase techniques such as coprecipitation processes, solvothermal approaches or sol gel methods.

Table 12.1 below lists common processes to manufacture nanomaterials.

Table 12.1: Common processes to manufacture nanomaterials with examples from the OECD Sponsorship programme on the safety testing of a representative set of nanomaterials

	Milling process	Inert gaz condensation	Pulsed laser ablation	Ion Sputtering	Spark (Arc discharge generation)	Chemical vapor depostition	Chemical Vapor Condensation	Spray pyrolisis	Laser Pyrolisis	Thermal Plasma Synthesis	Flame Synthesis	Low Temperature reactive synthesis	Coprecipitation process	Sol Gel Method	Microemusion process	Solvothermal/Hydrothermal Process	Template synthesis
Fullerene				x	x	x			x								
SWNT		x	x		x	x		x			x						
MWNT		x	x		x	x		x			x						
Silver nanoparticles		x	x		x								x		x	x	
Iron nanoparticles		x	x		x		x		x		x		x	x	x	x	x
Titaniun dioxide nanoparticles		x	x				x	x	x	x	x			x	x	x	
Aluminium oxide nanoparticles		x	x		x				x	x	x		x		x	x	
Cerium oxide nanoparticles		x		x				x		x	x		x	x	x	x	x
Zinc oxide nanoparticles			x			x		x		x	x		x				x
Silicon dioxide nanoparticles		x		x					x		x		x	x	x	x	
Dendrimers																	
Nanoclays													x	x			
Gold nanoparticles		x	x	x	x								x		x		x

13 INFORMATION ON POSSIBLE PARTICLE SHAPES, WITH PARTICULAR RELEVANCE TO DETERMINATION AND MANIPULATION OF CONSTITUENT PARTICLE SIZE DISTRIBUTION OF CERTAIN NANOMATERIAL CLASSES

13.1 General considerations

Information on particle shapes in commercial nanomaterials is not easily available mainly as this is often treated as confidential commercial information.

In this section extensive use was made of results from the project on "Inter-laboratory comparison of particle size distribution measurements applied to industrial pigments and fillers", and of internal data on the characterization of materials present in the Nanobiotechnology Laboratory of the JRC's Nanobiosciences Unit, including some representative nanomaterials from the JRC's repository.

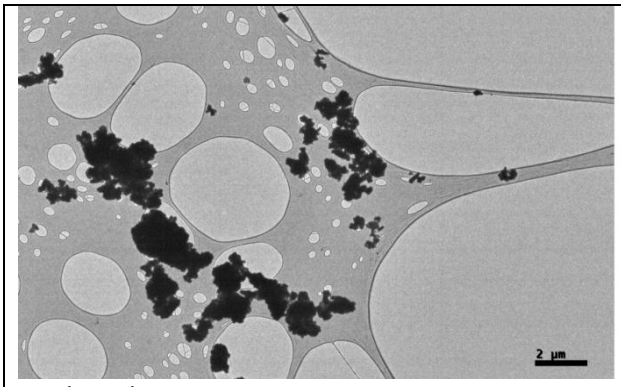
Nanoparticles can assume a wide variety of basic shapes from simple (almost) spherical ones, to rods of well-defined aspect ratio or triangular shapes, to elongated needles and fibers. In some cases the vast majority of particles present in a sample have a quite homogenous shape, while in others cases several different shapes can be present simultaneously. In more complex cases particles can appear as branched particle, thus rendering the classification of possible shapes even more complex.

13.2 Particle size and shape by Electron Microscopy

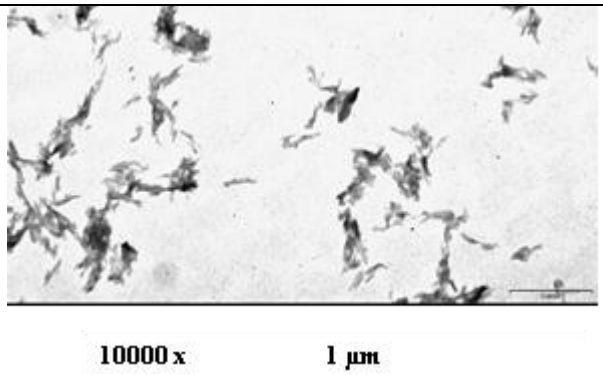
When measuring particle size with Electron Microscopy the quantity used to evaluate the size distribution needs to be chosen properly[42] and due consideration must be given to sampling and statistics of the analysed data.[61] In the case of quasi-spherical objects, the choice of the parameter is of minor importance: ECD (equivalent circle diameter), Area and Feret diameter are all quantities which can be used. In practical cases, ECD and Feret diameter are the most commonly chosen ones. For rod-shaped objects, length and diameter are required; in the case of discs, the diameter and thickness. The choice of the parameter is strongly related to the question of interest, therefore a "best choice" for all materials does not exist.

Below EM images are shown of several industrial materials used in paints in industrial applications (Figure 13.1).[E] Most of the samples show aggregation, but it is possible to identify the shape (and with some difficulties the size) of the constituent nanoparticles. In several cases the particles have a somewhat spherical shape: such as pigment red 101, rutile and anatase TiO_2, fumed silica, and (maybe) for Al-Co blue pigment. In two cases they appear as an elongated shape: pigment yellow 83 and pigment yellow 42. In one case the material is platelet-shaped (Pigment Metal 2).

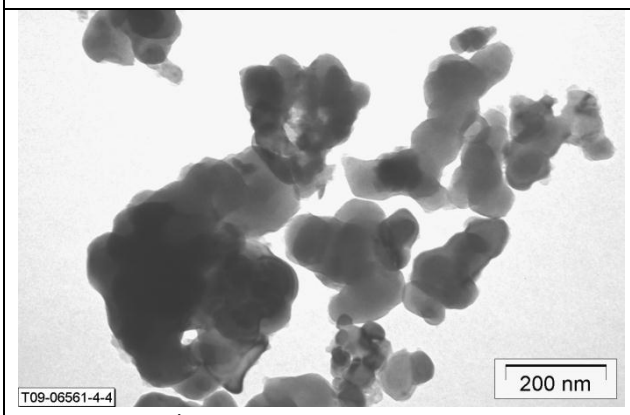
[E] Data from a work programme carried out by the JRC and Eurocolour (the association of European Pigments, dyes and fillers industry). A report is currently in preparation.



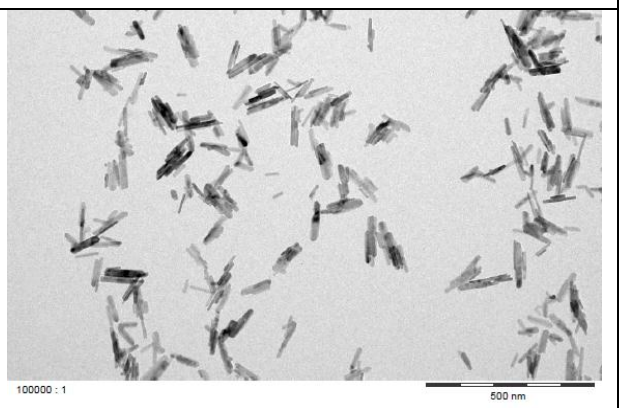
A. Al-CoBlue Pigment



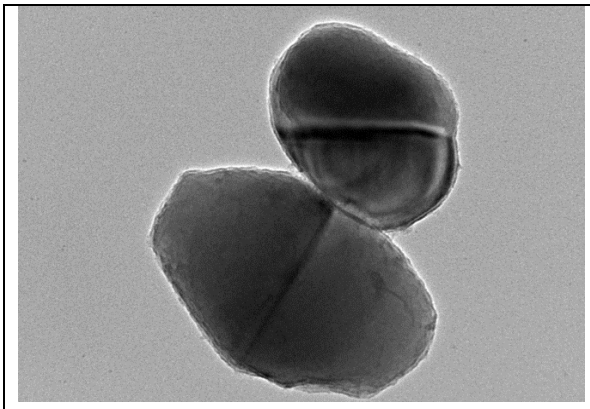
B. Pigment Yellow 83 transparent



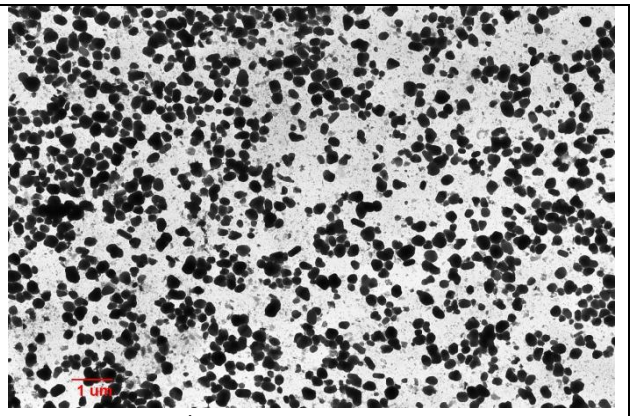
C. Pigment Red 101



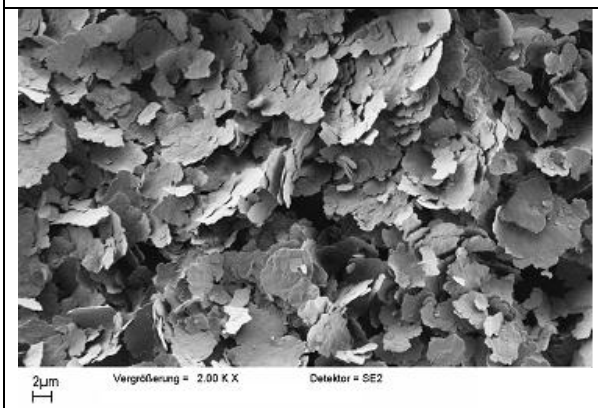
D. Pigment Yellow 42



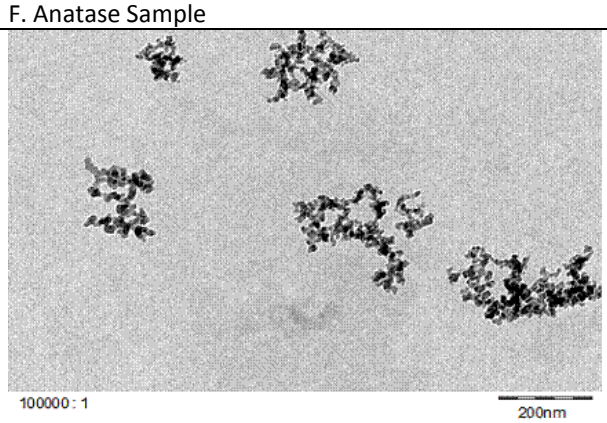
E. Rutile Sample



F. Anatase Sample



G. Pigment Metal 2



H. Fumed Silica

Figure 13.1: EM images of several industrial materials used in paints in industrial applications

In some cases (especially for Al-Co blue pigment) a higher magnification EM would be needed to assess whether the clusters seen in the image are single entities or aggregates of various primary particles.
The following table[F] shows the median value of the number size distribution of different materials, based on EM analysis, as provided by the material producers.

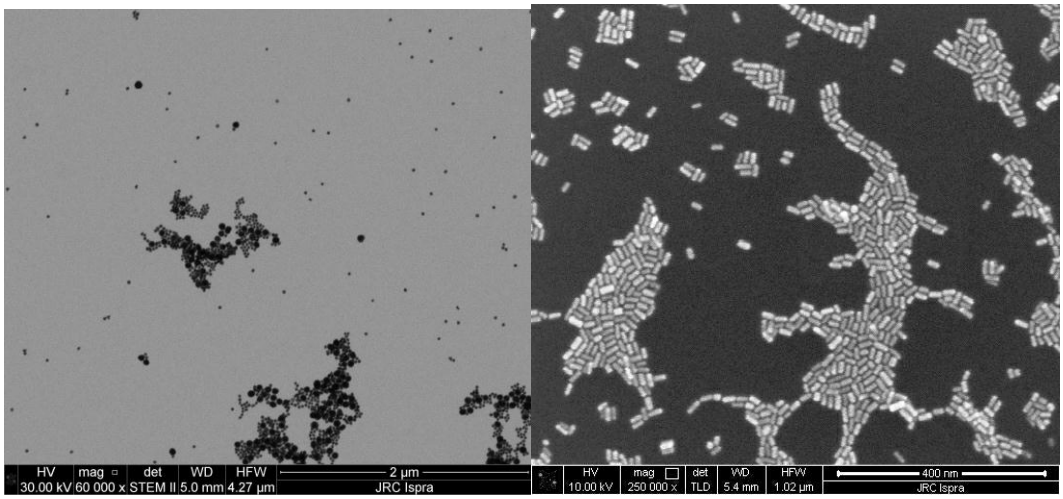
Material	Type or composition	Size information from the manufacturer/nm*
1 Al-Co-Blue	$CoAl_2O_4$	527
2 Pigment Yellow 83 transparent	Azopigment	47
3 Pigment Red 101	Fe_2O_3	249
4 Pigment Yellow 42	FeOOH	20
5 Rutile Sample	TiO_2	250
6 Anatase Sample	TiO_2	130
7 Pigment Metal 2	Cu/Zn alloy	
8 Fumed(pyrogenic) silicon dioxide	Silica	12

* Prior knowledge data based on EM analysis by material manufacturer; type of size parameter not specified

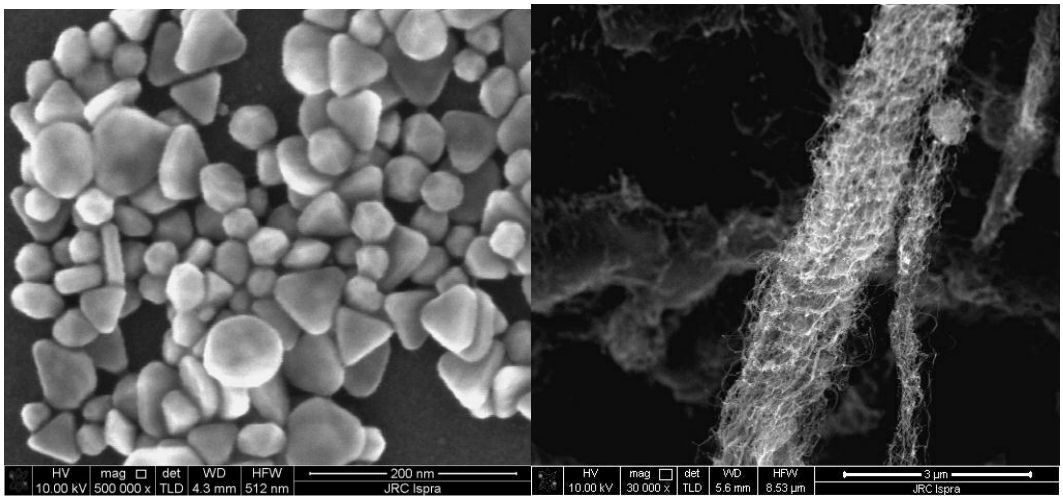
Figure 13.2 shows micrograph images taken from samples either produced or present in the JRC's Nanobiotechnology Laboratory obtained with scanning electron microscope either in transmission or reflection mode.
The images in Figure 13.2A and Figure 13.2B show gold nanoparticles synthesized in the nanobiosciences laboratory of JRC with a spherical shape and a diameter of 20nm and 60nm (Figure 13.2A) and nanorods of around 20 nm long and 5nm wide (Figure 13.2B). The bottom-up synthesis of gold nanoparticles is a very reliable and reproducible synthetic route that can be easily tuned to produce particles of a well defined size and shape (either spherical or rod-like).
The image in Figure 13.2C shows a sample of silver nanoparticles produced in the JRC laboratories. The sample has nanoparticles of sizes well below the 100nm limit, but of different shapes. From the image

[F] Data from a work programme carried out by the JRC and Eurocolour (the association of European Pigments, dyes and fillers industry). A report is currently in preparation.

some spherical, triangular and disk-like shapes can be identified. This image represent a good example of the difficulties inherent in trying to manipulate the particle size distribution of materials that are not spherical: in fact very often more than just one shape can be present in the sample under investigation. Finally, image in Figure 13.2D shows a bundle of carbon nanotubes with a typical fibre-like elongated shape with a nanometre-size width.



A. Gold nanoparticles of 20 and 60nm B. Gold nanorods



C. Silver nanoparticles D. Bundles of Carbon nanotubes

Figure 13.2: Micrograph images taken from samples either produced or present in the JRC's Nanobiotechnology Laboratory obtained with scanning electron microscope either in transmission or reflection mode

14 CONVERSION ABILITY BETWEEN DIFFERENT SIZE DISTRIBUTION METRICS

14.1 Introduction

As discussed in a previous report,[42] the term "particle size" is not very well defined and most particle sizing methods deliver method-defined properties. This is even true to a certain extent for imaging methods, which measure one or several of the external dimensions of a 2-dimensional projection of a 3-dimensional object[G]. Results from different sizing methods are therefore often not comparable, which may lead to incorrect material classification, disputes, and/or expensive re-testing. A possibility to convert results from a given sizing method to values comparable with the results of other sizing methods would therefore be highly beneficial.

The desire to convert different particle metrics is neither new nor unique to nanoparticles, but is inherent to all particle-sizing methods (e.g. Ref. 203). As many methods for size determination of larger particles are based on the same principles as those for nanoparticles (image analysis, sedimentation in a liquid, etc.) a significant amount of knowledge can be gained from the experience compiled over the last decades in the size determination of particles at the μm and mm scales.

Within the context of the EC definition, the term 'conversion' covers three separate issues, namely

a) conversion of equivalent diameters into external dimensions

b) conversion of intensity or volume based particle size distributions into number-based distributions

c) conversion of external surface area into median diameters.

Issue a) deals with the question to which degree it is possible to convert equivalent particle sizes as determined into reliable 'external dimensions' as required by the definition. This issue is relevant as several methods used for determining the size of nanoparticles (e.g. DLS, particle tracking analysis, disc sedimentation) do not measure external diameters but 'equivalent diameters', i.e. diameters of a sphere that has the same property as the material under investigation. For realistic, i.e. non-spherical materials, data obtained from these ensemble or fractionation methods differ significantly.

Issue b) addresses the question to which degree it is possible to convert the particle size distribution as determined by the various instruments into 'number-based size distributions' as required by the definition. This is relevant, as the signal used by several methods depends also on other parameters than the number of particles. Since the difference between methods may come from using a combination of different size metrics (volume-based vs. number based), reliable conversion algorithms would make ensemble methods like DLS or fractionation methods like CLS much more powerful.

[G] The issue is less severe for larger particles, where current imaging methods allow several pictures of a rotating particle, thus giving a good impression of the real shape. In the field of nanotechnology, alignment of platelets may severely bias size assessments.

Finally, issue c) deals with the issue whether it is possible to convert specific surface area to number based distributions of external dimensions, as required by the definition. The importance of this question comes from the fact that the EC definition of nanomaterial lists surface area as one criterion for a nanomaterial. In addition, measurement of surface area is well-established, relatively cheap and fast and surface area data are available for many materials. Being able to convert surface areas into at least median diameters (i.e. 50 % of particles are bigger than this diameter) would allow using surface area determination as a quick and easy screening method for the implementation of the definition.
This chapter discusses the feasibility of the conversions of type a), b) and c).

14.2 Conversion of equivalent diameters into external dimensions

The issue of conversion arises for all methods that do not measure the external diameter directly, i.e. for all methods not that are not based on direct imaging but probe other particle properties and convert these property values into diameters of particles that would have the same properties. These methods are in many cases ensemble methods, i.e. methods that combine signals from many individual particles, but also include methods like PTA and sp-ICP-MS. This section discusses the theoretical and practical limits of such conversions.
In ensemble methods, each individual particle gives rise to a fraction of the compound signal (e.g. light absorption after a certain time, sedimented mass at a certain height, scattered light). It is crucial to bear in mind that it is impossible for these methods to record the signal coming from a single particle – only the total or cumulative signal which comprises the signal parts from all particles measured at the same time is recorded. The signal generated by each particle depends on the size, but often also on factors like particle shape, density, composition and optical properties. The data evaluation therefore has the task to reconstruct the particle size from the sum of all the signals, which cannot be done without additional assumptions or information. Therefore, all data evaluation models usually assume a certain particle shape, but also assume that all particles have the same shape, density and composition. This means that a certain signal can be interpreted in different ways, depending on the assumptions made regarding shape, composition etc.
This fact is illustrated in a study on the signal generated by DLS from gold nanorods by Liu, Nikisha and Qun.[204] The authors investigated nanorods with a diameter of 25 nm and a length of 100 nm, i.e. a material clearly meeting the criteria of the EC nanomaterial definition. Particle size distribution measurements with DLS gave rise to two apparent peaks, one at 5-6 nm and one at 75 nm, hence two lengths corresponding neither to length nor to diameter of the nanorods. This confusing result can be understood when bearing in mind that DLS does not measure the size directly, but measures the diffusion coefficient and calculates the size of spheres that would diffuse at the same speed. The nanorods used in this study have in fact two orthogonal diffusion coefficients, one rotational (i.e. in the

direction of the axis of rotation) and one translational (perpendicular to the axis of rotation). The rotational diffusion coefficient of the nanorods used in the study is of the same size as the translational diffusion coefficient of 5 nm to 6 nm spherical particles, whereas the translational diffusion coefficient of the rods is the same as the one of 75 nm spherical particles.

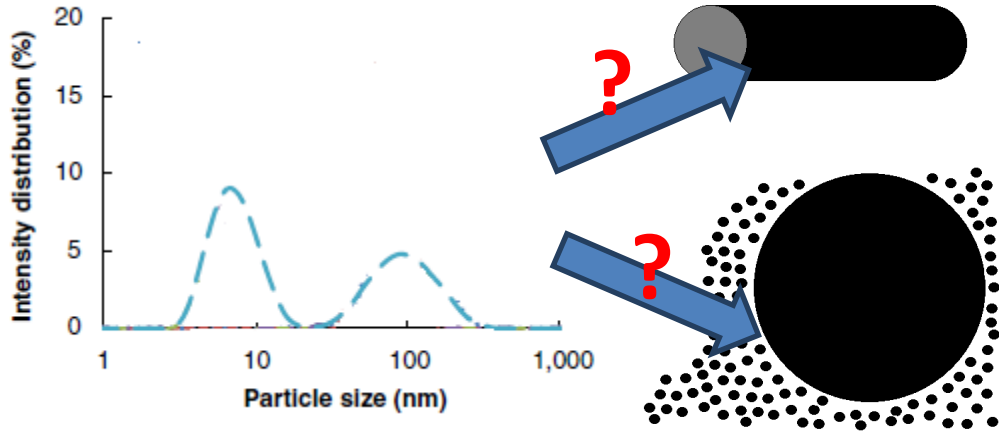


Figure 14.1: A certain DLS signal can come from nanorods but also from two different spherical populations; left side simplified from Ref. 204, right side showing the approximate underlying number-based distributions that could give rise to such a signal. Individual sizes of the particles are approximately to scale.

This means, based on assumptions on particle shape, two very different conclusions about the size and number of particles are obtained. Clearly, if other assumptions are made (e.g. triangular particles, etc.) then other conclusions might be drawn from the same DLS signal.

Real-life materials contain usually particles of different and non-regular shapes and sizes (see Figure 14.2), which results in several additional problems:

- The equations for the signal generation are often only developed for a certain shape, most often spheres. Even if all particles would have the same shape, it would in many cases not be possible to calculate the exact size metrics (length, width, height…) from the instrument signal.
- The final signal is a combination of the contributions from many particles with different shapes and sizes. Even if the equations for the signal generations were known for all shapes, one would have to know the shape of each individual particle in the measurement and the total number of particles in the measurement to obtain a size distribution, which would still not be unambiguous.
- The material properties influencing the signal generation are the same for all particles in the material. This may not be the case if, for example, the material consists of particles of different density or shape.

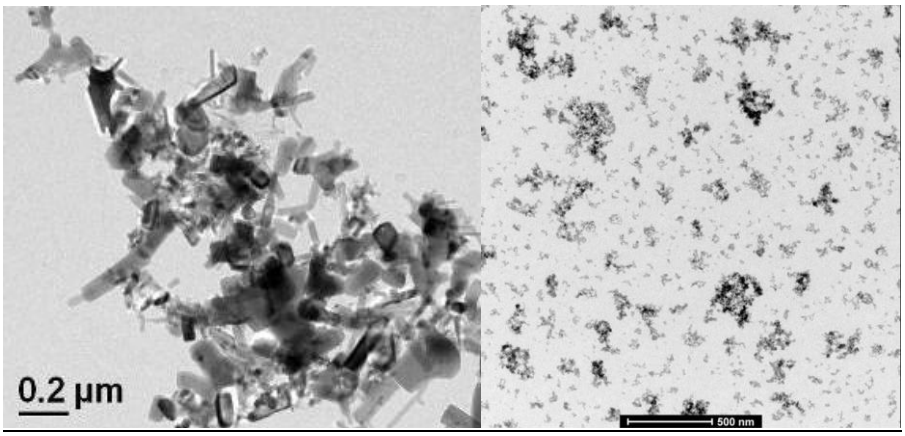


Figure 14.2: TEM image of a ZnO nanomaterial (left, from Ref. 205), SiO_2 nanomaterial (right; from Ref. 138])

The situation seems slightly more favourable for non-ensemble method that measure equivalent diameters of individual particles like PTA or sp-ICPMS[H]. Here the signal of each particle is recorded individually and, assuming specific particle properties like shape, density etc., is converted into an equivalent diameter for each individual particle. If the shape of a particular particle is known, the response of this particle can accurately be converted into external dimensions (e.g. knowledge of the volume of a sphere allows precise calculation of its diameter). An accurate conversion therefore requires the knowledge of the shape of each individual particle measured (a small sphere or a long rod may have the same volume). In practice, this is only possible if all particles have the same (regular) shape, as it is technically impossible to measure first the shape of one particle (e.g. by EM) and then introduce this particle into the other measurement instruments.

For these reasons converting equivalent diameters into 'external dimensions' is only possible with certain assumptions, or if the shape of the particles measured is known. In practice, this means that for unambiguous conversion all particles of a material must have the same (known, regular) shape.

14.3 Conversion of intensity-based into number-based particle size distributions

The second issue is whether (even assuming a simplified situation where all particles have the same shape) it is possible to convert the instrument output into number-based particle size distributions with sufficient accuracy to allow an assessment as to whether a material falls under the definition of 'nanomaterial' or not.

The signal intensity per particle of DLS, CLS, sp-ICP-MS and PTA decreases with decreasing particle size. The signal intensity of DLS and PTA is proportional to the particle diameter to the power of 6, in sp-ICP-

[H] Imaging methods do not fall into that category, as they measure dimensions directly (note that the Feret diameter is not an equivalent diameter).

MS it is proportional to the power of 3. This size-sensitivity is of crucial importance when measuring particles of different sizes simultaneously: in sp-ICP-MS, a particle with a diameter of 100 nm will give a signal that is roughly 40,000 times that of a 3 nm particle, whereas in DLS and PTA a 100 nm particle will give a signal that is 1,400,000,000 times that of a 3 nm particle. A reliable conversion of mass-based or intensity-based signals into number-based signals therefore requires accurate detection of a very weak signal in the presence of a very strong one. In DLS, CLS and sp-ICP-MS, small errors in the quantification of the signal which may be derived from incorrect subtraction of background noise, poor setting of instrumental baselines, improper noise cut-off or the intrinsic inaccuracy of the instrument, are magnified 1,000 to 1,000,000-fold, and may result in severe over- or underestimation of the number of small particles. This effect is illustrated in Figure 14.3.

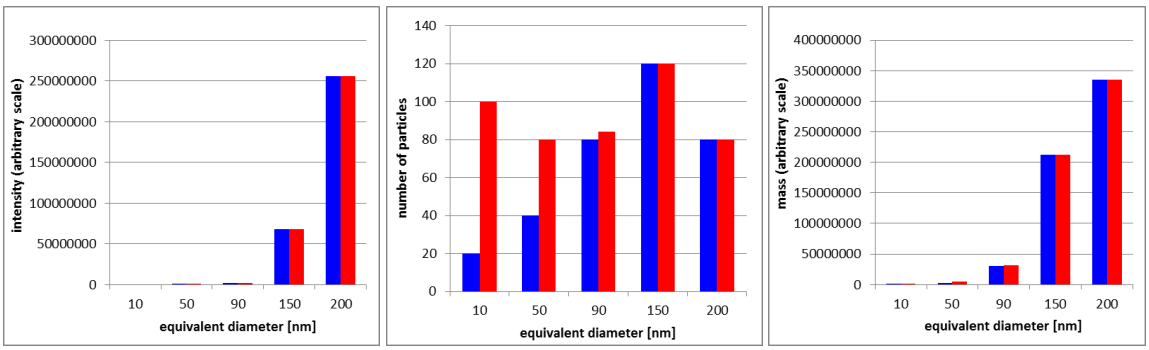


Figure 14.3: Two intensity-based size distributions (left) and particle-number based (middle) and mass based (right) size distributions derived from them. Note that a minute change (not visible in the graph) in the intensity for 10 and 50-nm particles (red columns) results in a large increase of small particles in the number based distribution, resulting in this case in a different classification of the material as nanomaterial or non-nanomaterial.

The nature of the bias in PTA is different: while it does not convert instrument noise into a huge number of particles, the limit of the contrast of the detector means that in the presence of big, very bright particles, smaller particles will not be visible. Based on these theoretical considerations, it is clear that the problem increases the broader the size distribution becomes. These theoretical considerations are confirmed by experiments: Patty and Frisken investigated the conversion of intensity-weighted to number weighted data for spherical particles and found that even at modest polydispersity (polydispersity index above 0.15) the conversion is not reliable.[206]

In addition, some of these methods have lower particle size limits for detection that are well above the minimum range value of 1 nm of the EC definition, i.e. they do not detect smaller particles at all, leading to the opposite effect of underrepresentation of small particles.

For these theoretical as well as practical reasons, handbooks of instrument manufacturers warn against conversion of intensity or mass-based results into number-based ones not only for nanoparticles, but also for particles at a larger scale.[207, 208, 209] As the underlying issues are the same for particles on the

nanometre- or micrometre scale, this long experience in conversion is a very strong indication that conversion between various metrics for distributions is not reliable.

These theoretical considerations were confirmed by two intercomparisons on spherical silica nanoparticles.[210, 211] Particle sizes were measured by DLS and CLS and reported both as intensity-based as well as volume-based diameters. Table 14.1 compares the relative standard deviations obtained.

Table 14.1: Comparison between intensity and volume-based relative standard deviation (RSD) from two intercomparisons on two different near-spherical silica particles (silica 1 = ERM-FD100, silica 2 = ERM-FD304 [Refs. 210,211]). Intensity-based and volume based pairs came from the same measurement.

	RSD of the intensity – based diameter [%]	RSD of the volume – based diameter%]
DLS – silica 1 [Ref. 210]	3.2	6.8
DLS – silica 2 [Ref. 211]	0.9	13.5
CLS – silica 1 [Ref. 210]	3.4	8.3
CLS – silica 2 [Ref. 211]	3.6	14.6

It should be noted that the averages for each material and method are based on the same measurements (the only difference is the conversion of the results), and therefore do not include any variation due to different measurement condition. Despite this highly controlled measurement setup and the high sphericity of the particles, converted (= volume-based) diameters scatter significantly more than intensity based ones, demonstrating that even for nearly perfect particles, conversion of the basis of the size distribution introduces significant variation. The slightly lower sphericity of silica 2 compared to silica 1 results in a markedly higher variation of the converted results, showing how sensitive conversion algorithms are to deviations from their assumption.

This practical experience is in line with the theoretical expectations and shows that, exactly as is the case for larger particles, conversion of different size metrics for the sizing of nanoparticles is - except in very specific cases normally not found for industrially produced nanoparticles - not reliable.

14.4 Conversion of number-based into mass-or volume based distributions

In many cases it is interesting to convert number-based distributions into mass-based ones, as materials are traded by mass and not by number of particles. With respect to nanoparticles, two different cases exist, namely conversion of number-based distributions of external dimensions into mass-based

distributions (applicable for imaging methods), and conversion of number-based hydrodydamic diameter into mass based distributions (PTA). As sp-ICP-MS immediately measures mass, no conversion is required for this method.

Imaging methods usually obtain detailed shape information of the individual particles. Commercial imaging software automatically produces a multitude of shape parameters that allow (as long as the particle is of a reasonably regular shape) calculation of a particle volume, and, if the density is known, calculation of the particle mass. While seemingly simple, two caveats exist in the case of nanoparticles:

- Imaging by EM only probes two of the three particle dimensions, so particle thickness often needs to be estimated. In addition, particles may preferably align in a certain way so that the image alone may be insufficient to estimate a particle volume. An example is platelets that by maximizing contact between the platelet and the carrier will present preferably the largest area for imaging. This means that additional information about particle shape is required to estimate the third particle dimension. Imaging by AFM is also affected by this problem, albeit that for AFM the vertical dimension (with respect to the substrate) is more reliable than the lateral dimensions (which are more affected by the shape of the AFM tip).
- Mass-based distributions are largely determined by large particles whereas number-based distributions tend to be dominated by small particles (see Figure 14.). Images for an accurate mass-based distribution therefore need to include a sufficient number of the large particles, which generally necessitates lower resolutions to probe a larger area. Lower resolutions in turn result in not being able to see many of the very small particles. This does not matter for mass-based distributions, as small particles do not contribute significantly to the mass-based distributions. The high resolution required for number-based distributions means that the estimate of the number of large particles is not accurate, which does not matter for number based distributions, as here the few large particles do not contribute much. Therefore, images used for number-based distributions are often not suitable for the estimation of mass-based distributions.

The situation is different for PTA, which measures the equivalent hydrodynamic diameter of individual particles. As explained in Section 14.2, knowledge of the shape of each individual particle is required for conversion, which in practice means that all particles must have the same (regular) shape.

14.5 Conversion of volume-specific surface area into number-based distributions of external dimensions

The current definition introduced a specific surface area of 60 m^2/cm^3 as an additional criterion to decide whether a material is a nanomaterial or not. This surface area of 60 m^2/cm^3 is based on the surface area of perfect, dense spheres of 100 nm nanometre diameter. However, many absorption

materials used in the laboratory and industry have volume specific surface areas of three to four times those specified in the definition and at the same time particle sizes of up to 1 mm.

Conversion of specific surface areas to number-based particle size distributions, or at least to the median of number based distributions would require knowledge of the shape of the particles as well as the particle size distribution.

- The specific surface of a particle depends strongly on the shape. For example, a cube of 100 nm length has nearly twice the surface area of a sphere of 100 nm diameter. Conversion of specific surface area therefore requires that all particles have the same shape.
- However, knowing the shape of the particles is not sufficient. Whereas particle size measurements determine the particle size distribution of an equivalent diameter, measurement of the specific surface area results only in one single number. Conversion of this number into sizes requires knowledge of the particle size distribution[I]. This means that using specific surface area data for the implementation of the current EC Definition still requires measurement of the particle size distribution[J].

These issues mean that specific surface area data cannot be converted into number–based distributions of external dimensions without prior knowledge about the material and without making a number of assumptions. Conversion into the median of number-based external dimensions is very approximate and is at best a crude screening tool.

14.6 Conclusion

Conversion of different size metrics as well as of distributions is a common problem for all methods of sizing of particles. Theoretical considerations as well as experience from sizing of both nanoparticles and larger particles show that such conversions generally do not result in reliable particle size distributions based on the converted data. This means that conversion of size metrics is generally not accurate enough for unknown materials where little or no previous knowledge on the material is available.

[I] An area of 0.13 μm^2 can be based on the area of one sphere of a diameter of 200 nm, 4 spheres of a diameter of 80 nm, 400 spheres of a diameter of 10 nm etc.

[J] Using specific surface area information could in principle be used to correct for a calibration bias in the measurement. In practice, modelling of the specific surface area from the biased size distribution will presumably introduce bigger biases than are incurred by calibration.

15 NANOSTRUCTURED MATERIALS

This chapter is dedicated to nanostructured materials. The first part (15.1) provides an overview about classes and definitions of nanostructured materials according to ISO and others classifications developed during the past decades.

In a second part (15.2), the most common manufacturing techniques for the production of nanostructured materials are summarised and information and examples for each method are given. Finally, the third part (15.3) describes methods for the characterisation of nanostructured materials.

15.1 Classes of nanostructured materials

The section provides an overview about classes of nanostructured materials, and their main features including surface and internal structure. ISO definitions and categories of nanostructured materials are explained first,[K] followed by other classification schemes according to various material properties. It should be noted here that since the EC Definition covers agglomerates and aggregates (composed of particles with one or more external dimensions at the nanoscale) it already covers certain types of nanostructured materials as defined by ISO.

ISO proposes the following classification scheme for nanostructured materials:[212]

15.1.1 Nanostructured material

Material having internal or surface structure in the nanoscale.

If external dimension(s) are in the nanoscale, ISO recommends using the term **nano-object**.[212]

ISO distinguishes between 5 categories of nanostructured materials:

15.1.2 Nanostructured powder

Powder comprising nanostructured agglomerates (agglomerate of nano-objects or agglomerate of nanostructured aggregates), nanostructured aggregates (aggregate formed from nano-objects), or other particles of nanostructured material (i.e., having internal or surface structure in the nanoscale), nanostructured core-shell particles (particle consisting of a core and shell(s), where the diameter of the core or the thickness of the shell is in the nanoscale), nanostructured capsules (shell with nanoscale thickness, which can enclose, fix, transport or release substances)

15.1.3 Nanocomposite

Solid comprising a mixture of two or more phase-separated materials, one or more being nanophase, including polymer matrix nanocomposite (nanocomposite with at least one major polymeric phase), polymer clay nanocomposite (polymer matrix nanocomposite with a nanostructured clay phase), metal

[K] Terms from the ISO Online Browsing Platform (https://www.iso.org/obp/ui/). Copyright remains with ISO.

matrix nanocomposite (nanocomposite with at least one major metallic phase), ceramic matrix nanocomposite (nanocomposite with at least one major ceramic phase)

15.1.4 Solid nanofoam

Meaning a solid matrix filled with a second, gaseous phase, typically resulting in a material of much lower density, with a nanostructured matrix, e.g. having nanoscale struts and walls, or gaseous nanophase consisting of nanoscale bubbles, or both

15.1.5 Nanoporous material

Solid material with nanopores

15.1.6 Fluid nanodispersion

Heterogeneous material in which nano-objects or a nanophase are dispersed in a continuous fluid phase of a different composition.

It includes nanosuspensions (heterogeneous material in which nano-objects or a nanophase are dispersed in a continuous fluid phase of a different composition), nano emulsions (fluid nanodispersion where the dispersed phase is a solid), liquid nanofoams (fluid nanodispersion filled with a second, gaseous nanophase, typically resulting in a material of much lower density), nano aerosols (fluid nanodispersion with gaseous matrix and at least one liquid or solid nanophase)

Some nanostructured materials described in the literature are not yet specifically defined by ISO in Ref. 212, for example nanofilms, nanoscale thin layers or nanostructured pattern or surfaces. ISO is currently working on a new document in order to define also other nanostructured material types.[213]

In addition to the ISO classification other classification schemes of nanostructured materials were proposed. These are described below.

Gleiter has proposed a detailed but complex classification schema for nanostructured materials according to their chemical composition and the dimensionality (shape).[214] According to the scheme, nanostructured materials can be divided into three categories according to the shape of the crystallites and the chemical composition:

- The first one comprises 'materials and/or devices with reduced dimensions and/or dimensionality in the form of (isolated, substrate-supported or embedded) nanometre-sized particles, thin wires or thin films'.[214] Examples are catalysts and semiconductor devices utilizing single or multilayer quantum well structures.
- The second category is corresponding to 'materials and/or devices in which the nanometer-sized microstructure is limited to a thin (nanometer-sized) surface region of a bulk material'.[214] Materials with enhanced corrosion resistance obtained by modification of a nanometer-thin surface region are examples of this category.

- The third one comprises 'bulk solids with a nanometer-scale microstructure'.[214] Materials assembled of nanometer-sized building blocks, for example crystallites, are examples of this category.

The classification of nanostructured material proposed by Siegel is based on the 'modulation dimensionality' of the material:[215]

- 0D (nanoclusters, nanospheres),
- 1D (multilayers),
- 2D (nanograined layers, films, plates, and networks)
- 3D (nanophase materials consisting of equiaxed nanometer sized grains).

Interestingly, nanofibres are not covered by this scheme.

Pokropivny and Skorokhod also proposed a classification according to the dimensionality of nanostructured materials.[216] According to these authors, nanostructured materials can be described as materials comprising building units with dimensions at the nanoscale size in at least one direction. The building units can be

- 0D: e.g., clusters and particles, nanospheres, fullerene, molecules, rings, grains
- 1D: e.g., nanotubes, nanofibres, nanowires
- 2D: e. g., nanoplates and layers

Here the dimensionality is related to the number of dimensions that are not at the nanoscale.

Nanostructured materials can be generated by assembling these building units, and 36 classes of nanostructured materials are defined by the authors of that scheme.[216]

15.2 Most common manufacturing techniques used for the production of nanostructured materials

Manufacturing techniques for nanomaterials including nanoparticles, nanotubes, core-shell nanoparticles, or composite nanospheres are described in Section 12, which includes also manufacturing techniques for certain constituents made from nanostructured materials as defined by ISO, such as fluid nanodispersions, or nanostructured powders. In this chapter manufacturing techniques for materials with structures at the nanoscale, but not regarded as nanomaterials according to the EC Definition of nanomaterial, are introduced. These materials will be called 'nanostructured materials' hereafter.

Two basic approaches are known to manufacture nanomaterials: 'top down' and 'bottom up' strategies (see Section 12 and Figure 15.1). The term 'top down' strategy applied to nanomaterial or nanostructured material synthesis is characterized by bulk material reduction or decomposition. The 'bottom up' strategy refers to nanoparticle synthesis by assembling atomic or molecular components.

One of the most common techniques used in nanostructured materials productions are patterning and etching which represents a top down strategy.[217] Typical techniques which utilise the bottom up

approach in the production of 2D and 3D nanostructures are e.g. sol gel methods,[218] and chemical or physical deposition techniques.[219]

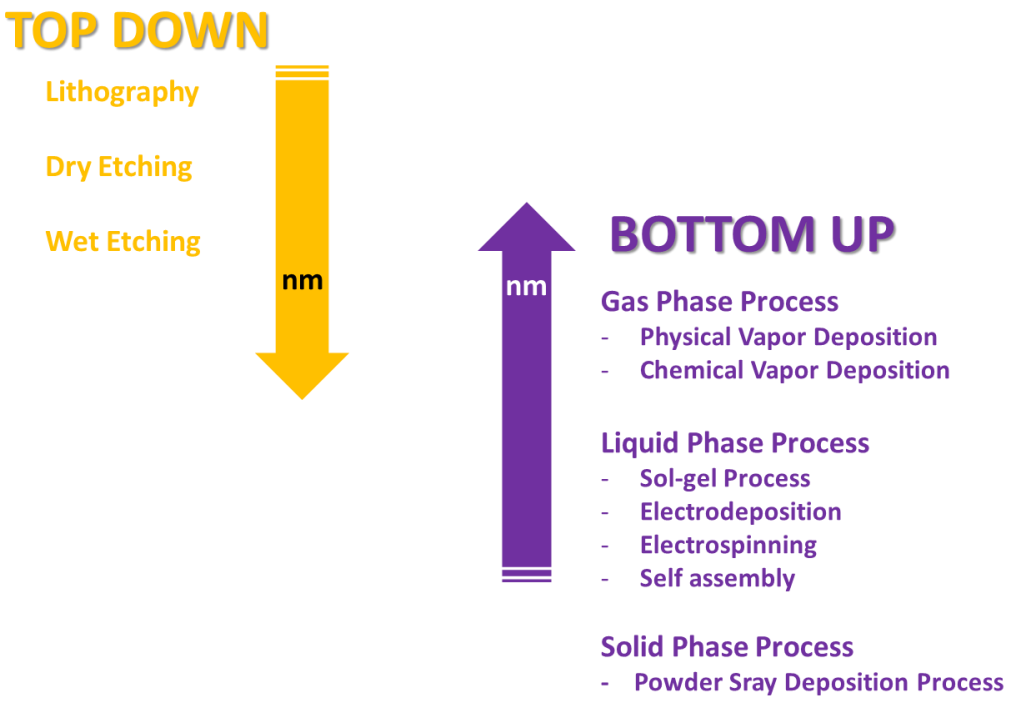


Figure 15.1: Approaches to produce nanostructured materials

15.2.1 Top down processes

The most widely used top down processes for production of nanostructured material are patterning/etching techniques such as lithography (the most employed), dry etching, or wet etching.

Lithography[217] is a technique which enables the creation of patterns, on the surface of a material, with a size ranging from few nanometres to millimetres. Depending on the use of the mask, lithography techniques can be divided in two groups: masked and mask free lithography.

In the first category (masked lithography), the major ones are:

- ***Photolithography*** (Figure 15.2) has been widely used in the manufacturing of MEMS (microelectromechanical system) devices, microchips and integrated circuit.[217] In this technique, a light sensitive polymer is used to ultraviolet light is used to define a pattern. The polymer can be a positive or negative photoresist. A positive photoresist becomes instable when exposed to UV light. The chemical structure of the resist changes under exposure to the UV light and would be removed with a solution called 'developper'. Negative resists behave in the opposite manner.

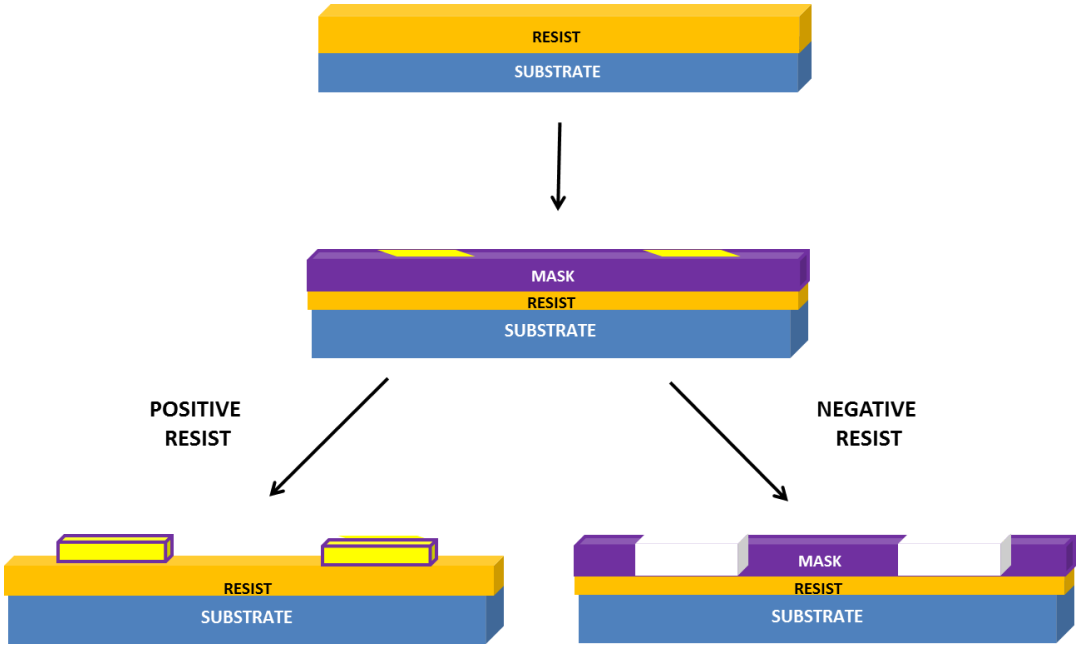


Figure 15.2: Photolithography

There are three main types of photolithography: **contact printing, proximity printing and projection printing.**[217] The two first ones are able to make pattern of few micrometres. The projection printing technique enables a resolution of 37 nm. In this system, an optical lens is used to project a deep UV-pattern from an excimer laser. Technologies as immersion lithography,[220] resolution enhancement technology[221] and extreme UV lithograph[222] are currently developed in order to improve the resolution of projection printing.

- ***Soft lithography***[217] is a collective name for techniques based on self-assembling and molding (Figure 15.3). This process requires inexpensive materials and does not need specialized equipment. In this technique, an organic polymer is used to generate patterns and structures without the use of the light or high energy particles. The four main known soft lithography processes are **printing/decal transfer, molding/embossing, phase-shifting edge lithography, nanoskiving**.[223]

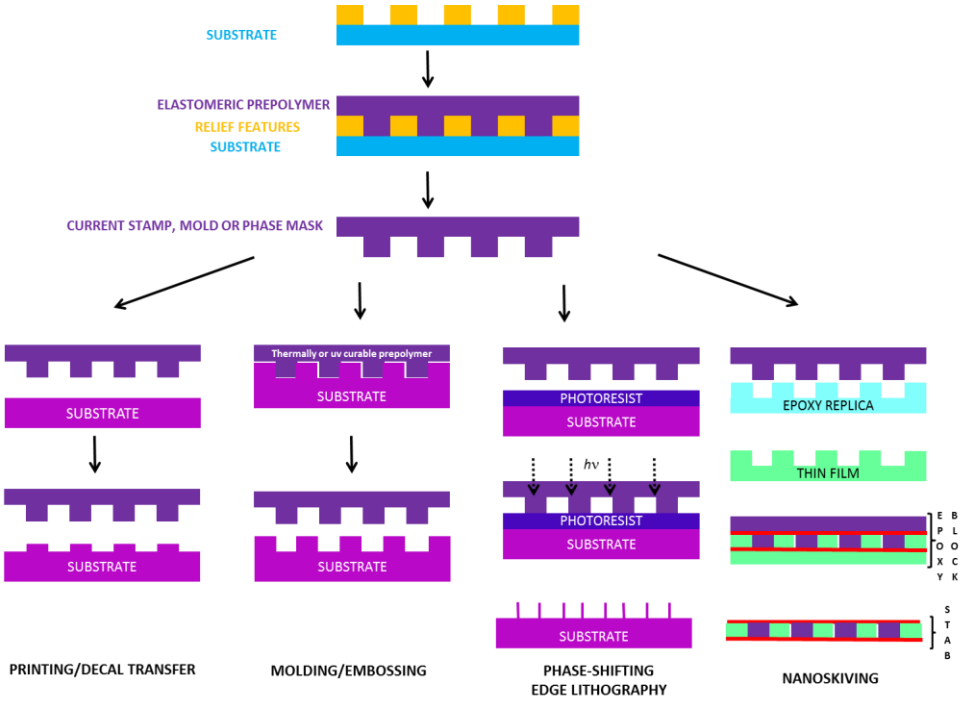


Figure 15.3: Soft lithography techniques

- ***Nano-imprint lithography*** (Figure 15.4) can create resist pattern, but it is possible also to imprint functional device structures in various polymers. In this process, a hard mold with a nanoscale relief features is pressed into a polymeric material at a controlled temperature and pressure as shown in a scheme above. This technique allows ultrahigh resolution (10 nm). The semiconductor and hard disk drive industries are investigating in this method for future high volume manufacturing of memory devices and patterned media. [224]

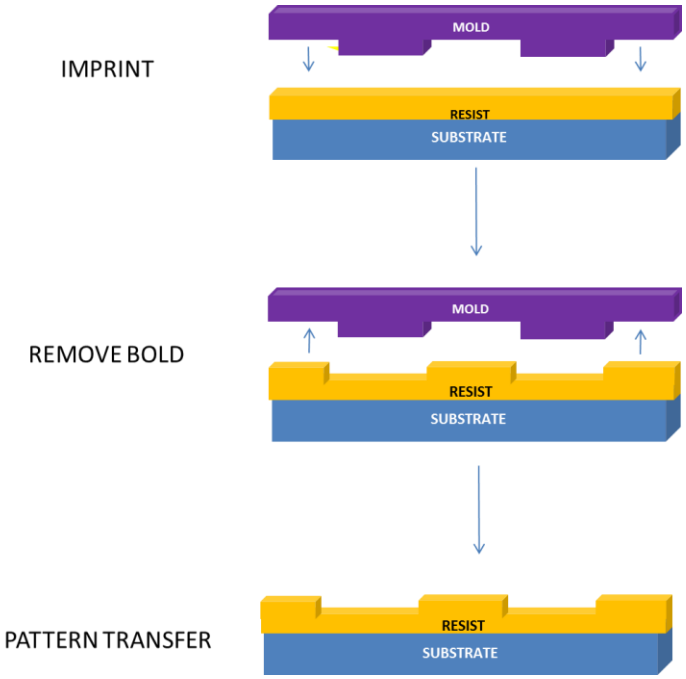


Figure 15.4: Nanoimprint Lithography

The mask free lithography techniques are

- ***Scanner beam lithography*** can be divided in three main classes[225]: scanner laser beams (250 nm resolution), ***focused electron beam*** (5 nm resolution), and ***focused ion beam*** (20 nm resolution). Electron beam lithography is a process in which an accelerated electron beam is focused on an electron-sensitive substrate to make an exposure. The focused ion beam lithography uses an accelerated ion beam instead of electron beam to write pattern directly onto a substrate. These processes are slow but could generate pattern with high resolution. The techniques could be applied in the production of integrated circuits, channels for nanofluidics, plasmonic lens. [217]
- ***Scanning Probe Lithography*** is a technique in which the surface could be manipulated with an atomic scale resolution. [217] The most common technique used is the ***dip-pen nanolithography*** process, in which nanoparticles or molecules could be deposited selectively onto a substrate. It could be achieved under ambient environment. One of the disadvantages of this process is the difficulty to generate reproducible structures between scans, and the challenge would be to scale up this technique.[225]

The dry etching processes are often plasma-based technique. The three basic dry etching methods are reactive ion etching (RIE), ion milling and vapour phase etching. ***Ion milling*** is a purely physical process which utilizes accelerated inert ions that strike perpendicular to the surface in order to create nanostructured surfaces. ***RIE etching***, also called ***ion-assisted etching***, is both a physical and chemical process. In this method, the reactive species react with the material only when the surfaces are 'activated' by the collision of incident ions from the plasma. ***Deep reactive ion etching (DRIE)*** is a technique in which passivation deposition and etching steps are performed sequentially in a two-step cycle. Dry etching can also be achieved a non-plasma technique if the etching gases are reactive enough like ***vapour-phase etching (VPE)*** processes. All these process are used to form electronic circuit on semiconductor industry.[226]

-***Wet etching*** is a technique in which material would be dissolved by chemical solutions in order to create nanostructured films. Wet etching works very well for etching thin films on substrates, and can also be used to etch the substrate itself. It is used in semiconductor and MEMS industry.[226]

15.2.2 Bottom up processes

With the bottom up methods,[141,142] nanostructured materials could be synthetized from the liquid, solid, or gas phase following physic chemical principles of atomic and molecular organization. Each process can be sub-classified based on the source of energy used for the nanomaterials production.

Gas Phase processes

Gas phase techniques are among the most common industrial methods for the production of nanomaterials but also nanostructured thin layers. These syntheses are simple, inexpensive, and could have high product yield.

In this method, nanostructured materials are synthetized from a vapour phase mixture.[142] Precursor would be evaporated to form a phase which is thermodynamically unstable relative to a solid material formation would induce a supersaturation situation, which is thermodynamically favourable for molecules reaction. If different conditions (i.e. degree of supersaturation, temperature, pressure, residence time…) are combined, particles would nucleate homogenously and enable formation of nanoparticles and would coat homogeneously the surface targeted.

15.2.3 Physical vapour deposition

In Physical vapour deposition (PVD) techniques, nanolayer is deposited by a transfer of a precursor material (source) to the substrate, both of which are in the same chamber deposition.

Thermal evaporation (Figure 15.5) is one of the most common methods to produce a nanocoating. By Appling this methods nanowires on a substrate as well as dendritic or fishbone shaped particles can be manufactured. In this technique the source material is sublimated at high temperatures and once it reaches the substrate surface, nucleation (this concept was explained in Section 12) and growth of nanostructure occurs. It is necessary to control the growth in order to choose size, size distribution, shape, crystal structure by parameter such as temperature, pressure, carrier gas, flow rate of carrier gas, substrate and evaporation time period.[227] This technique is ideal for non-refractory materials because of the temperature needed for the process.

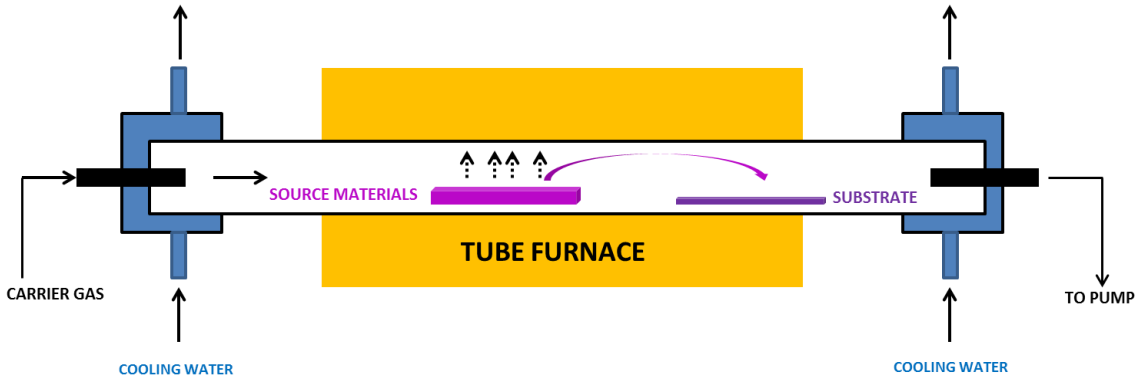


Figure 15.5: Thermal evaporation

Examples of nanomaterials which could be synthetized by this method are given below:

Metals : Fe_2O_3,[227] *CdO,*[227] *SnO,*[227] *SnO_2* [227]
Composites: SnO2/Pd,[228] *InN,* [228] *GaN,*[228] As_2O_3 [228]
Ceramics: SiO_2, [227] *TiO_2* [229]

Electron beam PVD is a versatile coating method which enables a thin layer formation with a large range of materials such as ceramic, oxide, metallic and offers high purity, structural and morphological control of film, and a high range of deposition rate.[230,231,232] The source are decomposed into molecules by the bombardment of electrons beam.[230] Then, material evaporated condenses of the surface of a substrate

to form a coating.[231] The fabrication of these nanostructured coating have many applications including space, turbine, optical, biomedical and auto industry. [230,231,232]

Examples of nanomaterials which could be synthetized by this method are given below:

Metals : Re, [230] *Cr,* [230] Mo, [230] *Au* [233]
Composites TiC, [230] *WCo,* [230] *YST (yttrium stabilized zirconia)* [230]
Ceramics: TiO_2 [232]
Carbon: layer of carbon [232]

Sputtering is a method in which high energy particles are generated and directed at the target. Atoms or molecules which were sputtered from the target are transported through a region of reduced pressure to the substrate.[226] The particles which bombard the target could be produced by different ways (direct current (dc), radio frequency (rf), Dc or Rf magnetron).[234] This process could be applied in the production of nano metals, semiconductors or insulators and this technique is the base of a lot of industrial coating activities, as it is scalable from small substrate to pieces of large areas. Moreover, with this processes, it is possible to achieve coating but also more complex nanostructured materials such as nanoripple, nanoholes, nanoneedles and nanodot periodic structures.[235,236]

Examples of nanomaterials which could be synthetized by this method are given below:

Metal: Fe,[236] *CuN,*[237] *Au*[238]
Composite: FeGaAs, [236] *Cu,*[237] CaF_2,[238] *LiF* [238]
Ceramics : Si, [238] SiO_2, [235] TiO_2, [238] *Hydroxyapatite*[239]
Carbon: HOPG (highly oriented pyrolytic graphite)[238]

Pulsed Laser Deposition (or ablation) is a process in which a laser beam is used as the excitation source to generate educts (atoms, molecules) from a solid surface. Plasma subsequently formed is deposited on the substrate.[240] Multitarget system could be also used. This technique is simple, enables the deposition of complex materials with preserved stoichiometry and allows to controlling the thickness of the desired coating.[240] Thin layers, nanowires or nanoclusters could be synthetized by this process.[228,240,241] Additionally by employing a laser spark atomizer highly mesopourous films can be produced.[242]

Examples of nanomaterials which could be synthetized by this method are given below:

Metal: Au,[243] *Sn,* [243] *Bi*[244]
Composite: FeNdB, [240] *GaAs,*[245] *LiF* [243]
Ceramic: ZrO_2, [218] *SiC,* [228] *Si,* [228] *LiMn* [242]

Molecular Beam Epitaxy is a technique in which atoms or clusters of atom are produced by heating up a solid form of the precursor in Ultra-High-Vacuum Chamber, to form high quality epitaxial structures with monolayer control.[246] The precursors could also be in the gas phase gaseous, and this technique is than called ***Chemical Beam Epitaxy.*** [247] This process is one of the most commonly used method to produce epitaxial layers of a wide range of materials such as oxides, semiconductors or metals.[246,247,248] This

technique enables a high purity of layer, allows a precise control of composition during growth, and it is an ideal way to synthesise a material layer by layer.[246,247]

Examples of nanomaterials which could be synthetized by this method are given below:

Metal: Au,[249] Ag,[250] Fe[251]
Composite: GaAs,[248] AlAs, [246] SiGe,[246] GaN [228], *ZnSe,*[252] *ZnCdSe*[252]
Ceramic: Si [253]

Arc Vapour Deposition is a method that uses the vaporisation from an electrode under arcing conditions as a source of precursors. A pulse or continuous high current and a low voltage electric current are needed.[254] The material can be vaporised from the molten surface of both electrodes thus two branches of this technique are recognised as ***Cathodic Arc Deposition*** and ***Anodic Arc Deposition***.[254] Arc Vapour Deposition technique provides a high vaporization rate but macroparticles of metals and liquid droplets could be produced too during the coating which could induce a non-homogeneous nanolayer.[254]

Examples of nanomaterials which could be synthetized by this method are given below:

Metal: deposition of adherent metal coating[254]
Composite: CrN, [254] *TiAlN,* [254] *TiCrN* [254]
Ceramic: TiN, [254] *TiC,* [254]
Carbon: carbon thin layer [255]

15.2.4 Chemical vapour deposition

Chemical Vapour Deposition (CVD, Figure 15.6) is a well-known process in which the reactant gases are introduced in a reaction chamber to decompose and react in a activate environment (heat, light, plasma) with the source materials to form the film on a substrate.[256] CVD reaction needs activation energy which could be provided by several processes.[256] These methods differ in the means by which chemical reactions are initiated. CVD covers techniques as ***Atmospheric Pressure Chemical Vapour Deposition (APCVD), Low Pressure Chemical Vapour Deposition (LPCVD), Metal-Organic Chemical Vapour Deposition (MOCVD), Plasma Assisted Chemical Vapour Deposition (PACVD) or Plasma Enhanced Chemical Vapour Deposition (PECVD), Laser Chemical Vapour Deposition (LCVD), Photochemical Vapour Deposition (PCVD), Chemical Vapour Infiltration (CVI),*** and ***Chemical Beam Epitaxy (CBE)***[257] It is one of the main processing technique for the deposition of thin films and coatings for a wide range of industrial applications.[257]

Examples of nanomaterials which could be synthetized by this method are given below:

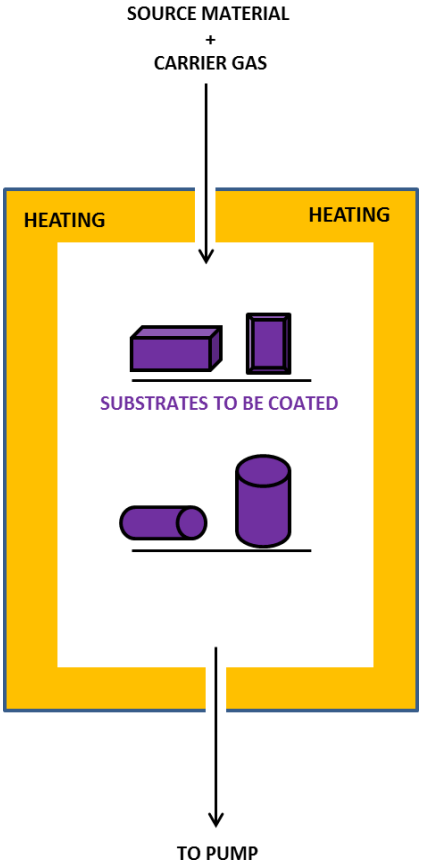


Figure 15.6: Chemical vapour deposition

Metal: W, [257] Mo, [257] Au, [257] Cu, [257] Pt, [257] Fe_2O_3 [256]
Composite: CrAlYt, [256] FeAl[256]
Ceramic: SiO_2[257] SiC, [257] TiN, [257] ZrO_2 [257]
Carbon: diamond film, [257] diamond like carbon (DLC) film[257]

15.2.5 Liquid phase processes

Sol-gel is a powerful method to prepare inorganic material such as glasses and ceramics. A dispersion of colloid particles (the sol) is formed by a hydrolysis of soluble precursor molecules.[247] Chemical reaction leads to form between those particles an infinite network of particles (the gel). High-purity nanomaterial could be synthetized by this method at low temperature.[258] Moreover, this process allows the formation of thin layers, membrane, monodisperse tubules or fibrils.[247,258] This process offers many advantages such as an excellent stoichiometry control of precursor solutions, possibility to customise produced microstructures or encapsulate elements, ability of layer deposition a large areas of substrates, and a simple and costless equipment.[247] The coating could be achieved by several techniques such as ***dip coating, angle-dependent dip-coating, capillary coating, roll coating, spin coating, flow coating.*** [247]

Examples of nanomaterials which could be synthetized by this method are given below:

Metal: V_2O_5, [258] *Co_3O_4,* [258] *WO_3* [258]
Composite: Au/SiO_2,[259] *Ag/SiO_2,*[259] *Ag/TiO_2* [259]

Ceramic: SiO_2,[258] TiO_2 [258]
Polymer and hybrid composite: Polystyrene,[258] Alginate–SiO_2,[260] Poly(vinyl alcohol)–poly(functional-siloxane)–SiO_2 [260]

Electrospinning is a simple, versatile technique to synthetize nanofibers from a rich variety of materials including polymers, composites, and ceramics. This process uses electrostatic forces to produce fine fibers from polymer solutions or melts and the fibers thus produced have a thinner diameter (from nanometer to micrometer) and a larger surface area than those obtained from conventional spinning processes. Furthermore, a DC voltage in the range of several tens of kVs is necessary to generate the electrospinning. Most of the polymers are dissolved in some solvents and then extracted from the solution and stretched by electricity spins fibers all in one continuous electricity field.[261] ***Electroblowing*** is a technique derivate from electrospinning which is combined with hot air blowing.[262] These techniques are particularly used in industry and are particularly attractive for academic research to develop artificial tissues.[261]

Polymer: Poly(ε-caprolactone),[261] Gelatin,[261] Poly(vinyl alcohol)[261]

Electrodeposition is a relatively inexpensive method which could be performed at low temperatures.[247]This method covers **electroplating** and **electroless plating**.[226] In the first one, the substrate would be placed in an aqueous electrolyte solution. When an electrical potential is applied between the substrate and the counter electrode in a liquid, a chemical redox reaction occurs, leading to the deposition of a layer.[226] The thickness of the film could be controlled by monitoring the consumed charge.[247] Electroless plating is an autocatalytic process in which the reduction of the metallic ions in the solution and the film coating could be formed out through the oxidation of a chemical compound present in the solution.[226] This method does not require any external electrical potential which in fact limits the possibility of controlling the thickness layer.[226]

Examples of nanomaterials which could be synthetized by this method are given below:

Metals: Au,[263] Pd,[263] Cu,[263] Fe,[263] Ni [263]
Composite: NiP, [263] FeNi, [263] CuSb,[263] CoNiP,[263] AuCuCd[263]

Anodization is another electrochemical process to synthesise nanostructured materials. It is a powerful approach to produce hollow nano-architectures.[264] Typical anodization includes alkaline cleaning, acid activation, and electrolyte anodizing.[265] The acid activation could be achieved in a mixture of nitric acid and hydrofluoric acid (HF) in order to remove oxide layer and surface contaminants.[265] Electrolyte anodization is performed in an electrochemical cell, which has a three electrode configuration composed of an anode, a cathode and a reference electrode. A current or a voltage is applied between anode and cathode to produce electrode reactions and leads to the formation of an oxide layer on the

anode surface. [265] The applications are various such as biosensing, immunosensor, solar cells or template for other synthesis.[264]

Ceramics: Titania dioxide nanotubes,[264] *nanoporous alumina surface,*[264] *nanoporous silica surface.* [264]

In nature, sophisticated nanostructures already exist.[266] These materials were made via ***Self-assembly***. Biologic molecules such as DNA, protein or peptide could form one, two or three dimensional nanostructures.[266] It is also possible to deposit a monolayer of molecules called Self Assembled Monolayer (SAM) on substrates [267] Dendrimers were already observed to form self-assembly monolayer.[268] Block copolymers could also self-assemble to form thin films.[269]

Examples of nanomaterials which could be synthetized by this method are given below:

Molecule layer: Alkanethiols,[267] *dialkyl disulfides,* [267] *dialkyl sulphide* [267]
Biologic molecules: DNA, [266] *phospholipid* [266]
Polymers: poly(styrene-b-methyl methacrylate)[269]

15.2.6 Solid phase processes

Thermal Spray process is a technique in which a coating is formed by stacking lamellae to a substrate from impact, flattening and solidification or impinging molten particles.[270] It was already commonly used for microparticle production, but to scale down to the nanometer range, the particle could not be thermal sprayed using the same process, and the injection force has to be increased.[270] Micrometer-sized agglomerates of nanoparticles, suspensions, or emulsions of nanoparticles, or salt of precursors could be used in order to achieve nanostructured coating.[271] With ***Plasma spraying***, the particles could be sprayed by a radio frequency or a direct current plasma jet in order to generate nanostructured coating. [270] In ***Wire Arc spray,*** the high electric arc energy from the two consumable wires heats gas inside a small vessel which is pressurized, a jet stream exits in a front of which the wires are positioned and then melted, atomized, and propelled to the substrate.[271] ***High velocity oxy fuel (HVOF***, Figure 15.7***)*** is a method in which combustion of reactants in order to create a very high velocity which is used to propel the particles to the substrate. This technique is particularly suitable for spraying nanosized particles.[270] ***Flame Spray*** is an oxy/acetylene combustion spraying technique in which a welding torch is used with the addition of a high velocity air stream to propel molten particles on a substrate.[270] **Detonation Gun (Dgun)** could be also used to spray particles in air at atmospheric pressure. The process is basically the same as in the flame spray method: a combustion and jet expansion is created by using acetylene and oxygen.[270]

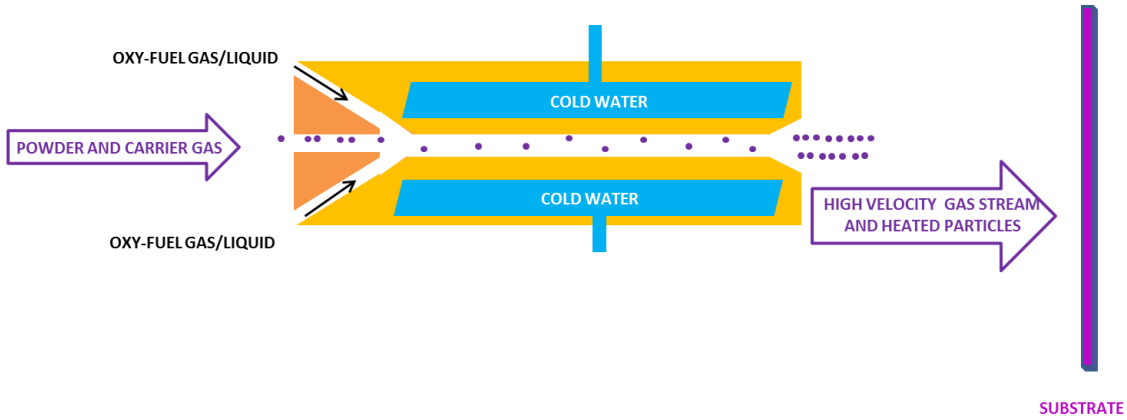


Figure 15.7: HVOF process

Examples of nanomaterials which could be synthetized by this method are given below:

Metal: Au,[272] *Cr_2O_3* [273]
Composite:Al_2O_3-CNT(Carbon nanotube),[274] *NiFe/SiO_2* [275]
Ceramic: TiO_2, [275] *$Al_2O_3TiO_2$* [275]

In situ formation of nanostructured materials, e.g. by heat treatments, can occur and leads to the formation of precipitated nano-objects in a solid matrix.[276] It is difficult to control the nanostructured synthesis in this type of synthesis. [276] Nevertheless, the use of the approach is ancient. For example, it was found that the Lycurgus cup, a cup dated from the fourth century A.D, contains gold and silver nanoparticles within the glass part to render its special optical properties.[277]

Alloys: Fe-Al-Cr, [277] *Ti-Cu-Ni-Sn-Ta* [278]

15.2.7 Summary

This chapter provided an overview about classification schemes for nanostructured materials (in particular but not exclusively according to ISO) and reviewed of most common manufacturing techniques to produce nanostructured materials, including examples of industry application for each method.

The most common top down manufacturing processes for nanostructured material are patterning /etching techniques as lithography (the most employed), dry etching, and wet etching. These approaches are commonly used in manufacturing of memory devices and semiconductor and MEMS industry. Bottom up processes are more diverse and cover academic and industry sectors as biomedical, space, automobile, coating industry.

15.3 Methods for the characterisation of nanostructured materials

15.3.1 Background

The current EC nanomaterial definition is based on particulate materials, and their external nanoscale dimensions. Other nanomaterial definitions, e.g. the CEN/ISO definition, include also materials with an

internal nanoscale structure. The latter approach turns the term nanomaterials into a much broader concept.

Whether or not to include nanostructured materials into a revised definition of nanomaterial is not discussed in this subsection of the report. Instead, a preliminary investigation into the available measurement methods for the characterisation of the internal nanoscale features of nanostructured materials is provided. This complements the information presented in Section 4 of the report (Update on relevant measurement methods) and the recent JRC Reference Report EUR 25404.[42]

In this section, the term nanostructured material is understood as 'material having internal or surface structure in the nanoscale' in ISO/TS 80004. [212]

15.3.2 Characterisation of internal structures in the nanoscale

15.3.2.1 Discrete vs continuous nanoscale features

Two major types of internal structure can be distinguished: those that are formed by a continuous phase (e.g. a very fine network of pores or thin layers) and those that are formed by a phase of discrete objects, precipitated or dispersed across the matrix.

In the former case, the nanostructured phase is difficult to separate or isolate from the nanostructured material, and size measurements in general need to be done *in-situ*, inside the nanostructured material, e.g. by tomographic techniques or by microscopy or micro-analysis of cross-sections of the nanostructured material.

In the latter case, one can imagine that for some materials the discrete nanophase is separated as nano-objects[212] from the matrix. If such treatment is possible, in a practicable manner, then further characterisation can be done with the techniques used for the analysis of the external dimensions of the nano-objects. The latter techniques are mentioned and discussed in Section 4 of this report, and will therefore not be discussed in this section.

15.3.2.2 Intentional incorporation vs accidental addition

The BSI publication PAS 139 'Detection and characterisation of manufactured nano-objects in complex matrices'[279] distinguishes between intentional incorporation and accidental addition of nano-objects in products. The absence of a priori knowledge about the nano-objects to be identified in the latter case makes their characterisation much more difficult and limits the number of available measurement methods. A very similar case is that of the nanostructured materials discussed here. The methods considered in the following most often require a priori knowledge about the nanophase or –structure to be characterised. It is therefore supposed that the nanostructure is obtained intentionally, e.g. by the incorporation or in-situ formation (e.g. Ref. 280) of nano-objects in the material's microstructure. It is also presumed that the intentionally created nanostructure is stable over time, at least to a certain

extent. The list of methods would be much shorter if the methods were supposed to be used for the detection of the presence of any (temporary) kind of nanostructure in a material or matrix.

15.3.3 Surface structure in the nanoscale

Surface modification of materials is often performed in the nano-regime. One can distinguish the application of uniform thin layers (e.g. a hard but thin (< 100 nm) coating to protect a softer substrate) or the application of patterned surface layers (e.g. anisotropic layers with lateral nanoscale features to obtain specific, e.g. electro-optical, responses).

The thickness of uniform coatings can be measured with a number of surface chemical analysis methods without nanoscale lateral resolution, if at least they give access to depth profiles. Some of these surface analytical techniques with low lateral resolution can also provide information about the dimensions of patterned surface layers, if these patterns have a periodic structure. However, if the patterned surface layer does not have a periodic structure, or it is a complex and long-range ordered pattern, then only methods with high lateral resolution can be used. Examples of these surface analytical techniques are given in Section 15.3.6.

15.3.4 Electron microscopy

15.3.4.1 Traditional electron microscopy

One of the emblematic artefacts of 'traditional' nanotechnology is the Lycurgus cup, a Roman glass vessel displaying unique optical effects.[281] The presence of Ag/Au alloy colloidal particles, and the resulting nanostructured nature of the material, formed *in-situ* in the glass through suitable heat treatments, was confirmed with (analytical) transmission electron microscopy.[282] The application of electron microscopy techniques in the characterisation of other, also more modern, nanostructured materials is abundantly illustrated in open literature.[283,284,285,286]

Whether TEM is used in a traditional bright-field or dark-field mode, or in more modern (e.g. high angle annular diffraction or scanning transmission) modes, in order to use TEM to reveal a nanostructure inside a material, a thin foil needs to be prepared that is electron-transparent. A plethora of sample preparation techniques have been developed, from simple grinding, polishing and dimpling with traditional polishing substances, to more advanced ion milling or cryo-sectioning methods. The sectioning and thinning procedure is very delicate and 'famous' for the introduction of artifacts in the finally observed image. And also after careful sample preparation, the electron beam itself can affect the sample during the observation and create point defects or even induce phase transformations (e.g. Ref. 287). SEM, as opposed to TEM, can also investigate the surface of nanostructured materials without requiring a great deal of sample preparation. However, to reveal internal (nano-)structure, beneath the material's surface, materialographic sections need to be prepared.

As with the TEM and SEM images obtained from particulate materials, image analysis methods can be used to obtain number-based size distributions of internal discrete features (particles, crystals, layers, …). However, it is not straightforward to estimate from the 2D-images of sections (whether from TEM or from SEM) the smallest dimension of an internal nanoscale feature, which extends below the surface of the section. For example, if a thin flake is not cross-sectioned perpendicular to the main lateral plane of the flake, then the nanoscale thickness of the flake may well be hidden and appear much larger. Unless use is made of consecutive sectioning and imaging, or even tomography (see Section 4) the 3D-dimensions need to be estimated from the 2D-images. For certain shapes this can be done, following special routines or numerical methods.[288]

15.3.4.2 Composition-based imaging modes in electron microscopy

Often, TEM and SEM instruments are equipped with energy- or wavelength dispersive x-ray spectrometers. These tools, and other more sophisticated accessories (e.g. electron backscatter diffraction, energy filtered EM or electron energy loss spectrometry) can be used to make images of the EM samples. Thanks to the chemical information acquired by these methods, they can reveal the existence of nanoscale features that are not detectable based on electron images only.

The application of electron microscopy to reveal internal structures in nanostructured materials is, to some extent, less affected by some of the issues of selectivity encountered when using EM to study the particle size distributions of nanoparticulate matter. The morphology of engineered nanostructures embedded inside a material matrix is often better defined and more stable than the structure of agglomerated and aggregated powders or powder suspensions. But this observation can certainly not be generalised, and it remains a concern whether the small volume of material seen in an EM image is representative for the material. For example, the dispersion of a pre-existing nanoparticle phase inside a composite material can be hampered by effects of sedimentation and agglomeration. This is one of the reasons why nanostructured materials are often produced '*in-situ*', meaning that the nanostructure originates from an initially homogeneous material that is (heat-)treated to induce the formation of a finely dispersed (nano-)structure (see the example of the Lycurgus cup, above, but also the oxide dispersion strengthened metallic materials described by e.g. Morris et al.[289]) It has also been suggested to combine the EM results, obtained on small volumes of sample, with SAXS data, which come from a larger measurement volume. The SAXS method relies actually on some basic information on the shape and size of the nanostructures, as can be obtained from EM, as input for the mathematical models that transform the raw SAXS data into, e.g., particle size distributions.[290]

15.3.4.3 Combined SEM-Focused ion beam instruments

Focused ion beams (FIB) are often used to prepare sections of materials, from which then electron transparent TEM foils are made. FIB can also be used to remove small amounts of material from an SEM sample inside the EM chamber. The combined SEM-FIB instruments allow one to make EM images from consecutive sections of the sample, systematically revealing, albeit in a destructive way, the interior structure of the test sample. This allows the study of features on the surface or on a cross-section of a sample that appear to be in the nanoscale, but which are bigger, as they extend below the surface of the sample.[291]

15.3.4.4 Electron microscopy on wet samples

A major obstacle for the use of traditional electron microscopy is the high-vacuum chamber in which the samples are placed for analysis. As discussed in Section 4 of this report, 'environmental' forms of electron microscopy provide a way out, at the expense however of spatial resolution and instrument cost.[292] Other solutions such as chemical treatments, fixation, encapsulation or cryogenic methods require more specific sample preparation, as described in a review paper by Dudkiewicz et al.,[293] and have been used to reveal delicate nanostructures, also in biological materials or food products.

15.3.4.5 Electron tomography

Electron tomography is not a (very) new technique, and exists in different forms (HAADF STEM or BF TEM or EFTEM or EELS based), some of which are mentioned already in Section 4 of this report. Nevertheless, it remains a technique reserved for highly specialised experts and laboratories. Ideally, tests are performed on samples of a specific (and very small) rod-shape, but production of these can be cumbersome or even impossible. Recently, a combined STEM and EDX tomography technique was presented, with the ability to capture the 3D distribution of chemical elements in small (nano-)particles, requiring no machining or milling of the sample (here: a 250 nm particle) into a special shape.[294] This technique has a larger field-of-view than other electron tomography techniques, but it still remains an expensive method for most industrial materials manufacturing companies. Wang et al.[295] report a number of limitations and artefacts, which obstruct the use of ET to measure the size of nanoparticles inside nanocomposites (here: Au particles in a SiO_2 matrix). Grenier et al demonstrate how ET and 3D-APT (see next paragraph) data need to be combined to obtain an accurate 3D characterization of nanoscale elements in nano-devices.[296]

15.3.5 3D-Atom probe tomography (3D-APT)

(3D) atom probe tomography, or atom probe field-ion microscopy, is a microanalytical technique based on field-evaporation (ions are pulled out from the sample by a strong electrical field). 3D-APT can be used to produce 3D-maps of the elemental composition of (very) small volumes of material, with a

spatial resolution that approaches the atomic resolution.[297,298] The technique can be used to provide improved traceability to results obtained with surface analytical techniques.[299]

3D-APT has a number of limitations: it can only be used on conductive materials and the number of 3D-APT instruments is limited. Also, sample preparation is challenging, as the technique typically requires the production of a needle-shaped sub-sample with very sharp tip (radius < 100 nm). The positioning of such sample in a larger object is challenging.[300] Moreover, the resulting sample volume is effectively too small to investigate structural features with a size around the value of 100 nm, critical for the EC nanomaterial definition. The representativeness of the results from such small volumes for larger amounts of material can be questioned and would lead to significant sampling uncertainty.

15.3.6 Surface analytical techniques

Baer et al.[301] have recently provided an overview of surface characterisation techniques for nanomaterials and nanoparticles. With respect to the sizing of features in nanostructured materials, some of the methods listed by Baer et al. have an imaging mode with a lateral resolution that meets the requirements for accurate nanoscale size analysis. This is relevant especially for those solid nanostructured materials from which one can prepare flat cross-sections. The methods named by Baer et al. are X-ray photoelectron spectrometry (XPS), Auger electron spectrometry (AES), Secondary ion mass spectrometry (SIMS), Low-energy ion scattering (LEIS), Medium-energy ion scattering (MEIS), Atomic force microscopy (AFM), Scanning tunnelling microscopy (STM), Sum frequency generation-vibrational spectrometry (SFG-VS) and Nuclear magnetic resonance (NMR). But a wide range of additional methods exist, such as Rutherford Backscattering (RBS), optical reflectometry, Raman spectroscopy, etc., most of which can be used to establish depth profiles with a depth resolution suitable to measure (indirectly) the thickness of nanoscale thin surface layers.

Examples of the application of two of these methods for depth profiling of nanoscale surface layers and for imaging of nanoscale surface patterns are the following:

15.3.6.1 Auger electron spectrometry (AES)

Auger electron spectrometry is based on the analysis of the energy of electrons emitted from the outermost atomic layers of a surface bombarded with incident electrons. In combination with e.g. ion beam sputtering, AES can be used to construct depth profiles of surface layers and coatings. BCR-261 is a certified reference material consisting of a thin, nanoscale tantalum pentoxide layer (30 nm or 100 nm, respectively) on a tantalum foil, of which the coating thickness was assessed with AES and other surface analytical techniques.

15.3.6.2 Scanning probe microscopy (SPM)

Atomic force microscopy as described in the JRC Reference Report EUR 25404 is a kind of SPM, with a probe that scans the surface of a sample to measuring its topology. If nanoparticles are deposited on a flat surface, the AFM will produce images of the nanoparticles and therefore give access to the morphological parameters, as described in Section 4 of this report. Similarly, AFM can produce images of cross-sections of nanostructured materials or of nanoscale patterned surfaces to reveal the dimensions of the nanoscale features. The analysis of unbound nanoparticles on a substrate is preferably performed in the non-contact AFM mode, to not drag the nanoparticles, and deform the image. Nanostructured materials, with fixed nanoscale surface patters, or embedded nanostructures, can also be imaged in the AFM's contact or tapping modes.

Other SPM probes can record signals that are related to the local physical or chemical properties of the sample. In this way, images can be obtained from nanostructures. Kimura et al.[302] show that SPM can also be used to detect nanoscale features up to 1 micrometre beneath the surface of a nanostructured material. These methods are limited to cases where the different phases in the nanostructure have significantly different physical properties (here stiffness).

15.4 Summary

Nanoscale features inside nanostructured materials can be analysed with a number of techniques, mostly members of the family of electron microscopy techniques. Available as scanning or transmission instruments, with chemical detection accessories, sectioning ion beams, or in tomography mode, electron microscopy provides a wide variety of possibilities. However, the results of EM studies critically depend on the preparation of representative and undistorted samples.

For the characterisation of thin surface coatings, a large number of depth-sensitive surface chemical analysis techniques are available. These techniques often do not require specific sample preparation, but their ability to determine film thickness depends on proper depth calibration, e.g. with existing reference materials.

Finally, for the assessment of surface patterns with lateral features in the nanoscale, there are a number of surface topographical techniques, most importantly scanning probe microscopy.

In general, the relevance and applicability of the above mentioned categories of techniques is dependent on the type of nanostructured material to be investigated, with particular challenges for softer materials (e.g. of biological origin), or materials stable only in wet conditions (e.g. suspensions of particles functionalised with nanostructured surface layers).

16 INFORMATION ON NATURAL OR INCIDENTAL MATERIALS UNDER THE DEFINITION OF DIRECT OR INDIRECT REGULATORY RELEVANCE

16.1 Introduction

Manufactured nanomaterials are used in many different applications, and thus can be released, e.g. via the use in consumer products, and found in the environment. In addition, nanomaterials are occurring in the environment naturally or incidentally. In general, naturally occurring nanomaterials are considered materials found in nature as a consequence of natural events (i.e. volcanic eruptions, ocean spray, forest fires or mineralization) with a particle size within the nano-range. Incidental nanomaterials are usually described in the literature as by-products occurring as a result of combustion or industrial processes. Natural and incidental nanomaterials are ubiquitous and occur in a broad variety of forms.

Currently there is no systematic description or classification of natural and incidental nanomaterials or on their prevalence, environmental fate and effects. The dynamics of formation and transformation of natural and incidental nanomaterials in the environment is poorly understood and the detection of nanomaterials in the environment, as well as their distinction and quantification, is still a challenge, regardless whether they are natural, incidental or manufactured. However, the distinction between natural, incidental and manufactured nanomaterials is relevant as nanomaterials are addressed in legislation explicitly (e.g. sector specific regulations such as cosmetic or food legislation) or in guidance documents, e.g. concerning REACH. The sector specific legislation includes so far a definition of nanomaterial focussing on engineered or intentionally manufactured nanomaterials, disregarding the potential presence of natural or incidental nanoparticles, while the Commission Recommendation on the definition of nanomaterial covers natural, incidental and manufactured nanomaterials, however without defining the term 'natural', 'incidental' or manufactured'. In some cases, the lack of a proper description or classification of these nanomaterials may lead to difficulties in the interpretation of the legislation.

This chapter summarizes information on natural and incidental nanomaterials, gives examples of their presence in the environment, and examines their direct or indirect relevance in the current European legislation. Finally, it introduces some methodological and analytical challenges to distinguish between engineered and natural/incidental nanomaterials present as background in the environment.

16.2 Naturally occurring nanomaterials

16.2.1 What are naturally occurring nanomaterials?

Literature provides examples for nanomaterials naturally occurring in the environment, but there is no clear definition of 'natural nanomaterial' or 'naturally occurring nanomaterial' available. Some information on what naturally occurring nanomaterials are can be derived from EU and non-EU regulatory texts.

16.2.1.1 Natural, incidental and manufactured nanomaterials in horizontal EU and non-EU legislation, guidance and normative context

The terms 'natural nanomaterial' or 'naturally occurring nanomaterial' may be derived from other terms defined in REACH. *Substances occurring in nature* are defined in REACH as *'substance: means a chemical element and its compounds in the natural state or obtained by any manufacturing process, …'* These substances are exempted from the obligation to register if they are included in Annex IV of REACH (such as glucose, starch, cellulose pulp) or fulfil the criteria of Annex V, which include e.g. minerals, ores, crude oil, coal if they are not chemically modified, as well as vegetable and animal fats, oils and waxes, and other substances such as coke, magnesia and glass. Furthermore, Annex V refers to substances which occur in nature. According the REACH definition, '*substances which occur in nature: means a naturally occurring substance as such, unprocessed or processed only by manual, mechanical or gravitational means, by dissolution in water, by flotation, by extraction with water, by steam distillation or by heating solely to remove water, or which is extracted from air by any means'* (Article 3(39)). Thus, one could argue that **a 'natural nanomaterial' is a material which fulfils the definition of nanomaterial (EC Recommendation) and simultaneously the REACH definition of 'substances which occur in nature'**. Following this argumentation, a nanomaterial obtained by mechanical fine grinding without application of chemical processes would still be considered natural.

Additional information on natural substances are provided in the guidance for the legislation for chemicals in Australia: the *National Industrial Chemicals Notification and Assessment Scheme,* in which the definition of naturally occurring chemical is equivalent to the definition of 'substance which occurs in nature' in REACH. The *Guide for importers and manufacturers of industrial chemicals in Australia* gives some examples on unprocessed chemicals occurring in a natural environment – chemicals extracted from plants, animals (milk and blood), minerals, crude oil – without applying any processing method, but the definition also applies to chemicals occurring in the nature that have been processed by certain methods that do not change their chemical composition. As in REACH, these methods include manual, mechanical and gravitational means. In addition, a descriptive list of extraction processes which are considered under this definition is also given in the Australian guidance, including filtration, centrifugation, sedimentation, cold pressing and sieving. Other extraction processes considered, and also mentioned in the European legislation, are extraction by dissolution in water, by flotation and by a process of heating to remove uncombined water, used to purify or concentrate chemical substances.[303]

In contrast to natural substances, the definition of *manufacturing* under REACH means *production or extraction of substances in the natural state*. No further details on accepted or exempted procedures are given, which may lead to difficulties in the interpretation of the term (see, e.g. case of registration of natural nanoclays in REACH given in the Commission Staff Working Document on the Second Regulatory Review on Nanomaterials).[137]

According to ISO, manufactured nanomaterials are *intentionally produced to have specific properties or composition,* this term is also included in the definition of engineered nanomaterials are *rationally designed, manufactured nanomaterials.*[9] This definition was also applied by the Working Party of Manufactured Nanomaterials (WPMN), a group of expert of the Organisation for Economic Co-operation and Development (OECD) that defined *manufactured nanomaterials* as *nanomaterials intentionally produced to have specific properties or specific composition.* Following this logic, natural or incidental nanomaterials would be all other nanomaterials not covered by the term *manufactured nanomaterials* as defined by OECD's WPMN.

16.2.1.2 Natural, incidental and manufactured nanomaterials in sector-specific EU legislation

Certain sectorial legislations in the EU have introduced a definition of nanomaterial tailored to the necessities of this sector. However, most of these definitions omit naturally or incidentally occurring nanomaterials and cover explicitly only *manufactured* or *intentionally produced* nanomaterials.

- The EU Biocidal Products Regulation (Regulation (EU) 528/2012) applies the recommended definition of nanomaterial by the European Commission, with slight modifications. The definition covers 'natural' nanomaterials, and as any other natural active substance used in a biocidal product, its origin, occurrence and geographical distribution shall be provided with the information required to support the approval of the active substance.[18] No further information, definition or guidance on what a naturally occurring nanomaterial is or on procedures for extraction are provided.
- Furthermore, the EU Cosmetic Products Regulation (Regulation (EU) 1223/2009)[16] uses in its definition of nanomaterial the term of '*intentionally manufactured*'. The legislative text does not further specify the meaning of intentionally manufactured.
- The Regulation on the provision of food information to consumers (FIC Regulation: Regulation (EU) 1169/2011)[17] defines 'engineered nanomaterial' referring to *intentionally produced material*; thus, excluding naturally and incidentally occurring nanomaterials.
- The new proposal for the regulation on medical devices[21,] amending the actual Directive 2001/83/EC on the Community code relating to medicinal products for human use[304], also suggests to include in the new Medical devices Regulation a definition for nanomaterials, and propose to introduce a definition for nanomaterials based on Commission Recommendation 2011/696/EU; thus, naturally occurring nanomaterials would be also included in the definition.

The term *intentionally produced material* was recently discussed by the Expert Group on the Provision of Food Information to Consumers – 'Preparation of a draft delegated regulation on the adaptation of the definition of 'engineered nanomaterials' in the FIC regulation'. The main purpose of this term in the context of nanomaterials is to exclude naturally occurring nanoparticles occurring in milk or blood from the definition. The draft Delegated Regulation is currently under discussion in the European Parliament.

Similarly to REACH, currently little guidance is available in the context of EU sector specific legislation on how to distinguish natural nanoparticles made for a specific purpose from so-called engineered nanomaterials, and how to separate them.

In the next section, a number of examples are provided of naturally occurring nanomaterials used or potentially used in scenarios regulated by different legislations.

16.3 Presence of nanomaterials in nature

Naturally occurring nanomaterials have been present in the nature for aeons. In fact, most natural biological processes occur at the nano-level; nature uses a range of low energy molecular interactions (e.g. *van der Waal forces, hydrogen bonds, electrostatic dipoles, fluidics and various surface forces*) to create structures with nanoscale features.[305] In nanobiotechnology, man has applied many of these nanoscale principles in a number of specific industrial purposes, e.g. anti-reflective coatings for solar cells, temperature biosensors or super-sticky tapes and glues. [306,307,308]

16.3.1 Naturally occurring nanomaterials and nanostructures in animals and plants

Nanoparticles and nanostructures are found in animals in a variety of special functions.[309] E. g. in insects, one can find nanostructures in moth's eyes, called corneal nipples, to absorb light more efficiently.[310] Structures of proteins in the nano-range, such as chitin multilayers in butterflies or beta-keratin nanofibers in penguins, are creating patterns that scatter light acting as a diffraction grid and inducing iridescence.[311] [312] Also, the presence of nanostructures in the exoskeleton of a type of hornets has been associated with the absorption and accumulation of light converted later into electricity, emulating solar cells.[313] Spider silk owes its strength to the molecular organisation at nanoscale level of fibroin, forming nanofibers.[314] Also recently, a new nano-structure creating a hydrophobic surface has been discovered in guillemots eggs providing strength and self-cleaning properties.

Similarly, plants have been an inspiration for man-made materials, especially to create new surfaces and films.[315,316] The most known example is the wax nanocrystals present in the lotus leaves providing a super hydrophobic water-repellent layer and self-cleaning effect.

16.3.2 Naturally occurring nanoparticles of geologic origin

The earth's crust, the oceans and the atmosphere are considered as one of the largest generators, reservoirs and distributors of naturally occurring nanoparticles.[317] One can distinguish aerosols, natural colloids, nano-minerals and -metals.

Aerosols

Dust storms are the largest source of environmental nanoparticles, mainly composed of mineral dust originating from soil erosion by the wind, iron and other metals, and anthropogenic pollutants. Another source of aerosols with nanoparticles are the volcanic emissions which contain heavy metal

nanoparticles produced by mechanical grinding.[318] The same mechanical process produces large quantities of nanoparticles in earthquakes.[317]

Natural colloids

Naturally occurring aquatic colloids have been defined as solid phase material with at least one dimension between 1 nm and 1 µm,[319] therefore some colloids present in the environment such as some inorganic colloids, humic substances, clays, metal sulphides, carbonates and large biopolymers that have external dimensions within the nano-range are included in this definition.

Ocean and water evaporation in general are a large reservoir of natural colloids present in fog, oceanic spray or clouds in which precipitates of calcium carbonates as well of halite and sulphate aerosol can be formed. Metal nanoparticle colloids can also occur in nature; (e.g., zinc ferrite nanoparticles that occurs naturally as hematite)[320] and can disperse in water forming magnetic nanocolloids or are released incidentally from drainage of mining sites.[321]

There is extensive research indicating that natural colloids can have a signifficant impact on environmental processes, such as transport of pollutants, patogens and nutrients and bioavailability. Findings in these studies may be an important source of information to understand the potential fate and behaviour as well as the potential risks associated to the presence of engineered, natural and incidental nanomaterials in the environment.[322, 323]

Nanominerals and mineral nanoparticles

As defined by Hochella et al.,[321] nanominerals are minerals that only exist in the nanoscale size range. There are some examples in nature of nanominerals as nanoclays, or iron and manganese oxyhydroxides. Nanoclays, for example, are on the market. Furthermore, one can find mineral nanoparticles in nature which are present in its equivalent bulk size as well. [321]

Nanominerals may have a biotic or abiotic origin, e.g. via nanobiomineralization and mineral weathering. In the process of nanobiomineralisation organisms such as viruses and bacteria produce nanoparticulate inorganic materials through redox reaction of aqueous ionic species. Some examples of this process are: the formation of magnetite nanoparticles by magnetotactic bacteria, i.e., bacteria that orient along the magnetic field lines of Earth's magnetic field or the synthesis of siliceous material by diatoms. Inhalation toxicity associated to worker exposure to silica during diatomaceous earth mining or to biogenic magnetite have been reported in the literature.[324,325] Similar redox reactions occurring in organisms are involved in mineral weathering process producing nanosized particles.[321]

Metals

Dynamic processes in nature may also enhance the natural transformation of metal ions into metal nanoparticles. In aquatic media, environmental conditions such as sunlight and the presence of dissolved organic matter have been suggested to be linked with the reduction of ionic metals with high reduction potential to its metallic nanoparticles, as in the case of Ag and Au.[326]

16.3.3 Naturally occurring nanomaterials in food and cosmetics

Natural nanomaterials in food

Nanomaterials are applied in food industry in form of nanoparticles, nano-emulsions or nanocomposites, and nanostructured materials are used in nanodelivery systems, formulation and packaging. However, not all nanoparticles found in food are intentionally manufactured; food can also be nanostructured either naturally or incidentally due to traditional manufacturing processes.

Milk is a typical example of a food which contains naturally occurring structures with external dimensions in the nanoscale. It contains proteins, carbohydrates and fats such as lactose, whey protein's aggregation in ricotta production, casein micelles or fat globules.[327] In addition, many traditional processes can create different nanostructures including nano-emulsions, surfactant's micelles, emulsion's bilayers, double or multiple emulsions, reverse micelles present in spreads, mayonnaise, cream, yoghurts or ice cream.

Naturally occurring nanoparticles have also a potential use in food packaging materials. An example is the use of nanoclays to reinforce polymeric materials, reduce permeability of gases, to enhance heat resistance and thermal stability or to enhance barrier properties (permeability). Studies have shown that the use of nanoclay as nanocomposites in plastic PET bottles of beer can increase the storage time.[328,329]

Impact of naturally occurring nanomaterials in EU food legislation

The use of natural nanoscale materials has been discussed due to its relevance in the regulatory context. In the Regulation on the provision of food information to consumers (FIC Regulation: Regulation (EU) 1169/2011),[17] the legal text provides a definition on engineered nanomaterials, excluding naturally and incidentally occurring nanomaterials and limiting the concept only to nanomaterials which are intentionally manufactured. Hence, the provisions for nanomaterials in food do not apply to naturally and incidentally occurring nanomaterials.

Furthermore, and according to "Questions and answers on the Commission Recommendation on the definition of nanomaterial" provided by the European Commission only nanomaterials with a defined, rigid shape (solid nano-objects) are covered by the EC nanomaterial definition, excluding, e. g., nano-emulsions: *[…] 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), and excludes nanostructured materials (i.e. solid products, parts or components) with an internal or surface structure in the range between 1-100 nm, such as computer chips).*[330]

In principle, the term 'intentionally manufactured nanomaterial' could also include naturally occurring nanoparticles when these have been deliberately created or selected for their properties and characteristics at the nanoscale form.

Natural nanomaterials in cosmetics

Engineered nanoparticles have been extensively used in the cosmetic sector because of their particular properties, e.g. as delivery agents, since nanoparticles can penetrate skin better; as antioxidants, for radical scavenging, and their use in anti-age creams for their capacity to conceal wrinkles due to optical reduction properties.[331] In contrast, the use of naturally occurring nanomaterials has been less explored in cosmetics. Minerals as TiO_2, ZnO or clay talc are naturally occurring materials; however, these minerals are used in cosmetics normally in the synthesized form (chemically processed) and therefore cannot be considered as natural nanomaterials.

In the recent years, researchers have been looking for promising applications of naturally occurring nanoparticles in this field. Plants have been revealed as a potential source of nanoparticles for cosmetic purpose. Recently, extraction and use of nanoparticles from English ivy roots (between 50 nm and 70 nm) have been claimed to be an alternative to the use of metal derived nanoparticles used as UV filters in sunscreen.[332]

Impact of naturally occurring nanomaterials in cosmetic legislation

In the Cosmetic Products Regulation (1223/2009) 'nanomaterial' *means an insoluble or biopersistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm.* The provisions for nanomaterials therefore do not apply to naturally and incidentally occurring nanomaterials. As for the EU food legislation, the term *intentionally manufactured material* leaves room for interpretation and in certain cases it might not be easy to distinguish them from natural or incidental nanomaterials.

16.4 Incidentally occurring nanomaterials

16.4.1 What are incidentally occurring nanomaterials?

Similar to the case of natural nanomaterials, there is no legal definition for 'incidental nanomaterials'. In the literature, incidental nanoparticles are often considered by-products of combustion or hot processes initiated or controlled by humans (anthropogenic). These nanoparticles are not always related to industrial activities, but also occur as a consequence of indoors activities, such as cooking.

It has been reported that carbon nanotubes and fullerenes are unintentionally/incidentally generated by combustion processes, e.g. from propane, stoves, wood fires and burning tires.[333] Generation of incidental nanoparticles by activities such as building demolitions, sandblasting, mining, metal working or even biomaterial degradation have been also described in the literature.[334]

16.4.2 Sources of incidental nanoparticles

Outdoors, aerosol pollution is a major source of local contaminant particulate matter that includes nanoparticles. Studies have shown that nanoparticles present in diesel and engine exhaust consist mainly in carbonaceous core, soot generated by incomplete combustion, usually showing spherical

shape that absorb other chemicals such as organic hydrocarbons, transition metal, surface nitrates and sulphates. Carbon nanotubes and nanofibres have also been detected in the environment near diesel combustion sources. Although nanoparticles from road vehicles represent a small mass fraction, they contribute 90% of the total number of particles generated by diesel combustion in contaminated environments.[335] The increase in particulate matter in the environment has caused concerns since particle pollution has been associated to an increase in cardiopulmonary diseases and mortality[336] and with cancer.[337]

Vehicles, however, are not the only source of nanoparticles from diesel exhaust. In a recent study, Kumar et al reviewed the emission of nanoparticles from a number of non–vehicle exhaust sources,[334] such as abrasion of tyres; industrial emissions; aircraft and ship emissions; particles generated from construction, demolition, and processing of concrete; from combustion during cooking, domestic biomass burning, forest fires and burning of agriculture residue, municipal waste incineration, cigarette smoking, heating units or cleaning processes. Some of the processes listed above also contribute to the generation of nanoparticles indoors.[318]

Airborne nanoparticles generated by high-energy mechanical methods such as milling, grinding, drilling or welding of naturally occurring materials for the preparation of powders or suspensions of nanoparticles may be incidentally release as dust or as aerosol droplets in the air,[338] and exposure to these nanoparticles may be relevant at the workplace.

Currently the Worker Protection Framework Directive 89/391/EEC,[339] does not stipulate specific provisions for nanomaterials; however, this directive applies fully to nanomaterials, and therefore it applies also the obligation of employers to manage the risk of exposure and ensure the worker protection. Incidental emissions at workplace can be reduced by the use of risk management measures such as engineering controls, personal protective equipment and proper packaging and storage.

Another potential source of incidental nanoparticles in the environment may be the formation of new nanoparticles from objects composed of bulk material or ionic solutions. This phenomenon was suggested by researchers that, in a recent study, demonstrated the generation of silver nanoparticles as a result of the reactions of oxidation and dissolution of silver ions from objects exposed to humid air, water or weathering processes.[322,340] Ultrafine particles may also be the result of gas photochemistry in air.

16.5 Distinction between engineered nanomaterials and natural or incidental nanomaterials: challenges

16.5.1 The need to distinguish between engineered and natural/incidental nanomaterials

As the market and applications of engineered nanomaterials increase, discharges of these nanoparticles are raising concerns about their impact on the environment and human health and therefore risk assessment, including exposure assessment, has become a task of a great relevance. Here, exposure

assessment for occupational health and release of engineered nanomaterials into soil and sediment are particularly important. For these purposes it is necessary to distinguish between natural (i.e., the background) and incidental/manufactured nanomaterials in order to understand the effects of the latter.

It can also be necessary to distinguish between manufactured/engineered, incidental and natural nanomaterials where legal provisions exist which apply to manufactured and/or engineered but not to incidental or natural nanoparticles. This is, e. g., the case for cosmetic products (Cosmetic Products Regulation 1223/2009, applies only to intentionally manufactured nanomaterials), biocidal products (Biocidal Products Regulation 528/2012 applies to natural and manufactured substances), food (Food Information Regulation 1169/2011, applies to engineered / intentionally produced nanomaterials).

16.5.2 Challenges to differentiate natural, incidental and engineered nanoparticles

There is information available on background concentration of nanoparticles found in the environment, e.g., in urban air,[341] and at the workplace.[342] However, quantification and distinction of manufactured nanomaterials from background levels of naturally occurring and incidental nanomaterials are challenging due to (i) the lack of validated and standardised methods for identification, separation and analysis of nanomaterials and (ii) because naturally or incidentally occurring nanoparticles may be present in higher concentrations than released engineered nanomaterials.

An example of is titanium dioxide. TiO_2 nanoparticles are produced in large quantities to be incorporated in a wide variety of products, for example as UV absorber pigment in sunscreens. The production of nanopowder TiO_2 is estimated to be approximately of 5.5-10 metric tons per year.[343] Furthermore, titanium minerals are one of the most abundant minerals in the Earth's crust, naturally present in different crystalline forms such as ilmenite, brookite, anatase and rutile. The high background concentration of natural TiO_2 nanoparticles makes it a challenge to distinguish natural from engineered nanoparticles in environmental studies.[344]

Workplace is a source of airborne incidental nanoparticles i.e. from welding fumes, grinding or bagging, and this may create challenges to differentiate these particles in facilities where engineered nanoparticles are produced. An example is given in Peters et al., in which it was possible to distinguish different incidental nanoparticles in a facility that produces lithium titanate metal oxide powder.[320]

In some cases particular characteristics indicative for the synthesis or manufacturing processes (size, morphology, chemical structure, surface functionalization or coatings), may help to differentiate engineered, incidental and natural nanomaterials. However, nanoparticles are dynamic in nature, and properties that indicate a manufacturing process may be modified by interaction with UV radiation, (in) organic ligands, through redox reactions, biotransformation, aggregation, coating with organic matter or natural surface functionalization. In this context, special care should be applied also in the sample

preparation and analysis to avoid modifications of these properties. In addition, nanomaterials in environmental samples are embedded in complex matrices therefore the distinction between natural, incidental and manufactured nanoparticles involves analysis of such samples.

Analogous arguments apply where it is necessary to distinguish manufactured, incidental and natural nanomaterials in products because of applicable regulatory provisions (see previous section). The current status of technology does not allow to make this distinction because of the lack of validated methods which would be necessary for regulatory purposes.

Some of the approaches currently used to analyse natural, incidental and manufactured nanoparticles in the environment are introduced in the next section.

16.5.3 Approaches to discriminate natural or incidental from manufactured nanoparticles

There is wide agreement that there is no unique method to measure the abundance and trace nanoparticles in the environment. Instead, detection and analytical workflows must be tailored according to the nanoparticle characteristics, surrounding environment and hypothesis to test.

Recently, Zänker and Schierz[345] have reviewed a number of techniques used to identify, quantify and distinguish engineered from naturally occurring nanoparticles, which involves sample preparation and treatment, detection and identification of particles to the quantification of the target nanoparticle.[344,346] Often, this involves coupling separation and several possibly complementary detection methods.

Some specific strategies can be used to distinguish the origin of nanomaterials in the environment. The use of elemental or isotopic ratios may be useful when detecting manufactured nanoparticles in an environment where their natural versions are present in high background concentrations, as is the case for some metal oxides.[347] Other features related with the manufacturing process including structural homogeneity, coating or surface modifications, compositional homogeneity or purity, rare or untypical elements associated with one or the other type of engineered nanoparticles, shifts in the isotopic distribution of the core element, or coated with bio-barcodes (e.g. oligonucleotides), can be applied for particular nanomaterials.[348] These approaches can be used in some cases to distinguish engineered, natural and incidental nanoparticles.[349]

17 INFORMATION ON MATRICES OF REGULATORY RELEVANCE IN WHICH CERTAIN MATERIALS MIGHT NEED TO BE ASSESSED UNDER THE DEFINITION, NOW OR IN THE FUTURE

17.1 Introduction

The use of nanotechnology in consumer products is increasing rapidly. According to the Project on Emerging Nanotechnologies Consumer Products Inventory,[350] as of October 2013, 1628 nano-based products are available in the global market. The largest main category is Health and Fitness, with a total of 788 products that includes products like cosmetics and sunscreens. Food and beverage products make up 12% of the products in the database. In the European market the trend is very similar since from 475 products containing nanomaterials available to European consumers, 42% belong to the category health and fitness and 6% to food and beverage.[351]

This rapid emergence of nanotechnology into consumer products has led to concerns as regards the potential risk for human health following consumer exposure. This risk is linked to the characteristic properties of certain nanomaterials that make them different from their macroscale counterparts and will be determined by the chemical composition of the nanomaterial, its physicochemical properties, the interactions with tissues and the potential exposure levels. Ingestion exposure via the gut, airborne exposure via the lungs and dermal exposure are the most important exposure routes that should be investigated for risk characterisation. Therefore, food and feed, textiles and cosmetic products are among the most important groups to be considered for regulatory purposes.[44] In addition, the future use of nanomaterials, especially for industrial purposes, raises specific concerns regarding their disposal at the end of their life cycle with the unavoidable release to the environment that may lead to indirect human exposure (e.g. via food chain or drinking water).

17.2 Results from EC's JRC survey

In the survey held by the EC's Joint Research Centre in support to the 2014 review of the EC definition of nanomaterial (see Section 7), the addressees were asked to provide information about relevant matrices for which a future need to determine the nanomaterial fraction could be envisaged. Consumer products, food and feed, food additives, food packaging, cosmetic products, biocidal products, pharmaceuticals and polymers have been identified as the target groups of regulatory relevance.

In more detail, to the question: 'For which matrices (consumer products, food and feed, cosmetics, biocides, substances, etc.) do you envisage or predict a future need to determine the nanomaterial fraction (i.e. volume or mass percentage of nanomaterial in the matrix, but not the size distribution) by in-situ measurements?', the following matrices were indicated in the replies:

- Consumer products, food and feed, food additives, food packaging cosmetic products, biocidal products, environmental matrices, plant protection products, pharmaceuticals, polymers in addition to measurements in test media and test organisms, while performing (eco)toxicity tests. In addition,

it should be clarified in the relevant legal frameworks if the definition should be applied to the bulk material or the material as present in the matrix (consumer product, food and feed etc.).

- Certain consumer products have nanoclaims, they need to be tested test whether such a claim is justified
- Tires, painted plastic
- In the future the detection of nanomaterials in products should be done for products containing nanomaterials which have been proven to have hazardous properties towards human health and/or the environment
- Soil products made from sewage sludge
- Waste water treatment plants (WWTP) effluents and sludge
- Various biota samples to address the fate of nanomaterials
- Various abiotic samples (sediment, sludge, water, air …) to assess the fate of nanomaterials

17.3 Nanomaterials in consumer products

17.3.1 Cosmetics

The Cosmetic Products Regulation[16] (see Sections 2 and 3) obliges the notification to the European Commission of a cosmetic product containing nanomaterials prior to placing it in the market. It also states that all ingredients present in the form of nanomaterials shall be indicated in the list of ingredients followed by the word 'nano' in brackets.

Nanopigments such as titanium dioxide (TiO_2) and zinc oxide (ZnO) are used in sunscreens for their capacity to reflect and scatter UV light thus protecting human skin against adverse effects of UV radiation. Nano-emulsions are preparations containing oil and water nanodroplets to increase the content of nutritious oils while preserving the transparency and the lightness of the formulas. They have become important as vehicles for the controlled delivery of cosmetics and for the optimised dispersion of active ingredients in particular skin layers. For instance, fragile active ingredients, like vitamins, are protected inside nano bubbles or liposomes that release the ingredient upon contact with the skin at the time of application. Silica nanoparticles are added to emulsions to improve the stability of the active ingredient within the formulation and enhance its delivery in cosmetics and dermal drugs. Nano-emulsions can be presented in a variety of formulations such as foams, creams, liquids and sprays. Some anti-ageing formulas and moisturisers include carbon based fullerenes and nano-gold in their formulations for their anti-oxidant and smoothing properties. Therefore, skin care products together with sunscreens and tooth paste are among the more relevant cosmetic matrices to take into account for regulatory purposes.

17.3.2 Food and Feed

As regards food and food-related products, Regulation (EU) 1169/2011 on the provision of food information to consumers[17] states that all ingredients present in the form of engineered

nanomaterials shall be clearly indicated in the list of ingredients followed by the word "nano" in brackets (see Sections 2 and 3). To market a novel food or ingredient, companies must apply to an EU country authority for authorisation, presenting the scientific information and safety assessment report. This regulation is at the moment under revision[19] and foresees a pre-market approval for foods modified by new production processes, such as nanotechnology.

One of the main areas of application of nanotechnology in food is food processing, reducing for instance the amount of fat or salt to promote healthy option. The development of nanostructured food such as ice creams, yogurts, creams or mayonnaise in which the use of nanodroplets allows creating for instance, a mayonnaise emulsion that has the same texture but with a fat content reduced in about 30%. This is also the case of nanograins of salt, thousand times smaller than normal table salt, which increases its surface area a million-fold and therefore reduces considerably the salt intake.

Another field of application of nanotechnology in food involves the use of nano-encapsulated food and feed additives that preserves the ingredients during processing and storage, controlling the release of additives and enhancing the uptake of supplements. One example is a colourless and tasteless beverage that contains nano-encapsulated ingredients or additives that could be activated by a consumer at a particular microwave frequency.[352]

Among the currently used nanomaterials in food products, nanosilver is used as an additive to prepare antibacterial wheat flour.[353] Nanosilica is used in food applications as anticaking agent to maintain flow properties in powder products and to thicken pastes. It has been found in powder products like milk powder, instant soups, powdered sauce and seasoning mixes, instant noodles, pancake and cake mixes and coffee creamers.[354] Another anticaking ingredient is titanium dioxide also used as white colorant in white-coloured sauces and dressings, in non-dairy creamers and in candies (e.g. marshmallows) sweets and chewing gums.

Food improvement agents (i.e. food additives, food flavourings, food enzymes),[355] food supplements, food contact materials, and feed additives[356] have to undergo an authorisation procedure before being placed on the market. The Commission is granting the authorisation only after a favourable opinion of the European Food Safety Authority (EFSA). For food additives,[357] and specific food contact materials (i.e. plastic), re-authorisation is requested for previously authorised products if significant change in production methods has occurred (including the use of nanotechnology).

In the specific case of feed additives authorisation, EFSA is assisted by the European Union Reference Laboratory for Feed Additives (EURL-FA) for the evaluation of the method of analysis for control purposes. The screening of the information available from already authorised products revealed that only 166 dossiers out of 289 contained information on particle size distribution and among them only 3% contained information below 1 μm. Therefore, with the existing information it is not possible to

determine whether already authorised feed additives contained nanomaterials. Additional information will be needed in future for the full assessment of new feed additives. As for food additives, it is expected that a re-authorisation of already authorised products will be requested in case of use of nanotechnology. In this frame the EURL-FA has received from EFSA a request to comment on the suitability of analytical methods applied by industry for the evaluation of specific products and to establish whether they fall under the definition of 'nano'.

Among other recommendations, the EFSA highlights the importance of developing methods to detect and measure ENMs in food/feed and biological tissues. Implementation of existing and future legislation will require reliable methods for the detection and quantification of nanoparticles in food and feed. At the moment, there is no single technique that can by itself provide a robust analytical method, especially considering the need to measure the number size distribution of nanoparticles introduced by the definition of nanomaterials.[358]

Finally, food packaging materials are the largest category of current nanotechnology applications for the food sector. Nanomaterials not only improve packaging properties but have antimicrobial properties and can monitor the condition of the food via the use of nanosensors. The main risk of consumer exposure to nanoparticles from food packaging is likely to be through potential migration of nanoparticles into food and drinks. Nanoclays are used in food packaging as diffusion barriers for instance in beverage packaging (e.g. PET bottles). Other examples are food containers made of plastic nano-silver composite and wrapping film containing nano zinc oxide for antimicrobial protection of food.[359]

17.4 Environment

Accidental or deliberate release of nanomaterials into the environment may come from point sources such as production facilities, landfills and wastewater treatment plants or from wear from materials containing nanoparticles. Whether the particles are released directly into water, soil or the atmosphere, they all end up in soil or water, either directly or indirectly for instance, via sewage treatment plants, waste handling or aerial deposition.[360]

Organisms can be either directly exposed to nanoparticles through exposure to air, soil or water or indirectly by consuming plants or animals which have accumulated them. One example is the exposure of humans to anthropogenic nanoparticle aerosols released into the atmosphere mainly from combustion processes reaching a concentration of about 10^6 particles per cm^3 of air. This implies that every hour, individuals breathe millions of nanoparticles, and it is estimated that at least half of these reach the alveolar region where the gas exchange takes place.[361] Moreover, nanomaterials are used in personal care products such as cosmetics and sunscreens, which can enter the environment on a continual basis from washing off of consumer products.[362]

Examples of methods that are successfully being used to measure nanoparticles in different environment include differential electrical mobility analysis or condensation nucleus counters (CPC) used for measuring aerosols, which can be applied to measure nanoparticles in the gas phase. Recent developments of aerosol mass spectrometry, in which particles are vaporized and the resulting ions analysed in a mass spectrometer have provided new alternative procedures to analyse nanoparticles in gas suspensions.[361] There are techniques for detection of nanoparticles in the liquid phase, such as optical chromophore counting, resonant light scattering and Raman scattering techniques, as well as the use of microscope techniques such as Scanning Transmission Electron Microscopy (STEM), or High Resolution Transmission Electron Microscopy (HRTEM). Using these techniques requires adequate sampling and sample preparation techniques which not only may modify the nanoparticle but could also prevent in-situ analysis of the nanoparticles in the environment they are in.

18 ANNEXES

18.1 Annex to Section 2

Table 1. Nanomaterial definition in the Cosmetic Products Regulation N. 1223/2009 versus new proposed definition (2013)

European Union: Cosmetic Products Regulation N. 1223/2009	*"Nanomaterial"* means an insoluble or biopersistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm.
Proposed new definition (July 2013)	"*Nanomaterial*" means an insoluble or biopersistent, material, manufactured to perform/fulfil a specific function or purpose, 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 1-100 nm. By derogation from point 2, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials. For the purposes of the definition provided above, '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.

Table 2. Nanomaterial definition in the Food Information to Consumer Regulation No 1169/2011 versus new proposed definition (DG SANCO, 2013)

Food Information to Consumer Regulation No 1169/2011	"Engineered nanomaterial" means any intentionally produced material that has one or more dimensions of the order of 100 nm or less or that is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic of the nanoscale. Properties that are characteristic of the nanoscale include: (i) those related to the large specific surface area of the materials considered; and/or (ii) specific physico-chemical properties that are different from those of the non-nanoform of the same material.
Proposed new definition (DG SANCO, 2013)	'Engineered nanomaterial' means any intentionally 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 dimension is in the size range 1 nm to 100 nm. By way of derogation: (a) food additives covered by the definition set out in the first paragraph shall not be considered as engineered nanomaterials, if they have been included in the Union lists referred to in Article 4 of Regulation (EC) No 1333/2008 by Commission Regulations (EU) No 1129/2011 and (EU) No 1130/201; (b) Fullerenes, graphene flakes and single-wall carbon nanotubes with one or more external dimensions below 1 nm shall be considered as nanomaterials.

	For the purposes of the definition set out in the first paragraph: — 'particle' means a minute piece of matter with defined physical boundaries, — '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, — 'aggregate' means a particle comprising strongly bound or fused particles; —'intentionally manufactured' means that the material is manufactured to perform/fulfil a specific function or purpose

Table 3. Overview of core elements of existing nanomaterial definitions.

Organization	Size range	Solubility	*Aggregates and Agglomerates	Distribution Threshold	**Intentionally manufactured/ Engineered	Novel properties
European Commission recommendation for a definition	1-100	No	Yes	50% by number	No	No
International Organization for Standardisation (ISO)	1-100	No	No	No	No	No
Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR)	1-100	No	No	0.15% by number	No	No
American Chemistry Council (ACC)	1-100	Yes	Yes	10% by weight	Yes	Yes
International Cooperation on Cosmetics Regulation (ICCR)	1-100	Yes	No	No	Yes	No
International Council of Chemical Associations (ICCA)	1-100	No	Yes	10% wt or more of nano-objects or 50 wt or more of aggregates/agglomerates consisting of nano-objects	Yes	No
German Chemical Industry Association (VCI)	1-100	No	Yes	10% weight of nano-objects	Yes	No

European Union Cosmetic Product Regulation (new proposed definition, 2013)	1-100	Yes	Yes	50% by number	Yes	No
Food information to Consumer Regulation (new proposed definition, 2013)	1-100	No	Yes	50% by number	yes	No
Biocides Regulation No 528/2012	1-100	No	Yes	50% by number	No	No
Medical Devices Regulation	1-100	No	Yes	50% by number	No	No
Switzerland	1-100	No	Yes	1% by number	No (but to be applied to synthetic nanomaterials)	No
France	1-100	No	Yes	50% by number	Yes	No
USA (FDA)	1-100	No	No	No	Yes	Yes
Taiwan	1-100	No	No	No	Yes	Yes
Korea	1-100	No	Yes (condensed nanoparticles)	No	No	No
China	1-100	No	No	No	No	Yes
Australia	1-100	No	Yes	10% by number	Yes	Yes
Canada	1-100	No	No	No	Yes	Yes

*'Yes' indicates agglomerates and aggregates explicitly addressed in the definition and 'no' not explicitly addressed.
**'Yes' indicates that the definition refers or applies to intentionally manufactured/engineered nanomaterials only, 'No' indicates that the definition does not specifically refers to manufactured/engineered nanomaterials.

18.2 Annexes to Section 7

Annex A to Section 7

Questions from the survey on experiences made during the implementation of the EC recommendation of a definition of nanomaterial

addressing experiences from relevant actors in the implementation of the definition (from industry associations, ECHA, EFSA, SANCO, …), including best practices and open challenges

Annex B to Section 7

Template for the provision of measured particle size distributions

Annex C to Section 7

Response statistics for the survey on experiences made during the implementation of the EC recommendation of a definition of nanomaterial

Annex D to Section 7

This annex contains the replies to the following questions of the survey Part B: Experience in the implementation of the definition:

- **B.16. Does your organisation make use of size distribution measurements of particulate materials?**
- **B.17. If yes, please list the methods which were used for these measurements. For each method listed, please identify the material(s) for which the method is used.**
- **B.18. Which of these methods are used by your organisation in-house?**
- **B.19. Are there borderline cases, i.e., materials for which it was difficult to decide whether they are nanomaterials according to the EC definition?**
- **B.20. If yes, please describe such borderline cases.**

Annex A to Section 7

Questions from the survey on experiences made during the implementation of the EC recommendation of a definition of nanomaterial addressing experiences from relevant actors in the implementation of the definition, including best practices and open challenges

A. Identification and general information about your organisation

A.1. Please give the name of the organisation for which you reply to this survey.

A.2. Please provide your name and your position in the organisation.

A.3. Please provide your email address for correspondence.

A.4. What is the type of your organisation?

A.6. How many employees does your company have?

A.7. In which country is your organisation principally based?

B. Your experience in the implementation of the definition

B.1. How would you describe your organisation's general experience with the implementation of the EC recommendation of a definition of nanomaterial?

B.2. Is the wording of the EC definition of nanomaterial clear and unambiguous?

B.3. If not, please explain why you do not consider the wording as clear or unambiguous.

B.4. Is it clear to which materials the EC definition of nanomaterials applies?

B.5. If not, please explain why it is not clear to which materials the EC definition of nanomaterials applies.

B.6. Are the individual elements (terms, thresholds, etc.) of the EC definition clear?

B.7. If not, please identify the elements that are unclear and give reasons.

B.8. Are you satisfied with the "Questions and Answers" section provided by the European Commission?

B.9. If not, how could the "Questions and Answers" section be improved?

B.10. Are you aware of any guidance on the implementation of the definition, other than the "Questions and Answers" section provided by the European Commission?

B.11. Please specify the guidance(s) that you are aware of

B.12. Is the guidance clear?

B.13. If not, please specify the elements of the guidance(s) which should be improved.

B.14. Has your organisation been facing issues in implementing the definition's specification on size distribution?

B.15. If yes, please describe these issues in more detail.

B.16. Does your organisation make use of size distribution measurements of particulate materials?

B.17. If yes, please list the methods which were used for these measurements. For each method listed, please identify the material(s) for which the method is used.

B.18. Which of these methods are used by your organisation in-house?

B.19. Are there borderline cases, i.e., materials for which it was difficult to decide whether they are nanomaterials according to the EC definition?

B.20. If yes, please describe such borderline cases.

B.21. Are you aware of measurement methods that have recently been developed or improved in a way that makes them a likely candidate method to help you implement the EC definition of nanomaterial in the near future?

B.22. If yes, please list the sources of your information (publications, reports, etc.) for each proposed method.

B.23. What level of resources do you use for the implementation of the EC definition of nanomaterial (e. g., manpower, instrumentation, consultancy, etc.)? Please add also a quantitative estimate of the most significant costs (person hours, instrument time, consumables etc.) for the type of material(s) that is (are) relevant for your organisation. Please specify the material(s).

B.24. Would you consider pragmatic solutions such as measurements of other, related material properties (e. g., specific surface area), and/or provision of information about the manufacturing process be acceptable as a substitute for size measurement for specific regulatory purposes?

B.25. If yes, please specify and give reasons.

B.26. Do you propose any change to the EC definition?

B.27. Please specify and/or give reasons for your answer to the previous question.

B.28. Here you can add any additional comments which you feel would be of particular use in the review process of the EC definition of nanomaterial.

C. Additional questions

C.1. Other than the EC definition of nanomaterial, are there any other relevant 'nanomaterial' definitions in the area (geographical or sectorial) relevant for your organisation?

C.2. If yes, provide a reference to this/these definitions and specify the most significant difference(s) between the EC definition of nanomaterial and the other definition(s).

C.3. Which recent scientific publications are particularly relevant for the implementation and review of the EC nanomaterial definition? (Max. 10 publications)

C.4. For which matrices (consumer products, food and feed, cosmetics, biocides, substances, etc.) do you envisage or predict a future need to determine the nanomaterial fraction (i.e. volume or mass percentage of nanomaterial in the matrix, but not the size distribution) by in-situ measurements?

D. Provision of measured particle size distributions[L]

D.1. Do you have reliably measured particle size distributions for materials with a large fraction of fine particles that provide a basis to decide whether or not the material should be classified as nanomaterial?

D.2. For how many materials will you provide us with the corresponding reliably measured particle size distribution?

D.3. [If the respondent indicated that measured particle size distributions could be provided, he was asked to download a template and to fill in the template individually for each material. This template can be found in Annex 7 B]

[L] The responses to Section D of the survey are included in Section 10 of this report.

Annex B to Section 7

Template for the provision of measured particle size distributions – please provide one file for each submitted distribution.

1. Specify your organisation (name, country). (If data are submitted collectively through a multi-member organisation, then the name of the member providing the data should also be given.)
2. Specify the material which you consider relevant for the discussion on the implementation of the definition and for which you provide a measured particle size distribution in this file.
3. **Attach or insert the actual measurement data.**
4. Please specify whether the provided data have been published. ☐ Yes ☐ No If yes, provide the full reference: …….. If no, please indicate whether the data can be used in the survey report only in aggregated form or in full and with reference to the source (organisation submitting the data). ☐ Data may be used in the report in aggregated form only. ☐ Full use is permitted, with reference to the organisation submitting the data.
5. Which measurement technique was used to measure the particle size distribution? ☐ Electron microscopy ☐ Light scattering technique ☐ Centrifugal liquid sedimentation ☐ Small-angle X-ray scattering ☐ Field flow fractionation ☐ Particle tracking analysis ☐ Atomic force microscopy ☐ X-ray diffraction ☐ BET analysis ☐ Other Please provide the additional information that specifies, within the general categories listed above, which method you used (e.g. scanning electron microscopy, dynamic light scattering – cumulants method, asymmetric flow field flow fractionation, …)
6. Please specify whether the used method was validated and the measurement uncertainty estimated.
7. Please specify in detail which kind of sample preparation steps were used.
8. Was the measurement made with reference to any national (DIN, AFNOR, BSI, ...) or international (CEN, ISO, ...) documentary standard method, or to another agreed, consensus protocol? ☐ Yes If yes, which standard method or agreed protocol was used? ☐ No If no, provide details/protocol used for the measurement method.
9. Are there any reference materials or other standard materials which were used to verify the results? ☐ Yes ☐ No If yes, specify the reference materials or other standard materials which were used to verify the results.
10. Has more than one method been used to measure the particle size distribution?

☐ Yes ☐ No If yes, which methods were used? Are the results obtained with different methods comparable? Please give an explanation.
11. The reported particle size distribution was: ☐ Mass based ☐ Intensity based ☐ Volume based ☐ Number based If any conversion was needed from mass/volume/intensity based size distribution data to particle number based distribution data please provide details on how the conversion was performed.

Annex C to Section 7

Response statistics for the survey on experiences made during the implementation of the EC recommendation of a definition of nanomaterial

Start date : 2013-08-09				
End date : 2013-09-27				
A Identification and general information about your organisation				
What is the type of your organisation?				
	Number of requested records	% Requested records(63)	% of total number records(63)	
Industry or trade association	22	35 %	35 %	
Private company	20	32 %	32 %	
EU Agency / EC Directorate General	1	2 %	2 %	
International organization	0	0 %	0 %	
Government authority	10	16 %	16 %	
Academic/research institution	6	10 %	10 %	
Non-governmental organization	2	3 %	3 %	
Other	2	3 %	3 %	
How many employees does your company have? *(Only for private companies)*				
Micro: <10	2	10 %	3 %	
Small: <50	0	0 %	0 %	
Medium: <250	1	5 %	2 %	
Large: >250	17	85 %	27 %	
In which country is your organisation principally based? *(Listed are only those with at least one reply.)*				
Austria	3	5 %	5 %	
Belgium	12	19 %	19 %	
Croatia	1	2 %	2 %	
Cyprus	1	2 %	2 %	
Czech Republic	4	6 %	6 %	
Estonia	2	3 %	3 %	
Finland	1	2 %	2 %	
France	2	3 %	3 %	
Germany	19	30 %	30 %	
Ireland	2	3 %	3 %	
Netherlands	2	3 %	3 %	
Spain	1	2 %	2 %	
United Kingdom	2	3 %	3 %	
None of the above	11	17 %	17 %	

B Your experience in the implementation of the definition				
Is the wording of the EC definition of nanomaterial clear and unambiguous?				
Yes	22	35 %	35 %	
No	41	65 %	65 %	
Is it clear to which materials the EC definition of nanomaterials applies?				
Yes	19	30 %	30 %	
No	44	70 %	70 %	
Are the individual elements (terms, thresholds, etc.) of the EC definition clear?				
Yes	23	37 %	37 %	
No	40	63 %	63 %	
Are you satisfied with the "Questions and Answers" section provided by the European Commission?				
Yes	24	38 %	38 %	
No	39	62 %	62 %	
Are you aware of any guidance on the implementation of the definition, other than the "Questions and Answers" section provided by the European Commission?				
Yes	36	57 %	57 %	
No	27	43 %	43 %	
Is the guidance clear?				
Yes	13	36 %	36 %	
No	23	64 %	64 %	
Has your organisation been facing issues in implementing the definition's specification on size distribution?				
Yes	44	70 %	70 %	
No	19	30 %	30 %	
Does your organisation make use of size distribution measurements of particulate materials?				
Yes	42	67 %	66.67 %	
No	21	33 %	33.33 %	
Are there borderline cases, i.e., materials for which it was difficult to decide whether they are nanomaterials according to the EC definition?				
Yes	30	71 %	48 %	
No	12	29 %	19 %	
Are you aware of measurement methods that have recently been developed or improved in a way that makes them a likely candidate method to help you implement the EC definition of nanomaterial in the near future?				
Yes	20	32 %	32 %	

No	43	68 %	68 %	
Would you consider pragmatic solutions such as measurements of other, related material properties (e. g., specific surface area), and/or provision of information about the manufacturing process be acceptable as a substitute for size measurement for specific regulatory purposes?				
Yes	36	57 %	57 %	
No	16	25 %	25 %	
No opinion	11	18 %	18 %	
Do you propose any change to the EC definition?				
Yes	53	84 %	84 %	
No	10	16 %	16 %	
C Additional questions				
Other than the EC definition of nanomaterial, are there any other relevant 'nanomaterial' definitions in the area (geographical or sectorial) relevant for your organisation?				
Yes	45	71 %	71 %	
No	16	25 %	25 %	
N/A	-	-		
D Provision of measured particle size distributions				
Do you have reliably measured particle size distributions for materials with a large fraction of fine particles that provide a basis to decide whether or not the material should be classified as nanomaterial?				
Yes	20	32 %	32 %	
No	43	68 %	68 %	

Annex D to Section 7

Replies to questions

B.16. Does your organisation make use of size distribution measurements of particulate materials?

B.17. If yes, please list the methods which were used for these measurements. For each method listed, please identify the material(s) for which the method is used.

B.18. Which of these methods are used by your organisation in-house?

B.19. Are there borderline cases, i.e., materials for which it was difficult to decide whether they are nanomaterials according to the EC definition?

B.20. If yes, please describe such borderline cases.

The table includes the responses of those addressees who answered question B.16 with "yes".

Methods used for the measurement of size distribution of particulate materials	**Material(s) for which the method(s) is/are used**	**Methods which are available in house**	**Observed borderline cases**
• Dynamic Light Scattering (DLS) • Nanoparticle Tracking Analysis (NTA NanoSight) • Scanning Mobility Particle Sizer Spectrometer (SMPS) • Transmission Electron Microscopy (TEM) • Disc Centrifuge (DC)	Silica (10, 34, 248 nm) Silver (15, 20, 50, 80, 110 nm) Gold (50, 250 nm) Silica (10, 34, 248 nm) Silver (10, 20, 50, 200 nm) Silver (15, 50, 110 nm) No experience yet, protocol in development	DLS, NTA, DC and SMPS	There is a strong dependency on the method used to determine the size distribution. Currently only microscopy techniques can reveal the primary particle sizes. A material may have a size distribution > 200 nm as measured with DLS, but whether this is the result of particles that are indeed larger than 200 nm or the result of aggregated or agglomerated particles can only be revealed using microscopy techniques.
The respondent did not indicate which methods are used.	*No material is given* *The respondent indicated that "Malvern" is used routinely. TEM as a scientific method, but not routinely applicable for all powders.*	*"Malvern" and TEM*	*The respondent indicated that there are borderline cases, but no example was given*
All contributing members of our organisation used the following methods for measurements: •Transmission electron microscopy (TEM) • Dynamic light scattering (DLS) • Nanoparticle tracking analysis (NTA) • Particle tracking analysis (PTA) • Scanning electron microscopy (SEM) • Atomic force microscopy (AFM) • Central particle sizing *(note: this method is unknown to JRC; probably CLS is meant)* • Gel permeation chromatography (GPC) • Condensation nuclear particle counter	*No material is given*	*All listed in the left column*	According to measurement methods, several borderline cases can arise. In the case of NTA/PTA, and where the measured modal size is close to 100nm, it is difficult to assess if a material is a nanomaterial according to the EC definition. For normal statistical measurements a number of samples are required in order to determine the exact typical value. To determine whether a material would fall under the definition large quantities of samples have to be tested. As an example, a member has reported cases in which about 52% of the counts where below 100nm for a test. In such cases it may be considered easier to decide that they fall under the definition although they might not. The characterisation of aggregates according to the EC definition often leads to borderline cases. Testing such materials does not help as it is impossible to break them apart and to test

• Micro-orifice uniform deposit impactor			whether the starting particles are nanomaterials. Finally, potential nanomaterials are often blended or compounded with plastics. As a result, it can be difficult to determine if a material is a nanomaterial unless the vendor is willing to provide specific information [1]. In addition, product manufacturers must often rely on the certificates of analysis provided by raw material vendors. Because no number based methods are currently validated, contradictory information is often obtained. As a result, the same raw material may be considered a nanomaterial or not depending upon the test method and assumptions selected by a vendor. [1] This circumstance is quite common for medical devices, where polymeric components often contain opacifiers, such as titanium dioxide, and colorants.
• CPS Disc Centrifuge, Lumisizer Disc centrifuge with CCD sensor, Malvern HPPS. Results were cross-checked qualitatively with Electron Microscopy pictures. • Laser diffraction Mastersizer 2000, CPS Disc Centrifuge. Results were cross-checked qualitatively with Electron Microscopy pictures	Nano-TiO_2 Nano-ZnO	To note that these methods are not used in house by companies, but were commissioned at specialised CROs for the preparation of dossiers by industry consortia.	none
Electron microscopy SLS DLS Centrifuge disk Specific surface area (VSSA)	*Not given*	Electron microscopy SLS DLS Centrifuge disk Specific surface area (VSSA)	none
• Laser diffraction using blue light • Sedimentation methods (e.g. Sedigraph) • Sieving	*Not given*	Laser diffraction and sedimentation methods are common in-house methods in the industrial minerals sector. Blue light laser diffraction equipment is only available in some large companies, not in SMEs.	• coarse materials with a nano-tail • materials with 1 or 2 dimensions in the nano range (e.g. phyllosilicates, elongated minerals) • minerals depending on the applied dispersion conditions to partly "break" the agglomerates/aggregates
SAMPLE PREPARATION: ISO 14887:2000 and OECD's "Guidance Notes on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials" (2012) CHEMICAL IDENTIFICATION: ICP (ICP-MS or ICP-OES) and Infrared (IR) spectroscopy PARTICLE SIZE DISTRIBUTION: by DLS / laser diffraction AND REM/TEM images (i.e. counting of particles). SURFACE AREA: For dry powders: BET; For suspensions: estimate surface area on the basis of particle size	Ag and ZnO, and probably applicable to most (precious) metals	Some are performed by the manufacturing/importing companies in-house; others are subcontracted to specialised laboratories. It depends on the equipment available in each company.	Pigments and so-called active forms

distribution (if shape of particles is more or less spherical). MORPHOLOGY: Digital light microscopic images AND TEM or REM to qualitatively describe the shape and the agglomeration behaviour of the particles.			
SEM,TEM,DC,DLC,LD,IG, DLS, SLS, SAX, DMA etc. Needless to say, it is needed to choose appropriate measurement method in accordance with sample conditions.	*Not given because of time constraints*	*Not given because of time constraints*	If generally speaking, our answer should be " No ", but if concretely speaking, it might be affected in specific case by measurement condition like size of parent population.
•laser light scattering but also gravitational sedimentation techniques • more important with regard to product quality are methods measuring the optical properties • disc centrifuge and XRD. With all these methods so-called “ensemble” properties are measured i.e. the sum of properties of “unbound” particles, agglomerates and aggregates. • SEM and TEM are mainly used to check shape and aspect ratio of crystallites (also often named in our industry as “primary particles”) and the state of dispersion in polymeric matrices, but normally not to measure particle size distribution.	White pigments and fillers tinting power, gloss in matrices which are close to real applications and depend very much on dispersion behaviour of pigments and fillers. ultrafine titanium dioxide grades	All of the methods mentioned here are used in-house by our organization. Nearly every method we are using is based on commercially available instruments but sample preparation, instrument set-up, result evaluation etc. is based on many years of experience in production processes, application know-how and personal skills. Sometimes details of methods and instrument adjustment e.g. to in-house standards are considered as proprietary know-how and will not be published or shared with customers or competitors. Consequently it is difficult or even impossible to compare results of outside-company measurements.	We are producing a huge variety of different grades of pigments and fillers depending on the needs of our customers. Accordingly these grades differ not only in their chemical composition (e.g. titanium dioxide vs. lithopone), but also in their crystal lattice (rutile vs. anatase), in their (primary) particle size and distribution, but mainly in their optical properties and dispersion behaviour corresponding to matrices in customer applications. It makes a big difference using standard measurement methods for determining particle size distribution compared to using same methods to determine a number based threshold based on diffuse definitions.
• TEM (in some cases with EDX) • AFM • DLS / NTA •TOF-SIMS • XPS • Chromatographic techniques • SAXS, WAXS • FT-IR Microscopy • Confocal Microscopy	• Ag, Au, TiO_2, CeO_2, Clay, Al_2O_3, SiO_2 • (Ag, SiO_2), • Ag, Au, TiO_2, CeO_2, Clay, Al_2O_3, SiO_2, CuO, Organic Nanoparticles); • Ag, Au, TiO_2, CeO_2, Clay, Al_2O_3, SiO_2, CuO, CNT, Organic Nanoparticles • Ag, Au, Clay • Organic Nanoparticles • SiO_2, Al_2O_3, Clay • Clay • Confocal Microscopy	Particle sizing techniques based on Brownian motion (e. g. Dynamic Light Scattering (DLS)), Nanoparticle Tracking Analysis (NTA)), microscopic techniques (e. g. REM, Confocal Microscopy; FT-IR coupled Microscope), Imaging Mass spectrometry (e. g. LA-ICP-MS, ToF-SIMS, MALDI-MS), Chromatographic	In some cases the results of complex, non spherical particle mixtures can be quite difficult to bring in line with the given definition.

		techniques (e.g. HDC-SP-ICP-MS, Hr-LC-MS/MS, GCxGC-ToF-MS), Flow cytometric techniques (FACS)	
•Transmission electron microscopy • Scanning electron microscope • Atomic Force Microscopy • Laser diffraction • Dynamic Light Scattering Hydrodynamic Chromatography • X Ray Disc Centrifuge Disc Centrifuge	Not given	All listed in the left column	Primary particle definition Remaining trace of catalyst A product produces for a Micrometric grade BUT with a Nanotail
The method selection normally depends on the material to be investigated and the task to be fulfilled. • Transmission electron microscopy (TEM) Focus on primary particles • Scanning electron microscopy (SEM) Showing mainly the "outer" particle dimensions, therefore not useful for primary particles, if no complete disintegration of agglomerates and aggregates can be reached. • Disc centrifuge (DC) Evaluation of the dispersion status of liquid paints or preparations • Laser diffraction (LD) Evaluation of the pigment powder, sample tested in solvents or airborne. For the measurement of the Nano range in an aerosol a combination with other methods (e.g. Scanning Mobility Particle Sizer – SMPS) may be necessary.	Not given	All methods mentioned in the left column beside SMPS.	Even imaging techniques like Transmission electron microscopy (TEM), which seem to deliver directly an overview about the particle state and a number size distribution are accompanied by several problems leading to uncertainties/deviations in the measurement results. The following factors have to be regarded as critical: - During the sample preparation a very small sample is taken and a dispersion step in a solvent with surfactants using ultrasonic treatment is necessary (applying strong shear forces). Nevertheless for organic pigments no complete disintegration of agglomerates/aggregates is reached. - The analysed images are two dimensional providing no information about the third dimension (depth). Thus systematic errors are present, especially for particle shapes deviating strongly from spherical (organic pigments are often platelet or needle shaped). - The images show strongly agglomerated and/or overlapping particles. Automatic image analysis is impossible, since primary particles would not be identified at all. Thus the analysis is done manually on a graphic tablet. The decision what structures are regarded as primary particles, strongly depends on the operator's opinion. Due to strong agglomeration and overlapping, often phantasy and intuition are needed. Some agglomerates are too complex to be analysed at all. Again it depends on the operator's opinion, to leave out and ignore such structures. For some pigments, all structures seen in the images are complex, and image analysis is impossible. The categorization of counted particles to "size classes" is done via. a graphic tablet combined with an evaluation software, which is commercially not available. As a consequence, we are using particle sizes obtained from TEM image analysis as relative measure, for comparison purpose only. Only for this purpose they are included in brochures and technical data sheets. It was never intended, and we think it is not advisable to be used as an absolute result for a decision like nano or not. As already described under B.5., no standardized and certified methods are

			currently available. Uncertainties/deviations between different labs are obvious and may cause a situation where results between between 20 and 80% by number have to be considered as “borderline cases”.
methods: DLS, DMA, SEM, EAB, AFM, TEM	particles: PSL, SiO_2, Au, Ag	DLS, DMA, SEM, EAB, AFM, TEM, BET	low purity nanomaterials: photocatalyst materials sometime contain two or more size distribution peak nanomaterial compound or mix: nanomaterials paint what about carbon black in tire manufacturing, there are quite a few nano carbon black
• CLS • DLS • TEM •XRD (averaged size) most of crystalline particle. PTA CC, CB, TD	Calcium carbonate, Carbon Black, Titanium Dioxide Calcium carbonate, Carbon Black, Titanium Dioxide CNT, TiO_2, C_{60}, .. most of crystalline particle.	all	Calcium Carbonate: TEM says 80 nm, while CLS says 120 nm. We do not know their uncertainty.
Powder/Dispersions: SEM/TEM with image analysis and/or DLS	*Not indicated*	Powder/Dispersions: SEM/TEM with image analysis and/or DLS	There were no borderline cases
• TEM analysis • Disc Centrifuge Photosedimentometer	Carbon Black primary particles (ASTM D3849) (carbon black aggregates)	The method(s) used varies by member companies.	No borderline cases
• TEM analysis Disc Centrifuge Photosedimentometer Photon Correlation Spectroscopy • Laser Diffraction Spectrometry	For carbon black aggregates (ASTM D3849) For carbon black agglomerates	All methods	No borderline cases
• Light scattering technique • BET analysis	Not indicated	All methods	No borderline cases
• DLS • AFM • SEM • TEM	• PPS, gold nanoparticles, SiO_2 nanoparticles • PPS, gold nanoparticles, SiO_2 nanoparticles • PPS, gold nanoparticles, SiO_2 nanoparticles • gold nanoparticles, SiO_2 nanoparticles	All methods	No borderline cases
Distributions (or a visual overview rather that complete measurements) are obtained from " • TEM, SEM • VSSA may be used as a screening technique " • SAXS used as a screening technique " • Liquid laser scattering (but care is needed regarding dispersion).	examples C.I. Pigment Blue 15, C.I. Pigment Red 57:1. C.I. Pigment Yellow 13 "	All used by our member companies and by external laboratories contacted	Unfortunately a large class of borderline cases occurs. Many samples analysed by TEM are such cases, as clearly nano and clearly not nano samples can be ruled out by cheaper analytics. Main TEM problems are: TEM results in a particle size distribution near the threshold TEM images cannot be evaluated due to problems with preparation, which occurs often for real-life material. TEM images give rise to the question if there might be preparation artifacts. Examples of these problems may be seen in our submission to DG ENT in July 2013. General problem related to a measurement strategy: How to ensure in CLS/FFF/AUC measurements that the degree of dispersion was sufficient. Validation by comparison to

			primary particle diameters expected from BET is a valid & strong criterion.
• DLS • NTA • A4F-Uv-vis • LIBD • A4F – LIBD • TEM	• TiO_2, Ag, Au, polystyrene beads (PS) • Ag, Au • PS • PS • PS • TiO_2, Ag, Au, CNT, fly ash, etc.	all	no
a) Laser Diffraction particle measure (Malvern Mastersizer). b) Electron Microscopy	Not indicated	Laser Diffraction Particle size measure (Malvern Mastersizer).	There is a big discussion, with several approaches; for example Iron oxides size discussion (very well known substances with a well study Safety Profile). Most Pigments are challenged as they are obtained as agglomerated and cannot be measured. Please refer to the conclusion and examples of JRC report: "Requirements on measurements for the implementation of the EC definition of "Nanomaterial". JRC concluded that for powder samples, as Pigments, there is a disagreement on the measures that leads to disagreement in the characterization as Nanomaterial or not.
DLS, DMA, SEM, EAB, and AFM	(Ag, Au, PS, ZnO, Silica)	all	No borderline cases
• TEM analysis Disc Centrifuge Photosedimentometer	For carbon black aggregates (ASTM D3849)	Disc Centrifuge Photosedimentometer	
• TEM • CLS/AUC • FFF • AFM • XRD	• all kinds of nanomaterials (paints, coatings, plastics, cosmetics, pharma, construction, catalysts) • products that are marketed as well-dispersed suspension or slurry (adhesives, pigment pastes, catalyst slurries) • available in-house, but not yet applied to nanodefinition issues • experimental stadium; test if one can overcome the 2D-limitation of TEM • for some particle classes a potentially cheaper alternative	all	Unfortunately a large class of borderline cases occurs. Many samples analysed by TEM are such cases, as clearly nano and clearly not nano samples can be ruled out by cheaper analytics. Main TEM problems are: TEM results in a particle size distribution near the threshold TEM images cannot be evaluated due to problems with preparation, which occurs often for real-life material. TEM images give rise to the question if there might be preparation artifacts. General problem related to a measurement strategy: How to ensure in CLS/FFF/AUC measurements that the degree of dispersion was sufficient. Validation by comparison to primary particle diameters expected from BET is a valid & strong criterion. See Brown et al 2013 Env. Health Perspect.
• particle size distribution by laser granulometry as standard test method for all products without nano relevance • REM - with counting of determined nano particles (no standard method)	No specific materials listed	Laser granulometry	*Note: this reply did not address borderline cases but general measurement difficulties*
We use Dynamic light scattering and currently attempting TEM. Our material has been manufactured for more than 40 years and been subjected to many academic studies where particle size has been characterised by more advanced techniques such as neutron diffraction and SAXS By the nature of the particle (low molecular mass - it is a layered magnesium silicate) techniques such as disc	Layered magnesium silicate – not specified further	none	Borderline cases not specified (relating to aggregates/agglomerates)

centrifuge and those monitoring Brownian motion do not work			
Laser Diffraction, DLS, Disc Centrifuge, Sedigraph, SEM, TEM	Material not specified	all	depending on definition of constituent particles (strongly fused non destructible aggregates of primary particles)
A large variety of methods are available internally (SEM, TEM, DLS…). Many of them are for research purposes and do not fulfil the criteria standard, validated and cost-efficient. DLS is readily available standard, however it cannot differentiate between particles and aggregates/agglomerates.	Material not specified	Primarily Dynamic Light Scattering (DLS).	The challenge exists for all materials we produce and handle in our daily business. It is necessary to agree on a single, robust measuring method as stated by the JRC: "As results of size measurements are method-defined, standardization of measurement methods is needed to ensure comparability between different laboratories. […] Summarising the current technical limitations, none of the currently available methods can determine for all kinds of potential nanomaterials whether they fulfil the definition or not" (JRC Report EUR 25404 EN, 2012).
• Brookhaven centrifuge (sedimentation in centrifugal field), laser diffraction - Cilas, Malvern, Dynamic light scattering (Malvern). Laser diffraction - Cilas, Malvern, Dynamic light scattering (Malvern). • Occasionally SEM or TEM	• TiO_2 • Iron oxides • TiO_2, Iron oxides	Brookhaven centrifuge (sedimentation in centrifugal field), laser diffraction - Cilas, Malvern, Dynamic light scattering (Malvern).	If crystallites of some titanium dioxide anatase pigments are measured by electron microscopy, they sometimes are and sometimes are not nanomaterials. Median particle size of some TiO2 anatase pigment types measured by electron microscopy and with considering crystallites as primary particles can be in range 100-120 nm. In such case some batches of the same material can be nano and some batches no, according to measurement results.
Electron microscopy, Dynamic light scattering (size measurement in liquids).	Not specified	Dynamic light scattering (DLS)	In different liquids nanoparticles may show different size distribution. For example, even if a powder was nanosized in its dry form, it may not be nanosized in liquid (aggregation may occur). If to use only DLS technique to determine the particle size distribution in liquid, then the fact that the aggregated particles were in nanosize in dry form (before mixing the particles with liquid) may not be discovered. In addition, DLS technique does not reliably show the size distribution in heterogeneous samples. In case of very polydisperse samples, larger particles dominate and usually small particles are not visible.
Aerosols: SMPS/FMPS (5-1000nm) DustMonitor (400nm-30µm) Powder/Dispersions: SEM/TEM with image analysis and/or DLS	Material not specified	All are used in-house	In some cases it is also with EM not possible to decide if you have a single particle or an aggregate of some. With DLS it is not possible to distinguish between agglomerates/aggregates and single particles.
• SEM (Scanning Electron Microscopy) • TEM (Trans Electron Microscopy) • BET (specific surface area) • DLS (Dynamic Light Scattering) • LD (Laser Diffraction) • XRD (X-ray Diffraction) • XRF (X-ray Fluorescence) • Liquid Photo Sedimentation (Disc centrifuge) • Helium Pyknometry (specific density)	all methods are used for all our particulate products, mainly metal oxides	all	Basically the definition makes out of nearly each fine particulate material a nano material. Most fine particulate substances consist of aggregates and agglomerates of primary particles or primary crystallites, resp. however, in any case these aggregates and the corresponding agglomerates are of sizes significantly above 100 nm. With granulometric methods only the external particle size can be determined whereas only with TEM and SEM (with appropriate resolution) you can also determine the primary particle sizes. VSSA cannot be used for the latter because all materials in question do not consist of mono disperse spherical particles.

18.3 Annexes to Section 8

Annex 8.1: List of relevant projects from FP7 and other European Research Programs

List of the relevant projects from compendium NanoSafety Cluster 2012-2013

The EU NanoSafety Cluster is a DG RTD NMP initiative to maximise the synergies between the existing FP6 and FP7 projects addressing all aspects of nanosafety including toxicology, ecotoxicology, and exposure assessment, mechanisms of interaction, risk assessment and standardisation.
Participation in the NanoSafety cluster is voluntary for projects that commenced prior to April 2009, and is compulsory for nano-EHS projects started since April 2009.

The NanoSafety Cluster compendium published in 2012 and 2013 documents the status of important projects on nanomaterial toxicity and exposure monitoring, integrated risk management, research infrastructure and coordination and support activities.
The abstracts describing the objectives of the projects are quoted from the European Commission's CORDIS website which hosts the project-related reports. They are included in the report to allow the reader to obtain a quick overview without the need to individually visit the project specific webpages. For more details on specific project results and final reports in case of concluded projects it is however recommended to consult the CORDIS information service, the European Nanosafety Cluster compendiums 2012 and 2013, or the project-specific websites.

The texts of the abstracts were quoted from the original European Commission websites according to the references.

Project already finished	Project that is going to finish in 2013	Long term project

No.	Acronym	Dates	Abstract [M]
1	ENNSATOX Engineered nanoparticle impact on aquatic environments: Structure, activity and toxicology	2009-2012	The use of engineered nanoparticles in cosmetics, pharmaceuticals, sensors and many other commercial applications has been growing exponentially over the past decade. EU and Member States research into the environmental impact of these materials, particularly in aquatic systems, is at an early stage. ENNSATOX addresses this deficit through a, comprehensive investigation relating the structure and functionality of well characterised engineered nanoparticles to their biological activity in environmental aquatic systems. An integrated approach will assess the activity of the particles in a series of biological models of increasing complexity. Parallel environmental studies will take place on the behaviour of the nanoparticles in natural waters and how they modify the particles' chemical reactivity, physical form and biological activity. An integrated theoretical model will be developed describing the environmental system as a series of biological compartments where particles transport between a) compartments by advection-diffusion and b) between phases by a transfer function. Following optimisation of the transfer functions a generic predictive model will be derived for the environmental impact of each class of nanoparticle in aqueous

[M] http://cordis.europa.eu/projects/home_en.html

			systems. A generalised understanding of the dependence of the nanoparticle biological activity on its structure and functionality will be obtained including the role and interaction of the biological membranes within organisms. ENNSATOX aims to generate: - exploitable IP (devices and ecotoxicology predictive software package); - set of standard protocols for assay of nanoparticle biological activity which can be later accredited; - global dissemination of results; - creation of an EU laboratory service; - tools and data to inform EU Regulation and the EC s code of conduct for responsible nanosciences and nanotechnologies research, ftp://ftp.cordis.europa.eu/pub/nanotechnology/docs/nanocode-recommendation-pe0894c08424_en.pdf.
2	ENPRA Risk assessment of engineered nanoparticles	2009-2012	Engineered Nanoparticles (ENP) are increasingly produced for use in a wide range of industrial and consumer products. Yet it is known that exposure to some types of particles can cause severe health effects. Therefore it is essential to ascertain whether exposure to ENP can lead to possible health risks for workers and consumers. We have formed a consortium of well-known scientists from European Universities and Research Institutes, with over 100 publications in the field of Nanotoxicology. Our aim is to develop an approach for the Risk Assessment of ENP (ENPRA). Their objectives are: (i) to obtain a bank of commercial ENP with contrasting physico-chemical characteristics and measure them; (ii) to investigate the toxic effects of ENP on 5 (pulmonary, hepatic, renal, cardiovascular and developmental) target systems and 5 endpoints (oxidative stress, inflammation; immuno-toxicity; fibrogenecity; genotoxicity) using in vitro animal/human models; (iii) to validate the in vitro findings with a small set of carefully chosen in vivo animal experiments; (iv) to construct mathematical models to extrapolate the exposure-dose-response relationship from in vitro to in vivo and to humans; (v) to use QSAR like models to identify the key ENP characteristics driving the adverse effects; (vi) to implement a risk assessment of ENP using the Weight-of-Evidence approach; (vii) to disseminate our findings to potential stakeholders. To harmonise the research activities between our EU group and the US, links with scientists from US Universities (Duke, Rochester) and Government Agencies (NIH/NIEHS, NIOSH and EPA) with on-going research in Nanotoxicology was established. Their objectives here were: (vii) to share information and agree on experimental protocols; (viii) to avoid duplication of work; (ix) to further validate the findings of this proposed study.
3	EuroNanoTox	2007-open	EURO-NanoTOX will serve as an entry portal for researchers and industry seeking critical toxicological data for nano-structured materials and wanting to develop research projects in this field. The portfolio of EURO-NanoTOX will be structured in the following way:

			1. Formulation of testing strategies for nanostructured materials 2. Sample pre-evaluation 3. in-vitro testing 4. ex-vivo testing 5. in-vivo testing
4	HINAMOX Health impact of engineered metal and metal oxide nanoparticles: Response, bio-imaging and distribution at cellular and body level	2009-2012	Metal oxide and metal NPs are particularly dangerous for two reasons: their special catalytic activity coming from the properties of their nano-interface may interfere with numerous intracellular biochemical processes and the decomposition of NPs and the ion leakage could heavily interfere with the intracellular free metal ion homeostasis, which is essential for cell metabolism. A very specific problem is the difficulty of localizing and quantifying them in cells. Obtaining dose effect relationships is not simple, because of the unknown amount of material present in affected cells. The following main points will be addressed in this proposal: * Design and synthesis of metal oxide and metal NPs, which can be traced by SPECT, PET, and fluorescence techniques and the appropriate characterization of these NPs. * Application of label-free techniques, such as IBM and EM to ensure that the radioactive and fluorescent constituents do not modify the cytological and organismic response by themselves. * Characterization of the uptake, distribution kinetics and NP release at the level of the organism. * Study of the interaction of NPs with plasma components forming complexes with NPs and the assessment of their possible impact on the uptake compared with that of bare or capped particles. * Quantification and localization of metallic NPs in immune competent cells is a key task for the establishment of proper dose-response correlations. A technique applicable with living cells as ultimate control will be IBM, capable of detecting single metal NPs in cells at different depths. * Development of sophisticated cell physiological approaches focusing on the determination of oxidative activity, cytokine production and adaptive processes concerning signalling pathways beyond standard vitality tests. The research project will indicate toxic levels of various NPs and sub-toxic effects will be investigated by analysing the signalling response of immune cells.
5	InLiveTox Intestinal, Liver and Endothelial Nanoparticle Toxicity Development and evaluation of a novel tool for high-throughput data generation	2009-2013	The InLiveTox project will form an interdisciplinary consortium at the European level, together with a key American research group to develop an improved in vitro model for the study of nanoparticle (NP) uptake, transport and cellular interaction, thus advancing our understanding of NP toxicity. Rather than repeat what has, or is being done in the field of aerosol NP and lung toxicology, InLiveTox will focus on the impact of NP exposure via ingestion, in the healthy and diseased gastrointestinal (GI) tract, vascular endothelium and liver. The key questions in this study are: (i) How do these tissues individually respond to NPs? (ii) How do the interactions between the different tissues modulate their responses? (iii) How does inflammation affect the toxicity of NPs and their ability cross the intestinal barrier? (iv) Which physico-chemical characteristics of NPs influence their

			uptake by intestinal epithelial cells and their subsequent interactions with endothelial and liver cells? The objective of InLiveTox will be to develop a novel modular microfluidics-based in vitro test system modelling the response of cells and tissues to the ingestion of NPs. Cell culture modules of target tissues such as the GI tract, the liver and the endothelium will be connected via a microfluidics system so that knock-on and cross talk effects between organs and tissues can be monitored. A major innovative aspect of the InLiveTox project pertains to the implementation of biological tissue models in a microfabricated compartmental cell culture system that allows multiple cell types to be addressed and investigated in combination. This system will be much easier, more convenient and ethically less questionable than animal testing, as well as more relevant than the in vitro single cell /co-culture models currently used. For this study, applications of the model will focus on NP toxicology, but the system could also be widely used in various applications of toxicology and pharmacology.
6	INSTANT Innovative Sensor for the fast Analysis of Nanoparticles in Selected Target Products	2012-2015	INSTANT will face the challenge of the detection, identification and quantification of engineered nanoparticles (ENPs) in complex matrices such as cosmetic products and engineered food and drinks. Therefore, new detection methods and technologies are mandatory. This is completely in line with the Call FP7-NMP.2011.1.3-1 which deals especially with innovative, practically implementable and cost effective measurement approaches for ENPs in complex matrices. Recently emerging ENPs include Ag, SiO2, TiO2, ZnO, and organic NPs. The Opinion of the Scientific Committee on the Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety released by the European Food Safety Authority (EFSA) (2009) also highlights the urgent need for such a tool. Accordingly, the interdisciplinary project INSTANT will develop an innovative and integrated technology for monitoring the exposure of consumers to ENPs using a label free opto-electrochemical sensor array in combination with novel recognition elements. The SME driven INSTANT will develop an innovative, cost effective, and easy to use analytical tool to extract, detect and identify ENPs typically used in cosmetic products (e.g. sunscreen, toothpaste, deodorant,...) and engineered food (e.g. instant soups, ketchup, ice cream,...) and drinks (e.g. fruit juice, energy drinks, bottled water,...). A crucial point of measuring in these complex matrices is the sample preparation and extraction. Therefore INSTANT will develop and integrate tailored extraction methods. Especially the size distribution of ENPs in the sample and the influence of the matrix on chemical and physical properties of the ENPs have to be taken into account. The INSTANT device will be designed to be used as a cost effective monitoring tool which is suitable for characterisation and classification of ENPs for the future implementation of quantitative structure-activity relationship studies.
7	ITS-NANO Intelligent testing strategy for engineered nanomaterials	2012-2013	The background, concept and objectives of ITS-NANO are straight forward. The volume of information on hazard characterisation of ENM is increasing fast. In parallel with the scientific development, regulation orientated initiatives are also taking place to identify needs. The ITS-NANO concept is 1: Gather targeted all scientific evidence, by literature search and communication with leading scientists. 2: Develop an initial assessment (document) of the available knowledge

			and the gaps, focussed on identifying knowledge level as how to develop an intelligent approach to grouping ENMs based their properties and their subsequent biological impacts in order to intelligently design next-generation nano-safety evaluation and risk assessment strategies. 3: Assemble stakeholders for presenting the initial assessment, having a dialog on how this relates to their aims/needs and how to make a consent driven strategy forward that ensures communication. 4: Revise the initial assessment document with the input from the stakeholder sent around for commenting, presenting the next draft for a smaller group for final commenting. 5: Publish it.
8	MARINA Managing Risks of Nanoparticles	2011-2015	While there are standard procedures for product life cycle analysis, exposure, hazard, and risk assessment for traditional chemicals, is not yet clear how these procedures need to be modified to address all the novel properties of nanomaterials. There is a need to develop specific reference methods for all the main steps in managing the potential risk of ENM. The aim of MARINA is to develop such methods. MARINA will address the four central themes in the risk management paradigm for ENM: Materials, Exposure, Hazard and Risk. The methods developed by MARINA will be (i) based on beyond-state-of-the-art understanding of the properties, interaction and fate of ENM in relation to human health and the quality of the environment and will either (ii) be newly developed or adapted from existing ones but ultimately, they will be compared/validated and harmonised/standardised as reference methods for managing the risk of ENM. MARINA will develop a strategy for Risk Management including monitoring systems and measures for minimising massive exposure via explosion or environmental spillage.
9	MembranenanoPart Modelling the mechanisms of nanoparticle-lipid interactions and nanoparticle effects on cell membrane structure and function	2013-2015	The central goal of our proposal is to develop physically justified models and computational tools to quantitatively describe and understand the molecular mechanisms of nanoparticle-cell membrane interactions, which we consider to be a crucial point in any predictive model of nanoparticle toxicity. We consider mechanisms of nanoparticle protein corona formation, the protective function of the membrane, nanoparticle uptake into the cell, and the effect of nanoparticles on the cell membrane. We plan to develop a consistent multiscale simulation scheme starting from nanoparticle-biomolecule interaction at the atomistic scale using molecular dynamics simulation, and then systematically constructing coarse-grained mesoscale models for simulating the structure and dynamics of the cell membrane perturbed by nanoparticles at the physiologically relevant time and length scales. We will develop and test a universal method for evaluating the rates of nanoparticle translocation through membranes and evaluate associated specific toxicity effects. Based on the information acquired from the simulations and analysed together with available experimental data, the toxicological impact will be deduced. We will apply our approach to a range of common engineered nanoparticles, relating their physicochemical properties such as size and shape, surface charge, hydrophobicity (logP), and plasma protein binding affinity to the toxicological effects and develop a test suite allowing to make toxicity prediction on the basis of purely computational or limited in vitro screening tests.
10	MODERN MODeling the EnviRonmental and human health effects of	2013-2015	Nano-sized materials are a common element in many industrial processes mainly due to their unique properties that lead to the production of high technology products. The widespread use of nanotechnology requires the consideration of the environmental and human health risks that may result from the introduction of engineered nanoparticles (eNPs) into the environment. Although toxic

	Nanomaterials (MODERN)		effects for certain types of eNP have been recently reported, there is still a lack of knowledge about their possible long-term effects in biological systems. **The project focuses on the understanding of the processes governing the interactions of nanoparticles with biological systems and their associated mechanisms of toxicity, which are essential for eNP safety assessment.** Information on the effects of well characterized eNPs will be obtained from literature and other data repositories. Targeted in vivo and in vitro experiments will be also carried out to overcome the limitations of data availability and for model validation. Computational methods will be applied to model both nanostructure-property relationships and the complex and highly non-linear nano-bio interactions and to diminishing the need for animal testing. The main goal of MODERN is to establish new modelling approaches suitable for relating nanotoxicity with the intrinsic molecular and physicochemical properties of eNPs at environmental exposure levels and to implement safe-by-design nanoparticle design strategies. This implies three specific objectives: (i) To apply computational models for the characterization of the structural and physicochemical properties leading to QNPRs and safe-by-design strategies for eNPs; (ii) To develop in silico models (QNAR) of biological activity of eNPs in the body and in the environment; (iii) To establish a categorization and hazard ranking protocol for eNPs based on structural similarity principles and in the analysis of their toxicological profiles.
11	ModNanoTox Modelling nanoparticle toxicity: principles, methods, novel approaches	2011-2013	ModNano-Tox will develop a number of well-documented and technically advanced models describing the behaviour of engineered nano-particles in organisms and the environment. Background to these models will be a thoroughly documented database, constructed based on: (1) an advanced evaluation of physicochemical properties of nano-particles and in silico modelling of their reactivity; and (2) assessment of the characterisation methodologies as well as toxicity protocols used to develop biological responses in toxicological studies. At the next level whole datasets will be evaluated for internal consistency and then compared with other relevant sets. The evaluation stage will be followed by development of toxicity models based at the individual organism level, using statistical and mechanistic models, in parallel with models predicting environmental fate. The toxicity and fate models will be integrated in mechanistic models to predict the long term risks of engineered nano-particles for populations under realistic environmental conditions. The risk assessment models will be developed in close collaboration with appropriate stakeholders and end users to ensure their suitability for practical use in relevant legislative contexts.
12	Nanodetector Ultrasensitive plasmonic detection of single nanoparticles	2012-2015	Controlled or uncontrolled disposing of nanoparticles in various components of man-made or biological matter may have wanted or undesired consequences. Developing the diagnostic tools to detect and characterize the grey goo is one of the challenges of nanotech-era. A development of general technology for detection and analysis of single nanoparticles in complex environment and a development of a laboratory prototype of the device based on this technology and its application are the goal of this project. The proposal is based on the new experimental phenomenon discovered recently by a project partner: single sub-wavelength objects give rise to giant optical signals in surface plasmon resonance microscopy. This provides a unique possibility for ultrasensitive on-line detection of engineered

			nanoparticles. Within the project a development of the device for detection of nanoparticles and its application for a number of practically important tasks will be performed. The work includes a development of theoretical description of the new effect, optimization of main components of the detection system, development of sophisticated software for effective image analyses and isolation of nanoparticle signals from background optical signals and noise. Preliminary experiments demonstrated a possibility to use surface modification to distinguish different types of nanoparticles. Within the project this approach will be used for identification of nanoparticles. Measurements will be performed in aqueous media as well as in air. Inorganic, plastic and protein nanoparticles will be examined. At the final step of the project monitoring of nanoparticles in simple (drinking water, mineral water, air) and complicated (wine, juice and other transparent non-colloidal drinks) will be performed. The end users will test the developed experimental system for monitoring of workplaces and waste during production of inorganic and protein nanoparticles.
13	NANODEVICE Novel concepts, methods, and technologies for the production of portable, easy-to-use devices for the measurement and analysis of airborne engineered nanoparticles in workplace air	2009-2013	The main project goal is to develop innovative concepts and reliable methods for characterizing ENP in workplace air with novel, portable and easy-to-use devices suitable for workplaces. Additional research objectives are - identification of relevant physico-chemical properties and metrics of airborne ENP; establishment of reference materials; - exploring the association between physico-chemical and toxicological properties of ENP; - analysing industrial processes as a source of ENP in workplace air; - developing methods for calibration and testing of the novel devices in real and simulated exposure situations; and - dissemination of the research results to promote the safe use of ENP through guidance, standards and education, implementing of safety objectives in ENP production and handling, and promotion of safety related collaborations through an international nanosafety platform.
14	NanoFATE Nanoparticle Fate Assessment and Toxicity in the Environment	2010-2014	Concept: NanoFATE has been conceived to fill knowledge and methodological gaps currently impeding sound assessment of environmental risks posed by engineered nano-particles (ENPs). Our vision is to assess environmental fate and risk of ENPs from high-volume products for which recycling is not an option; namely; fuel additive, personal care and antibacterial products. Two market ENPs from each product (CeO2, ZnO, Ag of varying size, surface and core chemistries) will be followed through their post-production life cycles i.e. from environmental entry as spent product, through waste treatment to their final fates and potential toxic effects. This will test the applicability of current fate and risk assessment methods and identify improvements required for a scientific assessment of ENPs at an early stage. Objectives: Such systematic study of the environmental fate and toxicity of selected ENPs will entail addressing 9 S&T objectives: 1: Design, tagging and manufacture of ENPs 2: Analysis of ENP interactions with abiotic and biotic entities 3: Generating predictive models for ENP exposure in waters and sludge-amended soils 4: Studying the fate and behaviour of ENPs through wastewater treatment 5: Determining acute and chronic ecotoxicity 6: Assessing effects of physico-chemical properties on ENP bioavailability

			7: Defining mechanisms of uptake, internal trafficking, and toxicity 8: Developing spatial RA model(s) 9: Improving understanding of ENP risks Methodology: The work plan is designed to progress beyond the state-of-the-art through focused work packages. While some objectives are delivered in single WPs, good cross WP integration will secure the key objectives of delivering new methods for quantifying ENP risks. Impact: NanoFATE will provide robust tools, techniques and knowledge needed by stakeholders to understand and communicate risks associated with different ENPs, including their environmental interactions and toxicity.
15	NanoHouse Life Cycle of Nanoparticle-based Products used in House Coating	2010-2013	NanoHOUSE intends to create a holistic and prospective view on the Environmental Health and Safety (EHS) impacts of nanoproducts used in house building, namely paints and coatings. The latter are using relatively high amounts of Engineered NanoParticles (ENPs) such as nano-Ag and nano-TiO2 which will be investigated. A new Life Cycle Thinking (LCT) approach will be developed gathering two complementary aspects: Investigation of risks and opportunities during the product life cycle as well as Life Cycle Analysis (ISO 14040). LCT will collect information on EHS impacts throughout all life cycle stages of the nano-products, identifying the data gaps which will guide the research work. NanoHOUSE will generate reliable scientific information for the missing data and will develop appropriate methods to analyse the potential EHS impacts of nano-products. NanoHOUSE first task will be to quantify the actual sources of ENPs during the use and ageing of actual coatings (weathering, renovation, demolition and final disposal). The project will then characterize the environmental compartments significantly impacted by ENPs released from nano-products, measure ENPs concentrations and states in those compartments, and investigate their fate in order to increase the knowledge regarding exposure to ENPs with a view to reducing the risks. NanoHOUSE will study the environmental behaviour and the toxicological effects of actually released ENPs (aged ENPs) and compare them with pristine ENPs. Finally, NanoHOUSE will improve the solutions for end of life treatments regarding ENPs release in the environment. Main outcomes of the project will be a scientific risk evaluation of nano-products used in building, solutions to improve their competitive and sustainable development by decreasing their potential to release ENPs, and contributions to standard tests for their certification. The NanoHOUSE consortium involves 5 research/academic partners and 4 industrial manufacturers of which 1 SME.
16	NanoImpactNet European network on the health and environmental impact of	2008-2012	Recent technological advances allow the targeted production of objects and materials in the nanoscale (smaller than 100 nm). Nanomaterials have chemical, physical and bioactive characteristics, which are different from those of larger entities of the same materials. Nanoparticles can pass through body barriers. This is interesting for medical applications, but it raises concerns about their health and

	nanomaterials		environmental impact. The objective of the NanoImpactNet is to create a scientific basis to ensure the safe and responsible development of engineered nanoparticles and nanotechnology-based materials and products, and to support the definition of regulatory measures and implementation of legislation in Europe. It includes a strong two-way communication to ensure efficient dissemination of information to stakeholders and the European Commission, while at the same time obtaining input from the stakeholders about their needs and concerns. The work plan shows six work packages (WPs: Human hazards and exposures, Hazards and fate of nanomaterials in the environment, Impact assessment, Communication, Integration and nomenclature, and Coordination and management). The work plan will be implemented over four years. Discussions about strategies and methodologies will be initiated through well-prepared workshops covering the WP topics. External researchers and stakeholders will be invited to participate. After these workshops, the researchers will collaborate to produce thorough reports and sets of guidelines reflecting the consensus reached. All of the leading European research groups with activities in nanosafety, nanorisk assessment, and nanotoxicology are represented in NanoImpactNet. All exposure routes, major disease classes and impact assessment approaches are represented within the network. It will coordinate activities within Europe. It will help implement the EU Action Plan for Nanotechnology and support a responsible and safe development of nanotechnologies in Europe.
17	Nanolyse Nanoparticles in Food: Analytical methods for detection and characterisation	2010-2013	The NanoLyse project aims to focus on the development of validated methods and reference materials for the analysis of engineered nano-particles (ENP) in food and beverages. The developed methods will cover all relevant classes of ENP with reported or expected food and food contact material applications, i.e. metal, metal oxide/silicate, surface functionalised and organic encapsulate (colloidal/micelle type) ENP. Priority ENPs have been selected out of each class as model particles to demonstrate the applicability of the developed approaches, e.g. nano-silver, nano-silica, an organically surface modified nano-clay and organic nano-encapsulates. Priority will be given to methods which can be implemented in existing food analysis laboratories. A dual approach will be followed. Rapid imaging and screening methods will allow the distinction between samples which contain ENP and those that do not. These methods will be characterised by minimal sample preparation, cost-efficiency, high throughput and will be achieved by the application of automated smart electron microscopy imaging and screening techniques in sensor and immunochemical formats. More sophisticated, hyphenated methods will allow the unambiguous characterisation and quantification of ENP. These will include elaborate sample preparation, separation by flow field fractionation and chromatographic techniques as well as mass spectrometric and electron microscopic characterisation techniques. The developed methods will be validated using the well characterised food matrix reference materials that will be produced within the project. Small-scale interlaboratory method performance studies and the analysis of a few commercially available products claiming or suspect to contain ENP will demonstrate the applicability and soundness of the developed methods.
18	NanoMICEX	2012-	The main aim of NANOMICEX project is to reduce the potential risk

	Mitigation of risk and control of exposure in nanotechnology based inks and pigments - NANOMICEX -	2014	upon workers exposure to the engineered nanoparticles employed in the operative conditions of the inks and pigments industry, by addressing at the health and environmental sequences associated with the inclusion of nano-additives within all stages of nanotechnology based products (production, use and disposal). To achieve it, new surface modifiers will be designed and developed to obtain less hazardous and more stable nanoparticles. The proposed work will focus on a selected set of nanoparticles relevant to the ink and pigment sector. Full characterisation will be carried out, followed by an exposure measurement in order to characterise and quantify any potential particle release in the production and processing activities. A comprehensive hazard assessment will allow the evaluation of effects on human and environmental models with comparisons between simple and modified nanoparticles carried out. Results from the assessment studies will be used to compile a risk assessment of the use of nanoparticles in the ink and pigment industry, and comparisons will be made with surface-modified nanoparticles. An evaluation of the effectiveness of risk management measures will be undertaken in order to select and design practical and cost effective strategies, which will be easy to implement in the real operative conditions. As part of this assessment, we will conduct a life cycle assessment, by evaluating their impacts during the whole process of manufacture, use and disposal of these products. The project results will involve industrial partners, providing an integrated strategy to mitigate the risk of workers dealing with nanoparticles, considering all relevant worker exposure scenarios. Furthermore, NANOMICEX will provide industrial stakeholders and the general public with appropriate knowledge on the risks of nanoparticles and nanoproducts, establishing synergies with the EU nanosafety infrastructure.
19	NanoMile Engineered nanomaterial mechanisms of interactions with living systems and the environment: a universal framework for safe nanotechnology	2013-2017	The NanoMILE project is conceived and led by an international elite of scientists from the EU and US with the aim to establish a fundamental understanding of the mechanisms of nanomaterial interactions with living systems and the environment, and uniquely to do so across the entire life cycle of nanomaterials and in a wide range of target species. Identification of critical properties (physico-chemical descriptors) that confer the ability to induce harm in biological systems is key to allowing these features to be avoided in nanomaterial production (safety by design). Major shortfalls in the risk analysis process for nanomaterials are the fundamental lack of data on exposure levels and the environmental fate and transformation of nanomaterials, key issues that this proposal will address, including through the development of novel modelling approaches. A major deliverable of the project will be a framework for classification of nanomaterials according to their impacts, whether biological or environmental, by linking nanomaterial-biomolecule interactions across scales (sub-cellular to ecosystem) and establishing the specific biochemical mechanisms of interference (toxicity pathway).
20	NANOMMUNE Comprehensive assessment of hazardous effects of engineered nanomaterials on the immune system	2008-2011	Engineered nanomaterials (ENs) present tremendous opportunities for industrial growth and development, and hold great promise for the enrichment of the lives of citizens, in medicine, electronics, and numerous other areas. However, there are considerable gaps in our knowledge concerning the potential hazardous effects of ENs on human health and the environment. Our EU-US partnership is committed to filling these knowledge gaps through a comprehensive assessment of ENs, with particular focus on effects on the immune system.

			The immune system is designed to respond to pathogens and foreign particles, and a core concept underpinning the current project is that the recognition versus non-recognition of ENs by immune-competent cells will determine the distribution as well as the toxicological potential of these materials. Our multidisciplinary consortium will focus on the procurement, synthesis and detailed physico-chemical characterization of representative categories of ENs, and the monitoring of potential hazardous effects using an array of in vitro and in vivo systems, as well as transcriptomic and oxidative lipidomic testing to determine specific nanotoxic profiles (signatures) of these materials. The final and integrative component of our research project is risk assessment of potential adverse effects of ENs on human health, and the dissemination of our findings. Through our comprehensive approach, which combines analytical procedures from many different disciplines and leading experts from several national institutes devoted to occupational and environmental safety, we aim to establish a panel of read-out systems for the prediction of the toxic potential of existing and emerging ENs, thus enabling a continuous and sustainable growth of the nanotechnologies. Overall, the results generated through this international program will contribute to the understanding and mitigation of possible adverse effects of nanomaterials.
21	NanoPolyTox Toxicological impact of nanomaterials derived from processing, weathering and recycling of polymer nanocomposites used in various industrial applications	2010-2013	The project NANOPOLYTOX will evaluate the toxicological impact of nanomaterials included in polymer nanocomposites, highly used in various industrial sectors, during their life cycle. The toxicological profile will be correlated with the changes in the physical and chemical properties of the nanomaterials during the artificial aging/weathering process of the polymeric nanocomposites. Raw nanomaterials and extracted nanomaterials will be characterized at different stages of their life cycle and their toxicity profiles will be obtained via in vitro and in vivo toxicity studies. The results from the in vivo studies will be used for the evaluation of the biological and environmental fate of nanomaterials. All the data generated during the project (physical, chemical and toxicological data) will be considered for the development of the novel LCIA methodology to apply to nanomaterials. These studies will also be taken into account for the selection of adequate digestion and extraction methods to separate the nanomaterials from the polymeric matrices. Moreover, optimization of these methods will facilitate the development of recycling techniques that will be applied in the end-stage of polymer nanocomposites. Disposal of the extracted toxic and/or innocuous nanomaterials will be carried out by mechanical and chemical recycling techniques. The chemical recycling technique will be based on a new separation method consisting of nanofibre filters to separate efficiently the raw nanomaterials from the polymeric matrices and re-use them in new applications. Finally, the nanofiber filters containing toxic nanomaterials will be immobilized in xerogel matrices by sol-gel processes and sintering.
22	NanoPUZZLES Modelling	2013-2015	Nanotechnology is rapidly expanding. However, some types of engineered nanoparticles can be toxic for living organisms and exhibit negative impact on the environment. Thus, the design of new

	properties, interactions, toxicity and environmental behaviour of engineered nanoparticles		nanomaterials must be supported by a rigorous risk analysis. Following the recommendations by the EU REACH system and regarding ethical aspects, the risk assessment procedures should be performed with possible reduction of living animal use. The main objective of the NanoPuzzles project is to create new computational methods for comprehensive modelling the relationships between the structure, properties, molecular interactions and toxicity of engineered nanoparticles. The methods will be based on the Quantitative Structure - Activity Relationship approach, chemical category formation and read-across techniques. Those methods have been widely used in risk assessment of other groups of priority chemicals. But, because of some specific reasons, they cannot be applied directly to nanoparticles. We will be developing novel methods within four complimentary areas ("puzzles"), namely: (i) evaluation of physico-chemical and toxicological data available for nanoparticles (NanoDATA), (ii) developing novel descriptors of nanoparticles' structure (NanoDESC), (iii) investigating interactions of nanoparticles with biological systems (NanoINTER), and (iv) quantitative structure - activity relationships modelling (NanoQSAR). Developed methods will be tested and verified for their technical viability by the collaborating industry representative. By implementing the NanoPuzzles methods, extensive animal testing would be significantly reduced. Moreover, the project will deliver the basis for categorising nanoparticles based on potential exposure, phys-chem, structural and toxicological properties. To maximise its impact, the project is going to cooperate with ModNanoTox, NanoTransKinetics, NanoSafety Cluster and NanoMedicine ETP.
23	NanoReTox The reactivity and toxicity of engineered nanoparticles: risks to the environment and human health	2008-2012	NanoReTox aims to identify the potential risks to the environment and human health posed by free engineered (i.e. manmade) nanomaterial by comprehensively addressing five key questions: - How does the environment into which nanoparticles are released affect their physicochemical properties and their bioreactivity? - How does this impact on their ability to interact with and/or penetrate mammalian and aquatic cells and organisms (bioavailability) and will bioavailability result in toxicity? - Is there a pattern of cellular reactivity and/or toxicity related to physicochemical properties, i.e. a hierarchy of activity? - What combination of conditions discovered in (1-3) above are most likely to pose a risk to human health and the environment? - How can this information be incorporated in a risk assessment model? The team of experts was assembled from across the EU and the US whose combined expertise can address these questions in depth, and therefore comprehensively cover the scope of research topic NMP-2007-1.3-2 Risk assessment of engineered nanoparticles on health and the environment.
24	NanosafePACK Development of a best practices guide for the safe handling and use of nanoparticles in packaging industries	2011-2014	The Nano-SafePACK project is to develop a best practices guide to allow the safe handling and use of nano-materials in packaging industries, considering integrated strategies to control the exposure to nano-particles (NP) in industrial settings, and provide the SMEs with scientific data to minimize and control the NP release and migration from the polymer nano-composites placed on the market. To achieve this aim, a complete hazard and exposure assessment will be conducted to obtain new scientific data about the safety of polymer composites reinforced using nano-meter-sized particles. The proposed

			work will focus on a selected set of nano-meter-sized materials (nano-clays and metal oxide NP) relevant to the packaging sector. Full characterisation will be carried out, followed by an exposure measurement in order to identify and quantify any potential particle release in the production and processing activities. A comprehensive hazard assessment will allow the evaluation of effects on human and environmental models, including the development of a NP migration and release index as a hazard indicator. Results from the exposure and hazard assessment studies will be used to compile a risk assessment of the use of NP in the packaging industry. An evaluation of the effectiveness of risk management measures will be undertaken in order to select and design practical and cost effective strategies, which will be easy to implement in the real operational conditions of industrial settings. In addition, as part of this assessment we will conduct a life cycle assessment of nano-composites, by evaluating their impacts during the processes of manufacture, use and disposal. The key aims of this project are aligned with the needs of the packaging industries in relation to the use of NP as nano-reinforcements the need to improve knowledge and guidance on safety issues for workers and consumers, which must be addressed prior to their widespread use.
25	NanoStair Establishing a process and a platform to support standardization for nanotechnologies implementing the STAIR approach	2012-2014	Standardization is one of the most adequate solutions to quickly capitalize and disseminate knowledge in reference documents, and have it implemented in the industry. It is very important in the field of nanotechnologies since the production of knowledge is very intensive. The overall objective of nanoSTAIR project is to build a sustainable process and platform in the field of nanotechnologies to support the transfer of knowledge gained through research to documentary standards in the context of the STAIR approach promoted by CEN-CENELEC. The project is organized around several activities that will boost the development of new documentary standards. A mechanism will be set up to identify, with a bottom-up approach, the opportunities for standardization from the results of research projects, co-funded by the European Commission or by National Research Programmes. This mechanism will be established using existing networks and initiatives such as NanoSafetyCluster or NANOfutures, as well as the network of the national standardization bodies in the various Member States. Then, the expression of the needs for standards from various stakeholders will be collected and resources from consortia sharing similar standardization opportunities will be pooled together to launch New Work Items Proposals (NWIP). The nanoSTAIR approach will be verified during the project thanks to 2 NWIP initiated. The consortium will provide assistance to select the right standardization umbrella (Technical Committee and Working Group at CEN or ISO level) As a result, nanoSTAIR will provide a set of procedures, a tool box and a practical guideline that will be useful to bridge the gap between research and standardization in nanotechnologies. nanoSTAIR will structure and ease the development of new documentary standards, and thus enable the European nanotechnology related industry to rapidly operate according to the state of the art and thus increase its competitiveness.
26	NanoSustain	2010-2013	Objective of the NanoSustain project is to develop innovative solutions for the sustainable design, use, recycling and final treatment of

	Development of sustainable solutions for nanotechnology-based products based on hazard characterization and LCA		nanotechnology-based products This will be achieved by a comprehensive data gathering and generation of relevant missing data, as well as their evaluation and validation, for specific nano-products or product groups in relation to their human health and environmental hazards and possible impacts that may occur during after-production stages. Although production of nano-materials is rapidly increasing, our knowledge about possible health and environmental effects associated with these materials is still rather poor. This lack of knowledge calls for more research. Due to their small size, nano-particles behave different than their chemical analogues. They can be taken up easily and in a unique way with possible adverse effects in man and organisms. Assessing their hazard is complex and needs new approaches and a close international cooperation. NanoSustain will address the questions, (1) how and to what degree society and the environment will be exposed to nano-materials and associated products, and (2) where do these particles end up? Expected results will improve our present knowledge on the impact and fate of these particles after entering economic and natural cycles. NanoSustain has mobilized the critical mass of expertise, resources and skills to tackle this complex issue. Based on results from hazard characterization, impact assessment and LCA, we will explore on a lab-scale new solutions for the design of selected nano-materials and associated products and their sustainable use, recycling and final treatment. As the concerned nanotech industry will actively participate in the planned project, NanoSustain will set the ground for the development of new sustainable products and industrial applications, and hence help to strengthen competitiveness of the European nanotechnology industry.
27	NanoTransKinetics Modelling basis and kinetics of nanoparticle interaction with membranes, uptake into cells, and sub-cellular and inter-compartmental transport	2011-2014	The prediction of biological (and in particular toxicological) impacts has, as its basic pre-requisite, the correct prediction of the sites of action and localization of the nano-particle in living organisms. We have identified the need for a paradigm shift in modelling these properties for nano-scale objects. The interactions between bare particles and organisms (cells, biological barriers) is radically different in the presence of proteins and lipids derived from the biological environment (the protein corona). The bare particle characteristic is therefore insufficient to describe the system. Similarly, nano-particles are trafficked and translocated between sites by active biological processes where traditional equilibrium principles for small molecules no longer apply. Nano-TransKinetics is firmly based on advanced high quality experimental data on the distribution of nano-particles in cells, across barriers, and (more limited) in vivo. We frame phenomenological models in a modular manner by abstracting the essential relevant principles of particle-protein (and matrix) interactions, cellular and barrier transport mechanisms of nano-particles, fitting them to experimental data. More detailed models allow for explicit checking of mechanisms and movements of individual particles into cells and across barriers. Enormous amounts of experimental data are now available to validate the models. A predictive capacity requires only simple input data on particle, corona and similar characteristics. The basis of these claims has been checked in preliminary studies, and a limited number of interactions, particles fluxes (and control parameters) between prescribed sites are sufficient to specify the system at each level of description. Resources (reaching

			far beyond the program itself) have been mobilised in experimental work in the Partners laboratories, and EU and US collaborations. The output will be predictive tools for use in nano-safety research and regulation and beyond.
	NanoValid Development of reference methods for hazard identification, risk assessment and LCA of engineered nanomaterials	2011-2014	The growing development, production and use of engineered nano-materials and associated products will increase exposure of both humans and ecosystems to these new materials. However, current knowledge is still incomplete and established test methods are as yet inappropriate to reliably assess the extent of exposure and risk of materials at the nano-scale. There is an urgent need to develop methods to overcome the current limitations of existing hazard and risk assessment schemes and to generate the body of reference data needed as the basis for regulative requirements and for measures to safeguard production, application and the disposal of nano-materials. The proposed project aims to mobilize the critical mass of international scientific knowledge and technical expertise required to address these questions. Current analytical and toxicity test methods and models will be put to test and subjected to rigorous intercalibration and validation. Where necessary, methods and test materials will be modified, adapted and validated, and new reliable reference methods developed, in cooperation with international standardisation bodies and the concerned industry, to support both pre and co-normative activities and to make the applicability of existing RA and LCA schemes to ENPs more reliable. The feasibility of validated measurement, characterization and test methods will be assessed by selected case studies to help the significant improvement of the performance of existing exposure monitoring systems as well as the development of new risk management and reduction strategies.
28	NEPHH Nanomaterials-related environmental pollution and health hazards throughout their life-cycle	2009-2012	The purpose of this project is to identify and rate important forms of nanotechnology-related environmental pollution and health hazards that could result from activities involved in nano-structures throughout their life-cycle, and to suggest means that might reduce or eliminate these impacts. Besides the positive multipurpose nano-reinforcement in materials and expanded devices applications, little is known about the environmental and health risks of certain manufactured nanomaterials. Initial research has indicated that nanomaterials can have a negative impact on human health and environmental pollution. For instance, carbon nanotubes may be more toxic than other carbon particles or quartz dust when being absorbed into the lung tissue; however, specific detailed research is required. More importantly, and fundamental to the success of nanotechnology, is the perceived safety of the technology by the public. As activity shifts from research to the development of applications, there exists an urgent need to understanding and managing the associated risks, but in particular to personnel working with these materials. To address these issues, an investigation of biological interactions of nanoscale and nanostructured materials on in vitro toxicological mechanisms is proposed. Further, an assessment of their impact on environmental pollution regarding water, soil and air is also proposed.
29	NeuroNano Do nanoparticles induce	2009-2012	As the use of nanoparticles becomes more prevalent, it is clear that human exposure will inevitably increase. Considering the rapidly ageing European population and the resulting increase in the incidence

	neurodegenerative diseases? Understanding the origin of reactive oxidative species and protein aggregation and mis-folding phenomena in the presence of nanoparticles		of neurodegenerative diseases, there is an urgent need to address the risk presented by nanoparticles towards neurodegenerative diseases. It is believed that nanoparticles can pass through the blood-brain barrier. Once in the brain, nanoparticles have two potential major effects. They can induce oxidative activity (production of Reactive Oxygen Species), and can induce anomalous protein aggregation behaviour (fibrillation). There are multiple disease targets for the nanoparticles, including all of the known fibrillation diseases (e.g. Alzheimer s and Parkinson s diseases). The factors that determine which nanoparticles enter the brain are not known. Nanoparticle size, shape, rigidity and composition are considered important, and under physiological conditions, the nature of the adsorbed biomolecule corona (proteins, lipids etc.) determines the biological responses. The NeuroNano project will investigate the detailed mechanisms of nanoparticle passage through the blood-brain barrier using primary cell co-cultures and animal studies. Using nanoparticles that are shown to reach the brain, we will determine the mechanisms of ROS production and protein fibrillation, using state-of-the-art approaches such as redox proteomics and isolation/characterisation of the critical pre-fibrillar species. Animal models for Alzheimer s diseases will confirm the effects of the nanoparticles in vivo. At all stages the exact nature of the nanoparticle biomolecule corona will be determined.
30	NHECD Nano health-environment commented database	2008-2012	We propose to use our recent advanced research results and build a novel and useful automatic database on the impact of nano-particles on health and environment, which will be hosted and maintained by an expert software company based in Europe. The strength and innovation is double folded: primarily in automatic extraction and understanding from free text, which is in particular suited to create a comprehensive database in the nano-particles area; and secondly creating automated tools for appropriate evolving ontology assisted by leaders in toxicity in Europe. The team has proven mathematical and computerized world level skills in the general area of Information Technology pertained to database, data warehouse and text mining on one hand, and in toxicity of nano-particles in particular on the other hand. The proposed database will be automatically and manually updated with state-of-the-art information, which will be automatically understood and extracted into a relational database and data warehouse that can be accessed by the public and agencies through the internet. These three tiers (information gathering, deep analysis, and presentation) will keep the database updated and easily used for complex queries. The database will serve a variety of communities, from regulators to scientists, companies, new activities and the general public with all aspects of toxicity from nano particles. The database and the internet site will also serve for expert information cooperation and exchange and for dissemination of information in this evolving domain, which has huge potential applications, where toxicity should be considered in advance.
31	QualityNano	2011-2015	Nano-scale objects interact with living organisms in a fundamentally new manner, ensuring that a fruitful marriage of nanotechnology and biology will long outlast short term imperatives. Therefore, investment in an infrastructure to drive scientific knowledge of the highest quality will have both immediate benefits of supporting the safety assessment of legacy nano-materials, as well as pointing towards future (safe)

			applications with the lasting benefits to society. There are immediate priorities, for few doubt that serious damage to confidence in nanotechnology, unless averted, could result in missed opportunities to benefit society for a generation, or more. QNano will materially affect the outcome, at this pivotal moment of nanotechnology implementation. The overall vision of QNano is the creation of a 'neutral' scientific & technical space in which all stakeholder groups can engage, develop, and share scientific best practice in the field. Initially it will harness resources from across Europe and develop efficient, transparent and effective processes. Thereby it will enable provision of services to its Users, and the broader community, all in the context of a best-practice ethos. This will encourage evidence-based dialogue to prosper between all stakeholders. However, QNano will also pro-actively seek to drive, develop and promote the highest quality research and practices via its JRA, NA and TA functions, with a global perspective and mode of implementation. QNano will also look to the future, beyond the current issues, and promote the growth and development of the science of nano-scale interactions with living organisms. By working with new and emerging scientific research communities from medicine, biology, energy, materials and others, it will seek to forge new directions leading to new (safe, responsible, economically viable) technologies for the benefit of European society.
32	REACHnano Easy-to-use tools to support the risk assessment of nanomaterials (EELP+)		The 'REACHnano' project aims to provide the industry and stakeholders with easy-to-use tools to support the risk assessment of nanomaterials along their lifecycle. It thus seeks to support the implementation of the REACH regulation with regard to nanomaterials and ultimately improve the protection of the environment and human health from risk. The project seeks to consolidate the knowledge base on nanomaterials-related risk and risk assessment. It will collect and evaluate the adequacy of the available information on the physicochemical, toxicological and ecotoxicological properties of nanomaterials and related exposure-, use- and risk-management measures. 'REACHnano' plans to develop a complete selection of standard testing models to be used in the risk characterisation process for nanomaterials and a complete description of the current exposure scenarios across the nanomaterials lifecycle. These will cover the existing operating conditions, efficient risk management measures and estimated exposure levels. The complete set of innovative tools supporting the risk assessment process, information exchange and the information search process will be made freely available in the form of a web-based toolkit and disseminated widely to stakeholders, including SMEs and competent authorities. Webinars, workshops and training will support use of the tools and thus implementation of the REACH regulation on nanomaterials.[N]
33	SANOWORK Safe Nano Worker Exposure Scenarios	2012-2015	The main goal of Sanowork project is to identify a safe occupational exposure scenario by exposure assessment in real conditions and at all stages of nano-materials (NM) production, use and disposal. In order to address this and more specifically the issues introduced by

[N] http://www.lifereachnano.eu/

			NMP.2011.1.3-2 call, we intend to: 1. Contain hazard and worker exposure potential by developing exposure mitigation strategy based on Prevention through Design approach. 2. Implement a rigorous exposure assessment in the workplace in order to evaluate the effectiveness of existing and proposed exposure reduction strategies. 3. Perform risk analysis off line and on site in order to identify substance product properties and operational condition that ensure a safer worker exposure scenario. 4. Assess COST/ EFFICIENCY of the proposed strategies on the basis of risk analysis results, materials/properties efficiency, risk transfer to insurance underwriter community. The Sanowork proposed risk remediation strategy will be applied to nano-material properties. The following representative pool of NM and nano-products have been selected: TiO2 and Ag (ceramic or textile photocatalytic/antibacterial surfaces); CNTs (polymeric nano-composites); organic/inorganic nano-fibers (nano-structured membranes for water depuration system). The strategy is addressed to mitigate risk by decreasing adverse health hazard and emission potential of nano-materials, setting back processes of transport to the point of entry. A sound balance between exposure and health hazards data, before and after the introduction of existing and proposed risk remediation strategies, will allow to evaluate the effectiveness of existing and proposed exposure reduction strategies. The cooperation with industrial key partners such as Plasmachem, Elmarco, GEA Niro, Colorobbia, Bayer will guarantee an accurate exposure assessment in the workplace.
34	Scaffold Innovative strategies, methods and tools for occupational risks management of manufactured nanomaterials (MNMs) in the construction industry	2012-2015	Manufactured nanomaterials and nanocomposites are being considered for various uses in the construction industry and related infrastructure industries, not only for enhancing material properties and functions but also in the context of energy conservation. Despite the current relatively high cost of nano-enabled products, their use in construction materials is likely to increase because of highly valuable properties imparted at relatively low additive ratios, rapid development of new applications and decreasing cost of base MNMs as they are produced in larger quantities. Thus the use of nano-products in the construction industry is a reality and can be expected to grow in the near future. Consequently, there is a general uncertainty with respect to health and safety risks and how to properly manage them to protect workers and be in compliance with OHS legislation. SCAFFOLD is an industrial oriented idea specifically addressed to provide practical, robust, easy-to-use and cost effective solutions for the European construction industry, regarding current uncertainties about occupational exposure to MNMs. This will be achieved by introducing a new paradigm to improve workers protection against NMs in construction, based on a novel holistic Risk Management approach (RMM). The aim of the SCAFFOLD project is to develop, test, validate and disseminate a new holistic, consistent and cost effective Risk Management Model (RMM) to manage occupational exposure to MNMs in the construction sector. This will be done by integration of a set of innovative strategies, methods and tools developed by the project into consistent state-of-the-art safety management systems.
35	SIINN Safe	2011-2014	The primary aim of the SIINN ERA-NET is to promote the rapid transfer of the results of nano-science and nanotechnology (N&N) research into industrial application by helping to create reliable conditions. In order

	Implementation of Innovative Nanoscience and Nanotechnology		to strengthen the European Research Area and to coordinate N&N-related R&D work, the project has the aim of bringing together a broad network of ministries, funding agencies, academic and industrial institutions to create a sustainable transnational programme of joint R&D in N&N. The commercial application of nano-materials (NMs) products is increasing rapidly, but one important question, the safety of NMs, still represents a barrier to their wide innovative use. Therefore the first priority of SIINN is to focus on developing a consolidated framework to address nano-related risks and the management of these risks for humans and the environment by investigating the toxicological behaviour of NMs. European R&D activities in N&N remain largely uncoordinated and fragmented, resulting in the sub-optimal use of available resources, such as human resources, research equipment and funding. Since available data on their toxicological behaviour is often scant, unreliable or contradictory, the SIINN Project will focus on ways of remedying this situation. After defining the criteria important for NM toxicology, the environmental health and safety (EHS) information currently available to Europe will be examined. Liaisons will strategically be established and maintained. They will network with organisations looking into the EHS of NMs within Europe and abroad with the aim of continually exchanging information with these. Available information will be examined for their reliability in respect of the assessment of the risks of NMs towards human health and to the environment and major knowledge gaps identified. At least two joint, transnational calls will be organised during the initial lifetime of SIINN in order to fill these gaps.
36	SMART-NANO Sensitive MeAsuRemenT, detection, and identification of engineered NANOparticles in complex matrices	2012-2016	SMART-NANO will develop an innovative, cost-effective technology platform that provides a total solution from sample-to-result for the detection, identification, and measurement of nanoparticles in complex matrices in Consumer Products, in Food, in the Environment and in situ in Biota. A key innovation is the miniaturized, application-specific, cartridge-based system integrating separation, detection, and quantification. On top of this CORE innovation, plug-in modules for sample preparation, high sensitivity size measurement, and hypersensitive identification, provide the necessary sensitivity and flexibility to this technology platform. Highly innovative approaches also lie in the supercritical CO2 isolation of Engineered Nanoparticles (ENPs) from complex matrices and the ICP-MS based hypersensitive identification of ENPs. A practical approach based on the development of the technology platform together with the development and field testing of methods and protocols will result in ready-to-use, cost-effective cartridges for immediate, widespread use in applications for real life detection and measurement of Engineered Nanoparticles. The consortium is led by a non-profit research organization whose mission is to transfer technological innovation to the market (CSEM), has a strong participation of a super-national research centre whose mission is to provide scientific support to EU policy makers (JRC), includes five SMEs (FeyeCon Carbon Dioxide Technologies, Postnova Analytics GmbH, Avid Nano Ltd, AHAVA Dead Sea Laboratories Ltd, ABICH S.r.l.) and a public research organization (Ru er Boakovi Institute) with a track record of excellence and innovation in the different analytical steps necessary to carry out the isolation, separation and measurement of nanoparticles in complex matrices.

			The SMART-NANO consortium has thus the required vision and experience to successfully execute this innovative project.
37	LICARA Life cycle approach and human risk impact assessment, product stewardship and stakeholder risk/benefit communication of nanomaterials	2012-2014	Nanomaterials have a great market potential for SMEs due to the high added values and the reduced batch sizes compared to their corresponding conventional bulk materials. Unless the benefits the introduction of nanomaterials is hampered due to the unknown human and ecological risks. It may take many years to fill all knowledge gaps. However, the SMEs have to address the various different aspects and perceptions of risks, in communication with the various stakeholders. For this reason, SMEs need guidance to assess the risks and the benefits of their nanoproducts in comparison with the conventional (non-nano) products. The main goal of this project is to develop a structured life cycle approach for nanomaterials that (1) enables to balance health/environmental risks of nanomaterials in view of paucity of data against their benefits and (2) that further allows a comparison with the risks and the benefits of the conventional (non-nano) products. This structured approach will be the base for a completely new service available to SMEs working with nanomaterials. As proof of principle and concrete benefit for the SMEs, LICARA will deliver guidelines to the members of the SME Associations to support them in their communication with regulators, clients and investors and to improve the production processes and/or applications of their specific nanoproducts. The consortium consists of 1 European and 3 national SME Associations, 2 SMEs, 3 RTDs and 1 project services company. The consortium is built in a way that the needed different expertise is represented. The partners are active in a range of (inter)national initiatives such as FP7 projects on life cycle assessment and risk assessment, OECD, ISO and CEN.

List of relevant FP7 projects not listed in compendium of the NanoSafety Cluster[o]

No.	Acronym	Dates	Abstract
1	NanoDiode Developing Innovative Outreach and Dialogue on responsible nanotechnologies in EU civil society	2013-2017	Stakeholder engagement and dialogue are essential to the responsible development of nanotechnologies in Europe. The European FP7 project NanoDiode, launched in July 2013 for a period of three years, establishes an innovative, coordinated programme for outreach and dialogue throughout Europe to support the effective governance of nanotechnologies. The project integrates vital engagement activities along the innovation value chain: at the level of research policy, research & development (R&D), and the diffusion of nanotechnology innovations in society. Importantly, it combines 'upstream' public engagement (by way of dialogues that integrate societal needs, ideas and expectations into the policy debate) with 'midstream' engagement (by organising innovation workshops at the level of the R&D practices that are at the heart of the research and innovation enterprise) and 'downstream' strategies for communication, outreach, education and training.
2	NANOPINION Monitoring public opinion on Nanotechnology in Europe	2012-2014	NANOPINION will provide a multi-tasking and enlivening online science-technology-social media-based platform for learning, information, outreach, dialogue and monitoring for young people, general public and consumer opinion on NT, realising the need for enhanced communication and dialogue between science and society for successful technology development and societal acceptance. A central dialogue arena of both physical and virtual aspects will be created to establish a dynamic outreach and dialogue model that will address the public in the high street via street knowledge and opinion labs, and other target groups in a variety of interactions in live events, online project portal, and web 2.0 tools. Controversial issues will be discussed on range of channels in order to establish a trustworthy and informed dialogue with the public. The engagements will be monitored continuously, and citizens opinions of NT will be gathered and traced using validated online and offline tools, thus providing clear direction and challenges driven by the citizens opinion regarding communication, NT fields, regulation, governance, research, social implications and education of NT. Past FP6/7 projects will be extensively used as prime knowledge, information and education resources for the project. NANOPINION will contribute to awareness and interest raising in the realm of NT, by engaging all age groups in the wider public in informing and discussion surrounding NT. We aim for the project to serve as an access bridge between FP7 and FP8 thus provides the EC with insights for policy framing concerning NT. The NANOPINION takes the debate to the outdoor arena dealing with "tough to reach" audience, that usually do not participate in science debates. Also, The project is going to

[o] http://cordis.europa.eu/projects/home_en.html

			offer experimental NT curriculum for high school that will carried out in EU, Associated countries and Russia. This curriculum will be used for a future baccalaureate/ A level/ matriculation program of study NT.
3	FISHTIO2 Titanium dioxide – the silent killer: finding the relevant biological target for exposure characterization and risk assessment of nanoparticles toxicity in fish model	2013-2017	The nano-ecotoxicological research is supported and promoted by European Commission. In 2005, the Action Plan "Nanosciences and nanotechnologies: An Action Plan for Europe 2005–2009" was adopted (European Commission, 2004). The European Commission clearly states the need for the new scientific experiments that will provide quantitative data on toxicology and ecotoxicology and allow for the risk assessments to be carried out on nanomaterials. In year 2006 the Chemicals Committee of the OECD has formed special Working Party on Manufactured Nanomaterials [WPMN]. One of the nanomaterials included in the OECD WPMN priority list is titanium dioxide (TiO2). Titanium dioxide nanoparticles (nano-TiO2) present the biggest ecotoxicological concern due to the rapid increase of anthropogenic input into the environment. Estimated environmental concentrations of nano-TiO2 in water range from 0.7 to 24.5 ng/mL. CURRENT AQUATIC EXOTOXICOLOGY TESTING OF NANO-TIO2 ARE NOT SUFFICIENT FOR THE RISK ASSESSMENT, as the testing is done by exposing the aquatic organisms to water suspension of nano-TiO2. Although the nano-TiO2 can be absorbed by the gills and skin of aquatic animals, the absorbed amount is insignificant compared to the potential of uptake through diet. Based on our previous research (Jovanovic et al., 2011, Jovanovic & Palic, 2012) we have classified nano-TiO_2 as a potent immunotoxin, and there have been no previous studies that have investigated synergistic effects of nano-TiO2 during co-exposure to pathogenic bacteria. Therefore, we propose to use multidisciplinary approach by combining immunology assays, bacterial challenge studies, gross pathology of the brain, kidney and liver, and next generation deep gene sequencing - in order to determine toxicological effects and relevant biological targets upon acute exposure to nano-TiO2 through diet. Such study will provide regulatory agencies with long-time sought relevant ecotoxicological data for performing the risk assessment.
4	MIRNANO Toxicogenomic studies on engineered carbon nanomaterials	2012- 2014	Toxicogenomic studies on engineered carbon nanomaterials Engineered nano-materials (ENM) are becoming an issue of great concern regarding their health effects. Different types of ENM are being used today in everyday consumer products as well as professional equipment such as medical devices. Several ENM, even those used in products that are already on the market, have been shown to be cytotoxic, geno-toxic and immune-toxic in experimental settings, but knowledge is still too scarce and inconsistent for efficient and accurate risk assessment on ENM exposure and the materials are still classified according to the toxicity of their respective bulk material. Carbon nanotubes (CNTs) are among the most utilized ENM and studies have indicated that certain types may have similar health effects as the well-known human carcinogen, asbestos. The toxic effects of CNTs have been investigated at several levels, but the genetic mechanisms behind these effects are still largely unknown. Toxico-genomics investigates the

			multifaceted genomic responses to xenobiotic substances in biological systems on a genome-wide level. Thus, toxico-genomic studies may reveal the genomic changes related to CNT exposure and may give insight into the mechanisms behind their hazardous effects. In this study genetic features such as mRNA and microRNA expression changes as well as histone modification patterns will be profiled on a genome-wide level in a bronchial epithelial cell line following exposure to various carbon nano-materials, including CNTs. Asbestos will be used as a positive control. This will enable the identification of early genomic changes which may elucidate the mechanism of action behind the cellular responses to these ENM and possibly reveal eventual toxic outcomes following exposure. Furthermore, the results are anticipated to lay a foundation for accurate risk assessment of CNTs.
6	NANOADJUST Metallic engineered nanomaterial in natural aquatic environments: data generation, management and integration into environmental exposure modelling		Metallic engineered nanomaterial in natural aquatic environments: data generation, management and integration into environmental exposure modelling The success of novel, new technologies often depends to a large degree on the public's risk-benefit perception. Engineered nanomaterials (ENMs) perceived risk is largely based on uncertainty as to their release and fate in the environment. The nanoADJUST project will develop expertise in the application of techniques and tools used to characterise and analyse the behaviour of metallic ENMs in natural aquatic media and integrate this expertise with environmental exposure modelling and risk management data requirements and processes. Data handling throughout the risk assessment (RA) process will be analysed and a statistical framework for the acquisition and management of nano-relevant data at all stages will be developed. Partitioning experiments in natural aquatic matrices shall address current research questions on ENM behaviour and fate, generating data for use in exposure modelling and RA. Fit-for-purpose analytical methodology shall be developed for quantification of nanoparticle related elemental concentrations in model experiments and aquatic environmental matrices. Within this work the concept of isotope tracer studies will be introduced into the emerging area of environmental based ENM research. Behavioural indicators or descriptors (i.e. partitioning likelihood distributions) shall also be developed for use in metallic ENM experimental analysis, exposure monitoring and risk assessment, and identification of organisms at risk of metallic ENM toxicity. The analytical and modelling expertise gained through this research work will complement RA projects related to other biological and chemical risks (pesticides, pathogens, etc.) at the researchers' European institution. It will also provide support and risk assessment expertise to other nano-related projects undertaken on an institutional and an EU level. The ability to generate, analyse and manage relevant ENM fate and behaviour data will support the high level risk modelling efforts under way within the EU.
8	INSIDEFOOD Integrated sensing	2009-2013	Integrated sensing and imaging devices for designing, monitoring and controlling microstructure of foods The main S&T objective of InsideFood is to provide

	and imaging devices for designing, monitoring and controlling microstructure of foods		technological solutions for sensing the microstructure of foods. The project will develop and combine X-ray nano- and microtomography, nuclear magnetic resonance spectrocopy, magnetic resonance imaging, optical coherence tomography, acoustic emission and time- and space-resolved reflectance spectroscopy. The techniques are correlated to understand the effect of microstructure on water and solute status, texture and optical properties and internal defects of food. In particular the consortium will consider fresh fruit, processed fruit and cereal products. The research is aimed to bring closer to the market on-line sensors for microstructure analysis and to provide tools for process design and optimization. To this end, data analysis algorithms are developed, including image processing, modelling and multivariate statistics. To reach the objectives, InsideFood joins research institutes with companies from the sensor, ICT and food sectors. 4 SMEs and 1 major food company participate in InsideFood. InsideFood is dedicated to dissemination of the project results, through a symposium, publications, IP development, a technology newsletter, a public website and a technology trade fare. The participating technology companies perform valorisation activities to implement the results in their product portfolio.
9	NANOREG A common European approach to the regulatory testing of nanomaterials	2013-2016	A common European approach to the regulatory testing of nanomaterials The innovative and economic potential of Manufactured Nano Materials (MNMs) is threatened by a limited understanding of the related EHS issues. While toxicity data is continuously becoming available, the relevance to regulators is often unclear or unproven. The shrinking time to market of new MNM drives the need for urgent action by regulators. NANoREG is the first FP7 project to deliver the answers needed by regulators and legislators on EHS by linking them to a scientific evaluation of data and test methods. Based on questions and requirements supplied by regulators and legislators, NANoREG will: (i) provide answers and solutions from existing data, complemented with new knowledge, (ii) Provide a tool box of relevant instruments for risk assessment, characterisation, toxicity testing and exposure measurements of MNMs, (iii) develop, for the long term, new testing strategies adapted to innovation requirements, (iv) Establish a close collaboration among authorities, industry and science leading to efficient and practically applicable risk management approaches for MNMs and products containing MNMs. The interdisciplinary approach involving the three main stakeholders (Regulation, Industry and Science) will significantly contribute to reducing the risks from MNMs in industrial and consumer products. NANoREG starts by analysing existing knowledge (from WPMN-, FP- and other projects). This is combined with a synthesis of

			the needs of the authorities and new knowledge covering the identified gaps, used to fill the validated NANoREG tool box and data base, conform with ECHA's IUCLID DB structure. To answer regulatory questions and needs NANoREG will set up the liaisons with the regulation and legislation authorities in the NANoREG partner countries, establish and intensify the liaisons with selected industries and new enterprises, and develop liaisons to global standardisation and regulation institutions in countries like USA, Canada, Australia, Japan, and Russia.
10	OBSERVATORYNANO European observatory for science-based and economic expert analysis of nanotechnologies, cognisant of barriers and risks, to engage with relevant stakeholders regarding benefits and opportunities	2008-2012?	ObservatoryNANO brings together leading EU organizations who collectively have expertise in the technological; economic; societal/ethical; health, safety, and environmental analysis of nanotechnologies. Its primary aim is to develop appropriate methodologies to link scientific and technological development of nanotechnologies with socio-economic impacts. Both of these aspects will be enhanced by expert opinion, making this project unique in providing relevant web-based reports in a common format across all sectors, considered by all criteria, and widely publicized. observatoryNANO will become an industry leading and opinion forming catalyst for nanotechnology in the EU. The purpose is to avoid the exaggerated socio-economic impact of nanotechnologies and place developments in a realistic time-frame. It will present a reliable, complete, and responsible science-based and economic expert analysis of peer-reviewed literature, patents, national funding strategies, investment trends, and markets; in combination with information derived from questionnaires, interviews and workshops with academic and industry leaders, investors, and other key stakeholders.
11	NANOINDENT Creating and disseminating novel nanomechanical characterisation techniques and standards	2008-2011	Creating and disseminating novel nanomechanical characterisation techniques and standards Our project aims to gather, improve, catalogue and present characterisation techniques, methods and equipment for nanomechanical testing. European-wide activities coordinated by a new virtual centre will improve existing nano-indentation metrology to reveal structure-properties relationship at the nano-scale. These methods are the only tools to characterise nanocomposite, nanolayer and interface mechanical behaviours in the nanometre range. This work will also lay down a solid base for subsequent efforts for defining and preparing new standards to support measurement technology in the field of nanomaterials characterisation. Steps include development of the classical and the dynamic nano-indentation method and its application to new fields, application of modified nano-indenters to new fields as scratching and wear measurement, firm and uniform determination of instrumental parameters and defining new standard samples for the new applications. The virtual centre will disseminate information based on a new Nanocharacterisation database built on two definite levels: on a broader level partners will inventory and process all novel

			nanocharacterisation techniques and, in narrower terms, they will concentrate on nanomechanical characterisation. This will be achieved through the synchronisation of efforts set around a core of round robins but the database will include data of other channels as parallel research work and literature recherché. Core activities comprise detailed dissemination activities. Indirect connections to the stakeholders by a webpage with a build-in interactive database will be complemented by direct events such as participation in workshops (oral and poster presentations), and regular technical reports in international journals. Activities above will lead to detailed descriptions of novel characterisation techniques.
12	NADETOX NAnomics in vitro DEvelopmental TOXcology	2012-2014	NAnomics in vitro DEvelopmental TOXcology The progress made in improving the development and the production of nanoparticles (NPs) is enormous. Metallic nanoparticles (mNPs) are among the most widely used types of NPs in electronics, foods, containers, pharmaceutical drugs, cosmetics and paints. This trend will lead to an ever-increasing presence of NPs in the environment. This scenario means that humans and the environment will be exposed to more and more nanotechnology-based products whose health risks and environmental impacts of NPs might outweigh their benefits. In order to promote prevention and safety in manufacturing and handling of NPs, NADETOX aims at evaluating the nanodevelopmental toxicity of selected metallic cobalt, silver and gold NPs extensively used in nano-medicine for cancer therapy. This novel and multidisciplinary research project is based on the 3Rs in vitro approach (Reduction, Refinement, Replacement), involving the development of a mechanistically-based alternative method, Frog Embryo Teratogenesis Assay-Xenopus (FETAX), and the combined use of peculiar advanced spectro-chemical, radio-analytical, biochemical and molecular biology techniques. These methods offer the opportunity to label NPs, avoiding surface modification, to localise and quantify them in organisms. NADETOX aims at the following goals: (i) Characterisation of NPs establishing their size and morphology. (ii) Study of stability and of the eventual release of metal ions from radiolabelled NPs in the reconstituted water medium suitable for the culture of Xenopus embryos (FETAX medium). (iii) Evaluation of embryolethality and teratogenicity of NPs by FETAX assay. (iv) Biokinetics studies to measure uptake, metabolic fate and bio-persistence of NPs in Xenopus at embryo and larva stages and structure diagnosis at DNA level, to get information on the genomic stability following formation of DNA-adducts.
13	SUN SUSTAINABLE NANOTECHNOLOGIES	2013- 2017	SUN (Sustainable Nanotechnologies) is the first project addressing the entire lifecycle of nanotechnologies to ensure holistic nanosafety evaluation and incorporate the results into tools and guidelines for sustainable manufacturing, easily accessible by industries, regulators and other stakeholders. The project will incorporate scientific findings from over 30 European projects, national and international research programmes and transatlantic co-operations to develop (i) methods and tools to predict nanomaterials exposure and

			effects on humans and ecosystems, (ii) implementable processes to reduce hazard and exposure to nanomaterials in different lifecycle stages, (iii) innovative technological solutions for risk management in industrial settings, and (iv) guidance on best practices for securing both nano-manufacturing processes and nanomaterials ultimate fate, including development of approaches for safe disposal and recycling. In summary, SUN stands for an integrated approach for the long-term sustainability of nanotechnologies through the development of safe processes for production, use and end-of-life processing of nanomaterials and products, as well as methods reducing both adverse effects and exposure to acceptable levels.
14	NanoDefine Development of an integrated approach based on validated and standardized methods to support the implementation of the EC recommendation for a definition of nanomaterial	2013-2017	Nanotechnology is a key enabling technology. Still existing uncertainties concerning EHS need to be addressed to explore the full potential of this new technology. One challenge consists in the development of methods that reliably identify, characterize and quantify nanomaterials (NM) both as substance and in various products and matrices. The European Commission has recently recommended a definition of NM as reference to determine whether an unknown material can be considered as 'nanomaterial' (2011/696/EU). The proposed NanoDefine project will explicitly address this question. A consortium of European top RTD performers, metrology institutes and nanomaterials and instrument manufacturers has been established to mobilize the critical mass of expertise required to support the implementation of the definition. Based on a comprehensive evaluation of existing methodologies and a rigorous intra-lab and inter-lab comparison, validated measurement methods and instruments will be developed that are robust, readily implementable, cost-effective and capable to reliably measure the size of particles in the range of 1–100 nm, with different shapes, coatings and for the widest possible range of materials, in various complex media and products. Case studies will assess their applicability for various sectors, including food/feed, cosmetics etc. One major outcome of the project will be the establishment of an integrated tiered approach including validated rapid screening methods (tier 1) and validated in depth methods (tier 2), with a user manual to guide end-users, such as manufacturers, regulatory bodies and contract laboratories, to implement the developed methodology. NanoDefine will be strongly linked to main standardization bodies, such as CEN, ISO and OECD, by actively participating in TCs and WGs, and by proposing specific ISO/CEN work items, to integrate the developed and validated methodology into the current standardization work.

List of relevant projects from *European Health Research Program* (EHRM)[P], *European Metrology Research Program* (EMRP)[Q] and *European Environment Life Program* (EELP+)[R].

15	NANEX Development of Exposure Scenarios for Manufactured Nanomaterials	2009-2010	Nanotechnology is a fast growing industry producing a wide variety of manufactured nanomaterials (MNMs) and numerous potential applications. Consequently, the potential for exposure to humans and the environment is likely to increase. Human exposure to MNMs and environmental release of these materials can occur during all the life cycle stages of these materials. For each stage of the life cycle of an MNM, exposure scenarios will need to be developed that effectively describe how exposure to humans and the environment occur and what measures are required to control the exposure. The aim of the NANEX project is to develop a catalogue of generic and specific (occupational, consumer and environmental release) exposure scenarios for MNMs taking account of the entire lifecycle of these materials. NANEX will collect and review available exposure information, focussing on three very relevant MNMs: - high aspect ratio nanomaterials - HARNs) (e.g. carbon nanotubes) - mass-produced nanomaterials (e.g. ZnO, TiO2, carbon black) - specialised nanomaterials that are currently only produced on a small scale (e.g. Ag)). The exposure information will include both quantitative (measurement results) and qualitative contextual exposure information (risk management measures). We will also review the applicability of existing models for occupational and consumer exposure assessment and for environmental release from these scenarios. We will carry out a small number of specific case illustrations and carry out a gap analyses of the available knowledge and data. Finally, we project knowledge will be disseminated to relevant stakeholders, taking into account other relevant activities that are taking place in this field.
16	MOD-ENP-TOX Modelling Assays Platform "MAP" for hazard ranking of engineered nanoparticles (ENPs)	2013-2015	MOD-ENP-TOX project is a multidisciplinary project aiming to accomplish the following objectives: (i) to develop a novel and rational Modelling Assay Platform (MAP) which can be used as a « Risk Indicator » tool to predict the toxicity of metal-based NPs (MeNPs), and (ii) to demonstrate the feasibility of a MAP prototype on a shortlist of MeNPs - which can be further developed to screen the toxicity of a large number ENPs. Based on the concept of Integrating Testing Strategies (ITS), the proposed generic MAP combines two main and complimentary paradigms: (1) a novel Computational Modelling Package (CMP) based on structural, mechanistic, as well as kinetic modelling tools and (2) an innovative high content screening (HCS) strategy that allows performing multiplexed streamlined assays for calibration, refinement and validation of the computed models. First a series of classification algorithms will be applied

[P] http://ec.europa.eu/eahc/index.html

[Q] http://www.emrponline.eu/

[R] http://ec.europa.eu/environment/life/

			to identify MeNPs with similar toxicity patterns, then computational modelling tools will be developed to establish a more rational relationship between MeNPs descriptors and their toxicity in a dynamic and quantitative way. An in-vitro/in-vivo HCS paradigm will be developed as a scalable assessment tool to calibrate and validate the predictive power of the CMP using subsets of the training set or independent set of MeNPs (validation set) respectively.
17	PRENANOTOX Predictive toxicology of engineered nanoparticles	2013-2015	The production of many consumer products based on manufactured NanoParticles (NPs) has led to a growing public and regulatory concern about the safety of nanomaterials. Since experimental toxicological testing of NPs, especially in vivo animal studies is costly and time-consuming, it is necessary to develop a novel research field and associated methods and tools to reach the goal of predictive nanotoxicology. The PreNanoTox consortium addresses three currently missing critical elements needed to develop a platform for predictive nanotoxicology and our suggested approach of providing them: (1) There is a current lack of unified large database We suggest to form this database by applying cutting edge information extraction tools on large repository of scientific articles; (2) There is a need for better understanding the underlying mechanisms of the primary interaction of NP with the cell membrane We suggest to apply appropriate theory and simulation assuming that the surface chemistry of a NP (affecting NPs surface reactivity, hydrophobicity, or surface electrostatics) as well as its other physical properties (e.g. size and shape) determine the strength of the non-specific adsorption of NPs to a cell surface, leading beyond a certain adhesion-strength threshold, to efficient uptake of the NPs; (3) There is a need to extend the traditional QSAR paradigm to the field of nanotoxicology This will be carried out by linking appropriate NP descriptors, with emphasis on those which determine the strength of adsorption of NPs to cells, with biological responses. The PreNanoTox consortium is made up of four research groups (from three scientific organizations), which lead in information technology, soft matter modelling, computational chemistry and in-vitro toxicology, yielding a synergetic output. This project will assist in safe designing of new engineered NPs as well as reducing the extent needed for empirical testing of toxicity.
18	E-NANOMAPPER A Database and Ontology Framework for Nanomaterials Design and Safety Assessment	2014-2017	eNanoMapper will develop a data management and analysis infrastructure together with ontologies supporting the safety assessment activities of the European Nanomaterials research and development community. The project will address the requirements of safety assessment of nanomaterials by providing databases, analysis tools and ontologies for risk assessment and linking them with existing resources in this area. The project plan involves close cooperation with NanoSafety Cluster members and other international organisations such as OECD, ISO/CEN, EC JRC, and ECHA. Their requirements will guide the development of tools for experimental design, model building, and meta analysis across multiple datasets. An ontology for nanomaterials will be developed to provide the following features: annotation of nanostructures and relevant biological properties, annotation of experimental model systems (e.g. cell lines), conditions, and

			protocols, complex search and reasoning capabilities, and the integration of data from existing nanotoxicology sources. Systematic physicochemical, geometrical, structural, and biological studies of nanomaterials are nearly absent in the public domain. The establishment of a universal standardisation schema and infrastructure for nanomaterials safety assessment is a key project goal. It should catalyze collaboration, integrated analysis, and discoveries from data organised within a knowledge-based framework. It will support the discovery of nanomaterial properties responsible for toxicity, the identification of toxicity pathways and nano-bio interactions from linked datasets, ontologies, omics data and external data sources. By interfacing with statistical and data mining tools we will be able to provide scientifically sound guidelines for experimental design as well as computational models for predicting nanotoxicity. These computational models will help to design safe nanomaterials and improve the risk assessment of existing nanoparticles.
19	FUTURENANONEEDS Framework to respond to regulatory needs of future nanomaterials and markets	2014-2017	Rapidly developing markets such as green construction, energy harvesting and storage, advanced materials for aerospace, electronics, medical implants and environmental remediation are potential key application targets for nanomaterials. There, nanotechnology has the potential to make qualitative improvements or indeed even to enable the technology. Impacts range from increased efficiency of energy harvesting or storage batteries, to radical improvements in mechanical properties for construction materials. In addition, concerns of these markets such as scarcity of materials, cost, security of supply, and negative environmental impact of older products could also be addressed by new nano-enabled materials (e.g. lighter aircraft use less fuel). FutureNanoNeeds will develop a novel framework to enable naming, classification, hazard and environmental impact assessment of the next generation nanomaterials prior to their widespread industrial use. It will uniquely achieve this by integrating concepts and approaches from several well established contiguous domains, such as phylontology and crystallography to develop a robust, versatile and adaptable naming approach, coupled with a full assessment of all known biological protective responses as the basis for a decision tree for screening potential impacts of nanomaterials at all stages of their lifecycle. Together, these tools will form the basis of a “value chain” regulatory process which allows each nanomaterial to be assessed for different applications on the basis of available data and the specific exposure and life cycle concerns for that application. Exemplar materials from emerging nano-industry sectors, such as energy, construction and agriculture will be evaluated via this process as demonstrators. The FutureNanoNeeds consortium is uniquely placed to achieve this, on the basis of expertise, positioning, open mindedness and a belief that new approaches are required.
20	NANOSOLUTIONS Biological Foundation for the Safety Classification of Engineered	2013-2017	The main objective of this research proposal is to identify and elaborate those characteristics of ENM that determine their biological hazard potential. This potential includes the ability of ENM to induce damage at the cellular, tissue, or organism levels by interacting with cellular structures leading to impairment of key cellular functions. These adverse effects may

	Nanomaterials (ENM): Systems Biology Approaches to Understand Interactions of ENM with Living Organisms and the Environment		be mediated by ENM-induced alterations in gene expression and translation, but may involve also epigenetic transformation of genetic functions. We believe that it will be possible to create a set of biomarkers of ENM toxicity that are relevant in assessing and predicting the safety and toxicity of ENM across species. The ENM-organism interaction is complex and depends, not simply on the composition of ENM core, but particularly on its physico-chemical properties. In fact, important physico-chemical properties are largely governed by their surface properties. All of these factors determine the binding of different biomolecules on the surface of the ENM, the formation of a corona around the ENM core. Thus, any positive or negative biological effect of ENM in organisms may be dynamically modulated by the bio-molecule corona associated with or substituted into the ENM surface rather than the ENM on its own. The bio-molecule corona of seemingly identical ENM cores may undergo dynamic changes during their passage through different biological compartments; in other words, their biological effects are governed by this complex surface chemistry. We propose that understanding the fundamental characteristics of ENM underpinning their biological effects will provide a sound foundation with which to classify ENM according to their safety. Therefore, the overarching objective of this research is to provide a means to develop a safety classification of ENM based on an understanding of their interactions with living organisms at the molecular, cellular, and organism levels based on their material characteristics.
21	GUIDENANO Assessment and mitigation of nano-enabled product risks on human and environmental health: Development of new strategies and creation of a digital guidance tool for nanotech industries	2013-2017	The main objective of GUIDEnano is to develop innovative methodologies to evaluate and manage human and environmental health risks of nano-enabled products, considering the whole product life cycle. A strategy to identify hot spots for release of nanomaterials (NMs) will be followed by decision trees to guide on the use of (computational) exposure models and, when necessary, design of cost-effective strategies for experimental exposure assessment. These will include on-site and off-site monitoring of industrial processes, use, accelerated aging, recycling and disposal set-ups. In all cases, there will be a strong emphasis on the transformation of NMs. Similarly, a tiered strategy to evaluate the environmental fate and the hazards for ecosystem and human health of NMs will be developed. The project will consider pristine synthesized NMs, transformed NMs released during the life cycle of the product, and interactions of the NMs with other substances in their host matrices and ubiquitous pollutants. The project will also develop innovative solutions to reduce identified risks. These will include safer-by-design approaches (to reduce NM hazard, reduce migration and release, or accelerate degradation when released), new technological solutions for exposure control measures, and solutions for waste minimization and treatment. These developments will be incorporated into an web-based Guidance Tool, which will guide the nano-enabled product developers (industry) into the design and application of the most appropriate risk assessment & mitigation strategy for a specific product. The correct implementation of this guidance will ensure that the risks associated to a nano-enabled product, throughout its whole life

			cycle, have been appropriately evaluated and mitigated to an acceptable level. This methodology will set up the basis for the certification (by an independent third party), as a risk communication tool addressed to regulators, insurance companies, and the society.
22	NANOMEGA Novel approach to toxicity testing of nanoparticles mimicing lung exposure. Possible protective effect of omega-3 acids	2010-2013	As nanotechnology and materials science have progressed, large quantities of engineered nanoparticles (NPs) have been produced. NPs promise to revolutionize our lifestyles by improving many industrial and consumer products. However, there is considerable concern about their unknown impact on human health: with their unique physicochemical properties (size less than 100 nm), NPs differ from the corresponding bulk material. Here we address the urgent need to determine the potential effects of NPs on human health and environmental safety. Our objectives are: a) To develop and optimize a novel approach to in vitro NP testing using an epithelial cell culture model that mimics in vivo interactions of particles with cells; b) To study mechanisms of NP toxicity using cardiovascular/cardiopulmonary cell models to identify specific markers of oxidative stress and their role in activating signal pathways associated with the inflammatory response, DNA damage and repair; c) To investigate protection by omega-3 fatty acids against inflammatory effects of NPs, and possible modulation of DNA repair, in an in vitro model. This research will provide information on mechanisms of action of metal oxide NPs, and specifically on their effect on risk of cardiovascular/cardiopulmonary diseases. The results will contribute to protecting European public health, and will be crucially important for formulating policy on safety of nanotechnology. The ambitious research tasks provide an excellent opportunity for the career development of Dr Rinna in this new field. By developing innovative techniques mimicking in vivo conditions, carrying out experiments on potential NP toxicity, and investigating how cells and DNA can be protected against injury, she will acquire an impressive range of expertise. By supervising master and co-supervising PhD students, she will improve her management and teaching skills and thus establish a base for a longer term position as a research team leader in Norway
23	NanoGENOTOX Facilitating the safety evaluation of manufactured nanomaterials by characterising their potential genotoxic hazard. (EHRM)	2009-2012	Human exposure to manufactured nanomaterials (MNs) used in consumer products may occur during several phases of their life cycle. The lack of scientific knowledge makes regulation difficult. The aim of the Joint Action is to establish a robust methodology to assess the potential genotoxicity of MNs and to generate data on the genotoxic effect of certain reference materials (www.nanogenotox.eu). The JA specific objectives are: • To obtain detailed physicochemical properties for each selected MN • To determine the influence of exposure media on MNs dispensability and to identify the optimum preparation protocols for the specific MNs • To generate in vitro genotoxicity data on MNs • To perform a round robin test on in vitro genotoxicity testing of MNs

			• To determine relevant doses and sampling time for biodistribution and in vivo genotoxicity studies, and to identify MN accumulation in organs for in vivo genotoxicity tests • To generate data from in vivo genotoxicity selected tests, and to assess the correlation between in vivo and in vitro results taking into account the kinetic results. Nanomaterials from the JRC repository were investigated Final deliverables available on-line on the website: http://www.nanogenotox.eu
24	MechProNO Traceable measurement of mechanical properties of nano-objects (EMPR)	2012-2015	Nano-objects such as carbon nanotubes can be used to improve products such as high strength concrete. However, to do this we need to be able to measure the mechanical properties of the nano-objects in order to fully exploit their novel features. The small size of nano-objects makes conventional measurement difficult, however atomic force microscopy can be used as it can visualise objects with very high resolution and measure physical properties. This project will develop measurement traceability for the mechanical properties of nano-objects such as nanoparticles, nanowires, nanoscale structures and composite materials through the development of test samples and techniques, as well as improved instruments. The improved reliability of measurements, and the ability to quantify properties such as the range of size of nano-objects with smaller uncertainty, will help improve products. Furthermore, the ability to identify new physical properties in a much more precise and systematic way should result in faster product development and quicker routes to market.
25	NanoChOp Chemical and optical characterisation of nanomaterials in biological systems (EMPR)	2012-2015	This project, which commenced in June 2012, will develop and validate methods to characterise nanomaterials in complex biological matrices. Through collaboration with nanomaterial developers, world leading academic research groups and analytical platform producers this research project will progress current state of the art to: • To develop a panel of fluorescent and non-fluorescent nanomaterials characterised in the native form for their physical and chemical and optical properties. • To develop and validate protocols for the dispersion of nanomaterials in a range of biological matrices • To validate the use of high accuracy methods for the physical characterisation of nanomaterials in a serum based biological matrix • To establish the feasibility of using emergent image based analysis systems for the physical characterisation of nanomaterials • To develop traceable measurements of fluorescent nanomaterial quantum yield in a serum based biological matrix • To develop and validate methods for the simultaneous physical and chemical characterisation of nanomaterials in a complex cell based biological matrix. • Establish the measurement uncertainty associated with

			nanomaterial fluorescence in nanobiotechnology applications
26	i-NANOTOOL LIFE+ Development of an interactive tool for the implementation of environmental legislation in Nanoparticle manufacturers (EELP+)	2013-2015	The objective of the i-NANOTOOL LIFE+ project is to contribute to the efficient implementation of environmental policy and legislation by companies involved in the production of nanomaterials, especially SMEs. It aims to help these companies access the most up-to-date information on the potential environmental impact of their activity and the current legislative requirements. The project will establish a complete and thorough compilation of current environmental regulations related to nanomaterials at European and national levels, in each country participating in the project - Spain, Portugal, Romania and Finland. It will also develop methodologies and tests to assess the environmental impact of nanomaterial and their production processes. To ensure nanomaterial producers can access the information they need, the project intends to develop an interactive platform. This will provide the latest information on the environmental impact of nanomaterials, related legislation and appropriate environmental management. It will also provide an environmental self-diagnosis e-tool for nanotechnology companies. The project hopes to extend the e-Tool throughout the EU. Ultimately, the project aims to enable companies manufacturing nanomaterials to successfully implement the most appropriate management techniques and meet the requirements of European and national environmental legislation. As well as reducing the environmental risks associated with nanotechnology, the project also seeks to contribute to the updating of environmental policy and legislation around nanotechnology. Expected results: The project expects to achieve the following results: • A complete compilation of current European environmental regulations related to nanomaterials; • A complete compilation of current national environmental regulations in each country participating in the project - Spain, Portugal, Romania and Finland; • Tests and methodologies to assess the environmental impact of nanomaterials and their production processes; • An innovative e-tool for assessing the environmental status of nanomaterial manufacturers and identifying appropriate management measures; • Awareness of the project and its outputs by 50% of nanomaterial manufacturers in Europe; • Use of the e-tool by 6% of the nanomaterial manufacturers in Europe and 50% of those in the participating countries; and • A reduction in the environmental impact of ENM manufacturing.
27	LIFE nanoRISK Best practices effectiveness, prevention and	2013-2016	The LIFE nanoRISK project aims to minimise environmental, health and safety (EHS) risks from exposure to engineered nanomaterials (ENMs). It hopes to do this by improving understanding of the risks associated with the release of ENMs to the environment by the polymer nanocomposite industry

	protection measures for control of risk posed by engineered nanomaterials (EELP+)		and identifying the most appropriate prevention and protection measures. The project will collate new information on the release rates of ENMs to air, water, wastewater and oil during their production, use and disposal. This will contribute to a complete description of the exposure scenarios throughout the nanocomposites' life cycle. It will also study the airborne behaviour of the target ENMs, notably their aggregation/agglomeration patterns and deposition factors. To identify the most appropriate Risk Management Measures (RMM) for controlling exposure to ENMs, the project will test potential RMMs at pilot scale. It will develop a compendium of testing protocols - based on international standards – and develop a nano-aerosols test chamber. RMMs tested in the chamber will include personal protective equipment (PPE), engineering techniques and organisational measures. The results will provide valuable data for determining whether a particular RMM is suitable, effective and feasible for a specific exposure scenario. The project's findings will help to strengthen the Library of RMM developed within the REACH Implementation Projects and improve the quality of Chemical Safety Assessments for nanomaterials. LIFE nanoRISK thus hopes to enable better implementation of the European REACH Regulation with regard to nanomaterials, and to reduce human and environmental risks from overexposure to nanoparticles. Expected results: • A complete description of ENM exposure scenarios across the nanocomposites' life cycle; • New information on the airborne behaviour of ENMs; • A compendium of at least ten testing protocols based on international standards to evaluate the effectiveness of RMMs in the workplace; • A prototype nano-aerosols test chamber to assess RMM performance at pilot scale; • A library of proven and technically feasible RMMs for mitigation and control of risks posed by ENMs; • Improved Chemical Safety Assessments of nanomaterials, facilitating better implement of the REACH Regulation; • A complete assessment report of ISO standards for PPE testing.

It is useful to mention here also the OECD Database on Research into Safety of Manufactured Nanomaterials which is a global resource that collects research projects that address environmental, human health and safety issues of manufactured nanomaterials. This database helps identify research gaps and assists researchers in future collaborative efforts. The database also assists the projects of the OECD's Working Party on Manufactured Nanomaterials (WPMN) as a resource of research information.

Annex 8.2: List of relevant scientific reviews/original articles

Title	Ref	Original abstract
A colloidal silica reference material for nanoparticles sizing by means of dynamic light scattering and centrifugal sedimentation.	O. Couteau, J. Charoud-Got, H. Rauscher, F. Franchini, F. Rossi, V. Kestens, K. Franks, G. Roebben, Particle and particle systems characterization, Vol. 27, p. 112-124 (2010)	IRMM-304 is a new nanoparticle reference material (RM) consisting of silica nanoparticles suspended in an aqueous solution, of which the particle size was characterized by dynamic light scattering (DLS) and centrifugal liquid sedimentation (CLS). The homogeneity and stability of IRMM-304 were confirmed and three method-specific mean particle sizes around a nominal particle size of 40 nm were assigned to the material. The characterization tests have revealed a systematic deviation between the measurement results obtained with DLS and CLS. The availability of IRMM-304 makes it possible to study this difference between methods. Several possible causes for differences between the DLS and CLS results are suggested and preliminarily investigated, such as the interaction between particle and suspending medium, the particle shape and the effect of polydispersity on the size averaging procedure. These investigations are one illustration of the potential role of IRMM-304 and other nanoparticle RMs in the development, comparison, improved understanding, and quality assurance of nanoparticle sizing methods.
A comparative study of submicron particle sizing platforms: Accuracy, precision and resolution analysis of polydisperse particle size distributions.	Will Anderson, Darby Kozak, Victoria A. Coleman, Åsa K. Jämting, Matt Trau, Journal of Colloid and Interface Science 405 (2013) 322–330	The particle size distribution (PSD) of a polydisperse or multimodal system can often be difficult to obtain due to the inherent limitations in established measurement techniques. For this reason, the resolution, accuracy and precision of three new and one established, commercially available and fundamentally different particle size analysis platforms were compared by measuring both individual and a mixed sample of monodisperse, sub-micron (220, 330, and 410 nm – nominal modal size) polystyrene particles. The platforms compared were the qNano Tunable Resistive Pulse Sensor, Nanosight LM10 Particle Tracking Analysis System, the CPS Instruments's UHR24000 Disc Centrifuge, and the routinely used Malvern Zetasizer Nano ZS Dynamic Light Scattering system. All measurements were subjected to a peak detection algorithm so that the detected particle populations could be compared to 'reference' Transmission Electron Microscope measurements of the individual particle samples. Only the Tunable Resistive Pulse Sensor and Disc Centrifuge platforms provided the resolution required to resolve all three particle populations present in the mixed 'multimodal' particle sample. In contrast, the light scattering based Particle Tracking Analysis and Dynamic Light Scattering platforms were only able to detect a single population of particles corresponding to either the largest (410 nm) or smallest (220 nm) particles in the multimodal sample, respectively. When the particle sets were measured separately (monomodal) each platform was able to resolve and accurately obtain a mean particle size within 10% of the Transmission Electron Microscope reference values. However, the broadness of the PSD measured in the monomodal samples deviated greatly, with coefficients of variation being _2–6-fold larger than the TEM measurements across all four platforms. The large variation in the PSDs obtained from these

		four, fundamentally different platforms, indicates that great care must still be taken in the analysis of samples known to have complex PSDs. All of the platforms were found to have high precision, i.e. they gave rise to less than 5% variance in PSD shape descriptors over the replicate measurements.
A comparison of atomic force microscopy (AFM) and dynamic light scattering (DLS) methods to characterize nanoparticle size distributions	Christopher M. Hoo, Natasha Starostin, Paul West, Martha L. Mecartney Journal of Nanoparticle Research 2008, Volume 10, Issue 1 Supplement, pp 89-96	This paper compares the accuracy of conventional dynamic light scattering (DLS) and atomic force microscopy (AFM) for characterizing size distributions of polystyrene nanoparticles in the size range of 20–100 nm. Average DLS values for monosize dispersed particles are slightly higher than the nominal values whereas AFM values were slightly lower than nominal values. Bimodal distributions were easily identified with AFM, but DLS results were skewed toward larger particles. AFM characterization of nanoparticles using automated analysis software provides an accurate and rapid analysis for nanoparticle characterization and has advantages over DLS for non-monodispersed solutions.
A complementary definition of nanomaterial	Wolfgang G. Kreyling, Manuela Semmler-Behnke, Qasim Chaudhry. Nanotoday, V 5(3), 2010, 165-168	In the wake of rapid developments in nanotechnologies and nanosciences, the need for an internationally agreed definition of a 'nanomaterial' has gained more urgency. A number of definitions are currently available. These are, however, mainly based on size parameter(s), and fall short in terms of applicability to particulate materials that only have a size fraction in the nano-scale, or that contain primary nanostructures in highly agglomerated or aggregated forms. To overcome these shortcomings, we are proposing a complementary definition based on volume specific surface area (VSSA) that can be used as a basis for classification and regulation purposes.
A critical review of the biological mechanisms underlying the in vivo and in vitro toxicity of carbon nanotubes; the contribution of physicochemical characteristics	Johnston, H.J., Hutchison, G.R., Christensen, F.M., Peters, S., Hankin, S., Aschberger, K., Stone, V. 2009 Nanotoxicology, vol. 4, no. 2, pp. 207-246. doi:10.3109/17435390903569639	This critical review of the available human health safety data, relating to carbon nanotubes (CNTs), was conducted in order to assess the risks associated with CNT exposure. Determining the toxicity related to CNT exploitation is of great relevance and importance due to the increased potential for human exposure to CNTs within occupational, environmental and consumer settings. When this information is combined with knowledge on the likely exposure levels of humans to CNTs, it will enable risk assessments to be conducted to assess the risks posed to human health. CNTs are a diverse group of materials and vary with regards to their wall number (single and multi-walled CNTs are evident), length, composition, and surface chemistry. The attributes of CNTs that were identified as being most likely to drive the observed toxicity have been considered, and include CNT length, metal content, tendency to aggregate/agglomerate and surface chemistry. Of particular importance, is the contribution of the fibre paradigm to CNT toxicity, whereby the length of CNTs appears to be critical to their toxic potential. Mechanistic processes that are critical to CNT toxicity will also be discussed, with the findings insinuating that CNTs can exert an oxidative response that stimulates inflammatory, genotoxic and cytotoxic consequences. Consequently, it may transpire that a common mechanism is responsible for driving CNT toxicity, despite the fact that CNTs are a diverse population of materials. The similarity of the structure of CNTs to that of asbestos has prompted concern surrounding the exposure of humans, and so the applicability of the fibre paradigm to CNTs will be evaluated. It is also necessary to determine the systemic availability of CNTs

		following exposure, to determine where potential targets of toxicity are, and to thereby direct in vitro investigations within the most appropriate target cells. CNTs are therefore a group of materials whose useful exploitable properties prompts their increased production and utilization within diverse applications, so that ensuring their safety is of vital importance
A multidisciplinary approach to the identification of reference materials for engineered nanoparticle toxicology.	Aitken RJ, Hankin SM, Tran CL, Donaldson K, Stone V, et al. 2008. Nanotoxicology 2:71–78	The development of reference materials for toxicology and metrology is a critical component in establishing testing strategies and methods for human health hazard assessment of nanoparticles. A UK-based project (REFNANO) employed a workshop-based process to identify a priority list of candidate reference materials to support the measurement, toxicology and risk assessment of engineered nanoparticles. Consensus was reached amongst leading UK experts in toxicology, metrology and risk assessment from academia, government and industry on: (i) A rationale for the selection and development of priority reference/test materials; (ii) a priority listing of reference/test materials; (iii) the quantities of materials needed and the matrix in which they are present; and (iv) a recommended set of characteristics to be determined for the reference/test materials. The project also identified a series of actions for the development and promulgation of reference materials for nanoparticles for further consideration, both nationally and internationally.
A new certified reference material for size analysis of nanoparticles.	A. Braun, V. Kestens, K. Franks, G. Roebben, A. Lamberty, T. P. J. Linsinger, J. Nanoparticle Research, Vol. 14, p. 1012-1023 (2012)	A certified reference material, ERM-FD100, for quality assurance and validation of various nanoparticle sizing methods, was developed by the Institute for Reference Materials and Measurements. The material was prepared from an industrially sourced colloidal silica containing nanoparticles with a nominal equivalent spherical diameter of 20 nm. The homogeneity and stability of the candidate reference material was assessed by means of dynamic light scattering and centrifugal liquid sedimentation. Certification of the candidate reference material was based on a global interlaboratory comparison in which 34 laboratories participated with various analytical methods (DLS, CLS, EM, SAXS, ELS). After scrutinising the interlaboratory comparison data, 4 different certified particle size values, specific for the corresponding analytical method, could be assigned. The good comparability of results allowed the certification of the colloidal silica material for nanoparticle size analysis.
A review of the in vivo and in vitro toxicity of silver and gold particulates: particle attributes and biological mechanisms responsible for the observed toxicity.	Johnston, H.J., Hutchison, G.R., Christensen, F.M., Peters, S., Hankin, S., Stone, V. 2010, Crit Rev Toxicol, vol. 40, no. 4, pp. 328-46. doi:10.3109/10408440903453074	This review is concerned with evaluating the toxicity associated with human exposure to silver and gold nanoparticles (NPs), due to the relative abundance of toxicity data available for these particles, when compared to other metal particulates. This has allowed knowledge on the current understanding of the field to be gained, and has demonstrated where gaps in knowledge are. It is anticipated that evaluating the hazards associated with silver and gold particles will ultimately enable risk assessments to be completed, by combining this information with knowledge on the level of human exposure. The quantity of available hazard information for metals is greatest for silver particulates, due to its widespread inclusion within a number of diverse products (including clothes and wound dressings), which primarily arises from its antibacterial behaviour. Gold has been used on numerous occasions to assess the biodistribution and cellular uptake of NPs following exposure. Inflammatory, oxidative, genotoxic, and cytotoxic consequences are associated with silver particulate exposure, and are

		inherently linked. The primary site of gold and silver particulate accumulation has been consistently demonstrated to be the liver, and it is therefore relevant that a number of in vitro investigations have focused on this potential target organ. However, in general there is a lack of in vivo and in vitro toxicity information that allows correlations between the findings to be made. Instead a focus on the tissue distribution of particles following exposure is evident within the available literature, which can be useful in directing appropriate in vitro experimentation by revealing potential target sites of toxicity. The experimental design has the potential to impact on the toxicological observations, and in particular the use of excessively high particle concentrations has been observed. As witnessed for other particle types, gold and silver particle sizes are influential in dictating the observed toxicity, with smaller particles exhibiting a greater response than their larger counterparts, and this is likely to be driven by differences in particle surface area, when administered at an equal-mass dose. A major obstacle, at present, is deciphering whether the responses related to silver nanoparticulate exposure derive from their small size, or particle dissolution contributes to the observed toxicity. Alternatively, a combination of both may be responsible, as the release of ions would be expected to be greater for smaller particles.
Analysis and Characterization of Manufactured Nanoparticles in Aquatic Environments.	Hassellöv M. and Kaegi, R Nanoscience and Nanotechnology: Environmental and human health implications. (Eds. Lead J.R. and Smith E.) Wiley 2009, p. 211-266	Chapter 6.
Analysis of currently available data for characterising the risk of engineered nanomaterials to the environment and human health – Lessons learned from four case studies	Aschberger, K., Micheletti, C., Sokull-Klüttgen, B., Christensen, F.M. 2011, Environment International doi:10.1016/j.envint.2011.02.005	their potential risk for the environment and human health. We have reviewed publicly available hazard and exposure data for both, the environment and human health and attempted to carry out a basic risk assessment appraisal for four types of nanomaterials: fullerenes, carbon nanotubes, metals, and metal oxides (ENRHES project 2009(1)). This paper presents a summary of the results of the basic environmental and human health risk assessments of these case studies, highlighting the cross cutting issues and conclusions about fate and behaviour, exposure, hazard and methodological considerations. The risk assessment methodology being the basis for our case studies was that of a regulatory risk assessment under REACH (ECHA, 2008(2)), with modifications to adapt to the limited available data. If possible, environmental no-effect concentrations and human no-effect levels were established from relevant studies by applying assessment factors in line with the REACH guidance and compared to available exposure data to discuss possible risks. When the data did not allow a quantitative assessment, the risk was assessed qualitatively, e.g. for the environment by evaluating the information in the literature to describe the potential to enter the environment and to reach the potential ecological targets. Results indicate that the main risk for the environment is expected from metals and metal oxides, especially for algae and Daphnia, due to exposure to both, particles

		and ions. The main risks for human health may arise from chronic occupational inhalation exposure, especially during the activities of high particle release and uncontrolled exposure. The information on consumer and environmental exposure of humans is too scarce to attempt a quantitative risk characterisation. It is recognised that the currently available database for both, hazard and exposure is limited and there are high uncertainties in any conclusion on a possible risk. The results should therefore not be used for any regulatory decision making. Likewise, it is recognised that the REACH guidance was developed without considering the specific behaviour and the mode of action of nanomaterials and further work in the generation of data but also in the development of methodologies is required.
Applications of particle-tracking analysis to the determination of size distributions and concentrations of nanoparticles in environmental, biological and food samples.	Gallego-Urrea JA, Tuoriniemi J, Hassellov M. 2011. Trends Anal. Chem. 30:473–83	Review. The manufacture of nanoparticles (NPs) and nanomaterial-based products is rapidly increasing and their possible occurrence in environment, food or biological tissue is becoming of concern for ecological and human health. However, there is a lack of suitable methods to analyse and to characterize NPs in low concentrations in complex matrixes. We compare several particle-tracking methods using video microscopy and a new technique called nanoparticle-tracking analysis (NTA). Video microscopy has been widely applied to investigate particle movement in biological samples, micro-rheology, and velocity profiles in fluids, whilst NTA was devised for determination of size distributions and concentrations in liquid samples. We critically discuss the advantages and the limitations of NTA for such application
Assessing Nanoparticle Toxicity	Annual Review of Analytical Chemistry Vol. 5: 181-205, 2012 DOI: 10.1146/annurev-anchem-062011-143134	The experimental considerations for performing in vitro nanoparticle toxicity studies, with a focus on nanoparticle characterization, relevant model cell systems, and toxicity assay choices are discussed. Additionally, three case studies (of silver, titanium dioxide, and carbon nanotube toxicity) are presented to highlight the important toxicological considerations of these commonly used nanoparticles.
Biological Interactions of Graphene-Family Nanomaterials – An Interdisciplinary Review	Vanesa C. Sanchez, Ashish Jachak, Robert H. Hurt, Agnes B. Kane, Chemical Research in Toxicology, 25 (1), 15-34 (2012)	Graphene is a single-atom thick, two-dimensional sheet of hexagonally arranged carbon atoms isolated from its three-dimensional parent material, graphite. Related materials include few-layer-graphene (FLG), ultrathin graphite, graphene oxide (GO), reduced graphene oxide (rGO), and graphene nanosheets (GNS). This review proposes a systematic nomenclature for this set of Graphene-Family Nanomaterials (GFNs) and discusses specific materials properties relevant for biomolecular and cellular interactions. We discuss several unique modes of interaction between GFNs and nucleic acids, lipid bilayers, and conjugated small molecule drugs and dyes. Some GFNs are produced as dry powders using thermal exfoliation, and in these cases, inhalation is a likely route of human exposure. Some GFNs have aerodynamic sizes that can lead to inhalation and substantial deposition in the human respiratory tract, which may impair lung defence and clearance leading to the formation of granulomas and lung fibrosis. The limited literature on in vitro toxicity suggests that GFNs can be either benign or toxic to cells, and it is hypothesized that the biological response will vary across the material family depending on layer number, lateral size, stiffness, hydrophobicity, surface functionalization, and dose. Generation of reactive oxygen species (ROS) in target cells is a potential mechanism for toxicity, although the extremely high hydrophobic surface area of some GFNs may also lead to significant interactions with

		membrane lipids leading to direct physical toxicity or adsorption of biological molecules leading to indirect toxicity. Limited in vivo studies demonstrate systemic biodistribution and biopersistence of GFNs following intravenous delivery. Similar to other smooth, continuous, biopersistent implants or foreign bodies, GFNs have the potential to induce foreign body tumours. Long-term adverse health impacts must be considered in the design of GFNs for drug delivery, tissue engineering, and fluorescence-based biomolecular sensing. Future research is needed to explore fundamental biological responses to GFNs including systematic assessment of the physical and chemical material properties related to toxicity. Complete materials characterization and mechanistic toxicity studies are essential for safer design and manufacturing of GFNs in order to optimize biological applications with minimal risks for environmental health and safety.
Characterization of Nanomaterials by Physical Methods	C.N.R. Rao and Kanishka Biswas Annu., Rev. Anal. Chem. 2009. 2:435–62,	Much progress in nanoscience and nanotechnology has been made in the past few years thanks to the increased availability of sophisticated physical methods to characterize nanomaterials. These techniques include electron microscopy and scanning probe microscopies, in addition to standard techniques such as X-ray and neutron diffraction, X-ray scattering, and various spectroscopies. Characterization of nanomaterials includes the determination not only of size and shape, but also of the atomic and electronic structures and other important properties. In this article we describe some of the important methods employed for characterization of nanostructures, describing a few case studies for illustrative purposes. These case studies include characterizations of Au, ReO_3, and GaN nanocrystals; ZnO, Ni, and Co nanowires; inorganic and carbon nanotubes; and two-dimensional graphene.
Characterizing manufactured nanoparticles in the environment: multimethod determination of particle sizes.	Domingos RF, Baalousha MA, Ju-Nam Y, Reid MM, Tufenkji N, et al. 2009. Environ. Sci. Technol. 43:7277–84	Sizes of stabilized (24 h) nanoparticle suspensions were determined using several state-of-the-art analytical techniques (transmission electron microscopy; atomic force microscopy; dynamic light scattering; fluorescence correlation spectroscopy; nanoparticle tracking analysis; flow field flow fractionation). Theoretical and analytical considerations were evaluated, results were compared, and the advantages and limitations of the techniques were discussed. No “ideal” technique was found for characterizing manufactured nanoparticles in an environmental context as each technique had its own advantages and limitations.
Classification of nanostructures by dimensionality and concept of surface forms engineering in nanomaterial science	Materials Science and Engineering C 27 (2007) 990–993 V.V. Pokropivny, V.V. Skorokhod	Various kinds of nanostructures are assayed and classified using dimensionality of the nanostructure itself and their components. Restricted set of nanostructure classes was suggested to build from the constituting elementary units, namely, 0D clusters and particles, 1D nanotubes and nanowires, 2D nanoplates and layers. Collection set of 36 main classes of nanostructures are presented, that in couple with size effects enable us to predict qualitatively the properties of nanostructured materials and nano-architectured nanodevises. Concept of “engineering of nanostructure surface forms” is advanced extending the concept of “grain boundaries engineering” in nanomaterial science and nanotechnology.
Count, size and visualize	Mater. Today 14:170–73. Malloy	Paper on Nanoparticle tracking analysis (NTA)

nanoparticles	A. 2011.	
Critical considerations for the determination of nanoparticle number concentrations, size and number size distributions by single particle ICP-MS	Francisco Laborda, Javier Jiménez-Lamana, Eduardo Bolea and Juan R. Castillo , J. Anal. At. Spectrom., 2013,28, 1220-1232 DOI: 10.1039/C3JA50100K	The metrological criteria for the implementation of the single particle inductively coupled plasma mass spectrometry (SP-ICPMS) methodology applied to nanoparticle size characterization and quantification have been investigated. The SP-ICPMS basis involves a process of counting events corresponding to individual nanoparticles, which requires (i) isolation of the contribution of the nanoparticles from that of the background/dissolved analyte, and (ii) avoiding the occurrence of multiple-nanoparticle events. A criterion based on three times the standard deviation of the continuous background (3σ) was selected as the threshold for discrimination of nanoparticle events from the background. Because the detectability of nanoparticles depends on both the size and number concentration, this 3σ criterion was also selected for detection of nanoparticles at the size detection limit and concentrations over the number concentration detection limit. However, at very low number concentrations, a less restrictive criterion must be used. The selection of a critical nanoparticle number concentration, based on the sample introduction and data acquisition parameters, allows the minimization of the occurrence of multiple-nanoparticle events, as well as controlling of the precision associated with the counting of nanoparticle events. Under such conditions, the standard uncertainty associated with the determination of number concentrations was 5%. The uncertainty for the determination of nanoparticle diameters was also studied, varying from 3 to 10% for diameters in the range of 100–40 nm, respectively. Reliable average number concentrations and sizes were obtained, although the number size distributions showed a significant broadening contribution due to the SP-ICPMS measurement process. The feasibility of SP-ICPMS for the implementation of the European Commission definition of "nanomaterial" was studied by analysing commercial silver nanoparticle suspensions.
Critical evaluation of nanoparticle tracking analysis (NTA) by NanoSight for the measurement of nanoparticles and protein aggregates.	Filipe V, Hawe A, Jiskoot W. 2010. Pharm. Res. 27:796–810	PURPOSE: To evaluate the nanoparticle tracking analysis (NTA) technique, compare it with dynamic light scattering (DLS) and test its performance in characterizing drug delivery nanoparticles and protein aggregates. METHODS: Standard polystyrene beads of sizes ranging from 60 to 1,000 nm and physical mixtures thereof were analysed with NTA and DLS. The influence of different ratios of particle populations was tested. Drug delivery nanoparticles and protein aggregates were analysed by NTA and DLS. Live monitoring of heat-induced protein aggregation was performed with NTA. RESULTS: NTA was shown to accurately analyse the size distribution of monodisperse and polydisperse samples. Sample visualization and individual particle tracking are features that enable a thorough size distribution analysis. The presence of small amounts of large (1,000 nm) particles generally does not compromise the accuracy of NTA measurements, and a broad range of population ratios can easily be detected and accurately sized. NTA proved to be suitable to characterize drug delivery nanoparticles and protein aggregates, complementing DLS. Live

		monitoring of heat-induced protein aggregation provides information about aggregation kinetics and size of submicron aggregates. CONCLUSION: NTA is a powerful characterization technique that complements DLS and is particularly valuable for analysing polydisperse nanosized particles and protein aggregates.
Detection and characterization of engineered nanoparticles in food and the environment – a review.	Tiede, K., Boxall, A., Lewis, J., David, H., Tear, S. and Hassellöv M. Food Additives and Contaminants 2008, Vol. 25, p. 1-27.	Nanotechnology is developing rapidly and, in the future, it is expected that increasingly more products will contain some sort of nanomaterial. However, to date, little is known about the occurrence, fate and toxicity of nanoparticles. The limitations in our knowledge are partly due to the lack of methodology for the detection and characterisation of engineered nanoparticles in complex matrices, i.e. water, soil or food. This review provides an overview of the characteristics of nanoparticles that could affect their behaviour and toxicity, as well as techniques available for their determination. Important properties include size, shape, surface properties, aggregation state, solubility, structure and chemical composition. Methods have been developed for natural or engineered nanomaterials in simple matrices, which could be optimized to provide the necessary information, including microscopy, chromatography, spectroscopy, centrifugation, as well as filtration and related techniques. A combination of these is often required. A number of challenges will arise when analysing environmental and food materials, including extraction challenges, the presence of analytical artifacts caused by sample preparation, problems of distinction between natural and engineered nanoparticles and lack of reference materials. Future work should focus on addressing these challenges.
Detection of fullerenes (C60 and C70) in commercial cosmetics.	Benn TM, Westerhoff P, Herckes P. 2011. Environ. Pollut. 159:1334–42	Detection methods are necessary to quantify fullerenes in commercial applications to provide potential exposure levels for future risk assessments of fullerene technologies. The fullerene concentrations of five cosmetic products were evaluated using liquid chromatography with mass spectrometry to separate and specifically detect C60 and C70 from interfering cosmetic substances (e.g., castor oil). A cosmetic formulation was characterized with transmission electron microscopy, which confirmed that polyvinylpyrrolidone encapsulated C60. Liquid-liquid extraction of fullerenes from control samples approached 100% while solid-phase and sonication in toluene extractions yielded recoveries of 27-42%. C60 was detected in four commercial cosmetics ranging from 0.04 to 1.1 μg/g, and C70 was qualitatively detected in two samples. A single-use quantity of cosmetic (0.5 g) may contain up to 0.6 μg of C60, demonstrating a pathway for human exposure. Steady-state modelling of fullerene adsorption to biosolids is used to discuss potential environmental releases from wastewater treatment systems.
Determination of particle size distributions and the degree of dispersion in nanocomposites (Review)	Nolte, H. , Schilde, C., Kwade, A, Composites Science and Technology Volume 72, Issue 9, 21 May 2012, Pages 948-958	Objective of this study was the investigation of measurement techniques to determine the quality of the dispersion process of nanoparticles in polymer composites. In order to prepare the matrix suspension, alumina nanoparticles were dispersed applying shear mixing techniques in a high performance laboratory kneader. The product quality in liquid state was determined by means of dynamic light scattering (DLS) and centrifugal sedimentation analysis (CSA). However, particle measurements in carrier fluids like epoxy resin are complex and challenging. Measuring

		values like particle size distribution and grade of homogeneousness are strongly influenced by the sample preparation and adjustments of the measuring device. Within this study the machine settings and the formulation was analysed systematically. Hereby an identification of the key parameters and an optimisation of the measuring process were possible. Additionally, the composite was cured and analysed by scanning electron microscopy (SEM). Finally all measuring techniques were evaluated and compared among each other. Thus, DLS is the fastest method to measure spherically particles in the liquid matrix, CSA allows a certain deviation from the spherical shape and SEM gives a qualitative impression of the final particle size in cured composite condition.
Ecotoxicity of nanosized TiO_2. Review of in vivo data	Menard A, Drobne D, Jemec A. . Environ. Pollut. 159:677–84, 2011.	This report presents an exhaustive literature review of data on the effect of nanoparticulate TiO(2) on algae, higher plants, aquatic and terrestrial invertebrates and freshwater fish. The aim, to identify the biologically important characteristics of the nanoparticles that have most biological significance, was unsuccessful, no discernable correlation between primary particle size and toxic effect being apparent. Secondary particle size and particle surface area may be relevant to biological potential of nanoparticles, but insufficient confirmatory data exist. The nanotoxicity data from thirteen studies fail to reveal the characteristics actually responsible for their biological reactivity because reported nanotoxicity studies rarely carry information on the physicochemical characteristics of the nanoparticles tested. A number of practical measures are suggested which should support the generation of reliable QSAR models and so overcome this data inadequacy.
Electrophoretic methods for separation of nanoparticles.	Surugau N, Urban PL. 2009. J. Sep. Sci. 32:1889–906	This article reviews progress in the application of electrophoretic techniques for the separation of nanoparticles. Numerous types of nanoparticles have recently been synthesised and integrated into different products and procedures. Consequently, analytical methods for the efficient characterisation of nanoparticles are now required. Several studies have revealed that gel electrophoresis can readily be used for separating nanoparticles according to their size or shape. However, many other studies focused on separation of nanoparticles by CE. In some cases nanoparticles could be separated by CZE, simply using pure buffer as the BGE. In other studies, buffer additives (most often SDS) were used, enabling fast separations of metallic nanoparticles by size. Other CE methods also allowed for separation of nanoparticle conjugates with biomolecules. Dielectrophoresis is yet another electrophoretic technique useful in separation and characterisation of nanoparticles; particularly nanotubes. Detection methods often used after electrophoretic separation include UV/Vis absorption and fluorescence spectroscopy. Examples of recent and relevant older reports are presented here. The authors conclude that electrophoretic methods for nanoanalysis can provide inexpensive and efficient tools for quality assurance and safety control; and as a consequence, they can augment transfer of nanotechnologies from research to industry.
Engineered Nanoparticles and Their Identification Among Natural Nanoparticles	H. Z¨anker and A. Schierz, Annu. Rev. Anal. Chem. 2012. 5:107–32	The more nanotechnology develops, the more likely the release of engineered nanoparticles into the environment becomes. Due to a huge excess of natural nanoparticles, the identification and quantification of engineered nanoparticles pose a big challenge to analysts. Moreover,

		identification in a qualitative sense and quantification by mass concentration alone are not sufficient, because the potential environmental hazard arising from engineered nanoparticles is controlled by many other properties of the particles. We discuss the most important methods of fractionation and detection of both natural and engineered nanoparticles, with a focus on the chemical nature of the particles, particle concentration, and particle size. Analyses should not rely on only one method; instead, several complementary methods should, if possible, be used. Coupled techniques should be further developed and increasingly applied. Dedicated techniques that are tailored to the search for a particular sort of engineered nanoparticles are more promising than universal approaches that search for any engineered nanoparticles.
European Regulation Affecting Nanomaterials - Review Of Limitations And Future Recommendations	Steffen Foss Hansen, Anders Baun, Dose-Response, 10:364–383, 2012 Formerly Nonlinearity in Biology, Toxicology, and Medicine Copyright © 2012 University of Massachusetts ISSN: 1559-3258 DOI: 10.2203/dose-response.10-029.Hansen	After learning about the potential risks associated with various specific nanomaterials, concerns have been raised about adequacy of existing regulation in Europe and what should be done to address any potential regulatory gaps related to nanomaterials. Understanding the limitations of the current regulation in regard to nanomaterials is a starting point in a democratic and transparent process towards adapting existing laws and facilitating an informed discussion about which kind of regulatory options best address the identified limitations. In the following we will introduce key pieces of European legislation affecting nanomaterials, analyse their limitations, and provide a number of recommendations on how these can be overcome. We find that, although nanomaterials are in principle covered by the scope of many of the existing legislative frameworks, it is often unclear, if current regulations are actually applicable when it comes to specific nanomaterials and their diverse applications. Main limitations seem to be: that requirements to do safety evaluations are triggered by production volumes by tonnage not tailored to the nanoscale, the profound lack of (eco)toxicological data, and that thresholds values and occupational exposure limits cannot be established with existing methodologies.
Evaluation of nanoparticle immunotoxicity	Dobrovolskaia, M. A.; Germolec, D. R.; Weaver, J. L. Nat. Nanotechnol. 2009, 4, 411–414.	The pharmaceutical industry is developing increasing numbers of drugs and diagnostics based on nanoparticles, and evaluating the immune response to these diverse formulations has become a challenge for scientists and regulatory agencies alike. An international panel of scientists and representatives from various agencies and companies reviewed the imitations of current tests at a workshop held at the National Cancer Institute in Frederick, Maryland. This article outlines practical strategies for identifying and controlling interferences in common evaluation methods and the implications for regulation
Flow field-flow fractionation for the analysis and characterization of natural colloids and manufactured nanoparticles in environmental systems: a critical review.	Baalousha M, Stolpe B, Lead JR. J. Chromatogr. A 1218:4078–103, 2011.	The use of flow field flow fractionation (FlFFF) for the separation and characterization of natural colloids and nanoparticles has increased in the last few decades. More recently, it has become a popular method for the characterization of manufactured nanoparticles. Unlike conventional filtration methods, FlFFF provides a continuous and high-resolution separation of nanoparticles as a function of their diffusion coefficient, hence the interest for use in determining particle size

		distribution. Moreover, when coupled to other detectors such as inductively coupled plasma-mass spectroscopy, light scattering, UV-absorbance, fluorescence, transmission electron microscopy, and atomic force microscopy, FlFFF provides a wealth of information on particle properties including, size, shape, structural parameters, chemical composition and particle-contaminant association. This paper will critically review the application of FlFFF for the characterization of natural colloids and natural and manufactured nanoparticles. Emphasis will be given to the detection systems that can be used to characterize the nanoparticles eluted from the FlFFF system, the obtained information and advantages and limitation of FlFFF compared to other fractionation and particle sizing techniques. This review will help users understand (i) the theoretical principles and experimental consideration of the FlFFF, (ii) the range of analytical tools that can be used to further characterize the nanoparticles after fractionation by FlFFF, (iii) how FlFFF results are compared to other analytical techniques and (iv) the range of applications of FlFFF for natural and manufactured NPs.
How physico-chemical characteristics of nanoparticles cause their toxicity: Complex and unresolved interrelations (Review)	Katrien Luyts, Dorota Napierska, Ben Nemery and Peter H. M. Hoet, Environ. Sci.: Processes Impacts, 2013, 15, 23	Recently engineered nanomaterials can be found in a range of commercial products, and without doubt more and more nanoparticles (NPs) will enter our environment. Unique – size dependent – properties not only determine their utility, but also their toxicity/hazardous properties. While size – based on the existing definitions – seems to be the most important property of NPs, it is now clear that other physico-chemical characteristics are (equally) important – and many of these characteristics are interrelated. In this overview, we try to link different critical characteristics separately with toxicity observed – in both in vitro and in vivo models – in order to unravel the complexity of the nano-related hazardous effects.
Hydrophilic/hydrophobic features of TiO2 nanoparticles as a function of crystal phase, surface area and coating, in relation to their potential toxicity in peripheral nervous system	Bolis, V. , Busco, C., Ciarletta, M., Distasi, C., Erriquez, J., Fenoglio, I., Livraghi, S., Morel, S. Journal of Colloid and Interface Science Volume 369, Issue 1, 1 March 2012, Pages 28-39	The hydrophilic/hydrophobic properties of a variety of commercial TiO 2 nanoparticles (NP), to be employed as inorganic filters in sunscreen lotions, were investigated both as such (dry powders) and dispersed in aqueous media. Water uptake and the related interaction energy have been determined by means of adsorption microcalorimetry of H 2O vapour, whereas dispersion features in aqueous solutions were investigated by dynamic light scattering and electrokinetic measurements (zeta potential). The optimized dispersions in cell culture medium were employed to assess the possible in vitro neuro-toxicological effect on dorsal root ganglion (DRG) cells upon exposure to TiO 2-NP, as a function of crystal phase, surface area and coating. All investigated materials, with the only exception of the uncoated rutile, were found to induce apoptosis on DRG cells; the inorganic/organic surface coating was found not to protect against the TiO 2-induced apoptosis. The risk profile for DRG cells, which varies for the uncoated samples in the same sequence as the photo-catalytic activity of the different polymorphs: anatase-rutile>anatase≫rutile, was found not to be correlated with the surface hydrophilicity of the uncoated/coated specimens. Aggregates/agglomerates hydrodynamic diameter was comprised in the ∼200-400nm range, compatible with the internalization within DRG cells.
Identification and characterization of organic nanoparticles in food.	Peters R, ten Dam G, Bouwmeester H, Helsper H, Allmaier G, et al. Trends Anal.	Interest in nanoparticles (NPs) has increased explosively over the past two decades. Using NPs, high loadings of vitamins and health-benefit actives can be achieved in food, and stable flavours as well as natural food-colouring dispersions can be developed. Detection and characterization

	Chem. 30:100–12., 2011.	of NPs are essential in understanding the benefits as well as the potential risks of the application of such materials in food. While many such applications are described in the literature, methods for detection and characterization of such particles are lacking. Organic NPs suitable for application in food are lipid-, protein- or polysaccharide-based particles, and this review describes current analytical techniques that are used, or could be used, for identification and characterization of such particles in food products. We divide the analytical approaches into four sections: sample preparation; separation; imaging; and, characterization. We discuss techniques and reported applications for NPs or otherwise related particle compounds. The results of this investigation show that, for a successful characterization of NPs in food, at least some kind of sample preparation will be required. While a simple sample preparation may be satisfactory for imaging techniques for known analytes, for other techniques, a further separation using chromatography, field-flow fractionation or ion-mobility separation is necessary. Subsequently, photon-correlation spectroscopy and especially mass spectrometry techniques as matrix-assisted laser desorption/ionization combined with time-of-flight mass spectrometry, seem suitable techniques for characterizing a wide variety of organic NPs.
Identification of the mechanisms that drive the toxicity of TiO_2 particulates; the contribution of physicochemical characteristics	Johnston, H.J., Hutchison, G.R., Christensen, F.M., Peters, S., Hankin, S., Stone, V. 2009, Part Fibre Toxicol, vol. 6, no. 33. doi: 10.1186/1743-8977-6-33	This review focuses on outlining the toxicity of titanium dioxide (TiO_2) particulates *in vitro* and *in vivo*, in order to understand their ability to detrimentally impact on human health. Evaluating the hazards associated with TiO_2 particles is vital as it enables risk assessments to be conducted, by combining this information with knowledge on the likely exposure levels of humans. This review has concentrated on the toxicity of TiO_2, due to the fact that the greatest number of studies by far have evaluated the toxicity of TiO_2, in comparison to other metal oxide particulates. This derives from historical reasons (whereby the size dependency of particulate toxicity was first realised for TiO_2) and due to its widespread application within consumer products (such as sunscreens). The pulmonary and dermal hazards of TiO_2 have been a particular focus of the available studies, due to the past use of TiO_2 as a (negative) control when assessing the pulmonary toxicity of particulates, and due to its incorporation within consumer products such as sunscreens. Mechanistic processes that are critical to TiO2 particulate toxicity will also be discussed and it is apparent that, in the main, the oxidant driven inflammatory, genotoxic and cytotoxic consequences associated with TiO_2 exposure, are inherently linked, and are evident both *in vivo* and *in vitro*. The attributes of TiO2 that have been identified as being most likely to drive the observed toxicity include particle size (and therefore surface area), crystallinity (and photocatalytic activity), surface chemistry, and particle aggregation/agglomeration tendency. The experimental set up also influences toxicological outcomes, so that the species (or model) used, route of exposure, experiment duration, particle concentration and light conditions are all able to influence the findings of investigations. In addition, the applicability of the observed findings for particular TiO_2 forms, to TiO_2 particulates in general, requires consideration. At this time it is inappropriate to consider the findings for one TiO_2 form as being representative for

		TiO_2 particulates as a whole, due to the vast number of available TiO_2 particulate forms and large variety of potential tissue and cell targets that may be affected by exposure. Thus emphasising that the physicochemical characteristics are fundamental to their toxicity.
In Vitro Cytotoxicity of Oxide Nanoparticles: Comparison to Asbestos, Silica, and the Effect of Particle Solubility	Brunner T, Wick P, Manser P, Spohn P, Grass R, Limbach L, Bruinink A, Stark W. Environ. Sci. Technol. 2006, 40, 4374-4381	Early indicators for nanoparticle-derived adverse health effects should provide a relative measure for cytotoxicity of nanomaterials in comparison to existing toxicological data. We have therefore evaluated a human mesothelioma and a rodent fibroblast cell line for in vitro cytotoxicity tests using seven industrially important nanoparticles. Their response in terms of metabolic activity and cell proliferation of cultures exposed to 0−30 ppm nanoparticles (μg g-1) was compared to the effects of nontoxic amorphous silica and toxic crocidolite asbestos. Solubility was found to strongly influence the cytotoxic response. The results further revealed a nanoparticle-specific cytotoxic mechanism for uncoated iron oxide and partial detoxification or recovery after treatment with zirconia, ceria, or titania. While in vitro experiments may never replace in vivo studies, the relatively simple cytotoxic tests provide a readily available pre-screening method.
Influence of size, surface area and microporosity on the in vitro cytotoxic activity of amorphous silica nanoparticles in different cell types	Virginie Rabolli, Leen C. J. Thomassen, Catherine Princen, Dorota Napierska, Laetitia Gonzalez, Micheline Kirsch-Volders, Peter H. Hoet, François Huaux, Hristine E. A. Kirschhock, Johan A. Martens and Dominique Lison Nanotoxicology, Volume 4, Issue 3, September 2010, Pages 307-318,	Identifying the physico-chemical characteristics of nanoparticles (NPs) that drive their toxic activity is the key to conducting hazard assessment and guiding the design of safer nanomaterials. Here we used a set of 17 stable suspensions of monodisperse amorphous silica nanoparticles (SNPs) with selected variations in size (diameter, 2335 nm), surface area (BET, 16-422 m 2/g) and microporosity (micropore volume, 0-71 μl/g) to assess with multiple regression analysis the physico-chemical determinants of the cytotoxic activity in four different cell types (J774 macrophages, EAHY926 endothelial cells, 3T3 fibroblasts and human erythrocytes). We found that the response to these SNPs is governed by different physico-chemical parameters which vary with cell type: In J774 macrophages, the cytotoxic activity (WST1 assay) increased with external surface area (αs method) and decreased with micropore volume (r2 of the model, 0.797); in EAHY926 and 3T3 cells, the cytotoxic activity of the SNPs (MTT and WST1 assay, respectively) increased with surface roughness and small diameter (r2, 0.740 and 0.872, respectively); in erythrocytes, the hemolytic activity increased with the diameter of the SNP (r2, 0.860). We conclude that it is possible to predict with good accuracy the in vitro cytotoxic potential of SNPs on the basis of their physico-chemical characteristics. These determinants are, however, complex and vary with cell type, reflecting the pleiotropic interactions of nanoparticles with biological systems. © 2010 Informa UK, Ltd.
Inhalation of poorly soluble particles. II. Influence of particle surface area on inflammation and clearance.	Tran CL, Buchanan D, Cullen RT, Searl A, Jones AD, Donaldson K: Inhal Toxicol 2000, 12:1113–1126.	In this article the volumetric overload hypothesis, which predicts the impairment of clearance of particles deposited in the lung in terms of particle volume, is re-evaluated. The degree to which simple expressions of retained lung burden explain pulmonary responses to overload was investigated using data from a series of chronic inhalation experiments on rats with two poorly soluble dusts, titanium dioxide and barium sulphate. The results indicated that the difference between the dusts in the level of inflammation and translocation to the lymph nodes could be explained most simply when the lung burden was expressed as total particle surface area. The shape of the statistical relationship for both lung responses indicated the presence of a

		threshold at approximately 200-300 cm(2) of lung burden. On the basis of this and other similar results, a hypothesis regarding a generic mechanism for the impairment of clearance and associated lung responses is proposed for such "low-toxicity" dusts.
Inhaled nanoparticles and lung cancer - What we can learn from conventional particle toxicology	Donaldson, K., Poland, C.A. Swiss Medical Weekly Volume 142, Issue JUNE, June 2012, Article numberw13547, (Review)	Manufactured nanoparticles (MNP) represent a growth area in industry where their interesting and useful properties bestow advantage over conventional particles for many purposes. This review specifically addresses the potential for lung cancer in those who might be exposed to airborne MNP. There is no strong evidence that MNP are carcinogenic and MNP come in a wide spectrum of materials, sizes, shapes and compositions and it is likely that the hazard will vary across different MNP types dependent upon their intrinsic properties. Low toxicity low solubility (LTLS) MNP are unlikely to pose a substantial cancer risk as they are not very biologically active. Nanoparticles with a more reactive surface may undoubtedly generate inflammation more readily and inflammation could be sufficiently intense to lead to secondary carcinogenesis via the oxidants and mitogens produced during inflammation. There is some evidence in vitro that MNP can gain access to the nucleus and the genetic material if specifically designed to do so by surface modification and that nanoparticles such as carbon nanotubes (CNT) can cause genetic aberrations by a primary mechanism additional to the inflammation-mediated one; these potential mechanisms require further study. High aspect ratio nanoparticles (HARN) are MNP that are fibre-shaped and analogously to asbestos might pose a special cancer hazard to the lungs, pleural and peritoneal mesothelium. Recent research suggests that the existing fibre pathogenicity paradigm is adequate for describing the hazard of HARN and that making the HARN of a non-biopersistent material or restricting the length could, via benign-by-design principles, allow safe HARN to be produced.
Interlaboratory comparison for the measurement of particle size and zeta potential of silica nanoparticles in an aqueous suspension,	A. Lamberty, K. Franks, G. Roebben, A. Braun, V. Kestens, T. Linsinger. Journal of Nanoparticle Research, Vol.13, p. 7317-7329 (2011)	The Institute for Reference Materials and Measurements has organised an interlaboratory comparison (ILC) to allow the participating laboratories to demonstrate their proficiency in particle size and zeta potential measurements on monomodal aqueous suspensions of silica nanoparticles in the 10–100 nm size range. The main goal of this ILC was to identify competent collaborators for the production of certified nanoparticle reference materials. 38 laboratories from four different continents participated in the ILC with different methods for particle sizing and determination of zeta potential. Most of the laboratories submitted particle size results obtained with centrifugal liquid sedimentation (CLS), dynamic light scattering (DLS) or electron microscopy (EM), or zeta potential values obtained via electrophoretic light scattering (ELS). The results of the laboratories were evaluated using method-specific *z* scores, calculated on the basis of consensus values from the ILC. For CLS (13 results) and EM (13 results), all reported values were within the ±2 $\|z\|$ interval. For DLS, 25 of the 27 results reported were within the ±2 $\|z\|$ interval, the two other results were within the ±3 $\|z\|$ interval. The standard deviations of the corresponding laboratory mean values varied between 3.7 and 6.5%, which demonstrates satisfactory interlaboratory comparability of CLS, DLS and EM particle size values. From the received test reports, a large discrepancy was observed in terms of the laboratory's quality assurance systems, which are equally important for the selection of collaborators in reference

		material certification projects. Only a minority of the participating laboratories is aware of all the items that are mandatory in test reports compliant to ISO/IEC 17025 (ISO General requirements for the competence of testing and calibration laboratories. International Organisation for Standardization, Geneva, 2005b). The absence of measurement uncertainty values in the reports, for example, hindered the calculation of zeta scores.
Interlaboratory Comparison of Size and Surface Charge Measurements on Nanoparticles prior to Biological Impact Assessment,	G. Roebben, S. Ramirez-Garcia, V. Hackley, M. Roesslein, F. Klaessig, V. Kestens, I. Lynch, M. C. Garner, A. Rawle, A. Elder, V. Colvin, W. Kreyling, H. Krug, Z. Lewicka, S. McNeil, A. Nel, A. Patri, P. Wick, M. Wiesner, Tian Xia, G. Oberdörster, K. Dawson, J. Nanoparticle Research, Vol. 13, p. 2675-2687, (DOI) 10.1007/s11051-011-0423-y (2011)	The International Alliance for NanoEHS Harmonization (IANH) organises interlaboratory comparisons of methods used to study the potential biological impacts of nanomaterials. The aim of IANH is to identify and reduce or remove sources of variability and irreproducibility in existing protocols. Here, we present results of the first IANH round robin studies into methods to assess the size and surface charge of suspended nanoparticles. The test materials used (suspensions of gold, silica, polystyrene, and ceria nanoparticles, with [primary] particles sizes between 10 nm and 80 nm) were first analysed in repeatability conditions to assess the possible contribution of between-sample heterogeneity to the between-laboratory variability. Reproducibility of the selected methods was investigated in an interlaboratory comparison between ten different laboratories in the USA and Europe. Robust statistical analysis was used to evaluate within- and between-laboratory variability. It is shown that, if detailed shipping, measurement, and reporting protocols are followed, measurement of the hydrodynamic particle diameter of nanoparticles in predispersed monomodal suspensions using the dynamic light scattering method is reproducible. On the other hand, measurements of more polydisperse suspensions of nanoparticle aggregates or agglomerates were not reproducible between laboratories. Ultrasonication, which is commonly used to prepare dispersions before cell exposures, was observed to further increase variability. The variability of the zeta potential values, which were also measured, indicates the need to define better surface charge test protocols and to identify sources of variability.
Issues in Particle Size Analysis.	R. Hogg, KONA Powder and Particle Journal No.26 (2008)	Important issues that arise in the acquisition, presentation and interpretation of particle size data are discussed. Presentation of size distributions as relative quantity versus size, representation of quantity by number, mass, etc., and procedures for inter-conversion of the different forms are described. Definitions of various kinds of average sizes are presented. Limitations on their use and the importance of precise definition are emphasized. Definitions of size for irregular particles, the role of particle shape and implications with regard to comparability of analytical results based on different principles and procedures are evaluated. Different types of measurement procedures are classified according to whether they involve measurements on individual particles, on separated classes of particles or on complete assemblages. The restrictions and constraints, such as size limitations and resolution which apply to these types, are discussed.
Measurement of nanoparticles by light-scattering techniques.	Brar SK, Verma M., Review, Trends Anal. Chem. 30:4–17, 2011.	Nanoparticles (NPs), due to their unique physical and chemical properties, especially their minute particle size (<100 nm), find applications in numerous industrial, commercial and consumer products. After their end-user applications, these NPs find their way into the environment and food products. The NPs so discharged need to be quantified accurately to

		determine their toxicity and exposure levels. At this time, there is a need to develop a unified method for their determination. There are plenty of techniques available in the market that were initially used for colloidal particles (e.g., microscopy, spectroscopy and the recent addition of magnetic resonance), but each of these techniques has a certain degree of uncertainty. Further, sample homogeneity, sample preparation, instrument-operating procedures, and statistical practices are likely to add to the complexity of the problem. In this context, this review attempts to understand the widely-used light-scattering techniques, including their theory, practice and real-world use in determination of NPs in environmental and food applications.
Measurements of nanoparticle number concentrations and size distributions in contrasting aquatic environments using nanoparticle tracking analysis	Gallego-Urrea JA, Tuoriniemi J, Pallander T, Hassellov M. Environ. Chem. 7:67–81, 2010	A feasibility study of nanoparticle tracking analysis (NTA) for aquatic environmental samples is presented here. The method has certain virtues such as minimum perturbation of the samples, high sensitivity in terms of particle concentration, and provision of number-based size distributions for aquatic samples. NTA gave linear calibration curves in terms of number concentration and accurately reproduced size measurements of certified reference material nanoparticles. However, the accuracy of the size distributions obtained with this method exhibited a high dependence on set-up parameters and the concentrations were shown to be strongly correlated with the refractive index of the material under examination. Different detection cameras and different data acquisition modes were compared and evaluated. Also, the effect of filtration of the samples was assessed. The size distributions for the contrasting environmental samples were fairly reasonable compared with other studies but an underestimation of small sizes was observed, which can be explained by a material-dependent lower detection limit in terms of size. The number concentrations obtained for the natural nanoparticles ranged from 0.5 to 20 × 108 particles mL^{-1} and correlated well with conventional turbidity measurements.
Measuring nanoparticles size distribution in food and consumer products: a review	Calzolai, L., Gilliland, D., Rossi, F., Food Addit. Conta.: Part A, 29, 1183 – 1193, (2012).	Nanoparticles are already used in several consumer products including food, food packaging and cosmetics, and their detection and measurement in food represent a particularly difficult challenge. In order to fill the void in the official definition of what constitutes a nanomaterial, the European Commission published in October 2011 its recommendation on the definition of 'nanomaterial'. This will have an impact in many different areas of legislation, such as the European Cosmetic Products Regulation, where the current definitions of nanomaterial will come under discussion regarding how they should be adapted in light of this new definition. This new definition calls for the measurement of the number-based particle size distribution in the 1-100 nm size range of all the primary particles present in the sample independently of whether they are in a free, unbound state or as part of an aggregate/agglomerate. This definition does present great technical challenges for those who must develop valid and compatible measuring methods. This review will give an overview of the current state of the art, focusing particularly on the suitability of the most used techniques for the size measurement of nanoparticles when addressing this new definition of nanomaterials. The problems to be overcome in measuring nanoparticles in food and consumer products will be illustrated with some practical examples.

		Finally, a possible way forward (based on the combination of different measuring techniques) for solving this challenging analytical problem is illustrated.
Mechanisms of silver nanoparticle release, transformation and toxicity: A critical review of current knowledge and recommendations for future studies and applications	Materials Reidy, B., Haase, A., Luch, A. Dawson, K., Lynch, I., Volume 6, Issue 6, 2013, Pages 2295-2350	Nanosilver, due to its small particle size and enormous specific surface area, facilitates more rapid dissolution of ions than the equivalent bulk material; potentially leading to increased toxicity of nanosilver. This, coupled with their capacity to adsorb biomolecules and interact with biological receptors can mean that nanoparticles can reach sub-cellular locations leading to potentially higher localized concentrations of ions once those particles start to dissolve or degrade in situ. Further complicating the story is the capacity for nanoparticles to generate reactive oxygen species, and to interact with, and potentially disturb the functioning of biomolecules such as proteins, enzymes and DNA. The fact that the nanoparticle size, shape, surface coating and a host of other factors contribute to these interactions, and that the particles themselves are evolving or ageing leads to further complications in terms of elucidating mechanisms of interaction and modes of action for silver nanoparticles, in contrast to dissolved silver species. This review aims to provide a critical assessment of the current understanding of silver nanoparticle toxicity, as well as to provide a set of pointers and guidelines for experimental design of future studies to assess the environmental and biological impacts of silver nanoparticles. In particular; in future we require a detailed description of the nanoparticles; their synthesis route and stabilisation mechanisms; their coating; and evolution and ageing under the exposure conditions of the assay. This would allow for comparison of data from different particles; different environmental or biological systems; and structure-activity or structure-property relationships to emerge as the basis for predictive toxicology. On the basis of currently available data; such comparisons or predictions are difficult; as the characterisation and time-resolved data is not available; and a full understanding of silver nanoparticle dissolution and ageing under different conditions is observed. Clear concerns are emerging regarding the overuse of nanosilver and the potential for bacterial resistance to develop. A significant conclusion includes the need for a risk-benefit analysis for all applications and eventually restrictions of the uses where a clear benefit cannot be demonstrated.
Measurement of the size of spherical nanoparticles by means of atomic force microscopy.	O. Couteau, G. Roebben, Measurement Science and Technology, Vol. 22, 065101 (2011)	Several techniques are nowadays available to determine the size distribution of nanoparticulate matter. Among these techniques, atomic force microscopy (AFM) is especially valuable because it can provide three-dimensional information on the shape of individual nanoparticles. This paper describes a new method to determine the size distribution of a population of spherical nanoparticles deposited on a hard substrate. The method is based on the acquisition and analysis of topographical AFM images. The size of individual nanoparticles is obtained by fitting the topographical region associated with the nanoparticle with a sphere. Tests on model systems based on nanoparticle reference materials consisting of polystyrene (PS) latex suspensions show promising results. The measured mean particle size is larger than the reference value, but this is a predictable effect of the AFM tip shape. Tests on a bi-modal mixture of two PS latex reference materials show the impact of the quality of the dispersion of the nanoparticles on the results obtained with the new technique.

Minimum physicochemical characterisation requirements for nanomaterial regulation.	Pettitt, M.E., Lead, J.R. Environment International Volume 52, February 2013, Pages 41-50	Appropriate characterisation of manufactured nanomaterials (NMs) is vital for many aspects of their synthesis, product formulation, toxicological testing and regulation. As the range and quantity of NMs in production has expanded, the interest in their potential environmental and toxicological consequences has grown. With this growth, there is increased need for clarity and rigour in characterising appropriate physicochemical parameters. Which physicochemical parameters should be characterised and under what conditions remains a topic of debate, along with the most appropriate techniques and methodologies to best describe any one characteristic. This review assesses the characterisation requirements of current and future regulatory frameworks for NMs, with specific focus on the incoming REACH framework of the EU. For regulatory compliance, characterisation requirements will be necessarily prescriptive. The minimum physicochemical parameters required to adequately describe NMs for regulatory purposes are proposed, along with a discussion of the most appropriate mechanisms to obtain those data in terms of the overarching delivery mechanism. Guiding principles for particle characterisation during the hazard testing required to comply with regulations are examined
Nanomaterial cytotoxicity is composition, size, and cell type dependent	Syed K Sohaebuddin, Paul T Thevenot, David Baker, John W Eaton and Liping Tang, Particle and Fibre Toxicology 2010, 7:22 doi:10.1186/1743-8977-7-22	Nanomaterials induce cell specific responses resulting in variable toxicity and subsequent cell fate based on the type of exposed cell. Our results indicate that the composition and size of nanomaterials as well as the target cell type are critical determinants of intracellular responses, degree of cytotoxicity and potential mechanisms of toxicity.
Nanomaterial Toxicity Testing in the 21st Century: Use of a Predictive Toxicological Approach and High-Throughput Screening	Andre Nel, Tian Xia, Huan Meng, Xiang Wang, Sijie Lin, Zhaoxia Ji and Haiyuan Zhang, Accounts Of Chemical Research 607, Vol. 46 (3), 2013, 607–621 ’	In this Account, we review the tools required for establishing predictive toxicology paradigms to assess inhalation and environmental toxicological scenarios through the use of compositional and combinatorial ENM libraries, mechanism-based HTS assays, hazard ranking, and development of nano-SARs. We will discuss the major injury paradigms that have emerged based on specific ENM properties, as well as describing the safer design of ZnO nanoparticles based on characterization of dissolution chemistry as a major predictor of toxicity.
Nanomaterials for environmental studies: Classification, reference material issues, and strategies for physico-chemical characterisation	Vicki Stone, Bernd Nowack, Anders Baun, Nico van den Brink, Frank von der Kammer, Maria Dusinska, Richard Handy, Steven Hankin, Martin Hassellöv, Erik Joner, Teresa F. Fernandes Science of the Total Environment 408 (2010) 1745–1754	NanoImpactNet is a European Commission Framework Programme 7 (FP7) funded project that provides a forum for the discussion of current opinions on nanomaterials in relation to human and environmental issues. In September 2008, in Zurich, a NanoImpactNet environmental workshop focused on three key questions: 1. What properties should be characterised for nanomaterials used in environmental and ecotoxicology studies? 2. What reference materials should be developed for use in environmental and ecotoxicological studies? 3. Is it possible to group different nanomaterials into categories for consideration in environmental studies? Such questions have been, at least partially, addressed by other projects/workshops especially in relation to human health effects. Such projects provide a useful basis on which this workshop was based, but in this particular case these questions were reformulated in order to focus

		specifically on environmental studies. The workshop participants, through a series of discussion and reflection sessions, generated the conclusions listed below. The physicochemical characterisation information identified as important for environmental studies included measures of aggregation/agglomeration/dispersability, size, dissolution (solubility), surface area, surface charge, surface chemistry/composition, with the assumption that chemical composition would already be known. There is a need to have test materials for ecotoxicology, and several substances are potentially useful, including TiO2 nanoparticles, polystyrene beads labelled with fluorescent dyes, and silver nanoparticles. Some of these test materials could then be developed into certified reference materials over time. No clear consensus was reached regarding the classification of nanomaterials into categories to aid environmental studies, except that a chemistry-based classification system was a reasonable starting point, with some modifications. It was suggested that additional work may be required to derive criteria that can be used to generate such categories, which would also include aspects of the material structure and physical behaviour.
Nanoparticle analysis and characterization methodology in environmental risk assessment of engineered nanoparticles	Hassellöv, M., Readman, J., Ranville, J. and Tiede, K., Ecotoxicology 2008. Vol. 17, p. 344–361	Environmental risk assessments of engineered nanoparticles require thorough characterization of nanoparticles and their aggregates. Furthermore, quantitative analytical methods are required to determine environmental concentrations and enable both effect and exposure assessments. Many methods still need optimization and development, especially for new types of nanoparticles in water, but extensive experience can be gained from the fields of environmental chemistry of natural nanomaterials and from fundamental colloid chemistry. This review briefly describes most methods that are being exploited in nano-ecotoxicology for analysis and characterization of nanomaterials. Methodological aspects are discussed in relation to the fields of nanometrology, particle size analysis and analytical chemistry. Differences in both the type of size measures (length, radius, aspect ratio, etc.), and the type of average or distributions afforded by the specific measures are compared. The strengths of single particle methods, such as electron microscopy and atomic force microscopy, with respect to imaging, shape determinations and application to particle process studies are discussed, together with their limitations in terms of counting statistics and sample preparation. Methods based on the measurement of particle populations are discussed in terms of their quantitative analyses, but the necessity of knowing their limitations in size range and concentration range is also considered. The advantage of combining complementary methods is highlighted.
Nanoparticles and metrology: A comparison of methods for the determination of particle size distributions	Coleman, V.A. , Jämting, Å.K., Catchpoole, H.J., Roy, M., Herrmann, J. Proceedings of SPIE - The International Society for Optical Engineering Volume 8105, 2011, Article number 810504	Nanoparticles and products incorporating nanoparticles are a growing branch of nanotechnology industry. They have found a broad market, including the cosmetic, health care and energy sectors. Accurate and representative determination of particle size distributions in such products is critical at all stages of the product lifecycle, extending from quality control at point of manufacture to environmental fate at the point of disposal. Determination of particle size distributions is non-trivial, and is complicated by the fact that different techniques measure different quantities, leading to differences in the measured size distributions. In this study we use both mono- and multi-modal dispersions of nanoparticle reference materials to compare

		and contrast traditional and novel methods for particle size distribution determination. The methods investigated include ensemble techniques such as dynamic light scattering (DLS) and differential centrifugal sedimentation (DCS), as well as single particle techniques such as transmission electron microscopy (TEM) and microchannel resonator (ultra high-resolution mass sensor). © 2011 Copyright Society of Photo-Optical Instrumentation Engineers (SPIE).
Nanoparticles in the Water Cycle. Chapter: Standardisation. pp. 207–31	Frimmel FH, Niessner R, eds. 2010. Berlin/Heidelberg: Springer. 239 pp. Gordalla BC, Standardisation. pp. 207–31	
Nanoparticulates (Chapter)	Hubbs, A., Porter, D.W., Mercer, R., Castranova, V., Sargent, L., Sriram, K., Haschek and Rousseaux's Handbook of Toxicologic Pathology 2013, Pages 1373-1419	This chapter describes the toxicological pathology of nanoparticulates, the tools which can help toxicologic pathologists evaluate nanoparticulate studies, and emphasizes unique features occurring in nanoscale dimensions. Nanoparticulates are particulates with at least one dimension less than 100. nm. The number and complexity of nanoparticulates is rapidly increasing due to improvements in nanotechnology, the technology that allows engineering in the nanoscale, and often from the atom up. Many products are in current use for diverse commercial purposes, with an estimated economic impact expanding into trillions of dollars in the near future. Recently, nanotechnology has expanded to include medical products, and the new products of nanomedicine include nanopharmaceuticals. Nanotoxicology, the study of the toxic effects of the new products of nanotechnology, lags far behind developments in nanotechnology and nanomedicine. Even fewer studies have investigated the toxicologic pathology of the products of nanotechnology. Nanoparticulates are generally more toxic on a mass basis than larger particles with the same composition. Nanoscaling of particulates increases surface area and thereby increases the dissolution rate of soluble particulates and increases inflammation associated with poorly soluble particulates. Nanosizing also facilitates movement between tissues and across intracellular barriers. In particular, nanoscaling permits extracellular transport in lymphatics, retrograde neuronal transport, and the use of nanoscale endocytic pathways for cell entry and intracellular transport. NPs are similar in size to subcellular structures, including components of the mitotic spindle, permitting critical interactions with these subcellular structures that are not possible with larger particulates. However, some of these unique features can be harnessed for new therapeutic and diagnostic products that allow targeted drug delivery and advanced imaging. Toxicologic pathologists will increasingly evaluate the safety of these new products of nanotechnology. An understanding of the toxicologic pathology of nanoparticulates plays a critical role in the safe development of nanotechnology
Nanoscale Reference Materials for Environmental, Health, and Safety Measurements: Needs, Gaps, and Opportunities.	A. B. Stefaniak, V. A. Hackley, G. Roebben, K. Ehara, S. Hankin, M. T. Postek, I. Lynch, Wei-En Fu, T. P. J. Linsinger, A. F. Thünemann, Nanotoxicology, Vol. 7, DOI:	The authors critically reviewed published lists of nano-objects and their physico-chemical properties deemed important for risk assessment and discussed metrological challenges associated with the development of nanoscale reference materials (RMs). Five lists were identified that contained 25 (classes of) nano-objects; only four (gold, silicon dioxide, silver, titanium dioxide) appeared on all lists. Twenty-three properties were identified for

	10.3109/17435390.2012.739664, p. 1325-1337 (2013).	characterisation; only (specific) surface area appeared on all lists. The key themes that emerged from this review were: 1) various groups have prioritised nano-objects for development as "candidate RMs" with limited consensus; 2) a lack of harmonised terminology hinders accurate description of many nano-object properties; 3) many properties identified for characterisation are ill-defined or qualitative and hence are not metrologically traceable; 4) standardised protocols are critically needed for characterisation of nano-objects as delivered in relevant media and as administered to toxicological models; 5) the measurement processes being used to characterise a nano-object must be understood because instruments may measure a given sample in a different way; 6) appropriate RMs should be used for both accurate instrument calibration and for more general testing purposes (e.g., protocol validation); 7) there is a need to clarify that where RMs are not available, if "(representative) test materials" that lack reference or certified values may be useful for toxicology testing and 8) there is a need for consensus building within the nanotechnology and environmental, health and safety communities to prioritise RM needs and better define the required properties and (physical or chemical) forms of the candidate materials.
Nanospecific Guidance in REACH: A Comparative Physical-Chemical Characterization of 15 Materials with Methodical Correlations	W. Wohlleben1, L. Ma-Hock2, V. Boyko3, G. Cox3, H. Egenolf3, H. Freiberger4, B. Hinrichsen5, S. Hirth5, R. Landsiedel6 Journal of Cermiac Science and Technology, Vol.4, No.2, Pages 93-104 DOI: 10.4416/JCST2012-00045, 2013	The REACH legislation deals with the Registration, Evaluation, Authorization and Restriction of Chemical substances in Europe. Here we provide a comparative physical-chemical characterization with standardized methods that adhere to the recent REACH guidance for nanomaterials. The suite of materials comprises two pigment-size reference materials, and 13 engineered nanomaterials, thereof four from the OECD sponsorship program. We report for each material on 20 endpoints for physical-chemical properties. Since toxicological effects and occupational exposures have already been reported for several of these materials, our results provide the basis for the exploration of grouping approaches. On the methodical level, our results provide a cross-validation where several methods report the same endpoint. This comparison reveals redundancies in the guidance and allows us to identify the most effective methods.
Nanotechnology and Sun Care A Risk Review	Staniland P., Nanotechnology and Sun Care A Risk Review, H and PC vol. 8(2) March/April 2013, 18-23	In the past few years, the use of nanomaterials in cosmetic products has been the cause of much debate, and has instigated a wide range of scientific studies. Large amounts of data have been considered in forming educated opinions on the safety of nanoparticles such as ‘nano’ versions of titanium dioxide (TiO2) and zinc oxide (ZnO) in sun care product manufacture. The vast majority of the data in the studies still recognises that ultrafine TiO2 and ZnO are safe for use in sun care applications, and offer excellent protection against UV radiation. Recent published opinions have brought a final definition of nanomaterials in cosmetic products closer, though a number of areas require further clarification. This article aims to review the studies and publications which have led to the formation of the current definitions in Europe, and highlight areas such as experimental methodology which require further consideration if comparable results are to be reported.
Nanotoxicity: challenging the myth of nano-specific toxicity	Ken Donaldson and Craig A Poland	The analysis of nanoparticle (NP) hazard is currently a major research pre-occupation for particle toxicologists since there is a pressing requirement for a comprehensive understanding of

	Current Opinion in Biotechnology 2013, 24:724–734	nanoparticle hazard because of the wide spectrum of NP varying in composition, shape and size that require testing for risk assessment. The Biologically Effective Doses (BEDs) of nanoparticles, the dose entity that drives toxicity include charge, solubility, contaminants, shape and the ability to translocate from the site of deposition in the lungs. We point out here that all of these modes of toxicity are relevant and described for conventional pathogenic particles. There is no evidence that particles below 100 nm, the threshold definition of a NP, show any step-change in their hazard meaning that there is no evidence of novel 'nano-specific hazard'. Therefore conventional particle toxicology data are useful and relevant to the determination of the nanoparticle hazard. Emphasis away from 'nano-specific effects' and the availability of hazard data from conventional particles will focus limited resource towards a full understanding of the NP hazard. This will lead to improved ability to identify and test for their effects and measure their toxicokinetics and so contribute to their risk assessment.
Nanotoxicity: Challenging the myth of nano-specific toxicity	Donaldson, K., Poland, C.A. Current Opinion in Biotechnology Volume 24, Issue 4, August 2013, Pages 724-734	The analysis of nanoparticle (NP) hazard is currently a major research pre-occupation for particle toxicologists since there is a pressing requirement for a comprehensive understanding of nanoparticle hazard because of the wide spectrum of NP varying in composition, shape and size that require testing for risk assessment. The Biologically Effective Doses (BEDs) of nanoparticles, the dose entity that drives toxicity include charge, solubility, contaminants, shape and the ability to translocate from the site of deposition in the lungs. We point out here that all of these modes of toxicity are relevant and described for conventional pathogenic particles. There is no evidence that particles below 100 nm, the threshold definition of a NP, show any step-change in their hazard meaning that there is no evidence of novel 'nano-specific hazard'. Therefore conventional particle toxicology data are useful and relevant to the determination of the nanoparticle hazard. Emphasis away from 'nano-specific effects' and the availability of hazard data from conventional particles will focus limited resource towards a full understanding of the NP hazard. This will lead to improved ability to identify and test for their effects and measure their toxicokinetics and so contribute to their risk assessment.
On the challenge of quantifying man-made nanoparticles in the aquatic environment.	Howard AG. 2010, J. Environ. Monit. 12:135–42	Technologies based on nanomaterials are developing daily, finding applications as diverse as new sensors for improved monitoring and detection, new medical imaging techniques, novel approaches to the treatment and remediation of contaminated land and green technologies for chemical production. An inevitable consequence of Man's exploitation of nanotechnology is both the deliberate and accidental release of manufactured nanomaterials into the environment. This presents the analytical science community with a challenge for which it is, at present, poorly prepared--the quantification of specific nanoparticles in the environment. The problem is the development of trace analysis methods targeted at solid phase species, rather than the dissolved species measured, for example, in a typical pesticide residue analysis. This will require the adoption of radically different approaches and techniques, many of which will be unfamiliar to the conventionally trained environmental analyst. This paper sets out to give a very brief overview of the techniques that are available, specifically questioning their suitability for the quantification of man-made nanoparticles in the aquatic environment. Suggestions are

		made as to how these techniques might be transferred from the characterization of synthetic products to the field of trace analysis. The analytical community is presented with a new frontier of environmental investigation that can only commence with the development of innovative approaches to the quantitative measurement of man-made nanomaterials in the environment.
Physico-chemical features of engineered nanoparticles relevant to their toxicity	Bice Fubini, Mara Ghiazza and Ivana Fenoglio., Nanotoxicology Volume 4, Issue 4, December 2010, Pages 347-363	Nanotoxicology studies require investigations of several physico-chemical aspects of the particle/body fluid interaction, here described by reviewing recent literature in the light of new experimental data. Current characterization mostly covers morphology and metric-related characteristics (form, chemical composition, specific surface area, primary particle size and size distribution), and is mandatory in any experimental study. To unveil toxicity mechanisms, several other physico-chemical properties relevant to (geno) toxicity need to be assessed, typically the release or quenching of radical/ROS (Reactive Oxygen Species), the presence of active metal ions, evidence of structural defects. Major tasks for physical chemists working on nanoparticles-induced genotoxicity are described with some examples: (i), Tailored preparation of the same material in different sizes; (ii) particle modification changing a single property at a time; and (iii) identification of appropriate reference materials. Phenomena occurring during the contact between nanoparticles and cellular media or biological fluids (dispersion, agglomeration/aggregation, protein adsorption) are discussed in relation to the surface properties of the nanoparticles considered. © 2010 Informa UK, Ltd.
Quantitative analysis of fullerene nanomaterials in environmental systems: a critical review	Isaacson CW, Kleber M, Field JA. Environ. Sci. Technol. 43:6463–74, 2009	The increasing production and use of fullerene nanomaterials has led to calls for more information regarding the potential impacts that releases of these materials may have on human and environmental health. Fullerene nanomaterials, which are comprised of both fullerenes and surface-functionalized fullerenes, are used in electronic, optic, medical, and cosmetic applications. Measuring fullerene nanomaterial concentrations in natural environments is difficult because they exhibit a duality of physical and chemical characteristics as they transition from hydrophobic to polar forms upon exposure to water. In aqueous environments, this is expressed as their tendency to initially (i) self-assemble into aggregates of appreciable size and hydrophobicity, and subsequently (ii) interact with the surrounding water molecules and other chemical constituents in natural environments thereby acquiring negative surface charge. Fullerene nanomaterials may therefore deceive the application of any single analytical method that is applied with the assumption that fullerenes have but one defining characteristic (e.g., hydrophobicity). Our findings include the following: (1) Analytical procedures are needed to account for the potentially transitory nature of fullerenes in natural environments through the use of approaches that provide chemically explicit information including molecular weight and the number and identity of surface functional groups. (2) Sensitive and mass-selective detection, such as that offered by mass spectrometry when combined with optimized extraction procedures, offers the greatest potential to achieve this goal. (3) Significant improvements in analytical rigor would result from an increased availability of well characterized authentic standards, reference materials, and isotopically labeled internal standards. Finally, the

		benefits of quantitative and validated analytical methods for advancing the knowledge on fullerene occurrence, fate, and behaviour are indicated.
Quantitative gold nanoparticle analysis methods: a review.	Yu L, Andriola A. Talanta 82:869–75, 2010	Research and development in the area of gold nanoparticles' (AuNPs) preparation, characterization, and applications are burgeoning in recent years. Many of the techniques and protocols are very mature, but two major concerns are with the mass domestic production and the consumption of AuNP based products. First, how many AuNPs exist in a dispersion? Second, where are the AuNPs after digestion by the environment and how many are there? To answer these two questions, reliable and reproducible methods are needed to analyse the existence and the population of AuNP in samples. This review summarized the most recent chemical and particle quantitative analysis methods that have been used to characterize the concentration (in number of moles of gold per litre) or population (in number of particles per mL) of AuNPs. The methods summarized in this review include, mass spectroscopy, electroanalytical methods, spectroscopic methods, and particle counting methods. These methods may count the number of AuNP directly or analyse the total concentration of element gold in an AuNP dispersion.
Rapid and quantitative sizing of nanoparticles using three-dimensional single-particle tracking.	Xu CS, Cang H,Montiel D, Yang H. J. Phys. Chem. C 111:32–35, 2007	We report the first application of three-dimensional (3D) single-particle tracking (SPT) to hydrodynamic size characterization of gold nanoparticles in water. Nanoparticles undergoing Brownian motion were dynamically locked at the focal point of a microscope objective, one at a time, by rapid counteractive movements of the sample container. The hydrodynamic radius was derived from the recorded trajectory of each individual nanoparticle. The directly measured size and size distribution using 3D-SPT were in agreement with those obtained using the conventional dynamic light scattering (DLS) and using transmission electron microscopy (TEM), respectively.
Rat pulmonary responses to inhaled nano-TiO_2: effect of primary particle size and agglomeration state	Alexandra Noël, Michel Charbonneau, Yves Cloutier, Robert Tardif Particle and Fibre Toxicology, 10:48 doi:10.1186/1743-8977-10-48, 2013	Compared to the controls, bronchoalveolar lavage fluids (BALF) showed that LA aerosols induced an acute inflammatory response, characterized by a significant increase in the number of neutrophils, while SA aerosols produced significant oxidative stress damages and cytotoxicity. Data also demonstrate that for an agglomeration state smaller than 100 nm, the 5 nm particles caused a significant increase in cytotoxic effects compared to controls (assessed by an increase in LDH activity), while oxidative damage measured by 8-isoprostane concentration was less when compared to 10–30 and 50 nm particles. In both SA and LA aerosols, the 10–30 nm TiO2 NP size induced the most pronounced pro-inflammatory effects compared to controls. Overall, this study showed that initial NP size and agglomeration state are key determinants of nano-TiO2 lung inflammatory reaction, cytotoxic and oxidative stress induced effects.
Reactivity of inorganic nanoparticles in biological environments: insights into nanotoxicity mechanisms	E.Casals, E Gonzalez and V F Puntes, Phys. D: Appl. Phys. 45, 443001, 2012, doi:10.1088/0022-3727/45/44/443001	Topical Review A deeper understanding of the behaviour of inorganic nanoparticles in biological media is needed not only to fully control and develop the potential of these materials but also to increase knowledge of the physical chemistry of inorganic materials when their morphology approaches that of molecular entities. Although this knowledge and control is not yet entirely acquired, industry and society are already using nanomaterials in greater quantities and in consumer products. As normally happens when something new arrives in society, the interest in the

		broader implications of this emerging technology has grown together with unfounded 'nanoeuphoria' and 'nanoscares'. In this context, only by understanding the mechanisms of the nano–bio interaction will it be possible to safely develop nanotechnology. In this review, we discuss on how nanoparticles behave once they are naturally or intentionally produced and are exposed to humans and the environment. The response of nanoparticles inside organisms or released to the environment is complex and diverse, and depends on a variety of parameters involved. Mainly, they may (i) be aggregated into microscopic particles or embedded in exposed materials; (ii) the surfaces of the nanoparticles, which determine their bioactivity, experience constant modifications; and (iii) nanoparticles may corrode and dissolve or they can suffer morphological modifications.
Reference materials and representative test materials: the nanotechnology case	G. Roebben, K. Rasmussen, V. Kestens, T. P. J. Linsinger, H. Rauscher, H. Emons, H. Stamm, J. Nanoparticle Research, Vol. 15, citation ID 1455, DOI 10.1007/s11051-013-1455-2 (2013).	An increasing number of chemical, physical and biological tests are performed on manufactured nanomaterials for scientific and regulatory purposes. Existing test guidelines and measurement methods are not always directly applicable to or relevant for nanomaterials. Therefore, it is necessary to verify the use of the existing methods with nanomaterials, thereby identifying where modifications are needed, and where new methods need to be developed and validated. Efforts for verification, development and validation of methods as well as quality assurance of (routine) test results significantly benefit from the availability of suitable test and reference materials. This paper provides an overview of the existing types of reference materials and introduces a new class of test materials for which the term 'representative test material' is proposed. The three generic concepts of *certified reference material*, *reference material (non-certified)* and *representative test material* constitute a comprehensive system of benchmarks that can be used by all measurement and testing communities, regardless of their specific discipline. This paper illustrates this system with examples from the field of nanomaterials, including reference materials and representative test materials developed at the European Commission's Joint Research Centre, in particular at the Institute for Reference Materials and Measurements (IRMM), and at the Institute for Health and Consumer Protection (IHCP).
Reference materials for measuring the size of nanoparticles	T. P. J. Linsinger, G. Roebben, C. Solans, R. Ramsch, Trends in Analytical Chemistry, Vol. 30, p. 18-27 (2011)	This article discusses the requirements for reference materials (RMs) for measuring the size of nanoparticles (NPs). Such RMs can be used for instrument calibration, statistical quality control or interlaboratory comparisons. They can come in the form of suspensions, powders or matrix-embedded materials [i.e. NPs integrated in a natural matrix (e.g., food, soil, or sludge)]. At present, uncertainty about the most suitable form of material, the most relevant measurands and the most useful metrological-traceability statement inhibits the production of NP RMs. In addition, the lack of validated methods and qualified laboratories to produce NP RMs present formidable challenges. Metal, inorganic and organic NPs are available, but most of them are intended to be laboratory chemicals. With the exception of latex materials, certified RMs are not available, although some metrology institutes have started to develop such materials for colloidal gold and silica particles.

Review of carbon nanotubes toxicity and exposure – assessment of the feasibility and challenges for human health risk assessment based on open literature	Aschberger, K., Johnston, H.J., Stone, V., Aitken, R.J., Tran, C.L., Hankin, S.M., Peters, S.A.K., Christensen, F.M. 2010, Crit Rev Toxicol, vol. 40, no.9, pp. 759-790. doi:10.3109/10408444.2010.506638	In our first feature article for summer 2011, SAFENANO's Associate Editor Sheona Peters provides an overview of a series of recently published articles that assess the feasibility and challenges of conducting a human health risk assessment for four different types of nanomaterials: carbon nanotubes, titanium dioxide nanoparticles, silver nanoparticles and carbon fullerenes.
Separation and characterization of nanoparticles in complex food and environmental samples by field-flow fractionation	von der Kammer F, Legros S, Larsen EH, Loeschner K, Hofmann T., Trends Anal. Chem. 30:425–36, 2011	The thorough analysis of natural nanoparticles (NPs) and engineered NPs involves the sequence of detection, identification, quantification and, if possible, detailed characterization. In a complex or heterogeneous sample, each step of this sequence is an individual challenge, and, given suitable sample preparation, field-flow fractionation (FFF) is one of the most promising techniques to achieve relevant characterization. The objective of this review is to present the current status of FFF as an analytical separation technique for the study of NPs in complex food and environmental samples. FFF has been applied for separation of various types of NP (e.g., organic macromolecules, and carbonaceous or inorganic NPs) in different types of media (e.g., natural waters, soil extracts or food samples). FFF can be coupled to different types of detectors that offer additional information and specificity, and the determination of size-dependent properties typically inaccessible to other techniques. The separation conditions need to be carefully adapted to account for specific particle properties, so quantitative analysis of heterogeneous or complex samples is difficult as soon as matrix constituents in the samples require contradictory separation conditions. The potential of FFF analysis should always be evaluated bearing in mind the impact of the necessary sample preparation, the information that can be retrieved from the chosen detection systems and the influence of the chosen separation conditions on all types of NP in the sample. A holistic methodological approach is preferable to a technique-focused one.
Size characterization of bentonite colloids by different methods.	Plaschke M, Schafer T, Bundschuh T, Manh TN, Knopp R, et al., Anal. Chem. 73:4338–47, 2001.	The size and shape of colloids released from a natural bentonite into a low-mineralized groundwater are investigated using various colloid characterization methods. For the applied methods such as atomic force microscopy (AFM), laser-induced breakdown detection (LIBD), photon correlation spectroscopy (PCS), and flow field-flow fractionation coupled to ICP-mass spectrometric detection (FFFF-ICPMS), the respective raw size data have to be corrected in order to consider chemical composition and shape of the colloids as well as instrumental artifacts. Noncontact mode AFM of the bentonite colloids shows disk-like shapes of stacked smectite platelets with a mean height-to-diameter proportion (aspect ratio) of ~1/10. A broad particle number size distribution is determined by image processing with a mean particle diameter of 73 nm. In agreement with AFM, a broad size distribution is also found by PCS and FFFF-ICPMS. Likewise, mean particle sizes found by LIBD (67 ± 13 nm) and FFFF-ICPMS (maximum in the number size distribution, ~70 nm) are in fair agreement with the AFM data. Somewhat higher values are obtained by PCS, where mean particle diameters of the intensity-weighted size distributions of larger than 200 nm are found (depending on the algorithm used

		for data processing). The influence of the disk-like particle shape on the results of the individual methods is discussed. As a conclusion, the application of different colloid characterization methods is a prerequisite to get complementary information about colloid size and shape, which is essential for the understanding of natural colloidal systems.
Size evaluation of gold nanoparticles by UV-vis spectroscopy.	Amendola V, Meneghetti M., J. Phys. Chem. C 113:4277–85, 2009	We present a method for the evaluation of the average size of gold nanoparticles based on the fitting of their UV−vis spectra by the Mie model for spheres. The method gives good results using a calibration of the dumping frequency of the surface plasmon resonance and accounting for the presence of nonspherical AuNP in solution by the Gans model for spheroids. It has been successfully applied to free and functionalized gold nanoparticles in various solvents with diameters in the 4−25 nm range. Despite the differences among samples, we found an accuracy of about 6% on the nanoparticles average size with respect to sizes measured by transmission electron microscopy (TEM). Moreover, the fitting model provides other information not available from TEM like the concentration of AuNP in the sample and the fraction of nonspherical nanoparticles, which is particularly useful for measuring aggregation processes. The fitting procedure and models are thoroughly discussed in the text, and the fitting programs are freely accessible on the web.
Size-dependent toxicity of metal oxide particles—A comparison between nano- and micrometer size	Hanna L. Karlsson, Johanna Gustafsson, Pontus Cronholm, Lennart Möller Toxicology Letters, Volume 188, Issue 2, 24 July 2009, Pages 112–118,	Toxicological studies have shown increased toxicity of nanoparticles (<100 nm) compared to micrometer particles of the same composition, which has raised concern about the impact on human health from nanoparticles. However, if this is true for a wide range of particles with different chemical composition is not clear. The aim of this study was to compare the toxicity of nano- and micrometer particles of some metal oxides (Fe_2O_3, Fe_3O_4, TiO_2 and CuO). The ability of the particles to cause cell death, mitochondrial damage, DNA damage and oxidative DNA lesions were evaluated after exposure of the human cell line A549. This study showed that nanoparticles of CuO were much more toxic compared to CuO micrometer particles. One key mechanism may be the ability of CuO to damage the mitochondria. In contrast, the micrometer particles of TiO2 caused more DNA damage compared to the nanoparticles, which is likely explained by the crystal structures. The iron oxides showed low toxicity and no clear difference between the different particle sizes. In conclusion, nanoparticles are not always more toxic than micrometer particles, but the high toxicity of CuO nanoparticles shows that the nanolevel gives rise to specific concern.
Size distribution of nanoparticles by dynamic light scattering. Comparison of Bayesian and Tikhonov inversion methods	Clementi, L.A. Vega, J.R., Orlande, H.R.B., Gugliotta, L.M. Inverse Problems in Science and Engineering Volume 20, Issue 7, October 2012, Pages 973-990	The diameter distribution of nanometric particles is estimated from multiangle dynamic light scattering (MDLS) measurements by solving an ill-conditioned nonlinear inverse problem through Tikhonov and Bayesian methods. For both methods, the data inputs are the angle-dependent average diameters of the particle size distribution (PSD), which are in turn calculated from the measured autocorrelation functions of the light intensity scattered by a dilute sample of particles. The performances of both methods were tested on the basis of: (i) two simulated polymer latexes that involved PSDs of different shapes, widths and diameter ranges; and (ii) two real polystyrene latexes obtained by mixing two well-characterized standards of narrow PSDs (of known nominal diameters and standard deviations). For PSDs exhibiting highly asymmetric

		modes, or modes of quite different relative concentrations, the Bayesian method produced PSD estimates better than those obtained through Tikhonov regularization.
Testing metal-oxide nanomaterials for human safety	Landsiedel, R., Ma-Hock, L., Kroll, A., Hahn, D., Schnekenburger, J., Wiench, K., Wohlleben, W. Advanced Materials Volume 22, Issue 24, 25 June 2010, Pages 2601-2627	Nanomaterials can display distinct biological effects compared with bulk materials of the same chemical composition. The physico-chemical characterization of nanomaterials and their interaction with biological media are essential for reliable studies and are reviewed here with a focus on widely used metal oxide and carbon nanomaterials. Available rat inhalation and cell culture studies compared to original results suggest that hazard potential is not determined by a single physico-chemical property but instead depends on a combination of material properties. Reactive oxygen species generation, fibre shape, size, solubility and crystalline phase are known indicators of nanomaterials biological impact. According to these properties the summarized hazard potential decreases in the order multi-walled carbon nanotubes $\gg$ CeO_2, ZnO > TiO_2 > functionalized SiO_2 > SiO_2, ZrO_2, carbon black. Enhanced understanding of biophysical properties and cellular effects results in improved testing strategies and enables the selection and production of safe materials.
The biological mechanisms and physicochemical characteristics responsible for driving fullerene toxicity	Johnston, H.J., Hutchison, G.R., Christensen, F.M., Aschberger, K., Stone, V. 2010, Toxicol Sci, vol. 114, no. 2, pp. 162-182. doi:10.1093/toxsci/kfp265	This review provides a comprehensive critical review of the available literature purporting to assess the toxicity of carbon fullerenes. This is required as prior to the widespread utilization and production of fullerenes, it is necessary to consider the implications of exposure for human health. Traditionally, fullerenes are formed from 60 carbon atoms, arranged in a spherical cage-like structure. However, manipulation of surface chemistry and molecular makeup has created a diverse population of fullerenes, which exhibit drastically different behaviours. The cellular processes that underlie observed fullerene toxicity will be discussed and include oxidative, genotoxic, and cytotoxic responses. The antioxidant/cytoprotective properties of fullerenes (and the attributes responsible for driving these phenomena) have been considered and encourage their utilization within the treatment of oxidant-mediated disease. A number of studies have focused on improving the water solubility of fullerenes in order to enable their exploitation within biological systems. Manipulating fullerene water solubility has included the use of surface modifications, solvents, extended stirring, and mechanical processes. However, the ability of these processes to also impact on fullerene toxicity requires assessment, especially when considering the use of solvents, which particularly appear to enhance fullerene toxicity. A number of the discussed investigations were not conducted to reveal if fullerene behaviour was due to their nanoparticle dimensions but instead addressed the biocompatibility and toxicity of fullerenes. The hazards to human health, associated with fullerene exposure, are uncertain at this time, and further investigations are required to decipher such effects before an effective risk assessment can be conducted.
The Challenge To Relate the Physicochemical Properties of Colloidal Nanoparticles to Their Cytotoxicity	Pilar Rivera-Gil, Dorleta Jimenez De Aberasturi, Verena Wulf, Beatriz Pelaz, Pablo Del Pino, Yuanyuan Zhao, Jesus M. De La Fuente, Idoia Ruiz De Larramendi,	In this Account we describe the physicochemical properties of nanoparticles (NPs) and how they can be determined and discuss their general importance for cytotoxicity. For simplicity, we focus primarily on in vitro toxicology that examines the interaction of living cells with engineered colloidal NPs with an inorganic core. Serious risk assessment of NPs will require additional in vivo studies. Basic physicochemical properties of nanoparticulate materials include colloidal

	Te_Ofilo Rojo, Xing-Jie Liang and Wolfgang J. Parak, Accounts Of Chemical Research, Vol. 46, No. 3 ’ 2013 ’ 743–749 ’	stability, purity, inertness, size, shape, charge, and their ability to adsorb environmental compounds such as proteins. Unfortunately, the correlation of these properties with toxicity is not straightforward. First, for NPs released either unintentionally or intentionally, it can be difficult to pinpoint these properties in the materials. Therefore, researchers typically use NP models with better defined properties, which don't include the full complexity of most industrially relevant materials. In addition, many of these properties are strongly mutually connected. Therefore, it can be difficult to vary individual properties in NP models while keeping the others constant.
The current state of engineered nanomaterials in consumer goods and waste streams: The need to develop nanoproperty-quantifiable sensors for monitoring engineered nanomaterials	Wise, K., Brasuel, M., Nanotechnology, Science and Applications Volume 4, Issue 1, 2011, Pages 73-86	Review. As nanomaterials are harnessed for medicine and other technological advances, an understanding of the toxicology of these new materials is required to inform our use. This toxicological knowledge will be required to establish the medical and environmental regulations required to protect consumers and those involved in nanomaterial manufacturing. Nanoparticles of titanium oxide, carbon nanotubes, semiconductor quantum dots, gold, and silver represent a high percentage of the nanotechnology currently available or currently poised to reach consumers. For these nanoparticles, this review aims to identify current applications, the current methods used for characterization and quantification, current environmental concentrations (if known), and an introduction to the toxicology research. Continued development of analytical tools for the characterization and quantification of nanomaterials in complex environmental and biological samples will be required for our understanding of the toxicology and environmental impact of nanomaterials. Nearly all materials exhibit toxicity at a high enough concentration. Robust, rapid, and cost effective analytical techniques will be required to determine current background levels of anthropogenic, accidental, and engineered nanoparticles in air, water, and soil. The impact of the growing number of engineered nanoparticles used in consumer goods and medical applications can then be estimated. This will allow toxicological profiles relevant to the demonstrated or predicted environmental concentrations to be determined.
The cytotoxic activity of amorphous silica nanoparticles is mainly influenced by surface area and not by aggregation.	Virginie Rabolli, Leen C.J. Thomassen, Francine Uwambayinema, Johan A. Martens, Dominique Lison, Toxicology Letters, Volume 206, Issue 2, 10 October 2011, Pages 197-203	The aggregation state of NP has been a significant source of difficulty for assessing their toxic activity and great efforts have been done to reduce aggregation of and/or to disperse NP in experimental systems. The exact impact of aggregation on toxicity has, however, not been adequately assessed. Here we compared in vitro the cytotoxic activity of stable monodisperse and aggregated silicon-based nanoparticles (SNP) without introducing a dispersing agent that may affect NP properties. SNP aggregates (180nm) were produced by controlled electrostatic aggregation through addition of KCl to a Ludox SM sol (25nm) followed by stabilization and extensive dialysis. The size of the preparations was characterized by TEM and DLS; specific surface area and porosity were derived from N 2 sorption measurements. Macrophage (J774) and fibroblast (3T3) cell lines were exposed to monodisperse or aggregate-enriched suspensions of SNP in DMEM in absence of serum. The cytotoxic activity of the different preparations was assessed by the WST1 assay after 24h of exposure. Parameters that determined the cytotoxic

		activity were traced by comparing the doses of the different preparations that induced half a maximal reduction in WST1 activity (ED 50) in both cell lines. We found that ED 50 (6-9µg/ml and 15-22µg/ml, in J774 and 3T3, respectively) were hardly affected upon aggregation, which was consistent with the fact that the specific surface area of the SNP, a significant determinant of their cytotoxic activity, was unaffected upon aggregation (283-331m 2/g). Thus studying small aggregated NP could be as relevant as studying disperse primary NP, when aggregates keep the characteristics of NP, i.e. a high specific surface area and a nanosize dimension. This conclusion does, however, not necessarily hold true for other toxicity endpoints for which the determinants may be different and possibly modified by the aggregation process.
The nanosilica hazard: Another variable entity	Dorota Napierska, Leen CJ Thomassen, Dominique Lison, Johan A Martens and Peter H Hoet Particle and Fibre Toxicology 2010, 7:39 doi:10.1186/1743-8977-7-39	Silica nanoparticles (SNPs) are produced on an industrial scale and are an addition to a growing number of commercial products. SNPs also have great potential for a variety of diagnostic and therapeutic applications in medicine. Contrary to the well-studied crystalline micron-sized silica, relatively little information exists on the toxicity of its amorphous and nano-size forms. Because nanoparticles possess novel properties, kinetics and unusual bioactivity, their potential biological effects may differ greatly from those of micron-size bulk materials. In this review, we summarize the physico-chemical properties of the different nano-sized silica materials that can affect their interaction with biological systems, with a specific emphasis on inhalation exposure. We discuss recent in vitro and in vivo investigations into the toxicity of nanosilica, both crystalline and amorphous. Most of the in vitro studies of SNPs report results of cellular uptake, size- and dose-dependent cytotoxicity, increased reactive oxygen species levels and pro-inflammatory stimulation. Evidence from a limited number of in vivo studies demonstrates largely reversible lung inflammation, granuloma formation and focal emphysema, with no progressive lung fibrosis. Clearly, more research with standardized materials is needed to enable comparison of experimental data for the different forms of nanosilicas and to establish which physico-chemical properties are responsible for the observed toxicity of SNPs.
The new toxicology of sophisticated materials: nanotoxicology and beyond.	Maynard, A., Warheit, D.B. & Philbert, M.A. 2011.120: S109-S129. Toxicological Sciences	Silica nanoparticles (SNPs) are produced on an industrial scale and are an addition to a growing number of commercial products. SNPs also have great potential for a variety of diagnostic and therapeutic applications in medicine. Contrary to the well-studied crystalline micron-sized silica, relatively little information exists on the toxicity of its amorphous and nano-size forms. Because nanoparticles possess novel properties, kinetics and unusual bioactivity, their potential biological effects may differ greatly from those of micron-size bulk materials. In this review, we summarize the physico-chemical properties of the different nano-sized silica materials that can affect their interaction with biological systems, with a specific emphasis on inhalation exposure. We discuss recent in vitro and in vivo investigations into the toxicity of nanosilica, both crystalline and amorphous. Most of the in vitro studies of SNPs report results of cellular uptake, size- and dose-dependent cytotoxicity, increased reactive oxygen species levels and pro-inflammatory stimulation. Evidence from a limited number of in vivo studies demonstrates largely reversible lung inflammation, granuloma formation and focal emphysema, with no progressive lung fibrosis. Clearly, more research with standardized materials is needed to enable

		comparison of experimental data for the different forms of nanosilicas and to establish which physico-chemical properties are responsible for the observed toxicity of SNPs.
The problem of regulating sophisticated nanomaterials.	Maynard, A., Bowman, D. & Hodge, G. 2011., Nature Materials 10: 554–57.	
The Role of Surface Functionality in Determining Nanoparticle Cytotoxicity	Sung Tae Kim, Krishnendu Saha, Chaekyu Kim, And Vincent M. Rotello,	In this Account, we discuss our research and that of others into how NP surface properties control interactions with biomolecules and cells at many scales, including the role the particle surface plays in determining in vivo behaviour of nanomaterials. These interactions can be benign, beneficial, or lead to dysfunction in proteins, genes and cells, resulting in cytotoxic and genotoxic responses. Understanding these interactions and their consequences helps us to design minimally invasive imaging and delivery agents.
Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective.	Auffan M, Rose J, Bottero JY, Lowry GV, Jolivet JP, Wiesner MR. 2009. Nat. Nanotechnol. 4:634–41	The regulation of engineered nanoparticles requires a widely agreed definition of such particles. Nanoparticles are routinely defined as particles with sizes between about 1 and 100 nm that show properties that are not found in bulk samples of the same material. Here we argue that evidence for novel size-dependent properties alone, rather than particle size, should be the primary criterion in any definition of nanoparticles when making decisions about their regulation for environmental, health and safety reasons. We review the size-dependent properties of a variety of inorganic nanoparticles and find that particles larger than about 30 nm do not in general show properties that would require regulatory scrutiny beyond that required for their bulk counterparts
Toxico-/biokinetics of nanomaterials (Review)	Archives of Toxicology Volume 86, Issue 7, July 2012, Pages 1021-1060 Landsiedel, R., Fabian, E., Ma-Hock, L., Wohlleben, W., Wiench, K., Oesch, F.de, Van Ravenzwaay, B.	Nanomaterials (NM) offer great technological advantages but their risks to human health are still under discussion. For toxicological testing and evaluation, information on the toxicokinetics of NM is essential as it is different from that of most other xenobiotics. This review provides an overview on the toxicokinetics of NM available to date. The toxicokinetics of NM depends on particle size and shape, protein binding, agglomeration, hydrophobicity, surface charge and protein binding. In most studies with topical skin application, unintentional permeation and systemic availability were not observed; permeation for some NM with distinct properties was observed in animals. Upon inhalation, low levels of primary model nanoparticles became systemically available, but many real-world engineered NM aggregate in aerosols, do not disintegrate in the lung, and do not become systemically available. NM are prone to lymphatic transport, and many NM are taken up by the mononuclear phagocyte system (MPS) acting as a depot. Their half-life in blood depends on their uptake by MPS rather than their elimination from the body. NMs reaching the GI tract are excreted with the faeces, but of some NM low levels are absorbed and become systemically available. Some quantum dots were not observably excreted in urine or in faeces. Some model quantum dots, however, were efficiently excreted by the kidneys below, but not above 5-6 nm hydrodynamic diameter, while nanotubes 20-30 nm thick and 500-2,000 nm long were abundant in urine. NM are typically not metabolized. Some NM cross the blood- brain barrier favoured by a negative surface charge
Ultrasonic dispersion of	Taurozzi JS, Hackley VA, Wiesner	Studies designed to investigate the environmental or biological interactions of nanoscale

nanoparticles for environmental, health and safety assessment issues and recommendations.	MR. 2011. Nanotoxicology 5:711729	materials frequently rely on the use of ultrasound (sonication) to prepare test suspensions. However, the inconsistent application of ultrasonic treatment across laboratories, and the lack of process standardization can lead to significant variability in suspension characteristics. At present, there is widespread recognition that sonication must be applied judiciously and reported in a consistent manner that is quantifiable and reproducible; current reporting practices generally lack these attributes. The objectives of the present work were to: (i) Survey potential sonication effects that can alter the physicochemical or biological properties of dispersed nanomaterials (within the context of toxicity testing) and discuss methods to mitigate these effects, (ii) propose a method for standardizing the measurement of sonication power, and (iii) offer a set of reporting guidelines to facilitate the reproducibility of studies involving engineered nanoparticle suspensions obtained via sonication.
Validation of dynamic light scattering and centrifugal liquid sedimentation methods for nanoparticle characterisation	O. Couteau, J. Charoud-Got, H. Rauscher, F. Franchini, F. Rossi, V. Kestens, K. Franks, G. Roebben, Particle and particle systems characterization, Vol. 27, p. 112-124 (2010)	A variety of techniques exists to analyse the size and size distribution of nanoparticles in a suspension. However, these nanoparticle characterisation methods have been rarely fully validated and appropriate reference materials with properly assigned SI traceable values are not easily found. This paper presents results of in-house validation studies of Dynamic Light Scattering (DLS) and Centrifugal Liquid Sedimentation (CLS) methods. During these studies, a silica nanoparticle reference material was tested under repeatability and intermediate precision conditions. The trueness of the DLS and CLS methods was investigated by measuring gold and polystyrene nanoparticle reference materials. Furthermore, for each method, an uncertainty budget has been established. Both method validation and estimation of reliable measurement uncertainties are prerequisites for the certification of new nanoparticle reference materials.
Validation of methods for the detection and quantification of engineered nanoparticles in food.	T.P.J. Linsinger, Q. Chaudhry, V. Dehalu, P. Delahaut, A. Dudkiewicz, R. Grombe, F. von der Kammer, E.H. Larsen, S. Legros, K. Loeschner, R. Peters, R. Ramsch g, G. Roebben, K. Tiede, S. Weigel, Food Chemistry, Vol. 138, p. 1959-1966 (2013)	The potential impact of nanomaterials on the environment and on human health has already triggered legislation requiring labelling of products containing nanoparticles. However, so far, no validated analytical methods for the implementation of this legislation exist. This paper outlines a generic approach for the validation of methods for detection and quantification of nanoparticles in food samples. It proposes validation of identity, selectivity, precision, working range, limit of detection and robustness, bearing in mind that each "result" must include information about the chemical identity, particle size and mass or particle number concentration. This has an impact on testing for selectivity and trueness, which also must take these aspects into consideration. Selectivity must not only be tested against matrix constituents and other nanoparticles, but it shall also be tested whether the methods apply equally well to particles of different suppliers. In trueness testing, information whether the particle size distribution has changed during analysis is required. Results are largely expected to follow normal distributions due to the expected high number of particles. An approach of estimating measurement uncertainties from the validation data is given.
Validity range of centrifuges for the regulation of nanomaterials: From classification to as-tested coronas	Wohlleben, W. Journal of Nanoparticle Research Volume 14, Issue 12, 2012, Article number1300	Granulometry is the regulatory category where the differences between traditional materials and nanomaterials culminate. Reported herein is a careful validation of methods for the quantification of dispersability and size distribution in relevant media, and for the classification according to the EC nanodefinition recommendation. Suspension-based techniques can assess

		the nanodefinition only if the material in question is reasonably well dispersed. Using dispersed material of several chemical compositions (organic, metal, metal-oxide) as test cases we benchmark analytical ultracentrifugation (AUC), dynamic light scattering (DLS), hydrodynamic chromatography, nanoparticle tracking analysis (NTA) against the known content of bimodal suspensions in the commercially relevant range between 20 nm and a few microns. The results validate fractionating techniques, especially AUC, which successfully identifies any dispersed nanoparticle content from 14 to 99.9 nb% with less than 5 nb% deviation. In contrast, our screening casts severe doubt over the reliability of ensemble (scattering) techniques and highlights the potential of NTA to develop into a counting upgrade of DLS. The unique asset of centrifuges with interference, X-ray or absorption detectors-to quantify the dispersed solid content for each size interval from proteins over individualized nanoparticles up to agglomerates, while accounting for their loose packing- addresses also the adsorption/depletion of proteins and (de-)agglomeration of nanomaterials under cell culture conditions as tested for toxicological endpoints.

Annex 8.3: List of relevant grey literature (reports/opinions)

Title	Ref.	Abstract/comment
Adverse Effects of Engineered Nanomaterials EXPOSURE, TOXICOLOGY, AND IMPACT ON HUMAN HEALTH	Edited by: Bengt Fadeel, Antonio Pietroiusti and Anna Shvedova ISBN: 978-0-12-386940-1, Elsevier, 2012	Critical review of the toxicity tests for nanomaterials
Certification of the equivalent spherical diameters of silica nanoparticles in aqueous solution.	ERM-FD304, K. Franks, A. Braun, J. Charoud-Got, O. Couteau, V. Kestens, A. Lamberty, T. Linsinger, G. Roebben, Report EUR 25018 EN, European Union, Luxembourg, ISBN 978-92-79-21867-5, 2012.	This report describes the certification of several equivalent spherical diameters of silica nanoparticles in aqueous solution, Certified Reference Material (CRM) ERM-FD304. The CRM has been certified by the European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE.
Certification of equivalent spherical diameters of silica nanoparticles in water.	ERM-FD100, A. Braun, K. Franks, V. Kestens, G. Roebben, A. Lamberty, T. Linsinger, Report EUR 24620 EN, European Union, Luxembourg, ISBN 978-92-79-18676-9, 2011.	This report describes the certification of the equivalent spherical diameters of silica nanoparticles suspended in aqueous solution, Certified Reference Material (CRM) ERM-FD100®. The CRM has been certified by the European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, BE. The intended use of this ERM-FD100 is to check the performance of instruments and methods that determine the particle diameter of nanoparticles (particle size ranging from approximately 1 nm to approximately 100 nm) suspended in a liquid medium. It is available in 10 mL pre-scored amber glass ampoules containing approximately 9 mL of suspension. The CRM was prepared from commercially available colloidal silica (Koestrosol 1530, Chemiewerk Bad Koestritz GmbH, DE). Certification of the CRM included testing of the homogeneity and stability of the ampouled diluted raw material, as well as the characterisation using an intercomparison approach. The material has been certified for the equivalent diameter of the silica nanoparticles in aqueous suspension using different methods. Certified values are the cumulants dynamic light scattering (DLS) intensity-weighted harmonic mean particle diameter, the line-start centrifugal liquid sedimentation (CLS) intensity-based modal (Stokes) particle diameter, the electron microscopic (transmission electron microscopy (TEM)/ scanning electron microscopy (SEM)) number-based modal particle diameter and the small angle X-ray scattering (SAXS) intensity-weighted average particle diameter. Indicative values have been established for the volume-weighted mean equivalent spherical diameter via the SAXS method and for the zeta potential via the electrophoretic mobility (ELM) method. Additional informational values are given for the volume-weighted mean diameter via the DLS method, and the pH value of the ERM-FD100 suspension. Uncertainties are expanded uncertainties estimated in

		accordance with the Guide to the expression of uncertainty in measurement (GUM) with a coverage factor of k = 2, corresponding to a confidence interval of about 95 %. An exception is the mean equivalent volume-weighted diameter determined by the SAXS method which has a coverage factor of 2.8
Comparative assessment of nanomaterial definitions and considerations for implementation	R.M. David, D.R. Boverhof, J.H. Butala, S. Clancy4, M. Lafranconi, C.M. Bramante, W.J. West http://nanotechnology.americanchemistry.com/Nanotechnology/Panel-Activities/Nanotechnology-Definitions/Nanotechnology-Panel-Presents-at-Society-of-Toxicology.pdf	The Nanotechnology Panel advocates for a regulatory definition of "nanomaterial" that clearly identifies materials of interest. Many regulatory bodies around the world have separately developed definitions of "nanomaterial" to identify materials of potential interest, resulting in inconsistent definitions among regulatory organizations. Such inconsistency complicates the regulatory process by impeding the ability of governments to share comparable information and increases compliance costs for industry. On March 13, 2013, the Nanotechnology Panel presented a poster titled Comparative assessment of nanomaterial definitions and considerations for implementation at the 52nd Annual Meeting of the Society of Toxicology. The panel conducted a comparative assessment of regulatory definitions of nanomaterials and identified inconsistencies that may impede communication and increase regulatory compliance costs. The panel makes recommendations concerning key properties that should be considered in any regulatory definition. In the poster, the Panel suggests the following core elements in a regulatory definition of "nanomaterial": Solid, particulate substances; Intentionally manufactured at the nanoscale; Consisting of "nano-objects" as defined by the International Organization for Standardization (ISO) but without the word "approximately" to describe the size range; A weight-based cut-off for ISO-defined nanomaterial content; Consideration of aggregates and agglomerates of nanomaterials; and, Exclusion of aggregates and agglomerates if they cannot be readily broken down into nano-objects. These elements can be used as a first step to identify "nanomaterials" that may be of regulatory interest. These recommendations are designed to reduce ambiguity and confusion so stakeholders can have a clear understanding of how to comply with regulation. Further evaluation of materials identified by this definition will involve consideration of hazard, use patterns, and potential human and environmental exposures on a case-by-case basis.
Concern-driven integrated approaches to nanomaterial testing and assessment – report of the NanoSafety Cluster Working Group 10	Nanotoxicology, 2013; Early Online, 1–15, ISSN: 1743-5390 print / 1743-5404 DOI: 10.3109/17435390.2013.802387	The outcome of NanoSafety Cluster Working Group 10, this commentary presents a vision for concern-driven integrated approaches for the (eco-)toxicological testing and assessment (IATA) of NM. Such approaches should start out by determining concerns, i.e., specific information needs for a given NM based on realistic exposure scenarios. Recognised concerns can be addressed in a set of tiers using standardised protocols for NM preparation and testing. Tier 1 includes determining physico-chemical properties, nontesting (e.g. structure–activity relationships) and evaluating existing data. In tier 2, a limited set of in vitro and in vivo tests are performed that can either indicate that the risk of the specific concern is sufficiently known or indicate the need for further testing, including details for such testing. Ecotoxicological testing begins with representative test

		organisms followed by complex test systems. After each tier, it is evaluated whether the information gained permits assessing the safety of the NM so that further testing can be waived. By effectively exploiting all available information, IATA allow accelerating the risk assessment process and reducing testing costs and animal use (in line with the 3Rs principle implemented in EU Directive 2010/63/EU). Combining material properties, exposure, biokinetics and hazard data, information gained with IATA can be used to recognise groups of NM based upon similar modes of action. Grouping of substances in return should form integral part of the IATA themselves.
Considerations on a definition of nanomaterial for regulatory purposes.	G. Lövestam, H. Rauscher, G. Roebben, B. Sokull-Klüttgen, N. Gibson, J.-Ph. Putaud, H. Stamm, JRC Reference Report, EUR 24403 EN, European Union, Luxembourg, ISBN 978-92-79-16014-1, 2010.	The aim of this report is to review and discuss issues and challenges related to a definition of 'nanomaterial', and to provide practical guidance for a definition for regulatory purposes. The report suggests that a definition for regulatory purposes should: • only concern particulate nanomaterials, • be broadly applicable in EU legislation, and in line with other approaches worldwide, • use size as the only defining property. This calls both for a clarification of the meaning of the word 'material' and a clear definition of the nanoscale limits. Enforceability of the definition requires the adoption of instructions on how such limits can be applied for nanoscale materials with size distributions. Size-derived properties, nanoscale materials incorporated in a matrix and the origin of the material are also points that should be considered. It is clear that any definition will have implications within the context in which it is used and may need adaptation for specific regulations or directives. It should therefore be emphasised that adoption of a definition will also involve policy choices, and accordingly will entail political decisions.
Engineered Nanoparticles: Nanometrology Status and Future Needs within Europe.	M. Stintz, F. Babick, G. Roebben, Co-Nanomet Consortium, ISBN: X, 2011	Engineered nanoparticles (ENPs) are particles designed and produced to have all external dimensions in the nanoscale (between approximately 1 nm and 100 nm). To profit fully from the potential of ENPs and to responsibly deal with the HSE concerns requires the development of scientifically sound classification methods for nanoparticles, distinguishing them in terms of their production method, but also in terms of their basic physico-chemical characteristics and properties. In this report, Co-Nanomet has addressed the related measurement issues with the purpose of identifying and promoting metrology solutions for both airborne and suspended ENPs.
Environmental, Health, and Safety Research Strategy	Report, National Nanotechnology Initiative 2011, National Science and Technology Council Committee on Technology, http://nano.gov/node/681	Nanotechnology safety benefits everyone, from lab researchers and factory workers to the consumers of products enabled by this emerging technology. Accordingly, the Federal Government has developed the 2011 NNI Environmental, Health, and Safety (EHS) Research Strategy, a comprehensive approach to ensuring the safe, effective, and responsible development and use of nanotechnology. The NNI 2011 EHS Research Strategy provides guidance to the Federal agencies that produce the scientific information for risk management, regulatory decision-making, product use, research planning, and public outreach. The core research areas providing this critical information are (1) Nanomaterial Measurement Infrastructure, (2) Human Exposure Assessment, (3) Human Health, (4) Environment, (5) Risk Assessment and Risk Management Methods, and (6) Informatics and Modelling. Consideration of ethical, legal, and societal implications (ELSI) of nanotechnology were also woven into the strategy.

European Consultation on Metrological Traceability, Standards and Dissemination of Metrology in Industrial Nanotechnology.	L. Pendrill, O. Flys, K. Dirscherl, G. Roebben, Co-Nanomet Consortium, ISBN: 978-0-9566809-8-3, 2011.	In support of sustainable growth in the nanomanufacturing industry, a CO-NANOMET [EU FP7 CSA-CA 218764] consultation of both the means and content of the dissemination of metrology to the workplace has been made during 2010. Programmes encouraging industry to innovate by exploiting nanometrology through local networking and through various documentary standards (i.e. written standards, i.e. norms) are found to be promising means of disseminating nanometrology to industry. These are two examples of how a European innovation infrastructure can provide support throughout the innovation process – from initial idea, through design, manufacture, conformity assessment and marketing – through to the finished product. An innovation infrastructure in nanometrology will network and integrate actors in each field – from policy makers to metrologists - so that these can work together to tackle the major challenges.
European Nanometrology 2020	T. Burke, R. Leach, R. Boyd, M. Gee, D. Roy, A. Yacoot, H.-U. Danzebrink, T. Dziomba, L. Koenders, L.E. Depero, K. Carneiro, K. Dirscherl, V. Morazzani, J. Lausmaa, L. Pendrill, A. Pidduck, G. Roebben, A. Sánchez, W.E.S. Unger, A. Proykova, Co-Nanomet Consortium, 2011. http://www.nano.org.uk/files/home/european-nanometrology.pdf	As a strategic guidance, this document contains a vision for European nanometrology 2020; future goals and research needs, building out from an evaluation of the status of science and technology in 2010. It incorporates concepts or the acceleration of European nanometrology, in support of the effective commercial exploitation of emerging nanotechnologies
Identification of Knowledge Gaps and Strategic Priorities for Human and Environmental Hazard, Exposure and Risk Assessment of Engineered Nanomaterials	ITS- nano report. WP2 Nanosafety cluster	The report synthesises information from numerous reviews and reports (worldwide coverage) to identify existing knowledge, areas of particular strengths of expertise, and prioritise areas in which further research should be undertaken. With respect to hazard, this expert opinion is supplemented by an assessment of the literature presented as 'heat maps' to indicate the research areas where publications have been most numerous. This information will be used as a background to the discussion at the stakeholder meetings and moderated to take into account the expert opinion of leading researchers in the field as well as various stakeholders. This is intended as a flexible/adaptable approach to knowledge gap identification which can be updated in future as new evidence becomes available. As this is the baseline information which feeds into the ITS, this will help maintain the responsiveness and longevity of the ITS which must be adaptable in order to remain responsive and current for both regulators, industry and researchers in future.
Inter-laboratory comparison of particle size distribution measurements applied to industrial pigments and	Gilliland G, Hempelmann U (editors), JRC Scientific and Policy Report (in preparation)	

fillers.		
Interpretation and implications of the European Commission's definition on nanomaterials	RIVM Report 601358001, 2012-06-29 Bleeker EAJ, Cassee FR, Geertsma RE, de Jong WH, Heugens EHW, Koers-Jacquemijns M, van de Meent D, Oomen AG, 151434395 Popma J, Rietveld AG, Wijnhoven SWP	The Dutch ministries have requested RIVM to interpret the meaning and implications of the Recommendation from a scientific perspective and to consider the implications for use in legislation. This report provides the basis for discussions by policy makers and stakeholders on the use and further implementation of the recommended definition in national and international legal frameworks.
Introductory Guide to Nanometrology.	P.-E. Hansen, G. Roebben, F. Babick, R. Boyd, A. Braun, I. Busch, H.-U. Danzebrink, L. Depero, K. Dirscherl, T. Dziomba, E. Eriksson, K. Franks, M. Gee, N. M. Jennett, V. Kestens, L. Koenders, M. Krumrey, J. Lausmaa, R. Leach, L. Pendrill, A. Pidduck, S. Put, D. Roy, M. Stintz, R. Turan, A. Yacoot, Co-Nanomet Consortium, ISBN 978-0-9566809-1-4, 2010.	This Guide introduces the reader to the science of measurements at the nanoscale, that is nanometrology. It is aimed at researchers in the nanotechnology area, for whom the metrology aspect is new, and at metrologists, interested in knowing about the specifics of metrology at the nanoscale. The Guide does not give an exhaustive review of the field. Rather it is intended to increase the general awareness of nanometrology, and its basic challenges. In a first section, three main questions are addressed: 1. What is (nano)metrology? 2. Why is nanometrology important? 3. What are the main challenges for nanometrology? The Guide continues with a section on the meaning of a number of generic metrology concepts. In the third section, the Guide illustrates some of the identified nanometrological challenges with practical examples and case studies from three different application areas (thin films, surface structures and nanoparticles). A final subsection is devoted to the emerging issue of metrology for nanobiotechnology.
Proceedings of the European Workshop 'Metrology for nanoparticle characterisation: Instruments, Standard Methods and Reference Materials'.	28-29 April, 2010, Nuremberg, eds. M. Stintz, G. Roebben, Co-Nanomet Consortium, ISBN 978-0-9566809-3-8, 2010.	This scientific workshop was intended to bring together stakeholders involved in engineered nanoparticle (ENP) metrology, such as manufacturers of ENPs, (eco)toxicologists, developers of measurement techniques, representatives of standardisation bodies and authorities. They discussed and assessed the current metrology status with regard to: - relevant measurands (depending on the context of the measurement); - available measurement techniques, their limits and fields of application; - the dissemination of such techniques to science, industry and public authorities; - traceability and measurement uncertainty; - standardisation; - reference materials Based on this the workshop participants tried to reveal current and future needs for engineered nanoparticles (ENP) metrology in the following sessions: A Fundamentals of ENP characterisation; B Objectives of ENP characterisation; C1 ENPs in liquid media: sizing; C2 ENPs in liquid media: interfacial properties; D Characterisation of airborne particles; E Reference materials; F Sizing and material characterisation

NanoGenoTox project deliverables DELIVERABLE 4.1: Summary report on primary physicochemical properties of manufactured nanomaterials used in NANOGENOTOX. DELIVERABLE 4.2: Transmission Electron Microscopic characterisation of NANOGENOTOX nanomaterials. DELIVERABLE 4.3: Crystallite size, mineralogical and chemical purity of NANOGENOTOX nanomaterials. DELIVERABLE 4.4: Determination of specific surface area of NANOGENOTOX nanomaterials. DELIVERABLE 4.5: Surface charge, hydrodynamic size and size distribution by zetametry, dynamic light scattering (DLS) and small-angle X-ray scattering (SAXS) in optimized aqueous suspensions for titanium and silicon dioxide. DELIVERABLE 4.6: Dustiness of NANOGENOTOX nanomaterials using the NRCWE small rotating drum	March 2013, available on the website http://www.nanogenotox.eu/	Safety evaluation of manufactured nanomaterials by characterisation of their potential genotoxic hazard. Research performed on the representative series of nanomaterial NM-XXX form the JRC Repository

and the INRS Vortex shaker. March 2013, 2028 KB DELIVERABLE 4.7: Hydrochemical reactivity, solubility, and biodurability of NANOGENOTOX nanomaterials. March 2013, 2496 KB DELIVERABLE 5: In vitro testing strategy for nanomaterials. DELIVERABLE 6: Characterisation of manufactured nanomaterials for their clastogenic/aneugenic effects or DNA damage potentials and correlation analysis. DELIVERABLE 7: Identification of target organs and biodistribution including ADME parameters. March 2013, 2959 KB MILESTONE REPORT 2: Determination of acute toxicity of TiO2, SiO2, and CNT nanomaterials of the NANOGENOTOX Joint Action Plan. MILESTONE REPORT 2: Evaluation of the determination of Ti in tissues.		

Nanomaterial in consumer products; detection, characterisation and interpretation	Oomen, A.G., Bennink, M., van Engelen, J.G.M. & Sips, A.J.A.M 2011, Netherlands National Institute for Public Health and the Environment. RIVM Report 320029001/2011	The aim of the present study is to investigate nanomaterials in consumer products and to put the applicability of the analytical techniques and the acquired results in a risk assessment perspective. To that end, 25 consumer products with and without a nanoclaim were selected, purchased and analysed, and the results were put in a broader perspective. Electron microscopic analysis is used as this is the only current approach to directly image and visualize nanomaterials in samples and is regularly used for nanomaterials in a scientific setting. Other techniques are being developed to detect nanomaterials in consumer products. An overview of the techniques that can be used to detect nanomaterials and the infrastructure of these techniques should become available from the European Framework Project QNano, which will probably start early 2011. RIKILT, part of Wageningen University and Research Centre, is a Dutch partner in this project.
Nanomaterials and REACH Background Paper on the Position of German Competent Authorities	http://www.bfr.bund.de/cm/349/nanomaterials-and-reach.pdf	The present background paper reflects the position of the German federal authorities on the regulation of nanomaterials (NMs) under REACH. It is intended as a basis for preparing decision-making routes for political processes responses to from outsiders (e.g. Bundestag deputies or NGOs). With respect to the imminent negotiations on the regulation of NMs under REACH in the EU it is intended to explain and justify the position of the German competent authorities. This paper also deals with the regulatory need for ultrafine fibres and particles. If required the document will be adapted to fit the current discussions and knowledge.
Nanomaterials under REACH. Nanosilver as a case study	RIVM Report 601780003/2009 M.E.J. Pronk et al. http://www.rivm.nl/bibliotheek/rapporten/601780003.pdf	This report describes a hypothetical registration of nanosilver under the new EU REACH regulation on chemicals, taking into account the ongoing discussions within the REACH Competent Authorities and its Subgroup on Nanomaterials on how REACH applies to nanomaterials (as described in documents of this subgroup dated December 2008-March 2009). The case study on nanosilver is purely a scientific exercise, with the aim to generate recommendations for future policy guidance on how to deal with first generation nanomaterials under REACH. Given this, it is stressed that this report does not pretend to provide a complete overview of all available toxicity data on (nano)silver, and is as such not to be used for an actual registration under REACH.
Nanometrology	Nanoforum Report, 2006, http://www.co-nanomet.eu/content/Co-nanomet%20protected%20documents/training%20and%20resources/library/Eighth%20Nanoforum%20Report_%20Nanometrology.pdf	The concept of nanometrology is introduced and defined in the first part of this report. Its importance is highlighted through an introduction to size – property relationships, which is the key aspect of nanometrology. Nanotechnology and nanosciences exploit and study new phenomena that appear when some characteristic structure of a material is in the nanometre size range. It is obvious that a key element of nanometrology is to measure dimensions in the range 1 to about 100 nm (with precision reaching 0.1 nm nowadays), and to correlate the measured size with properties. This introduction is followed be a review of European institutions and companies active in nanometrology, which shows that there are not that many organisations involved with nanometrology considering the importance of the field. In nanometrology there are two main issues to consider: precise measurement of sizes in the nanometre range, and adapting existing or developing new methods to characterise properties as a function of size. A direct consequence of this is obviously developing methods to characterise sizes based on evaluation of properties, and to compare sizes measured using various methods. This report provides a comprehensive review of characterisation

		methods, properties that are size dependent in a range of materials, as well as characteristic dimensions. It also provides a classification of nanostructures and dimensions that characterise them with regard to possible applications.
Nanosafety research at CSIRO	http://www.csiro.au/org/Understanding-nanosafety.html	Nanosafe Australia, a nationwide network of toxicologists and risk assessors, convened by Assoc. Prof Paul Wright at RMIT, aims to support government, industry and NGOs in their efforts to understand the occupational and environmental health and safety issues surrounding nanotechnology products and their manufacturing processes. The work of Nanosafe Australia informs risk assessment processes by identifying hazards, characterising dose-response relationships, and assessing potential exposures. Overall, Australian researchers appear to be participating actively in international nanosafety efforts.
Nanoscale reference materials.	G. Roebben, G. Reiners, H. Emons, in Nanotechnology Standards, Eds V. Murashov, J. Howard, Springer Science+Business Media, New York, NY, ISBN – 978-1-4419-7852-3 (2011)	Globalisation of both science and trade has increased the relevance of the comparability of measurement data whether in research, industry or regulatory contexts. Reference materials (RMs) are essential tools in the quest for comparable and reliable measurement results, a quest which laboratories, worldwide, are tasked with every day. An explicit acknowledgement of the importance of RMs in today's measurement systems is found, for instance, in the laboratory accreditation standards, such as ISO/IEC 17025
Nanotechnologies - Methodology for the classification and categorization of nanomaterials, providing a comprehensive, globally harmonised methodology for classifying nanomaterials.	ISO/TR 11360:2010,	ISO/TR 11360 introduces a system called a "nano-tree", which places nanotechnology concepts into a logical context by indicating relationships among them as a branching out tree. The most basic and common elements are defined as the main trunk of the tree, and nanomaterials are then differentiated in terms of structure, chemical nature and other properties.
Nanotoxicology: Progress toward Nanomedicine, Second Edition	Book, March 3, 2014 by CRC 512 Editor(s): Nancy A. Monteiro-Riviere; C. Lang Tran, IN PRESS	Book with two chapters which are relevant: Risk Assessment of Engineered Nanomaterials: State of the Art and Roadmap for Future Research, Danail Hristozov, Laura MacCalman, Keld Alstrup Jensen, Vicki Stone, Janeck Scott-Fordsmand, Bernd Nowack, Teresa Fernandes, and Antonio Marcomini Issues Related to Risk Assessment of Nanomaterials, Maureen R. Gwinn
REACH Implementation Project Substance	JRC Advisory Report, 2011, http://ec.europa.eu/environm	The objective of the project 'Substance identification of nanomaterials' (RIP-oN 1) was to evaluate the applicability of existing guidance and, if needed, to develop specific advice on how to establish the substance

Identification of nanomaterials (RIP-oN 1)	ent/chemicals/nanotech/pdf/report_ripon1.pdf	identity of nanomaterials.
Requirements on measurements for the implementation of the European Commission definition of the term nanomaterial.	T.P.J. Linsinger, G. Roebben, D. Gilliland, L. Calzolai, F. Rossi, N. Gibson, C. Klein, JRC Reference Report, EUR 25404 EN, European Union, Luxembourg, ISBN 978-92-79-25603-5 (print), 2012.	This report describes the requirements for particle size measurements of nanomaterials based on the definition. It discusses the related generic measurement issues and reviews the capabilities of the measurement methods currently available. Moreover, it illustrates with practical examples the measurement issues that remain to be solved.
Regulating Nanotechnologies: Risk, Uncertainty and the Global Governance Gap	Robert Falkner, London School of Economics and Nico Jaspers, Global Environmental Politics, 12(1), February 2012, pp. 30-55.	This paper reviews the emerging debate on nanotechnology risk and regulatory approaches, investigates the current state of international cooperation and outlines the critical contribution that a global governance approach can make to the safe development of nanotechnologies.
Responsible Use of Nanotechnologies	Federal Ministry for the Environment, Nature Conservation and Nuclear Safety 2010. Report and recommendations of the German NanoKommission 2011, http://www.bmu.de/fileadmin/bmu-import/files/english/pdf/application/pdf/nano_schlussbericht_2011_bf_en.pdf	The present report summarises the discussions and out-comes of the NanoKommission's second dialogue phase from 2009 to 2011. During this period the NanoKommission comprised eighteen permanent members representing a variety of stakeholder groups. The members' work was supported by four Issue Groups, each consisting of 20-25 members representing ministries and public authorities, research and industry, environmental, consumer and women's organisations, trade unions and churches. An additional Working Group comprising NanoKommission members, research scientists and representatives of government authorities was set up to address the concept of "Sustainable Nanotechnologies –Green Nano".
Risk Assessment of Products of Nanotechnologies, Scientific Committee on Emerging and Newly Identified Health Risks	Report SCENIHR, http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_023.pdf	The SCENIHR was asked: To identify and assess new information and update the opinions of the SCENIHR on potential risks of products of nanotechnologies, in particular, with respect to characterisation, eco-toxicology and toxicology as well as exposure assessments.
Scientific Committee on	Opinion no. 129, March 2013	After a SCCNFP opinion, 2,2'-methylene-bis-(6(2H-benotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol) S79

Consumer Safety SCCS OPINION ON 2,2'-Methylene-bis-(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol) (in nano form only) COLIPA n° S79	http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_129.pdf	was requested for inclusion in Annex VII, part 1 – List of UV filters which Cosmetic Products may contain – to Council Directive 76/768/EEC. As a result of the recast of the European Cosmetic Directive (76/768/EEC) into the Cosmetic Products Regulation (EC No. 1223/2009) a new description will be necessary for this chemical. According to the description stated in the Cosmetic Products Regulation under article 2 this material could fulfil the definition of a nanomaterial. Based on this new definition supplementary data on the material on its nano form, was submitted by the applicant. According to the applicant, the current submission II takes into account the available information on the nano-form of this ingredient.
Scientific Committee on Consumer Safety SCCS ADDENDUM to the OPINION SCCS/1489/12 on Zinc oxide (nano form) COLIPA S76	Opinion no. 137, July 2013, http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_137.pdf	In January 2013, Cosmetics Europe1 submitted a document in which they proposed their own – broader - interpretation of the characteristics laid out in the scientific opinion on zinc oxide in nano form. In particular, they proposed the purity requirements to be reduced to 96% (as data on one of the material with 96% purity were provided in the submission), the median diameter of the particle number size distribution to be accepted when it is greater than 30 nm, the possible coatings to be extended to all (authorized or not prohibited) cosmetic ingredients, and the omission of the solubility specification. Both the Commission's services and the Member States expressed doubts regarding this interpretation, and therefore seek a clarification from the SCCS
Scientific Committee on Consumer Safety SCCS OPINION ON Titanium Dioxide (nano form) COLIPA n° S75	Opinion no. 136, July 2013 http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_136.pdf	The SCCNFP opinion from 2000 (SCCNFP/0005/98) is on micro-crystalline preparations of TiO2 and preparations of coarse particles. However, since this opinion, new scientific data on nanosized particles including, TiO2 has become available. Therefore, the SCCP considers it necessary to review the safety of nanosized TiO2 in the light of recent information. Also, a safety assessment of nanosized TiO2, taking into account abnormal skin conditions and the possible impact of mechanical effects on skin penetration needs to be undertaken". Supplementary information on nanosized Titanium dioxide was submitted following a meeting with stakeholders on 1 October 2008, where data requirements were agreed.
SINN, Deliverable 2.6, Consolidated Framework for EHS of Manufactured Materials, July 2013	Karl Höhener, Juergen Hoek	
Small is different: a science perspective on the regulatory challenges of the nanoscale The Expert Panel on Nanotechnology Science Advice	Council of Canadian Academies, 2008	This report summarizes the work of the Expert Panel on Nanotechnology (the panel) established by the Council of Canadian Academies (the Council), to assess "…the state of knowledge with respect to existing nanomaterial properties and their health and environmental risks, which could underpin regulatory perspectives on needs for research, risk assessment and surveillance."

Specific Advice on Exposure Assessment and Hazard/Risk Characterisation for Nanomaterials under REACH (RIP-oN 3)	Aitken, R.A, Bassan, A., Friedrichs, S., Hankin, S.M., Hansen, S.F., Holmqvist, J., Peters, S.A.K., Poland, C.A., Tran, C.L.. RNC/RIP-oN3/FPR/1/FINAL.	The REACH Implementation Projects on Nanomaterials (RIP-oNs) seek to provide scientific and technical advice on key aspects of the implementation of REACH with regard to nanomaterials. The objectives of the RIP-oN 3 project were to: 1) develop advice on how to do exposure assessment for nanomaterials within the REACH context to cover i) development of Exposure Scenarios, ii) evaluation of operational conditions and risk management/mitigation measures and iii) exposure estimation, and; 2) to develop ideas for how to conduct hazard and risk characterisation for nanomaterials. The latter will involve threshold/non-threshold considerations. The approach taken was largely driven by the contract specifications and comprised a step wise, evidence based approach, on which guidance changes were developed. The project was implemented through a series of specified and linked tasks (A, B1-B4, C1 – C3, and D).
Specific Advice on Fulfilling Information Requirements for Nanomaterials under REACH (RIP-oN 2)	Hankin S.M., Peters S.A.K., Poland C.A., Foss Hansen S., Holmqvist J., Ross B.L., Varet J. and Aitken R.J. RNC/RIP-oN2/FPR/1/FINAL	The REACH Implementation Projects on Nanomaterials (RIP-oNs) seek to provide scientific and technical advice on key aspects of the implementation of REACH with regard to nanomaterials. The objectives of the RIP-oN 2 project, undertaken by a consortium led by the Institute of Occupational Medicine, were to develop specific advice on i) how REACH information requirements on intrinsic properties of nanomaterials can be fulfilled, including the appropriateness of the relevant test methods (and dosimetry) for nanomaterials and outline, when relevant, possible specific testing strategies and ii) the information that is needed for safety evaluation and risk management of nanomaterials and, in particular, if information is needed beyond or in addition to the current information requirements listed in REACH Annexes VI-X.
Synthetic Amorphous Silicon Dioxide (NM-200, NM-201, NM-202, NM-203, NM-204): Characterisation and Physico-Chemical Properties. JRC Repository: NM-series of Representative Manufactured Nanomaterials	Kirsten Rasmussen, Agnieszka Mech, Jan Mast, Pieter-Jan De Temmerman, Nadia Waegeneers, Frederic Van Steen, Jean Christophe Pizzolon, Ludwig De Temmerman, Elke Van Doren, Keld Alstrup Jensen, Renie Birkedal, Marcus Levin, Signe Hjortkjær Nielsen, Ismo Kalevi Koponen, Per Axel Clausen, Yahia Kembouche, Nathalie Thieriet, Olivier Spalla, Camille	The present report presents the physico-chemical characterisation of the synthetic amorphous silicon dioxide (SiO2, SAS) from the JRC repository: NM-200, NM-201, NM-202, NM-203 and NM-204. NM-200 was selected as principal material for the OECD test programme "Testing a representative set of manufactured nanomaterials". *Note: these materials are hosted in the JRC Nanomaterials Repository (http://ihcp.jrc.ec.europa.eu/our_activities/nanotechnology/nanomaterials-repository/list_materials_JRC_rep_oct_2011.pdf)*

	Giuot, Davy Rousset, Olivier Witschger, Sebastian Bau, Bernard Bianchi, Boris Shivachev, Douglas Gilliland, Francesca Pianella, Giacomo Ceccone, Giulio Cotogno, Hubert Rauscher, Neil Gibson and Hermann Stamm, 2013 EUR 26046 EN ISBN 978-92-79-32323-2 ISSN 1831-9424 doi:10.2788/57989	
Toward Advancing NanoObject Count Metrology: A Best Practice Framework Short Running Title: How to Count Nanoparticles to Meet Regulatory Demands	Scott C. Brown, Volodymyr Boyko, Greg Meyers, Matthias Voetz and Wendel Wohlleben, EHP Report. 27/09/2013 http://ehp.niehs.nih.gov/wp-content/uploads/121/9/ehp.1306957.pdf Supply information: http://ehp.niehs.nih.gov/wp-content/uploads/121/9/ehp.1306957.s001.pdf	This review highlights current particle size metrology challenges faced by the chemical industry due to these emerging number percent content thresholds, provides a suggested best practice framework for nano-object identification and identifies research needs as a path forward.
Toxic Effects of Various Modifications of a Nanoparticle Following Inhalation	Report BAUA, O. Creutzenburg, 2013 Final report of the project "Toxic Effects of Various Modifications of a Nanoparticle Following Inhalation" – Project F 2246	This project aimed at comparing the toxic effects of the triple TiO2 UV TITAN M212, TiO2 UV TITAN M262 and TiO2 P25 coded in the European nanomaterials repository with NM-103, NM-104 and NM-105. These differ in crystal structure (rutile; rutile; 80 % anatase/20 % rutile) and surface modifications (with silicone □ hydrophobic; with glycerol □ hydrophilic; untreated □ hydrophilic, respectively) suggesting a different toxic potential after uptake in lungs. Wistar rats were exposed to aerosol concentrations of 3, 12 and 48 mg/m3 mimicking exposure scenarios at workplaces (6 hours/day, 5 days/week for 28 days) while controls inhaled clean air. (Overview of scientific literature on Toxic effect of TiO_2)
VCI position on the definition of the term nanomaterial for use in	VCI Verband der CHemischen Industrie e.V. (German Chemical Industry Association).	Proposition of an alternative definition.

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What Defines Nanomaterials?	Prepared for: National Institute for Public Health and the Environment (RIVM) Prepared by: ARCADIS NEDERLAND BV EU 2011/S120-199032, 2012.	The aim of this project is to determine which physicochemical properties make a nanomaterial different from a 'conventional' material and at what size these properties are changed to 'nanospecific' properties. These size thresholds can then be used to prepare the Dutch input into the foreseen review of nanomaterial definition. This work is being done within the context of the European Commission (2011) definition of "nanomaterial"

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European Commission
EUR 26567 EN – Joint Research Centre – Institute for Health and Consumer Protection

Title: **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**

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Luxembourg: Publications Office of the European Union

2014 – 288 pp. – 21.0 x 29.7 cm

EUR – Scientific and Technical Research series – ISSN 1018-5593 (print), ISSN 1831-9424 (online)

ISBN 978-92-79-36600-0 (pdf)
ISBN 978-92-79-36601-7 (print)

doi:10.2788/36237

Abstract

In October 2011 the European Commission (EC) published a Recommendation on the definition of nanomaterial (2011/696/EU). The purpose of this definition is to enable determination when a material should be considered a nanomaterial for regulatory purposes in the European Union. In view of the upcoming review of the current EC Definition of the term 'nanomaterial' and noting the need expressed by the EC Environment Directorate General and other Commission services for a set of scientifically sound reports as the basis for this review, the EC Joint Research Centre (JRC) prepares three consecutive reports, of which this is the first. This Report compiles information concerning the experience with the definition regarding scientific-technical issues that should be considered when reviewing the current EC definition of nanomaterial. Based on this Report 1 and the feedback received, JRC will write a second, follow-up Report 2. In that report the JRC will provide its assessment of the scientific-technical issues compiled in Report 1, in relation to the objective of reviewing the current EC nanomaterial definition. In a third report JRC will provide recommendations to improve content and the implementation of the EC Definition as well as related communication aspects.

LB-NA-26567-EN-N

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ISBN 978-92-79-36600-0