ERA-NET SIINN
Safe **I**mplementation of **I**nnovative
Nanoscience and **N**anotechnology

Deliverable D2.6: Consolidated Framework for EHS of Manufactured Nanomaterials

Final Version

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Content

0 Introduction

Background of the consolidated framework

The European ERANET "Safe Implementation of Innovative Nanoscience and Nanotechnology" (SIINN) has as its primary aim the promotion of the rapid transfer of the results of nanoscience and nanotechnology research into industrial applications by helping to create reliable conditions.

Further information regarding the SIINN project can be found at:

http://www.siinn.eu/en/

In order to strengthen the European Research Area and to coordinate nano-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.

The commercial application of manufactured nanomaterial (MNM) products is increasing rapidly, but one important question, the safety of MNMs, 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 MNMs**.

This consolidated framework for environmental, health and safety (EHS) issues represents one of the key deliverables of the SIINN project and is intended to be used for dissemination and exploitation purposes towards:

- European and national policy-makers,
- European and national stakeholders and decision-makers for EHS related issues, precautionary actions and regulations,
- Industry and industrial associations and interest groups,
- Researchers working in the field of N&N or nanosafety,
- Relevant ETPs (such as NANOfutures ETIP) and clusters (e.g. the NanoSafety cluster),
- The European Commission,
- International organisations such as the OECD, ISO, CEN, etc.,
- Industrial unions such as CEFIC,
- Specialists for workers safety, human health and the environment,
- Non-governmental organisations (NGO),
- Insurance companies, risk assessment and validation organizations,
- The Partners and Associate Partners of SIINN.

Purpose of this document

The purpose of this document is to present to the reader in a condensed and accessible form a gateway to basic information and definitions for nanomaterials, the identification of: best practices, synergy potentials and the elaboration of recommendations for future collaborations on the strategic and operational level addressing MNM EHS. This includes precautionary measures, pre-normative work, steps towards regulations as well as common actions and projects.

It will also define common activities based on the deliverables of WP1 and WP3 such as an overview of best practices for workers' safety and environmental protection, iden-

tification of knowledge gaps and recommendations for improvements. These activities will cover among others:

- Safe handling of nano objects in process- and product R&D,
- Safe processes, products and transport,
- Safe nano products,
- Safe end-of-pipe processes,
- Standard operational procedures (SOP).

The document has been designed to be open for necessary changes, additions and updates. It is not intended to be comprehensive and final. As a living document it has thus undergone several consultation and consolidation rounds among a broad range of key stakeholders in the field. Consequently, the information depicted in the following chapters is only a snapshot in time, and subject to further changes, as appropriate.

Guidance for the reader

Environmental and health safety will be abbreviated as EHS, and manufactured nanomaterials as MNM throughout this whole document.

The document is intended as a summary that functions as an introduction to and overview of the selected topics. However, it is not meant to replace in-depth full-length texts that are necessary for the respective specialists.

In order to fulfill its purpose for the readers of the respective target groups, this document is organized in a way to give a guideline how to enter into the topic of EHS, and to give substantial hints for further reading or own researches.

To this end, the document starts with a **glossary**, which provides a comprehensive review of definitions, methods, their sensitivity and use. It is intended to form a common, harmonized ground of understanding and vocabulary for all stakeholders involved in nanotechnologies with a focus on manufactured nanomaterials (MNM).

In its second chapter, the document gives an introductory section on **risk assessment** with a focus on MNM, which is intended as an overview of ongoing activities, focussing on the relevant components of risk in general. The overview does not aim to be complete, and it is recognized that there is a continuous production of new studies in this area.

The third chapter focuses on specific **precautionary measures**, which can be applied as a bridging element until definitive rules and regulations in the field of EHS of MNM are available.

After that, a chapter on **good practices** is following, which is offering information on solutions that may or may not work for a given system, according to an 80:20 rule. Accordingly, this chapter summarizes currently applied good practices solutions in the field of MNM.

Chapter 5 builds a bridge to issues relating to **standardisation and assessment recommendations**, which will have an ever increasing influence of the implementation of MNM in the market.

Chapter 6 takes reference to the increasingly important topic of regulatory issues connected to nanomaterials research, production, and use in different technological fields and market segments.

In chapter 7, specific sources of information and **data sources** are compiled, which are intended to offer the reader an entry point for further information regarding relevant topics in the field of EHS of MNM.

As a very specific topic of SIINN, chapter 8 depicts the dedicated **SIINN Roadmap**. In order to prepare future transnational calls based on a common transnational RTD program, SIINN has developed a road map and strategy towards such common programs. Both have been elaborated based on the identified gaps as well as the state-of-the-art, as elaborated within this report. The three calls for transnational projects generated from the SIINN network are also listed in this chapter.

1 Glossary

1.1 Introduction

The Glossary provides a selected review of the most important definitions, methods, their sensitivity and use. It is intended to form a common, harmonized ground of understanding and vocabulary for all stakeholders involved in nanotechnologies with a focus on manufactured nanomaterials (MNM). Objective and efficient discussions about EHS of MNM shall thus be enabled. References are provided in sufficient detail for the reader for further work on the topic. The Glossary treats the major issues of EHS of Nanoparticles. Within the sections, the information is ordered alphabetically.

1.2 Selected working definitions relevant for “nanomaterials” by different organizations / countries

In the field of nanomaterials, many different institutions are working, involving different focal points on definitions and wording: standardization organizations, regulators, scientists, economic or health organizations, industries, and others.

Therefore, quite often a given subject has more than one definition.

The definitions/descriptions of nanomaterials formulated so far have

i) given a general size frame for nanomaterials in both external and internal dimensions and

ii) referred to the unique physico-chemical characteristics of the specific material under discussion.

Whilst such broad definitions can be scientifically justified, they are not easy to apply within the context of a regulatory framework.

For regulatory purposes (*see ISO TS 80004-1 (2008); 2011/696/EU; JRC Reference Report 2012, EUR 25404 EN*) a definition of ‘nanomaterial’ should ideally fulfill the requirements of being:

• a single definition broadly applicable in EU legislation and policies,

• legally clear and unambiguous,

• enforceable through agreed measurement techniques and procedures, and

• in line with other approaches worldwide.

The definition will be used primarily to identify materials for which special provisions might apply (e.g. for risk assessment or ingredient labeling). Those special provisions are not part of the definition but of specific legislation in which the definition will be used.

According to EC, nanomaterials are not intrinsically hazardous per se but there may be a need to take into account specific considerations in their risk assessment. Therefore, one purpose of the definition is to provide clear and unambiguous criteria to identify materials for which such considerations apply.

It is only the results of the risk assessment that will determine whether the MNM is hazardous and whether or not further action is justified. Taking into account this observation, for the further evaluation of the results during the SIINN project, the highest importance was devoted to the EU, ISO and OECD WPMN information regarding the “nanomaterials” definition.

In this context it is worth noting that ISO and OECD also consider materials with an inner structure in the size of nanometers.

1.2.1 Nanomaterial

1. 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. 2. 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 %. By derogation from point 1, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials	***Commission recommendation of 18 October 2011 on the definition of nanomaterial. 2011/696/EU***
A material should be considered as falling under the definition in point 1, 2011/696/EU, 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 in point 1, 2011/696/EU, even if the material has a specific surface area lower than 60 m^2/cm^3.	***JRC Reference Report 2012, EUR 25404 EN Requirements on measurements for the implementation of the European Commission definition of the term "nanomaterial"***
Material with one or more external dimensions, or an internal structure, on the nanoscale, which could exhibit novel characteristics compared to the same material without nanoscale features *NOTE: Novel characteristics might include increased strength, chemical reactivity or conductivity.*	***British Standards Institution, PAS (Publicly Available Specification) 71: Vocabulary Nanoparticles*** ***EU SCCP: EU Scientific Committee on Consumer Products (SCCP),*** *18 December 2007, Safety of nanomaterials in cosmetic products*
Any form of a material composed of discrete functional parts, many of which have one or more dimensions in the nanoscale.	***EU SCENIHR The EU Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR)*** *– 29 November 2007*
Material with any external dimension in the nanoscale or having internal or surface structure in the nanoscale *Note: This generic term is inclusive of nano-object and nanostructured material.*	***ISO-CEN : ISO TS 80004-1***
Material which is either a nanoobject or is nanostructured.	***OECD-WPMN: Considerations on a Definition of Nanomaterial for Regulatory Purposes, 2010***

A material made up of nanostructures between 1 and 100 *nanometres* (or billionths of a metre) in size. These nanostructures can be *nanoparticles* , nanotubes (such as carbon nanotubes) or nano-crystals.	***CSI 2007 Glossary***
An insoluble or biopersistent and intentionally manufactured material with one or more external dimensions, or an internal structure, in the na-noscale.	***European Cosmetic Products Regulation, Regulation (EC) No 1223/2009 on cosmetic products. – OJ L 342, 22.12.2009, p. 59***
Any intentionally produced material in the nanoscale or is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions in the nanoscale.	***EU (Novel Foods)***
An Engineered Nanomaterial (ENM) is any inten-tionally produced material in the nanoscale.	***ACC-The American Chemistry Council***
Industrial materials intentionally produced, manufac-tured or engineered to have specific properties or specific composition, in the nanoscale.	***Australia - NICNAS***
Manufactured material (MNM) at or within the nanoscale in at least one spatial dimension, or is smaller or larger than the nanoscale in all spatial dimensions and exhibits one or more nanoscale phenomena properties or specific composition, in the nanoscale.	***Canada***
Materials having structures in the nanoscale properties or specific composition, in the nanoscale.	***Denmark***
In the frame of the Swiss Action Plan Synthetic Nanomaterials and the application of the Precau-tionary Matrix the Swiss Authorities recommend including particulate materials up to 500nm (maxi-mum of size distribution of primary particles) into the assessment of the nanorelevance of MNM. This recommendation is based on two considerations: • In size distributions of MNM with a maximum at 500nm, still a large fraction of the MNM can be in the low nm range • Nanospecific biological interactions can occur up to <300 nm [Gehr, 2010].	***Switzerland (Swiss Action Plan Synthetic Nanomaterials)***
Materials having structured components in the nanoscale.	***The UK***

Materials in the nanoscale and are deliberately engineered i.e. not natural or unintentional by-products of other processes, and are 'free' within any environmental media at any stage in a product's life-cycle.	***The UK (DEFRA)***
Engineered nanoscale material is any particle, substance, or material that has been engineered to have one or more dimensions in the nanoscale.	***US-EPA***

1.2.2 Nano-object

A nano-object is a material with at least one, two or three external dimensions in the nanoscale range of 1 to 100 nm	***International Standards Organization, 2008 (ISO/TS 27687:2008) and 2010 (ISO/ CD TS 80004-1:2010)***
Material with one, two or three external dimensions in the nanoscale. Note: Generic term for all discrete nanoscale objects.	***ISO/TC 229 and CEN ISO/TS 27687***
Material confined in one, two, or three dimensions at the nanoscale.	***OECD-WPMN: Considerations on a Definition of Nanomaterial for Regulatory Purposes, 2010***

1.2.3 Nanoparticle

A particle having one or more dimensions of the order of 100nm or less	***UKPAS71 document UK***
A discrete entity which has three dimensions of the order of 100 nm or less.	***EU Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) – 29 November 2007***
Nano-object with all three external dimensions in the nanoscale.	***CEN ISO/TS 27687***
Particle with one or more dimensions at the nanoscale.	***EU Scientific Committee on Consumer Products (SCCP), 18 December 2007, Safety of nanomaterials in cosmetic products***
Nano-object with all three external dimensions in the nanoscale. *NOTE: If the lengths of the longest and the shortest axes of the nano-object differ significantly (typically by more than three times) the terms nanorod or nanoplate are intended to be used instead of the term nanoparticle*	***ISO/TS 276871***

1.2.4 Nanoplate

Material confined in one, two, or three dimensions at the nanoscale. Nano-object with one external dimension in the nanoscale and the two other external dimensions significantly larger *NOTE 1: The smallest external dimension is the thickness of the nanoplate.* *NOTE 2: The two significantly larger dimensions are considered to differ from the nanoscale dimension by more than three times.*	***CEN ISO/TS 27687*** ***ISO/TS 276874***

1.2.5 Nanofibre

Nano-object with two similar external dimensions in the nanoscale and the third dimension significantly larger. *NOTE: Types of nanofibres include nanowhiskers, nanorods and nanowire.*	***CEN ISO/TS 27687***
Nanoparticle with two dimensions at the nanoscale and an aspect ratio of greater than 3:1.	***British Standards Institution, PAS (Publicly Available Specification) 71: Vocabulary - Nanoparticles***

1.2.6 Nanorod

Solid nanofibre.	***CEN ISO/TS 27687***
A discrete entity which has two dimensions that are of the order of 100 nm or less, and one long dimension.	***EU Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) – 29 November 2007***
Straight solid nanofibre.	***British Standards Institution, PAS (Publicly Available Specification) 71: Vocabulary - Nanoparticles***
Nano-object with two similar external dimensions in the nanoscale and the third dimension significantly larger than the other two external dimensions *NOTE 1: The largest external dimension is the length of the nanorod and is not necessarily in the nanoscale.* *NOTE 2: The two similar external dimensions are considered to differ in size by less than three times and the significantly larger external dimension is considered to differ from the other two by more than three times.* *NOTE 3: A nanorod can take any cross-sectional shape consistent with the dimensional limits of the definition.*	***ISO/TS 276875***

1.2.7 Nanotube

Hollow nanofibre.	***CEN ISO/TS 27687 PAS 71 Steering Group British Standards Institution, PAS (Publicly Available Specification) 71: Vocabulary - Nanoparticles***
A discrete hollow entity which has two dimensions of the order of 100 nm or less and one long dimension.	***The EU Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) – 29 November 2007***
Hollow nanorod.	***ISO/TS 276875***

1.2.8 Nanowire

Electrically conducting or semiconducting nanofibre.	***CEN ISO/TS 27687***
Conducting or semi-conducting nanofibre.	***British Standards Institution, PAS (Publicly Available Specification) 71: Vocabulary - Nanoparticles***
Elongated structure with only two dimensions in the nanoscale and with properties that allow for the transmission of energy.	***British Standards Institution, PAS (Publicly Available Specification) 131: Terminology for medical, health and personal care applications of nanotechnology***

1.2.9 Nanostructured material (NSM)

Having an internal or surface structure at the nanoscale.	***OECD-WPMN: Considerations on a Definition of Nanomaterial for Regulatory Purposes, 2010***
Any structure 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.	***The EU Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) – 29 November 2007, the existing and proposed definitions relating to products of nanotechnologies***
Having a structure at the nanoscale. *NOTE: Agglomerates and aggregates of nanoparticles are examples of nanostructured particles.*	***British Standards Institution, PAS (Publicly Available Specification) 71: Vocabulary Nanoparticles***

Possessing a structure comprising contiguous elements with one or more dimension in the nanoscale but excluding any primary atomic or molecular structure *NOTE 1: An example of a primary atomic or molecular structure is the arrangement of atoms in a crystalline solid.* *NOTE 2: The use of the term contiguous implies that a sphere of approximately 100 nm diameter, inscribed in a nanostructured material, will intersect more than one element of the structure.*	***British Standards Institution, PAS (Publicly Available Specification) 131: Terminology for medical, health and personal care applications of nanotechnology.***

Nanostructured materials (NSM) include <u>agglomerates</u> and <u>aggregates</u> which may be defined as:

An **agglomerate** is a group of nano-objects and/or aggregates held together by weak forces, such as Van der Waals forces or electrostatic forces in which the resulting external surface area is similar to the sum of the surface areas of the individual components;	***EFSA, 2009, CEN ISO/TS 27687, ISO/TC 24/SC 4-Particle characterisation***
"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;	***COMMISSION RECOMMENDATION of 18 October 2011 on the definition of nanomaterial*** *(Text with EEA relevance)*
An **Aggregate** is a group of nano-objects held together by strong forces, such as those associated with covalent or metallic bonds where the resulting external surface area may be significantly smaller than the sum of calculated surface areas of the individual components.	***EFSA, 2009, CEN ISO/TS 27687, ISO/TC 24/SC 4-Particle characterisation***
"**Aggregate**" means a particle comprising of strongly bound or fused particles.	***COMMISSION RECOMMENDATION of 18 October 2011 on the definition of nanomaterial*** *(Text with EEA relevance)*

1.3 Nanoscale

Overview of nanoscales used in existing working definitions of nanomaterials:

Organization / Country	Nanoscale
ISO-CEN (*ISO/TC 229 and CEN ISO/TS 2768719*)	Approximately 1 nm to 100 nm
OECD *OECD-WPMN:, 2010*	Typically between 1 nm and 100 nm
EU SCENIHR 2007	In the order of 100 nm or less
EU SCCP 2007	In the order of 100 nm or less
EU (Cosmetic Products)	1 nm to 100 nm

EU (Novel Foods)	In the order of 100 nm or less
ACC	Typically between 1 nm and 100 nm
Australia (NICNAS)	Typically between 1 nm and 100 nm
Canada	1 nm to 100 nm
Denmark	In the 1-100 nm range
The UK *British Standards Institution, PAS71*	Less than 100 nm
The UK (DEFRA)	Up to 200 nm (in two or more dimensions)
US-EPA	Generally, but not exclusively, below 100 nm and above 1 nm

Table 1: Nanoscales used in existing working definitions

1.4 Manufactured nanomaterials (MNMs)

1.4.1 Definition of MNMs according to OECD-WPMN

(Considerations on a Definition of Nanomaterial for Regulatory Purposes, 2010)

Nanomaterials intentionally produced to have specific properties or specific composition.

1.4.2 List of relevant manufactured nanomaterials adopted by OECD

(OECD: Series on the Safety of Manufactured Nanomaterials No. 27, 2010)

Class	**MNM** Nanomaterials by parent substance	**Definition**
Carbon products	**Fullerenes**	Any closed-cage structure having more than twenty carbon atoms consisting entirely of three-coordinate carbon atoms J. Chem. Inf. Comp. Sci. [7], 35, 969-978, *NOTE Also referred to as buckyball and buckminsterfullerene.* *British Standards Institution, PAS (Publicly Available Specification) 71: Vocabulary — Nanoparticles* A fullerene with 60 carbon atoms (C60) is sometimes called buckminsterfullerene. *British Standards Institution, PAS (Publicly Available Specification) 134:2007. Terminology for carbon nanostructures*
	Single-walled carbon nanotubes (SWCNTs)	Single-walled carbon nanotubes (CNT) also known as 'buckytubes' with a cylindrical nanostructure in the form of a tube and an engineered CNT typically has a nanoscale thick wall, geometrically shaped similar to a Buckyball, with a nanoscale diameter, and a length that may exceed 100 nm.
	Multi-walled carbon nanotubes (MWCNTs)	Carbon nanotube (CNT) with a multilayer wall.

Metal oxides	**Titanium dioxide (TiO_2)** **Aluminium oxide(Al_2O_3)** **Cerium dioxide (CeO_2)** **Zinc oxide (ZnO)** **Silicon dioxide (SiO_2)**	The metal oxides are common in their bulk, non-nanoparticulate forms, and they are now being produced in nanosized forms that capitalize on their enhanced properties.
Metals	**Silver nanoparticles(Ag)** **Iron nanoparticles (Fe)** **Gold nanoparticles (Au)** **Bimetallic nanoparticles Fe-Pd, Fe-Ni, Fe-Ag**	Nanoparticulate zerovalent metals
Nanoclays	**Nanoclays**	Filler substance, mainly consisting of nano-scale platelets of the mineral montmorillonite, which occurs in clay.
Dendrimers	**Dendrimers**	Synthetic, three-dimensional macromolecule built up from a monomer, with new branches added in a step-by-step fashion until a symmetrical branched structure is created *NOTE: Where there is perfect branching, the particle is referred to as a dendrimer; where the branching is imperfect, it is referred to as hyperbranched.* *PAS 71 Steering Group* *British Standards Institution, PAS (Publicly Available Specification) 71: Vocabulary — Nanoparticles* Repeatedly branched macromolecule *NOTE Dendrimers can be configured as a sphere, partial sphere or wedge structure (i.e. dendritic wedge).* *British Standards Institution, PAS (Publicly Available Specification) 131: Terminology for medical, health and personal care applications of nanotechnology*

Table 2: Relevant manufactured nanomaterials adopted by OECD

1.5 MNMs Physical-chemical properties of relevance for EHS

Environment, Health, Safety issues adopted by OECD *(OECD: Series on the Safety of Manufactured Nanomaterials No. 27, 2010)* and overview of analytical methods suitable for their characterization.

1.5.1 Aggregation/agglomeration

The terms agglomeration and aggregation are often used interchangeably to describe the attractions that hold together a collection of particles. However, it has been suggested that it is more appropriate to consider nanoparticle aggregation and agglomeration as distinct phenomena (c.f. *Recommendation of European Commission. Last updated 18 October 2011*) with agglomerates formed by clusters of particles that are held together by electrostatic interactions, whereas aggregates are formed from covalently fused or sintered particles that are not easily sepa-

rated [Oberdörster, 2007]. Aggregation and agglomeration can occur due to a number of deliberate and accidental mechanisms [Schneider, 2008]. When hierarchical assemblies, aggregates and agglomerates are included in the determination of the size, their presence induces a shift to larger sizes.

Analytical methods suitable for aggregation/agglomeration measurement

Name/ Acronym / Spatial resolution or LOD	Referenced documents
Atomic force microscopy / **AFM** / ~0.1 nm	ISO/20998-1:2006 ISO/13322-1:2004 ISO/TS 13762:2001 ISO/AWI TS 10797 ISO/AWI TS 10798 ISO/AWI TR 13014 ENRHES *Engineered Nanoparticles: Review of Health and Environmental Safety, 2009* RNC/RIP-oN2/FPR/1/FINAL, Specific Advice on Fulfilling Information Requirements for Nanomaterials under REACH (RIP-oN 2) Final Project Report, 01 July 2011 JRC Reference Reports, Joint Research Centre, EU, 2010 SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), Opinion on the scientific basis for the definition of the term "nanomaterial", 8 December 2010 OECD WPMN (ENV/JM/MONO(2010)46
Electron microscopy/ **SEM** / 1 nm to 1μm	
Scanning transmission electron microscopy / **STEM** / < 0.1 nm	
Transmission electron microscopy / **TEM** / > 0.1 nm	
X-ray diffraction / **XRD** / 1-3 wt%	
Scanning tunneling microscopy / **STM** / resolution of ~1 nm or better.	
Small angle neutron scattering / **SANS**	

Table 3: Analytical methods suitable for aggregation/agglomeration measurement

1.5.2 Chemical composition

The chemical composition, in terms of elemental composition and chemical structure, is an intrinsic property of all materials and it is consequently an important parameter influencing the behaviour of nanoparticles. Nanoparticles can have very different chemical compositions, from completely inorganic, e.g. metals (iron, nickel, zinc, titanium, gold, silver, palladium, iridium, and platinum), and metal oxides (titanium oxide, zinc oxide, silica, iron oxide, etc.), to entirely organic (fullerenes, CNT, nanopolymers, biomolecules).

Analytical methods suitable for chemical composition measurements

Name/ Acronym **Spatial resolution or LOD are missing in many cases. If this is intentional, it should be mentioned above.**	**Referenced documents**
X-ray diffraction / **XRD** / 1-3 wt%	OECD Guidance Manual for testing (OECD, 2009) Preliminary Guidance Notes on Sample Preparation and Dosimetry for nanomaterials (OECD, 2010) ENRHES *Engineered Nanoparticles: Review of Health and Environmental Safety, 2009* RNC/RIP-oN2/FPR/1/FINAL, Specific Advice on Fulfilling Information Requirements for Nanomaterials under REACH (RIP-oN 2) Final Project Report, 01 July 2011
Analytical electron microscopy / **AEM** / H 0.5nm	
Nuclear magnetic resonance spectroscopy and Pulsed field gradient / **NMR**	
X-ray photoelectron spectroscopy / **XPS** / H1 mm	
Auger Electron Spectroscopy / **AES** / H1–2nm	
Atomic Absorption Spectrometry Analysis / **AASA**	
Proton Induced X-Ray Emission Analysis / **PIXE**	
Energy Dispersive X-Ray Fluorescence Analysis/ **XRF**	
Inductively-coupled plasma combined with the selectivity and sensitivity of optical emission spectrophotometry or mass spectrometry **/ICP-OES /ICP-MS /** Detailed analysis of the main components, as well as trace impurities	
Chemical force microscopy / **CFM**	
The aerosol time-of-flight mass spectrometry / **ATOF-MS**	
Electron paramagnetic resonance and electron spin resonance spectroscopies / **EPR** / **ESR**	
Fourier transform infrared spectroscopy / **FTIR**	
Raman Spectroscopy / **RS**	
Transmission electron microscopy / **TEM** / **TEM-EDS** (energy dispersive spectrometry)	

Table 4: Analytical methods suitable for chemical composition measurements

1.5.3 Crystal structure

A crystal structure is composed of a pattern, a set of atoms arranged in a particular way, and a lattice exhibiting long-range order and symmetry. Many materials with the same chemical composition can have different lattice structures, and exhibit different physico-chemical properties. Several structural investigations on inorganic nanoparticles indicate that also the crystal lattice type may have an important role on the overall structure of nanoparticles, because of the very high portion of surface atoms with respect to the bulk lattice. The size reduction may create discontinuous crystal planes that increase the number of structural defects, as well as disrupt the electronic configuration of the material, with possible toxicological consequences.

Analytical methods suitable for crystal structure measurements

Name/ Acronym / Spatial resolution or LOD	Referenced documents
Atomic force microscopy / **AFM /** ~0.1 nm	ISO/AWI TS 10797 ISO/AWI TS 10798 ISO/NP TS 10812 ISO/DTR 10929, ISO 27628:2007, ISO/13322-1:2004 ISO/13322-2:2006 BS EN 13925-1:2003 BS EN 13925-2:2003 BS EN 13925-3:2005
Electron microscopy / **SEM** / 1 nm to 1μm	
Scanning transmission electron microscopy / **STEM** / < 0.1 nm	
Transmission electron microscopy / **TEM** / > 0.1 nm	
X-ray diffraction / **XRD** / 1-3 wt%	
Scanning tunneling microscopy / **STM** / resolution of ~1 nm or better.	
Small angle neutron scattering / **SANS**	

Table 5: Analytical methods suitable for crystal structure measurements

1.5.4 Crystallite or grain size

Crystallites are small, often microscopic crystals that, held together through highly defective boundaries, constitute a polycrystalline solid. Crystallites are often also referred to as **grains**, but they are not equal, a grain can contain several crystallites. **Crystallite size** is the average size of the particle whereas the particle size denotes the individual size of the particle. **Grain size** is the average diameter of the grains.

Crystallite size is usually measured from **X-ray diffraction patterns (XRD)** and grain size by other experimental techniques like **transmission electron microscopy (TEM)**.

BS EN 13925-1:2003; BS EN 13925-3:2005; ISO/AWI TS 10797; RNC/RIP-oN2/ FPR/1/FINAL, 01 July 2011.

1.5.5 Octanol-water partition coefficient (KOW)

A coefficient representing the ratio of the solubility of a compound in octanol (a non-polar solvent) to its solubility in water (a polar solvent). The higher K_{OW}, the more non-polar the compound. Log K_{OW} is generally used as a relative indicator of the tendency of an organic compound to adsorb to soil. Log K_{OW} values are generally inversely related to aqueous solubility and directly proportional to molecular weight."

U.S. Environmental Protection Agency, 2009, Glossary of technical terms: U.S. Environmental Protection Agency, access date May 24, 2011 http://www.epa.gov/oust/cat/tumgloss.htm.

1.5.6 Photocatalytic activity and radical formation potential

Photocatalytic activity (PCA) is the ability of a material to create upon exposure to light electron–hole pairs, which generate highly reactive free radicals (e.g. hydroxyl radicals: OH) on the material surface able to undergo secondary reactions. Photocatalytically active materials are semiconductors. Titanium dioxide is a semiconductor of this kind. The photocatalytic decomposition of water on the surface of a TiO_2 nanoparticle results in the generation of free radicals on the surface which in turn react with organic matter. This is an example of the potential of a given nanomaterial to generate free radicals.

Photocatalytic activity is highly material dependent. Within materials, it is size dependent. It can be enhanced or completely switched off by treating the surface of the material or by introducing dopants. Thus, while photocatalytic activity is very relevant for risk assessment, it is not a property that all nanomaterials will have.

RNC/RIP-oN2/B3/2/FINAL; Sapanbir S Thind et al 2012 Nanotechnology, 23, 475706; ENV/JM/MONO(2009)20/REV; ISO 22197-1:2007(restricted to some forms of NM).

1.5.7 Protein corona (in vitro: conditioned nanoparticles)

The concept of Differential Adsorption or Protein Corona Formation means that the physico-chemical properties of nanomaterials upon contact with media in specific body compartments (*e.g.,* respiratory tract, GI-tract, blood, extra/intracellular fluid) determine which proteins/lipids adsorb on and desorb from the surface in a dynamic process; this coating then in turn determines the biodistribution of NPs across barriers and in target tissues or cells [Lundquist, 2008].

Analysis of such formation of a protein corona in plasma showed the existence of an inner “hard corona” with stable and very slowly exchanging proteins, and an outer weakly interacting protein layer rapidly exchanging with free proteins [Oberdörster, 2010]. Upon translocation to specific organs the formation of new coronas is to be expected. Research of these phenomena is a high priority, for understanding the fate and effects of nanomaterials.

The functional groups play an important role in the formation of nanoparticle-protein corona. [R. Podila, 2012]. The protein corona may influence cellular uptake, inflammation, accumulation, degradation and clearance of the nanoparticles. Furthermore, the nanoparticle surface can induce conformational changes in adsorbed protein molecules which may affect the overall bio-reactivity of the nanoparticle. [Saptarshi, 2013; Tenzer, 2013];

Further research will determine how similar or dissimilar is the formation of the hard corona for different types of NPs, and how different is the corona formation in relevant media other than plasma. The importance of protein corona for purposes of targeted drug delivery across barriers, as well as for toxicity testing (use of dispersant media, including proteins, prior to testing) needs to be evaluated.

1.5.8 Redox activity

Redox reactions can occur abiotically or biologically, and may alter a nanomaterial's physico-chemical properties including surface area, surface charge, and chemical composition, which in turn can affect the material's potential to aggregate, size, toxicity and mobility. Redox reactions are the basis of chemical transformations of inorganic and organic species and the precipitation and dissolution of inorganic substances that influences their sequestration and mobility.

Hence measurement of the redox potential would be potentially meaningful for nanomaterials which can participate in electron transfer or uptake. Chemically stable inorganic nanomaterials in physiological redox conditions do not appear to exhibit cytotoxicity in vitro, whereas nanomaterials with strong oxidative (e.g. CeO_2, Mn_3O_4 and Co_3O_4) or reductive powers (e.g. FeO, Fe_3O_4, AgO and CuO) can be cytotoxic and genotoxic towards biological targets in vitro [Auffan, 2009]. Standard electrochemical methods, such as **cyclic voltammetry**, may be used to study the redox activity of nanomaterials.

Referring to the redox potential of MNM, OECD WPMN have highlighted that redox reactions are the basis of chemical transformations of inorganic and organic species and the precipitation and dissolution of inorganic substances that influences their sequestration and mobility (ENV/JM/MONO(2010)46). Hence, OECD suggests that measurement of the redox potential would be potentially meaningful for nanomaterials which can participate in electron transfer or uptake.

1.5.9 ROS generation potential

The relationship between Reactive Oxygen Species generation and ecotoxicity has been studied to a lesser extent. Whilst it has been demonstrated that ROS generation and oxidative stress is an important factor to assess nanomaterial toxicity, not all nanomaterials exhibit the electronic configurations or surface properties that allow spontaneous (extrinsic) or a cellular (intrinsic) ROS generation; particle interactions with cellular components could generate ROS during these interactions.

Referring to the Cell-free ROS/RNS production capacity, further research required into the relationship between ROS/RNS generating capacity and (eco)toxicological effects of nanomaterials, as well as the development of standard measurement methods for nanomaterials (RNC/RIPoN2/ B4/2/FINAL). The R&D requirement includes basic research to establish the relevance of the property and applicability of methods, the validation of methods and the development of Standard Operating Procedures (SOP).

A number of methods have been identified for detecting ROS generation from nanomaterials, under both abiotic conditions and in cells:

RNC/RIP-oN2/B3/2/FINAL, or [Hotze, 2008].

Analytical methods suitable for ROS generation measurements

Name/ Acronym
Electron spin resonance / ESR (spin trap / spin probe based approaches)
XTT (XTT is a tetrazolium derivative) assay
Electron paramagnetic resonance / EPR (spin trap / spin probe based approaches)
Spectrofluorimetry
Singlet Oxygen Sensor Green / SOSG
Dithiothreitol (DTT) assay / DTT
Furfuryl alcohol assay / FFA assay
High-performance liquid chromatography /HPLC combined with electrochemical or UV detectors, pulse radiolysis, fluorometric methods, ESR, spectrophotometric detection, capillary electrophoresis (CE), and CL based determination methods
Fluorogenic probes / DCFH and dihydrorho-damine-123 (DHR-123),
ROS responsive nanosensor, based on PEBBLE (Probes Encapsulated By Biologically Localised Embedding) technology

Table 6: Analytical methods suitable for ROS generation measurements

1.5.10 Shape/aspect ratio

A detailed description of the **physical shape** of the nanomaterial should be provided using terms such as spheres, fibres, tubes or plates.

Guidance for Notifiers Handbook REQUIREMENTS FOR NOTIFICATION OF NEW INDUSTRIAL NANOMATERIALS: Guidance on new chemical requirements for notification of industrial nanomaterials

Shape is important as it is the variation of the hydrodynamic radius between spherical particles and oblong ones (larger for the latter) with the same mass, which triggers a variation in their mobility and diffusion in both gas and liquid phases. The second effect is that the shape influences the deposition and adsorption kinetics in biological media.

Aspect ratio: Ratio of the longest Feret's diameter of a particle to the shortest perpendicular.

Adapted from BS 2955:1993, *Glossary of terms relating to particle technology*, by PAS 71 Steering Group
PAS 71 Steering Group: British Standards Institution, PAS (Publicly Available Specification) 71: Vocabulary — Nanoparticles

Analytical methods suitable for shape/aspect ratio measurements

Name/ Acronym / Spatial resolution or LOD (Level of Detail)	Referenced documents
Atomic force microscopy / **AFM /** ~0.1 nm	ISO/13322-1:2004 FDIS ISO/NP TS 10868 ISO/AWI TS 10797 ISO/AWI TS 10798 ISO/AWI TR 13014 ISO/DTR 10929 ISO/DTS 11888 ENRHES *Engineered Nanoparticles: Review of Health and Environmental Safety, 2009* RNC/RIP-oN2/FPR/1/FINAL, Specific Advice on Fulfilling Information Requirements for Nanomaterials under REACH (RIP-oN 2) Final Project Report, 01 July 2011 JRC Reference Reports, Joint Research Centre, EU, 2010 SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), Opinion on the scientific basis for the definition of the term "nanomaterial", 8 December 2010 OECD WPMN (ENV/JM/MONO(2010)46
Electron microscopy / **SEM** / 1 nm to 1μm	
Scanning transmission electron microscopy / **STEM** / < 0.1 nm	
Transmission electron microscopy / **TEM** / > 0.1 nm	
X-ray diffraction / **XRD** / 1-3 wt%	
Scanning tunneling microscopy / **STM** / resolution of ~1 nm or better.	
Dynamic light scattering / **DLS/** 3 nm - μm	
Static Light Scattering **SLS**	
Field Flow Fractionation **FFF /** Flow FFF: 1nm -1 μm; Sed FFF: 50nm-1 μm	
FFF-ICP-MS **FFF-Confocal Microscopy** **FIFFF-SLS** **SedFFF-DLS**	

Table 7: Analytical methods suitable for shape/aspect ratio measurements

1.5.11 Size/size distribution

Size of a particle as determined by a specified measurement method

PAS 71 Steering Group
British Standards Institution, PAS (Publicly Available Specification) 71: Vocabulary — Nanoparticles
The size distribution of a material should be presented as size distribution based on the number concentration (i.e. the particle number) and not on the mass concentration of a nanomaterial product as a small mass concentration may contain the largest number fraction.

Recommendation of European Commission. Last updated 18 October 2011

Analytical methods suitable for size/size distribution measurements

Name/ Acronym / Spatial resolution or LOD	Referenced documents
Atomic force microscopy / **AFM /** ~0.1 nm	FDIS ISO/15900:2009 ISO 28439:2011 ISO/21501-1:2009 ISO/13318-1:2001 ISO/13322-1:2004 ISO/TS 13762:2001 ISO/13320:2009
Differential mobility Analyzer / **DMA** / 3 nm to μm particles	

Field flow fractionation / **FFF** / Flow FFF 1 nm - 1μm; Sed FFF 50 nm -1μm	ISO/22412:2008 ISO/13321:1996 ISO/22412:2008, ASTM E2490-09 ISO/13321:1996 ISO/20998-1:2006 ISO/21501-2:2007 BS EN 13925-1:2003 ISO/AWI TS 10797 ISO/NP TS 10868 ISO/AWI TR 13014 ISO/DTR 10929 ISO/CD 12025 ECHA, 2008. R.7a ISO/TS 13762:2001 JRC Reference Reports, Joint Research Centre, EU, 2010 SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), Opinion on the scientific basis for the definition of the term "nanomaterial", 8 December 2010 OECD WPMN (ENV/JM/MONO(2010)46)
Hydrodynamic Chromatograph / **HDC**	
Electron microscopy / **SEM** / 1 nm to 1 μm	
Scanning mobility particle Sizer / **SMPS**	
Scanning transmission electron microscopy / **STEM** / < 0.1 nm	
Single particle mass Spectrometer / **SPMS**	
Size exclusion Chromatograph / **SEC**	
Transmission electron Microscopy / **TEM** / > 0.1 nm	
X-ray diffraction / **XRD** / 1-3 wt%	
Dynamic light scattering (photon correlation spectroscopy or quasi elastic light scattering) / **DLS (PCS, QELS)** / 3 nm - μm	
Fluorescence correlation spectroscopy (Confocal microscopy)/ **FCS** / ~200nm	

Table 8: Analytical methods suitable for size/size distribution measurements

1.5.12 Surface area (&porosity)

Surface area is the measure of how much exposed area a solid object has, expressed in square units. The reduction in size to the nanoscale is accompanied by an inherent increase in the surface-to-volume ratio, and therefore a greater proportion of entities at the surface compared to the bulk (non-nanoscale) material. Increase in surface area increases reactivity and sorption behaviour.

Porosity or **void fraction** is a measure of the void (i.e., "empty") spaces in a material, and is a fraction of the volume of voids over the total volume, between 0–1, or as a percentage between 0–100%

Analytical methods suitable for Surface area (&porosity) measurements

Name/ Acronym	Comments / Information	Referenced documents
Brunauer Emmett Teller / **BET**	The BET-method allows surface area or porosity measurements within pores or other nanostructures as small as about 1 nm. The density of only the material without the empty spaces in between is required. Thousands of m^2 g–1 A limitation of the BET-method is that it is only applicable to powders and/or dry solid materials and not to nano-materials embedded in solids and suspensions.	ENV/JM/MONO (2009)20/REV ISO/9277:2010 disperse or porous solids ISO/18757:2005 fine ceramic materials TSI - Measuring Nanoparticle Exposure Application Note NSAM-001

Epiphaniometer	Monitoring environmental aerosols However, it has not been used widely in assessing aerosol exposure, possibly due to its use of a radioactive source, and its complexity of use.	
Monitors for lung deposable particle surface areas (NSAM, Discmini, Partector,Nanocheck)	The effect of initial aerosol charge, the composition of the material, presence of aggregates and the effect of particle shape have to be considered. Sampled particles are charged, collected in an electrically isolated filter and the charge rate measured. Monitor measure the surface area of particles (reported as mm^2 cm^{-3}) deposited in the lung.	

Table 9: Analytical methods suitable for Surface area (&porosity) measurements

1.5.13 Surface chemistry (surface modification)

The term **surface chemistry** is often used in the context of surface chemical composition, and is somewhat a broad and non-specific term which does not predispose itself to 'quantitative' characterisation according to a single comparable metric or measurand. Surface chemistry includes elements of solubility equilibrium, catalytic properties, surface charge, and surface adsorption and desorption of molecules from solution, amongst others. Most of these properties are functions of the atomic or molecular composition of the surface and the physical surface structure. Chemical purity, functionalisation and surface coating are also important aspects to take into account.

Surface modification of a nanomaterial can either be done **by coating, functionalisation or other means,** which may be **chemical** (organic, inorganic or both) or **physical** (e.g. irradiation, surface attrition). Purposely applied and environmentally acquired coatings can have a major impact on nanomaterial interaction with biological systems.

Analytical methods suitable for surface chemistry measurements

Name/ Acronym / Spatial resolution or LOD	Referenced documents
Analytical electron microscopy / **AEM** / Spatial resolution H0.5nm	RNC/RIP-oN2/FPR/1/FINAL, Specific Advice on Fulfilling Information Requirements for Nanomaterials under REACH (RIP-oN 2) Final Project Report, 01 July 2011
Chemical force microscopy / **CFM /** Identifying the nature of individual atoms	
X-ray photoelectron spectroscopy / **XPS** / Spatial resolution H1 mm / Atomic composition of layers from 1–10nm	
Auger Electron Spectroscopy / **AES** / H1–2nm	
Secondary ion mass spectrometry / **SIMS /** Atomic composition of layers from 1–3nm	

Table 10: Analytical methods suitable for surface chemistry measurements

A good overview is given at:
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2841528

1.5.14 Surface charge/zeta potential

Surface charge is the electric charge present at an interface. There are many different processes which can lead to a surface being charged, including adsorption of ions, protonation/deprotonation, and the application of an external electric field. Surface charge causes a particle to emit an electric field, which causes particle repulsions and attractions, and is responsible for many colloidal properties.

Zeta potential Electrostatic potential at the slipping plane (which marks the region where the liquid molecules surrounding the particle first begin to move with respect to the surface) relative to the potential in the bulk solution

[Hunter, 1993]
British Standards Institution, PAS (Publicly Available Specification) 71: Vocabulary - Nanoparticles

Analytical methods suitable for surface charge/zeta potential measurements

Name/ Acronym / Spatial resolution or LOD	Referenced documents
Capillary electrophoresis/**CE**	ISO/CD 13099-1 ISO/CD 13099-2 ISO/AWI TR 13014 RNC/RIP-oN2/FPR/1/FINAL, Specific Advice on Fulfilling Information Requirements for Nanomaterials under REACH (RIP-oN 2) Final Project Report, 01 July 2011
Zeta potential Indicates the degree of repulsion between adjacent, similarly charged particles in a dispersion	
Zeta potential measurement, combined with Dynamic Light Scattering (**DLS**)	
Zeta Potential Nanoparticle Tracking Analysis (Z-NTA) adds measurements of electrostatic potential to simultaneous reporting of nanoparticle size, light scattering intensity, fluorescence and count, and does so particle-by-particle.	-

Table 11: Analytical methods suitable for surface charge/zeta potential measurements

1.5.15 Water solubility/dispersability

The solubility of a chemical in water may be defined as the maximum amount of the chemical that will dissolve in pure water at a specified temperature. Above this concentration, two phases will exist if the organic chemical is a solid or a liquid at the system temperature: a saturated aqueous solution and a solid or liquid organic phase. Aqueous concentrations are usually stated in terms of weight per weight (ppm, ppb, g/kg, etc.) or weight per volume (mg/L, moles/L, etc.) - [Lyman, 1990].

- The property of water solubility is considered to be very relevant and applicable to nanomaterials. It has been suggested that the measurand of interest (beginning with a pre-determined unit of particles in a standardised solution

and temperature) is to measure the mass proportion of nanomaterials which are held in solution, and whether this mass diminishes after a set period of time, or; determine the amount of time required for mass to diminish by X% (*ENV/JM/MONO(2009)20/REV*);

- Distinguish between solubilisation and dispersion. Water solubility has the potential to increase in the nano-size range. For nanomaterials, it can be difficult to distinguish between when a substance is dispersed and when it is dissolved due to its small particle size. It is important to recognise that solubility and dispersibility are different and distinct phenomena, with different implications on testing and characterisation, and it is important to differentiate between them. It should also be ensured that no undissolved material contributes to what is being measured. Update to guidance was proposed regarding the difference between solubilisation and dispersion and a recommendation to elucidate between the two for nanomaterials. However, as highlighted in *ENV/JM/MONO(2009)20/REV*, specific methods to determine dispersion stability remain to be determined.

1.6 Exposure to manufactured nanomaterials

1.6.1 Benchmark exposure level

Criterion for evaluation of exposure

Glossary on particles and further links, IFA-Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung

1.6.1.1 Benchmark Dose or Concentration

An exposure due to a dose or concentration of a substance associated with a specified low incidence of risk, generally in the range of 1% to 10%, of a health effect; or the dose or concentration associated with a specified measure or change of a biological effect.

ORD Exposure Factors Handbook, Exposure Factors Glossary, http://www.epa.gov/ncea/efh/report.html

1.6.1.2 Benchmark Dose Limit

A statistical lower confidence limit on the dose or concentration at the **Benchmark Dose or Benchmark Concentration** (BMD or BMC), respectively.

Risk Assessment Glossary, http://www.epa.gov/risk/index.htm

1.6.1.3 Benchmark materials

Standard, or a set of standards, used as a point of reference for evaluating the level of toxicity. Benchmarks may be drawn from a firm's own experience, from the experience of other firms in the industry, or from legal requirements such as environmental regulations.

Ex: To place any pulmonary response to exposure to a given nanomaterial in perspective, results should be compared to those for particles of well-defined toxicity. Such benchmark materials could include nano-sized TiO_2, carbon black, or crystalline silica. These benchmark materials should be characterized for surface area and particle number per mass, as well as for particle size and with respect to chemical purity and crystallinity to allow comparisons to be made using a variety of dose metrics [Oberdörster, 2005].

1.6.1.4 Ecotoxicological Benchmarks

Numerical values that represent concentrations of contaminants in abiotic media (sediments, water, soil) or tissues of plants and animals above which concentrations those contaminants are expected to cause harm.

Ecological Risk Assessment Glossary of Terms,
http://www.epa.gov/Region5/superfund/ecology/glossary.html

1.6.2 Detection limit

The lowest concentration of a chemical that can reliably be distinguished from a zero concentration.

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

1.6.3 Dose (for chemicals that are not radioactive)

In terms of monitoring exposure levels: the amount of a substance to which a person is exposed over some time period. Dose is a measurement of exposure. Dose is often expressed as milligram (amount) per kilogram (a measure of body weight) per day (a measure of time), e.g. when people eat or drink contaminated water, food, or soil. In general, the greater the dose, the greater the likelihood of an effect. An "exposure dose" is how much of a substance is encountered in the environment. An "absorbed dose" is the amount of a substance that actually got into the body through the lungs, eyes, skin, stomach, or intestines.

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

ORD Exposure Factors Handbook, Exposure Factors Glossary,
http://www.epa.gov/ncea/efh/report.html

In terms of health effects: the amount of a substance available for interaction with metabolic processes or biologically significant receptors after crossing the outer boundary of an organism. The potential dose is the amount ingested, inhaled, or applied to the skin. The applied dose is the amount of a substance presented to an absorption barrier and available for absorption (although not necessarily having yet crossed the outer boundary of the organism). The absorbed dose is the amount crossing a specific absorption barrier (e.g., the exchange boundaries of skin, lung, and digestive tract) through uptake processes. Internal dose is a more general term denoting the amount absorbed without respect to specific absorption barriers or exchange boundaries. The environmental impacts and health effects related to the air pollution in the form of particulate matter have been directly linked to the so called “deposited dose” which can be measured by monitoring the inhaled and exhaled particle concentrations. The amount of the chemical available for interaction by any particular organ or cell after oral ingestion or intravascular injection is termed the "deliverable dose" for that organ or cell.

Glossary of health, exposure, and risk assessment terms,
http://www.epa.gov/ttn/atw/hlthef/hapindex.html

M. Geiser and W.G. Kreyling, Deposition and biokinetics of inhaled nanoparticles, Particle and Fibre Toxicology 2010, 7:2
http://www.particleandfibretoxicology.com/content/7/1/2

T. Hussein, J. Löndahl, P. Paasonen, A. Koivisto, T. Petäjä, K. Hämeri, M. Kulmala, Modeling regional deposited dose of submicron aerosol particles, Science of The Total Environment, 2013, 458–460, Pages 140–149
http://www.sciencedirect.com/science/article/pii/S0048969713004439

1.6.4 Dose - response relationship

The relationship between the doseof a substance and the resulting changes in body function or health (response).

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

The dose-response relationship is a fundamental concept in toxicology and the basis for measurement of the relative harmfulness of a chemical. A dose-response relationship is defined as a consistent mathematical and biologically plausible correlation between the number of individuals responding and a given dose over an exposure period.

TOXICOLOGY AND EXPOSURE GUIDELINES, UNL Environmental Health and Safety, 2012 (402) 472-4925 ·
http://ehs.unl.edu/documents/tox_exposure_guidelines.pdf

1.6.4.1 Dose response curves

A dose-response relationship is represented by a dose response curve. The curve is generated by plotting the dose of the chemical versus the response in the test population. Dose-response curves provide valuable information regarding the potency of the compound.

TOXICOLOGY AND EXPOSURE GUIDELINES, UNL Environmental Health and Safety, 2012 (402) 472-4925 ·
http://ehs.unl.edu/documents/tox_exposure_guidelines.pdf

1.6.4.2 Dose terms

In toxicology, studies of the dose given to test organisms are expressed in terms of the quantity administered:

- **Quantity per unit mass (or weight)**. Usually expressed as milligram per kilogram of body weight (mg/kg)
- **Quantity per unit area of skin surface**. Usually expressed as milligram per square centimeter (mg/cm^2)
- **Volume of substance in air per unit volume of air**. Usually given as microliters of vapor or gas per liter of air by volume (ppm). Particulates and gases are also given as milligrams of material per cubic meter of air (mg/m^3).

TOXICOLOGY AND EXPOSURE GUIDELINES, UNL Environmental Health and Safety 2012 (402) 472-4925.
http://ehs.unl.edu/documents/tox_exposure_guidelines.pdf

1.6.5 Exposure

Contact with a substance by swallowing, breathing, or touching the skin or eyes. Exposure may be short-term (acute exposure), of intermediate duration, or long-term (chronic exposure).

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

Terminology adopted by the International Society of Exposure Analysis (ISEA), in which exposure is defined as “the contact between an agent and a target,” that is, the human as the target and a contact at an exposure surface over an exposure period. Knowledge and control of exposure to MNMs is critical in risk assessment and management.

Zartarian, V., Bahadori, T. and McKone, T. 2005, "Adoption of an official ISEA glossary", J. Expos. Anal. Environ. Epidemiol., vol. 15, pp. 1-5.

Engineered Nanoparticles: Review of Health and Environmental Safety ENRHES, 2009 http://ihcp.jrc.ec.europa.eu/whats-new/enhres-final-report

1.6.5.1 Acute exposure

Contact with a substance that occurs once or for only a short time (up to 14 days) (compare with intermediate duration exposure and chronic exposure).

Agency for Toxic Substances and Disease Registry (ATSDR) http://www.atsdr.cdc.gov/glossary.html

Engineered Nanoparticles: Review of Health and Environmental Safety ENRHES, 2009 http://ihcp.jrc.ec.europa.eu/whats-new/enhres-final-report

1.6.5.2 Chronic exposure

Contact with a substance that occurs over a long time (more than 1 year) (compare with acute exposure and intermediate duration exposure)

Agency for Toxic Substances and Disease Registry (ATSDR) http://www.atsdr.cdc.gov/glossary.html

Engineered Nanoparticles: Review of Health and Environmental Safety ENRHES, 2009 http://ihcp.jrc.ec.europa.eu/whats-new/enhres-final-report

1.6.6 Exposure assessment

The process of finding out how people come into contact with a hazardous substance, how often and for how long they are in contact with the substance, and how much of the substance they are in contact with.

Agency for Toxic Substances and Disease Registry (ATSDR) http://www.atsdr.cdc.gov/glossary.html

Engineered Nanoparticles: Review of Health and Environmental Safety ENRHES, 2009 http://ihcp.jrc.ec.europa.eu/whats-new/enhres-final-report

Sources of information also include standards from ISO (ISO, 2007; 2008) and the British Standards Institution (BSI, 2007), reports from the Organisation for Economic Co-operation and Development (OECD, 2010) and outputs from European projects such as NanoSafe2 (http://www.nanosafe.org; accessed 4th September 2010), as well as the general scientific literature.

1.6.7 Exposure concentration

It is recommended that a minimum of three exposure levels be used. Information regarding the actual anticipated exposure levels in humans would be useful in determining the exposure concentration range to be evaluated. However, such information for nanoparticles is often lacking. In all cases, similar exposure concentrations of the test and benchmark materials should be used, and the various dose metrics discussed above should be considered when choosing the exposures for the benchmark and test materials. It is recommended that the

highest concentration chosen should exhibit toxicity with the benchmark material [Oberdörster, 2005].

1.6.8 Exposure-dose reconstruction

A method of estimating the amount of people's past exposure to hazardous substances. Computer and approximation methods are used when past information is limited, not available, or missing.

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

1.6.9 Exposure duration

For intratracheal instillation or pharyngeal aspiration, a single exposure to the nanomaterial is sufficient for Tier 1 studies. Caveat: consider high dose and bolus effect! For inhalation, a two week exposure is recommended, although shorter exposures, perhaps at higher concentrations, should be done if this mimics human exposures [Oberdörster, 2005].

1.6.10 Exposure investigation

The collection and analysis of site-specific information and biologic tests (when appropriate) to determine whether people have been exposed to hazardous substances.

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

Sources of information also include standards from ISO (ISO, 2007; 2008) and the British Standards Institution (BSI, 2007), reports from the Organisation for Economic Co-operation and Development (OECD, 2010) and outputs from European projects such as NanoSafe2 (http://www.nanosafe.org), as well as the general scientific literature.

1.6.11 Exposure limits

Established concentrations which, if not exceeded, will not generally cause adverse effects to the exposed population.

Health impacts of ultrafine particles, **Desktop literature review and analysis,** *L. Morawska, M. R Moore, Z. D Ristovski, Department of the Environment and Heritage, September 2004, ISBN 0 6425 5055 7*

http://www.environment.gov.au/resource/health-impacts-ultrafine-particles

1.6.12 Exposure metrics

There are three main metrics, all of which could have some utility in measuring exposure to nanoparticles. These are: i) **mass concentration** (units mg m^{-3}); ii) **number concentration** (units m^{-3}) and; iii) **surface area concentration units** (units m^2 m^{-3}). A case may be made for the use of any of these metrics under certain circumstances.

Important NOTES:

- *The issues of metrics should not be decided on exposure assessment issues alone, toxicological information needs to be carefully considered;*
- *At this time it is not possible to make a definitive statement concerning which of the metrics are the most appropriate for nanoparticles. In relation to measuring exposure, the best available guidance at this time is that measurements should encompass assessment of at least mass, but where possible also number and/or surface area concentration; in addition, measurements of size distribution is also discussed. (RNC/RIP-ON3/B3/4/FINAL).*

1.6.13 Exposure pathway

The route a substance takes from its source (where it began) to its end point (where it ends), and how people can come into contact with (or get exposed to) it. An exposure pathway has five parts: a source of contamination (such as an abandoned business); an environmental media and transport mechanism (such as movement through groundwater); a point of exposure (such as a private well); a route of exposure (eating, drinking, breathing, or touching), and a receptor population (people potentially or actually exposed). When all five parts are present, the exposure pathway is termed a completed exposure pathway.

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

Engineered Nanoparticles: Review of Health and Environmental Safety ENRHES, 2009, http://ihcp.jrc.ec.europa.eu/whats-new/enhres-final-report.

1.6.14 Exposure registry

A system of ongoing follow-up of people who have had documented environmental exposures.

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

1.6.15 Exposure routes

(including occupational, environmental and consumer exposure scenarios)

1.6.15.1 Dermal (Skin absorption)

Skin contact can occur during the handling of liquid suspensions of nanoparticles or dry powders. Skin absorption is much less likely for solid bound or matrixed nanomaterials.

The interaction of NPs with skin is an open and controversially discussed topic. Nevertheless, size is regarded to play an important role for skin penetration. Besides particle size, the surface chemistry of the particles and the presence of other excipients in the formulations contribute to skin absorption. Shape, coating, purity, presence of catalysts, extent of agglomeration and agglutination of the nanoparticles could influence the amount permeating the skin, and the toxicity of the MNMs. Furthermore, the state of the skin influences penetration (hydration) and the mechanical stress is of outmost importance. Four pathways of penetration across the skin have been identified depending on physicochemical properties of the compound: intercellular, transcellular, and two transappendageal, through hair follicles and sweat glands. Quantitative data are needed be-

cause there is evidence that NPs can pass through the skin in particular conditions such as wounds, flexures sites and lesions. Finally, to investigate the interaction between new MNMs and the human skin the researchers have to take into consideration several exposure variables, such as anatomical exposure sites, extension of the exposition area, time of exposition, chronic and repeated exposure, presence of skin diseases, and the role of cleanser and penetration enhancer.

WIREs Nanomed Nanobiotechnol 2011 3 463–478 DOI: 10.1002/wnan.146, Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, 2011, 3, 463–478, 2011: http://onlinelibrary.wiley.com/doi/10.1002/wnan.146/abstract.

International Archives of Occupational and Environmental Health 2009, 82, 9, pp 1043-1055, http://link.springer.com/article/10.1007/s00420-009-0458-x/fulltext.html.

1.6.15.2 Ingestion

As with any particulate, ingestion can occur if good hygiene practices are not followed. However, exposure via ingestion is particularly relevant due to the inclusion of MNMs in food, food packaging and oral medicines. The intestinal epithelial layer represents the initial gate that ingested MNMs must pass to reach the body. The MNMs have to pass the glycocalix, the cell membrane and the cells to reach the subepithelial fascia. From there, they might reach the blood capillaries, and only from there be transported to the rest of the body by the circulatory system.

Ingested nanoparticles could be harmful to health:

http://phys.org/news/2012-02-ingested-nanoparticles-health.html

Oral exposure to polystyrene nanoparticles affects iron absorption, G. J. Mahler, M. B. Esch, E. Tako, T. L. Southard, S. Archer, R. Glahn, M. L. Shuler, Nature Nanotechnology 7, 264–271, (2012).

1.6.15.3 Inhalation

Inhalation is the most common route for exposure to airborne particles. The human respiratory tract consists of three major regions. The uppermost region is the extrathoracic region. The middle portion is the tracheobronchial region, and the innermost portion is the alveolar region. The uptake of inhaled particles by our body is determined by where they deposit in the respiratory tract. In industrial hygiene workplace monitoring, it is common to sample aerosols according to their deposition in a specific region of the respiratory tract. This is often referred to as size-selective health hazard sampling. The criterion for size-selective sampling depends on the aerosol being sampled. Examples: for coal dust, the health effects relate to the deposition deep in the alveolar regions of the lung, so the respirable fraction of the aerosol is the metric of interest. In contrast, the throracic fraction of the aerosol is of interest for sampling cotton dust. The teflon nanoparticles pass through epithelium, basal membrane and to the circulation. Therefore inhaled nanoparticles may migrate from the lungs to the circulation. 18nm platinum nanoparticles translocate from lung to liver after 6h. Larger platinum particles did not. [Oberdörster, 2001] After 1, 3, 5, and 7 days exposure to carbon nanoparticles labeled with 13C (CMD = 36 nm; GSD = 1.66) translocate to cerebrum, cerebellum, olfactory bulbs. [Oberdörster, 2004]. According to ICRP Model, 1994 - Nose Breathing: NPs of 100-5nm and NPs < 5nm deposit mainly in the alveolar and nasal regions, respectively. Nanoparticles around 20nm deposit mainly in the alveolar region. Approximately 5nm particles deposit mainly nasal, pharyngeal and laryngeal tract. Clearance of nanoparticles from the lung is more slowly than fine particles. More nanoparticles translocate to interstitial sites and to regional lymph nodes compared to the larger particles. Inhaled nanoparticles may migrate from the lungs to the circula-

tion. Nanoparticles phagocytized by interstitial macrophages: this causes delay of clearance.

NSAM-001appnote[TSI 2010].pdf
The ICRP (International Commission of Radiological Protection) Publication 66 Human Respiratory Tract Model for Radiological Protection (HRTM) (ICRP-66, 1994)
U.S. Environmental Protection Agency (1996).
Air Quality Criteria for Particulate Matter (Final Report, Oct 2004)
http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=87903#Download

1.6.15.4 Injection

Exposure by accidental injection (skin puncture) is also a potential route of exposure, especially when working with animals or needles.

Note: The most studied route in occupational exposure is “inhalation”. However the exposure studies by other routes are becoming increasingly important. A systematic review on applied production methods, products and their handling must be compiled before any specific recommendations can be given related to the probability of exposure.

BIA-Workshop "Ultrafine aerosols at workplaces" (BIA-Report 7/2003e)

http://www.dguv.de/ifa/Publikationen/Reports-Download/BIA-Reports-2002-bis-2004/BIA-Report-7-2003/index-2.jsp

P. Borm et al., The potential risks of nanomaterials: a review carried out for ECETOC,Particle and Fibre Toxicology 2006, 3, 11,
http://www.particleandfibretoxicology.com/content/3/1/11

1.6.16 Exposure devices

Devices used to assess exposure to nanomaterials or nano-size aerosols can be subdivided into devices that monitor (on-line) a chemical substance or aerosol by “near or quasi” real-time detection and devices that sample (time-aggregated) chemical substances or aerosols on a substrate, followed by off-line analysis.

Safety of Nanomaterials along Their Lifecycle: Release, Exposure, and Human Hazards, *eds. Wendel Wohlleben, Thomas A.J. Kuhlbusch, Jürgen Schnekenburger, Claus-Michael Lehr,* CRC Press, Dec 4, 2014 - *Science* - 472 pages

Nanoparticle exposure at nanotechnology workplaces: A review, Kuhlbusch et al. Particle and Fibre Toxicology 2011, 8:22,
http://www.particleandfibretoxicology.com/content/8/1/22

1.6.17 Intermediate duration exposure

Contact with a substance that occurs for more than 14 days and less than a year (compare with acute exposure and chronic exposure).

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

1.6.18 Respiratory tract

The respiratory tract is one of the organ systems with vital functional elements in constant, direct contact with the environment. The human respiratory tract has a vast internal surface area and a very thin air-blood tissue interface facilitating

gas exchange and blood oxygenation functions. Moreover, this system has evolved with a series of structural and functional barriers to deal with inhaled particulates before being conducted deeply into the lung. The human respiratory tract consists of three sequential regions, assisting the filtration effect, including nasopharyngeal, tracheobronchial and the pulmonary regions. However, considering the vast internal surface area of the alveoli and airways with an approximate total of 150 m^2 which facilitates broad access of inhaled materials to the lung tissue, this system cannot always deal adequately with the wide range of airborne materials that may occur in urban or occupational environments. Depending upon the physicochemical characteristics of inhaled materials, the respiratory system can be considered as a site of toxicity for pulmonary toxicants and following absorption, a pathway for inhaled chemicals to reach other organs distant from the lung and elicit their toxic effects at extrapulmonary sites.

Particle-Lung Interactions, CRC Press, Feb. 18, 2000, ed. Peter Gehr, Joachim Heyder.

S. Bakand, A. Hayes, F. Dechsakulthorn, *Nanoparticles: a review of particle toxicology following inhalation exposure, Inhalation Toxicology, 2012; 24(2): 125–135.* *http://informahealthcare.com/doi/abs/10.3109/08958378.2010.642021*

1.7 Environmental fate and behavior

Note: The focus of this document is on manufactured nanomaterials (MNM). However, consideration may also be given to ultrafine particles and incidental nanoparticles related to the by-products of combustion, vaporization etc. and/or naturally occurring particles in the environment, generating a background against which emissions of MNMs will have to be measured and monitored. Current research has indicated that it can be difficult to differentiate between the nanoparticle of concern and other particles. Emphasis should be given to review comparable standards and methods for general particle assessment and identification.

Nanotechnology Safety and Health Program, National Institutes of Health, 2012.

P. Borm et al., The potential risks of nanomaterials: a review carried out for ECETOC,Particle and Fibre Toxicology 2006, 3, 11,.

http://ec.europa.eu/health/ph_risk/documents/ev_20040301_en.pdf

Expert recommendations:

- Development of a nomenclature for intermediate and finished MNMs, as an international effort
- Assignment of a universally recognized CAS Number to MNMs
- Sources of NPs not directly related to MNMs or their production have to and will be included in the discussion of health effects induced by Nanoparticles.

European Commission. Community Health and Consumer Protection. 2004. Nanotechnologies: A Preliminary Risk Analysis on the Basis of a Workshop Organized in Brussels on 1–2 March 2004 by the Health and Consumer Protection Directorate General of the European Commission. *http://europa.eu.int/comm/health/ph_risk/documents/ev_20040301_en.pdf*

1.7.1 Bioaccumulation

Bioaccumulation refers to the accumulation of a toxic substance in an organism at a rate greater than that at which the substance is lost. Thus, the longer the biological half-life of the substance the greater the risk of chronic poisoning, even if environmental levels of the toxin are not very high.

1.7.2 Bioaccumulation of nanomaterials

The potential for nanomaterials to bioaccumulate in living organisms and to enhance the bioaccumulation of other toxic substances may pose severe risks to human health and, by extension, possibly to other animals. At present there is limited information to identify the most critical properties that are likely to lead to bioaccumulation or bio-magnification of MNMs. However, ready uptake, biostability and poor clearance are likely to be the driving factors.

http://toxics.usgs.gov/definitions/bioaccumulation.html.

1.7.3 Contaminant

A substance that is either present in an environment where it does not belong or is present at levels that might cause harmful (adverse) health effects.

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

1.7.4 Contaminant concentration

The amount of a substance (contaminant) present in a certain amount of soil, water, air, food, blood, hair, urine, breath, or any other media.

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

1.7.5 Environmental factors

Environmental factors may contribute to the response for a given chemical. For example, such factors as air pollution, workplace conditions, living conditions, personal habits, and previous chemical exposure may act in conjunction with other toxic mechanisms.

TOXICOLOGY AND EXPOSURE GUIDELINES (Revised 1/03)
http://ehs.unl.edu/documents/tox_exposure_guidelines.pdf

1.7.6 Environmental media and transport mechanism

Environmental media include water, air, soil, and biota (plants and animals) that can contain contaminants. Transport mechanisms move contaminants from the source to points where human exposure can occur. The environmental media and transport mechanism is the second part of an exposure pathway.

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

1.7.7 Half-time ($t_{½}$)

The time it takes for half the original amount of a substance to disappear. In the environment, the half-time is the time it takes for half the original amount of a substance to disappear when it is changed to another chemical by bacteria, fungi, sunlight, or other chemical processes. In the human body, the half-time is the time it takes for half the original amount of the substance to disappear, either by being changed to another substance or by leaving the body. In the case of radioactive material, the half life is the amount of time necessary for one half

the initial number of radioactive atoms to change or transform into another atom (that is normally not radioactive). After two half lives, 25% of the original number of radioactive atoms remains.

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

1.7.8 Hazard

A source of potential harm from past, current, or future exposures.

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

1.7.9 Hazardous substance release and health effects database (HazDat)

The scientific and administrative database system developed by ATSDR to manage data collection, retrieval, and analysis of site-specific information on hazardous substances, community health concerns, and public health activities.

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

1.7.10 Hazardous waste

Potentially harmful substances that have been released or discarded into the environment.

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

1.7.11 Nanopollution

Generic name for all waste generated by nanodevices or during the nano-materials manufacturing process.

1.7.12 Potential environmental processes affecting nanomaterials

Agglomeration / Disagglomeration	The process by which nanomaterials come together or spread apart within their existing environment
Association with biotic/abiotic suspended particulate material	Processes whereby nanomaterials interact with other materials in the environment around them e.g. via adherence, sorption etc.
Complete mineralisation	The conversion of a carbon-containing nanomaterial to an inorganic state via biotic and abiotic decomposition
Deposition	The settling of nanomaterials from within a solution, suspension mixture or vapour, e.g. from an aerosolised form into water
Diffusion	The net transport of nanomaterials from a region of higher concentration to one of lower concentration by random molecular motion
Dissolution	Process whereby a solid nanomaterial dissolves into a solvent to yield a solution

Re-suspension	The renewed suspension of insoluble nanomaterials after they have been precipitated, e.g. from on a surface into gas or from sediment into water
Settling / Sedimentation	Process whereby nanomaterials in suspension/solution to settle out of the fluid in which they are entrained
Speciation	Association of a nanomaterial with other molecular or ionic dissolved chemical substances
Transformation	Process whereby a nanomaterial undergoes either a biological or chemical transformation
Accumulation	Nondegradable MNMs can accumulate into the cells and/or organs and exert damage effect. MNMs accumulated in organisms at the lower trophic level can transfer to higher trophic level animals with the occurrence of biomagnification varying depending on the specific food chain studied.

Table 12: Potential environmental processes affecting nanomaterials

1.7.13 PBT profile

For the purpose of environmental hazard identification of nanomaterials, the PBT profile (persistence, bioaccumulation, toxicity) is of major importance as defined by REACH (http://ec.europa.eu/enterprise/sectors/chemicals/files/reach/review2012/benefits-final-report-part-b_en.pdf)

1.7.14 Persistence

Persistence can be defined as the property of continuation of existence of a chemical/material. Persistence or accumulation is considered a risk factor for hazardous effects in the long-term. Persistence is used primarily in a risk assessment context, to define chemicals or materials that are retained in the body or in the environment, although it could also be applied to durable products. Insoluble, non degradable nanomaterials would have a high priority for risk assessment as (bio)persistence/accumulation may be associated with chronic hazardous effects. In this respect persistence can be considered as the opposite of solubility or (bio)degradability.

1.7.15 Risk

The probability that something will cause injury or harm.

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

Chance of something happening that will impact on objectives

AS/NZS 4360:2004 Definitions

Effect of uncertainty on objectives

ISO 31000 Definitions (ISO/IEC Guide 73)
AS/NZS ISO 31000:2009

Risk is a measure of the probability that harm will occur under defined conditions of exposure to a chemical

John Duffus & Howard Worth, *Hazard and Risk,* IUPAC Educators' Resource Material http://old.iupac.org/publications/cd/essential_toxicology/IUPACTOX4.pdf

A probability function f_P of exposure and hazard [Krug, 2011]:

$R = f_P$ {Exposure, Hazard}.

H.F. Krug, P. Wick, Nanotoxicology: an interdisciplinary challenge. Angew. Chem. Int. Ed Engl. 50, 1260-1278, 2011.

1.7.16 Risk assessment

Risk assessment requires information on both the potential hazard, the release of the substance into the environments and the likelihood and/or degree of resulting short- and long-term exposure.

ENV/JM/MONO(2012)8 ***IMPORTANT ISSUES ON RISK ASSESSMENT OF MANUFACTURED NANOMATERIALS, Series on the Safety of Manufactured Nanomaterials, No. 33***

1.7.17 Risk assessment framework

The classical risk assessment framework includes four main steps: hazard identification, hazard characterisation including dose-response assessment, exposure assessment, and risk characterization

NRC (1983). Risk Assessment in the Federal Government: Managing the Process. Committee on the Institutional Means for Assessment of Risks to Public Health, Commission on Life Sciences, National Research Council. Washington, D.C.: National Academy Press, 191 pp.
ENV/JM/MONO(2012)8 ***IMPORTANT ISSUES ON RISK ASSESSMENT OF MANUFACTURED NANOMATERIALS, Series on the Safety of Manufactured Nanomaterials, No. 33***

1.7.18 Risk characterization

Estimation of the incidence and severity of the adverse effects likely to occur in a human population or environmental compartment due to actual or predicted exposure to a substance, and may include "risk estimation", i.e. the quantification of that likelihood.

European Commission JRC 2003a.

Risk characterization is the final part of risk assessment where all the information gathered during the first three steps of risk assessment come together

CCA. 2008. Small Is Different: A Science Perspective On The Regulatory Challenges of the Nanoscale. Ottawa: The Council of Canadian Academies.

1.7.19 Risk management

Coordinated activities to direct and control an organisation with regard to risk
ISO 31000 Definitions (ISO/IEC Guide 73)

Culture, processes and structures that are directed towards realizing potential opportunities whilst managing adverse effects

AS/NZS 4360:2004 Definitions

Note: In the Standard, the expressions “risk management” and “managing risk” are both used. In general terms, “risk management” refers to the architecture (principles, framework and process) for managing risks effectively, and “managing risk” refers to applying that architecture to particular risks.

1.7.20 Risk management framework

Set of components that provide the foundations and organizational arrangements for designing, implementing, monitoring, reviewing and continually improving risk management throughout the organisation

ISO 31000 Definitions (ISO/IEC Guide 73)

Set of elements of an organisation’s management system concerned with managing risk

AS/NZS 4360:2004 Definitions

1.7.21 Risk management policy

Statement of the overall intentions and direction of an organisation related to risk management

ISO 31000 Definitions (ISO/IEC Guide 73)

1.7.22 Risk management plan

Scheme within the risk management framework specifying the approach, the management components and resources to be applied to the management of risk

ISO 31000 Definitions (ISO/IEC Guide 73)

1.8 Toxicity

Nanotoxicology is the field which studies potential health risks of nanomaterials.

Toxicity is the degree to which a substance can damage a living or non-living organisms. Toxicity can refer to the effect on a whole organism, such as an animal, bacterium, or plant, as well as the effect on a substructure of the organism, such as a cell (cytotoxicity) or an organ (organotoxicity), such as the liver (hepatotoxicity). By extension, the word may be metaphorically used to describe toxic effects on larger and more complex groups, such as the family unit or society at large.

1.8.1 Toxicity tests

The design of any toxicity test incorporates:

- a test organism, which can range from cellular material and selected strains of bacteria through higher order plants and animals
- a response or biological endpoint, which can range from subtle changes in physiology and behavior to death
- an exposure or test period
- a dose or series of doses.

The objective is to select a test species that is a good model of humans, a response that is not subjective and can be consistently determined for a given dose, and a test period that is relatively short.

TOXICOLOGY AND EXPOSURE GUIDELINES (Revised 1/03)
http://ehs.unl.edu/documents/tox_exposure_guidelines.pdf

1.8.2 Toxic dose low (TDLO)

The lowest dose of a substance introduced by any route, other than inhalation, over any given period of time, and reported to produce any toxic effect in humans or to produce tumorigenic or reproductive effects in animals.

TOXICOLOGY AND EXPOSURE GUIDELINES (Revised 1/03)
http://ehs.unl.edu/documents/tox_exposure_guidelines.pdf

1.8.3 Toxic concentration low (TCLO)

The lowest concentration of a substance in air to which humans or animals have been exposed for any given period of time that has produced any toxic effect in humans or produced tumorigenic or reproductive effects in animals.

TOXICOLOGY AND EXPOSURE GUIDELINES (Revised 1/03)
http://ehs.unl.edu/documents/tox_exposure_guidelines.pdf

Toxicology is the branch of medical and biological science studying the nature, adverse effects, detection, and treatment of poisons on living organisms. A fundamental principle of toxicology is that any substance is poisonous if given in a large amount. From the study of cancer-causing substances, carcinogens, it appears that there are some materials for which there is no safe dose, no level of exposure below which they do not cause cancer.

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

1.8.4 Toxicological profile

A document that examines, summarizes, and interprets information about a hazardous substance to determine harmful levels of exposure and associated health effects. A toxicological profile also identifies significant gaps in knowledge on the substance and describes areas where further research is needed.

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

1.9 Health effects of manufactured nanomaterials

Adverse health effect

A change in body function or cell structure that might lead to disease or health problems

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

1.9.1 Health effects of MNMs as the basis for pathophysiology and nano-toxicology:

Possible MNM effects	Possible pathophysiological outcomes
ROS generation	Protein, DNA and membrane injury, oxidative stress
Oxidative stress	Phase II enzyme induction, inflammation, mitochondrial perturbation
Mitochondrial perturbation	Inner membrane damage, permeability transition (PT), pore opening, energy failure, apoptosis, apo-necrosis, cytotoxicity
Inflammation	Tissue infiltration with inflammatory cells, fibrosis, granulomas, atherogenesis, acute phase protein expression (e.g., C-reactive protein)
Uptake by reticulo-endothelial system	Asymptomatic sequestration and storage in liver, spleen, lymph nodes, possible organ enlargement and dysfunction
Protein denaturation, degradation	Loss of enzyme activity, auto-antigenicity
Uptake in the cell nucleus	DNA damage, nucleoprotein clumping, autoantigens
Uptake in neuronal tissue	Brain and peripheral nervous system injury
Perturbation of phagocytic function, ‘‘particle overload,’’ mediator release	Chronic inflammation, fibrosis, granulomas, interference in clearance of infectious agent
Endothelial dysfunction, effects on blood clotting	Atherogenesis, thrombosis, stroke, myocardial infarction
Generation of neoantigens, breakdown in immune tolerance	Autoimmunity, adjuvant effects
Altered cell cycle regulation	Proliferation, cell cycle arrest, senescence
DNA damage	Mutagenesis, metaplasia, carcinogenesis

Table 13: Health effects of MNMs as the basis for pathophysiology and nano-toxicology

Note: *it has to be emphasized that not all nanoparticles produce these adverse health effects - the toxicity of nanoparticles depends on various factors, including: exposure, nanoparticle chemistry, size, shape, aggregation, crystallinity, surface functionalization, electromagnetic properties etc. In addition, the toxicity of any nanoparticle to an organism is determined by the individual’s genetic*

complement, which provides the biochemical toolbox by which it can adapt to and fight toxic substances.

1.9.2 Other terms used in relation to MNMs health effects:

1.9.2.1 Absorption

The process of taking in. For a person or an animal, absorption is the process of a substance getting into the body through the eyes, skin, stomach, intestines, or lungs.

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

1.9.2.2 Aerosols

Mixtures of solid or liquid particles with air. Because of potential damage to the human respiratory tract, aerosols are the subject of intensive research. Air can carry particles in the size range of particles, of which air itself consists, up to over 100 microns. Of primary importance for human health are particles <10 µm.

1.9.2.3 Bronchoalveolar lavage (BAL) damage markers – BAL profile

This method samples the cells and fluid from the bronchoalveolar space and allows the assessment of inflammation by quantification of cell numbers and types and components of the fluid phase. In addition, considerable extra information can be gained by various *ex vivo* manipulations of the BAL cells, e.g., gene expression, phagocytic potential, etc. Other BAL damage markers include BAL lactate dehydrogenase levels (as a measure of cytotoxicity), BAL protein levels (increases in BAL fluid protein concentrations generally are consistent with enhanced permeability of vascular proteins into the alveolar regions, indicating a breakdown in the integrity of the alveolar-capillary barrier), and BAL alkaline phosphatase levels (as a measure of Type 2 alveolar epithelial cell toxicity). Methodologies for cell counts, differentials, and pulmonary biomarkers in lavaged fluids have previously been described [Oberdörster, 2005].

1.9.2.4 Carcinogens

Chemicals that are associated with lung cancer.

TOXICOLOGY AND EXPOSURE GUIDELINES (Revised 1/03)
http://ehs.unl.edu/documents/tox_exposure_guidelines.pdf

1.9.2.5 Catalysis/Catalyst

Catalysis is the change in rate of a chemical reaction due to the participation of a substance called a **catalyst**. Catalysts can be either heterogeneous or homogeneous, depending on whether a catalyst exists in the same phase as the substrate. Heterogeneous catalysts act in a different phase than the reactants. Homogeneous catalysts function in the same phase as the reactants, but the mechanistic principles invoked in heterogeneous catalysis are generally applicable. While such improved activity is most advantageous for an industrial application, the same catalytic activity may contribute to a most aggressive form of long-term toxicity: Following a rapid uptake into cells, such chemically active particles may interfere with cellular metabolism by catalyzing specific reactions within the cytosol. While a normal toxic agent exerts a dose and mass-related effect on the corresponding organism, a catalytically active material may repeat its chemical interaction with the host over and over again. Catalytic activity

therefore greatly enhances the potency of such toxins, especially if the material has a long-term persistence within the host organism.

PAC, 1996, 68, 149 (A glossary of terms used in chemical kinetics, including reaction dynamics (IUPAC Recommendations 1996)) [Limbach, 2007].

1.9.2.6 Cell proliferation

Increased cell division plays a key role in pathological responses and can be determined in epithelial or mesothelial cells by uptake of labeled nucleotide precursors, such as tritiated thymidine or BrdU (5-bromo-2'deoxyuridine). Recommended experiments are designed to measure the effects of particle exposures on airway and lung parenchymal cell turnover in rats following exposures [Oberdörster, 2005].

1.9.2.7 Dosimetry

Since mass may not be the proper dose metric for comparing the toxicity of fine vs. ultrafine particles, characterization of the test material should also include surface area per mass and particle number per mass. For practical purposes, dose could be monitored as mass delivered/animal or mass inhaled/animal and then be converted easily to a surface area or particle number dose as necessary, provided the correlation between these three particle parameters is available [Oberdörster, 2005].

1.9.2.8 Experimental models

Experimental models account for most of what we know about the health effects of nanoparticles. They can be classified in 2 categories:

- *in vitro* models (studies of isolated cultured cells);
- *in vivo* (studies of the whole animal) or ex vivo models (studies of isolated tissues).

In vitro models models study the interaction between particles and cultured cells and the resulting biological response

In vivo and ***ex vivo*** models are more difficult to use to study nanoparticles, because of their cost (there is very little research of this type in public laboratories) and the problems encountered in generating stable nanometric aerosols (rapid aggregation or agglomeration of some newly emitted nanoparticles). The conclusions are consistent with and reinforce those of the in vitro models. They allow us to identify some types of tissue (inflammation, fibrosis) and systemic (cardiovascular or central nervous system) responses. They do not currently allow a categorical response about a possible carcinogenic effect. They do, however, provide complementary information about how nanoparticles penetrate the organism.

1.9.2.9 Genotoxicity

Denoting a substance that by damaging DNA may cause mutation or cancer.

1.9.2.10 Medical Dictionary for the Health Professions and Nursing © Farlex 2012Histopathology

Description of the general effects of treatments on the lungs should include endpoints such as presence of dust-laden macrophages, cellular infiltrates and hyperplastic changes in the epithelium. It is recommended that the entire respiratory tract be evaluated for adverse pathological effects. This would include the upper respiratory tract – the nose, larynx and upper airways; the lower respirato-

ry tract and lymph nodes; and the pleural region. Histopathological observations in a Tier 1 process would focus primarily on inflammatory responses and the development of fibrosis. Fibrosis can be determined in lung tissue by specific staining of collagen in histopathological slides, or by qualitative and quantitative histopathology [Oberdörster, 2005].

1.9.2.11 Inhalation

Inhalation is used as a method of testing exposure of the respiratory tract for hazard identification and to obtain dose-response data. Physicochemical characterization of the generated aerosol is essential [Oberdörster, 2005].

1.9.2.12 Intratracheal instillation

Intratracheal instillation of nanomaterial suspended in an appropriate vehicle is considered an acceptable method for pulmonary exposure to evaluate the relative toxicity of the test material [Oberdörster, 2005].

1.9.2.13 Lethal dose low (LDLO)

The lowest dose, other than LD50 of a substance introduced by any route, other than inhalation, which has been reported to have caused death in humans or animals.

TOXICOLOGY AND EXPOSURE GUIDELINES (Revised 1/03)
http://ehs.unl.edu/documents/tox_exposure_guidelines.pdf

1.9.2.14 Lethal dose fifty (LD50)

A calculated dose of a substance which is expected to cause the death of 50 percent of an entire defined experimental animal population. It is determined from the exposure to the substance by any route other than inhalation.

TOXICOLOGY AND EXPOSURE GUIDELINES (Revised 1/03)
http://ehs.unl.edu/documents/tox_exposure_guidelines.pdf

1.9.2.15 Lethal concentration low (LCLO)

The lowest concentration of a substance in air, other than LC50, which has been reported to cause death in humans or animals.

TOXICOLOGY AND EXPOSURE GUIDELINES (Revised 1/03)
http://ehs.unl.edu/documents/tox_exposure_guidelines.pdf

1.9.2.16 Lethal concentration fifty (LC50)

A calculated concentration of a substance in air, exposure to which for a specified length of time is expected to cause the death of 50 percent of an entire defined experimental animal population.

TOXICOLOGY AND EXPOSURE GUIDELINES (Revised 1/03)
http://ehs.unl.edu/documents/tox_exposure_guidelines.pdf

1.9.2.17 Mortality

Death. Usually the cause (a specific disease, a condition, or an injury) is stated.

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

1.9.2.18 Mutagen

A substance that causes mutations (genetic damage).

Agency for Toxic Substances and Disease Registry (ATSDR)

http://www.atsdr.cdc.gov/glossary.html

1.9.2.19 Mutagen

Mutagens are agents that cause changes (mutations) in the genetic code, altering DNA. The changes can be chromosomal breaks, rearrangement of chromosome pieces, gain or loss of entire chromosomes, or changes within a gene.

TOXICOLOGY AND EXPOSURE GUIDELINES (Revised 1/03)
http://ehs.unl.edu/documents/tox_exposure_guidelines.pdf

1.9.2.20 Nanomaterial-based catalysts

Nanomaterial-based catalysts are usually heterogeneous catalysts broken up into metal nanoparticles in order to speed up the catalytic process. Metal nanoparticles have a higher surface area so there is increased catalytic activity because more catalytic reactions can occur at the same time. Nanoparticle catalysts can also be easily separated and recycled with more retention of catalytic activity than their bulk counterparts. These catalysts can play two different roles in catalytic processes: they can be the site of catalysis or they can act as a support for catalytic processes.

Work programme 2013 (European Commission C(2012) 4536 of 09 July 2012) Annex 7 to the Decision NMP for CAP_en[1].pdf

1.9.2.21 Oxidative stress markers – ROS/RNS

Reactive oxygen and nitrogen species have been implicated in DNA damage and induction of inflammatory cytokines and growth factors. A cellular BAL fluid levels of gluthathione, total antioxidants, or nitrate/nitrite (a measure of nitric oxide production), lipid peroxidation of lung tissue, or *ex vivo* measurement of ROS/RNS from BAL cells can be employed to monitor oxidant generation and oxidant stress [Oberdörster, 2005].

1.9.2.22 Tumor

An abnormal mass of tissue that results from excessive cell division that is uncontrolled and progressive. Tumors perform no useful body function. Tumors can be either benign (not cancer) or malignant (cancer).

Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

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- Glossary on particles and further links: IFA-Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung
- ORD Exposure Factors Handbook, Exposure Factors Glossary, http://www.epa.gov/ncea/efh/report.html
- Risk Assessment Glossary, http://www.epa.gov/risk/index.htm
- Glossary of health, exposure, and risk assessment terms, http://www.epa.gov/ttn/atw/hlthef/hapindex.html
- TOXICOLOGY AND EXPOSURE GUIDELINES, UNL Environmental Health and Safety, 2012 (402) 472-4925 · http://ehs.unl.edu/documents/tox_exposure_guidelines.pdf
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For exposure assessment and exposure investigation: Sources of information also include standards from ISO (ISO, 2007; 2008) and the British Standards Institution (BSI, 2007), reports from the Organisation for Economic Co-operation and Development (OECD, 2010) and outputs from European projects such as NanoSafe2 (http://www.nanosafe.org; accessed 4th September 2010), as well as the general scientific literature.

2 Risk assessment

2.1 Introduction

There are many operative definitions of risk, which do not necessarily harmonize. For the purpose of risk assessment for human health and the environment, a definition such as the ones given in chapters 1.7.15 to 1.7.22 of this report or the one suggested by OHSAS (Occupational Health & Safety Advisory Services) is relevant [OHSAS, 2007]. This defines risk as the product of the probability of a hazard resulting in an adverse event, times the severity of the event ("Risk is a combination of the likelihood of an occurrence of a hazardous event or exposure(s) and the severity of injury or ill health that can be caused by the event or exposure(s)".

In other words, **Risk is a probability function of both Exposure and Hazard**, see also [Krug, 2011].

What is then risk assessment? A generally agreed upon definition is not available, but within the community where risk assessment for human health and the environment is performed and observed, it is seen as “a process by which scientists evaluate the potential for adverse health or environmental effects from exposure to naturally occurring or synthetic agents” [Society of Toxicology, 1998]. The goal of this activity is to provide risk managers (authorities, health and safety personnel etc.) with a science-based foundation for decision making in management of agents possibly affecting health and environment.

The principal stages of risk assessment are depicted in the flow chart in the Figure 1 below and can be summarized as follows:

- Hazard identification
- Examination of dose-response relationships (hazard characterization)
- Exposure assessment
- To highlight uncertainties in the determination of hazards and dose-response relationships
- To evaluate possible modes (mechanisms) of actions for the hazard of concern.

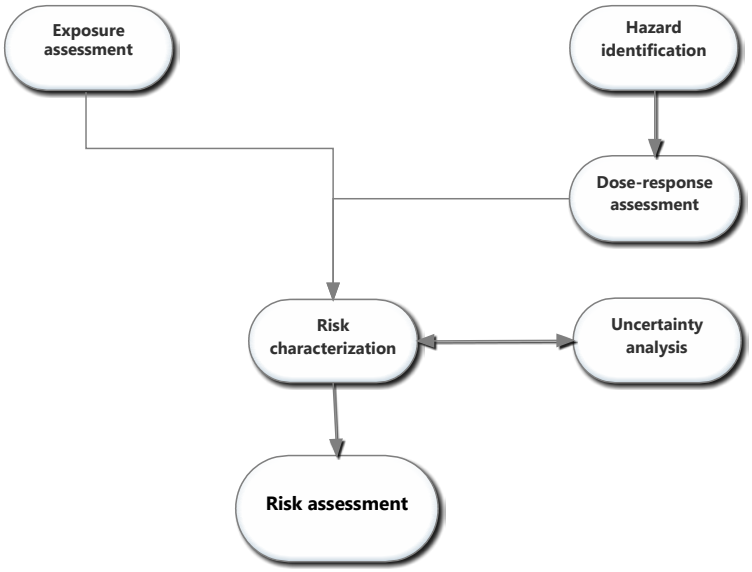

Figure 1: Flow chart depicting the different processes that are involved in risk assessment of agents for health and the environment. Importantly, there is no risk unless both exposure and hazard criteria are present.

A health risk assessment typically evaluates the evidence within several areas of studies (such as human studies, animal studies, cellular studies, modelling and *in silico* studies; “lines of evidence”). Obtaining data from different types of studies make it thus possible to integrate the various pieces of data, to perform an integrative risk assessment.

Due to advances in scientific knowledge as well as in modelling and measuring techniques, the procedures currently used for human and environmental risk assessment are required and expected to change substantially in the near future. Regarding risk assessment for human health, the approach currently used is hazard-driven with strong reliance on the use of laboratory inbred rat strains and to a lesser extent, inbred mouse strains as test species. Over time, these tests have been increasingly standardised by the introduction of good laboratory practice and ICH (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) or OECD (Organisation for Economic Co-operation and Development) test guidelines. Some *in vitro* tests, in particular for genotoxicity and topical effects have been added. Many of the tests in current use are written into legislative requirements for the approval of various types of products. To address uncertainties due to the need for extrapolations from animal experiment data to characterise effects that may occur in humans, conservative standard default values (also called assessment factors, uncertainty factors, or default factors) are common practice.

There are both political and scientific reasons behind a change in the way that human risk assessment is conducted. The primary changes that are proposed are:

- A paradigm shift from a hazard-driven process to one that is exposure-driven
- A progressive reduction of tests using laboratory animals.

The latter change is also reinforced by the REACH legislation of 2006 with the aim that animal testing is a last resort (Regulation (EC) No 1907/2006 concerning the registration, evaluation, authorization and restriction of chemicals). In turn, this ambition is founded on “The three Rs (3Rs)” guiding principle for animal use in testing (Replacement, Reduction, Refinement; [Russel and Burch, 1959]).

Risk assessment can be performed according to different approaches, that are either qualitative (stating risk in terms like ´low´, ´intermediate´, ´high´ etc.); quantitative (giving e.g. a probability number for the risk in question); or a mixture of the two. Furthermore, the assessments can be founded in different analytical frameworks.Specific considerations for **risk assessment** of nanomaterials have recently been published by OECD, in the new OECD "Series on the Safety of Manufactured Nanomaterials No. 33: IMPORTANT ISSUES ON RISK ASSESSMENT OF MANUFACTURED NANOMATERIALS" [OECD, 2012].

The sections below give an overview of the key activities in risk assessment, as well as an introduction to specific challenges for risk assessment of MNM.

2.2 Environmental (ecological risk assessment)

Environmental risk assessment tries to estimate the likelihood or probability of an adverse outcome or event due to pressures or changes in environmental conditions resulting from human activities. This is based on the comparison between an indicator of exposure (e.g. Predicted Environmental Concentration or PEC) and an indicator of effect (e.g. a Predicted No Effect Concentration or PNEC) or an ecotoxicological end point (e.g. a No Observed Effect Concentration (NOEC) or an EC/LC50) [Straub, 2002]. Risk estimation is simply calculated as the ratio between these indicators (e.g. PEC/PNEC or TER: Toxicity Exposure Ratio).

The procedures for ecological risk assessment according to the requirements of European chemical regulations are described in detail in official documents such as the Technical Guidance Document (TGD) on risk assessment [European Commission, 2003] and, for plant protection products (PPPs), in the Annex VI (Uniform Principles) of the Directive 91/414 [European Commission, 1991]. Even if the details of the procedures are different, the conceptual approaches are similar.

A PEC for each environmental compartment (water, sediment, air, soil, biota) is generally estimated using multimedia modelling approaches applied to standardized environmental scenarios such as the local, regional and continental scenarios proposed by the TGD or the European agricultural scenarios proposed by the FOCUS group for PPPs [EFSA, 2004]. Experimental monitoring data may be used if available and suitable.

Also a PNEC must be calculated for each environmental compartment, using available ecotoxicological data [Straub, 2002]. The traditional procedure is based on a relatively reduced data set corrected by application factors (AF) related to the degree of uncertainty and the amount of information available. If suitable information is available, a PNEC may be also derived using the Species Sensitivity Distribution (SSD) approach or higher tier data (mesocosm or field data) that improve the ecological realism of the assessment [Yi, 2002]. In some cases, if toxicological information is lacking, approximated extrapolation approaches may be applied (e.g. equilibrium partitioning method for soil and sediments).

2.3 Human health risk assessment

Risk assessment of human exposure to chemical substances or physical agents comprises the two different but complementary approaches [ECETOC, 1995]:

1. The toxicological assessment, which is based on experimental data;
2. The epidemiological approach, which studies human response to exposures and bases its judgment on observations in human populations.

Toxicological evaluation of substances requires knowledge on the toxic effects (hazard identification) seen at different exposure times via routes relevant to the common use of the substance. Organ specificity and other relevant endpoints like fertility, pre- and postnatal toxicity or carcinogenicity, their dose-response and the No Observed Effect Level (NOEL) are identified by appropriate repeated dose studies in animals. In vitro testing and information on the mode of action of the adverse effect can contribute important pieces of information on the toxic potential of a chemical substance.

Epidemiological evaluations either start with observed exposures (new, increased or accidental), and relate these to observed health effects, or from observed changes in health of populations and relating these to suspected exposures. Once a toxic potential of a substance is recognized and it is expected to be related to certain health effects, the health effects need to be observed and measured in exposed or potentially exposed populations. Often industrial workers are the first to be in contact with a toxic substance.

2.4 Exposure assessment

Since toxic effects are dose dependent, knowledge of the extent and duration of exposure is an integral part of the risk assessment process [WHO, 1994]. Exposure defines the amount of a chemical to which a population or individuals are exposed via inhalation, oral and dermal routes. Animal or human exposure is usually defined as the daily dose, e.g., in mg of the chemical/kg body weight/day. This daily dose may result from

oral, inhalation or dermal exposure or as a sum thereof. Ultimately, it is the dose, which reaches the cellular target over a given time period that results in the toxicological response. Thus, the toxic potency of a chemical is the product of the interrelated external, internal, and target doses.

For evaluation of time dependency of exposure it is essential to know whether the substance is rapidly excreted or metabolized or accumulating in the body. The relevant time frame is depending on this knowledge. But also in ubiquitous exposures occurring through e.g. air or water pollution the exact exposure need to be measured or estimated, as in these cases there are no unexposed individuals or populations.

2.5 Mode of action

Modes of action identify the biochemical pathways that link exposure to a stressor to any outcome (immediate or eventual) [Crump, 2011].

The mode of action for a stressor that causes chronic toxicity can be considered to consist of three parts:

- Initial (primary) interactions between the stressor and biological components;
- Intermediary (secondary) stage(s) as a consequence of the initial interaction(s); these may include irreversible steps and/or additional effects resulting from prolonged exposure to the stressor
- Late (tertiary) stage(s) in which evident effects on health are manifested.

The links between these stages must be understood if the relevance of data from animal or in vitro models to man is to be considered. There is a good understanding of the modes of action of a number of highly toxic chemicals such as dioxins [Sewall, 1995]. In the case of MNM, such studies are presently mostly lacking.

2.6 Toxicity testing

The early onset and persistence of pulmonary lesions in rodents suggest that subchronic (90 days) or short-term *in vivo* data may be predictive of the long-term effects of some nanomaterials [Oberdörster, 2010]. Technically, because of the high propensity of hydrophobic nanomaterials to agglomerate, preparation of adequate aerosol dispersions suitable for inhalation studies is immensely difficult. As a result, alternative exposure methods (e.g., intratracheal instillation, intratracheal inhalation, pharyngeal/laryngeal aspiration) have been used. Although inhalation is the preferred toxicity method of testing nanoparticles, it appears that, with appropriate dosing schemes and match controls, some of these alternative methods are reasonable alternatives to more costly and time consuming inhalation studies and may be useful for hazard identification or establishing the relative toxicity ranking among different nanomaterials for further testing [Han, 2012]. To date, carefully monitored tests are used to establish the inhalation toxicity of airborne nanoparticles. However, only very recently a new ISO standard was adapted to ensure that the results of such tests are reliable and harmonized worldwide [ISO, 2010]. This standard establishes a battery of inhalation toxicity testing chamber monitoring, including a differential mobility analyzing system (DMAS), for determining particle number, size, size-distribution, surface area and estimated mass dose, as well as morphological examination using transmission electron microscopy (TEM) or scanning electron microscopy (SEM) equipped with an energy dispersive X-ray analyzer (EDXA) for chemical composition. More such standards for toxicity testing are needed involving different exposure routes and specialised in different classes of MNMs.

2.7 Computational methods

Recent developments both within and outside the EU promote risk assessment activities that reduce or even abolish the use of animals in safety testing. In addition, the required testing of an ever increasing number of chemicals during shorter time periods also put additional stress on traditional testing methods. Consequently, a number of alternative approaches are getting more frequently used where appropriate. These methods include in vitro tests, optimised in vivo tests and also computational methods. The latter include e.g. biokinetic modelling, SARs (structure activity relationships) and QSARs (quantitative structure activity relationships).

QSARs are mathematical models that are used for prediction of toxicological behaviour of chemicals based on knowledge from a library of chemical structures. The assumption is that similarity in chemical structure also predicts the effects in a biological system. A number of QSAR models have been developed and tested both for ecotoxicology [Altenburger et al. 2003] and human toxicology purposes [Bernauer et al. 2005; Natarajan and Basak 2011]. Of special interest for nanotoxicology is the potential to use QSARs for grouping of materials [Burello and Worth 2011], which is needed in a setting where novel nanomaterials are constantly developed.

The potential usefulness of QSARs for toxicological testing has also been recognized by OECD and its Working Party on QSARs. This WP has released a tool box for the validation of QSARs [OECD 2004], which was followed by further guidance at the EU-level [ECHA 2008]. Lately, it has been recommended by OECD [OECD, 2012] to include methods like QSAR in the future planning of risk assessment.

2.8 Specific needs and challenges for MNM risk assessment

Although it is likely that substantial efforts have been done by various stakeholders regarding MNM risk assessment, there are not many outcomes that are available in the open scientific literature. It is anyway possible that such assessments have been performed, e.g. by competent authorities and also by the industry that manufactures, uses, or disposes of MNM and MNM containing products. The needs for proper risk assessment are on the other hand stated in many documents (including *inter alia* [Maynard, 2006]; [Borm, 2006]; [Savolainen, 2010]; [Klaine, 2012]; [Kuempel, 2012]), as well as the challenges for MNM risk assessment, whether they are specific or not.

In general, it is considered that the currently available toxicology tests and assays are appropriate also for hazard evaluation of MNM (e.g. [Oberdörster, 2005]; [OECD, 2008, 2010]; [Kuempel, 2012]). However, certain factors may influence the toxicity of MNM relative to larger particles, or to the dissolved form of the chemicals in question.

There are a number of challenges that make RA of MNM unique and possibly also to some extent more complicated than RA of traditional chemicals in solution and of particles of larger size than nanosized particles. Without prioritization, some of the major challenges include (see also Table 14 for an overview of challenges and corresponding needs and knowledge gaps):

- engineered nanomaterials are sophisticated materials that currently appear in many forms, made up of many different chemical elements, and sometimes in combination with other materials. It is likely that the development of new materials will proceed even faster than until today, leading to that the repertoire of materials needing assessment becomes forbiddingly large. Thus, there is a need to find ways to perform predictive risk assessment, based on e.g. grouping principles that can include a number of materials.
- A given ENM exist along a value chain with its specific life cycle, from the initial research stages to the final disposal. To what extent is the material existing at the

nanoscale along this chain, and to what extent are humans and/or the environment actually exposed to the material?

- A number of physical-chemical properties of ENM characterize these materials. Although some knowledge has accumulated during the last years, the respective properties´ influence on the toxicity of ENM needs further investigation.
- An ENM in an environmental setting (water, soil and sediment, or air) or in an organism such as the human will be exposed to different kind of matrices (which can be both organic and inorganic). A significant challenge deals with understanding the effects of these matrices on the properties of the ENM.

Challenge	Corresponding needs and knowledge gaps
Exposure assessment data for products entire life cycle	- Value chain characterization - MNM behaviour during product manufacture, use, aging, disposal
Relevant detection and characterization	- Behaviour in complex media - Methods for realistic concentrations - Noise (background) levels - Behaviour in different organismal environments
Realistic hazard assessment	- Toxikokinetic modelling in organisms - Effects due to matrix interaction - Long term, low dose and persistency effects - High throughput and high content data for endpoint identification and mode of action - Relevant controls - Relevant dose metrics
Risk assessment approach development	- Improved exposure assessment - Case-by-case vs grouping approaches - Quantified RA methods - Uncertainty analyses

Table 14: Overview of challenges and corresponding needs and knowledge gaps

2.9 Characterisation methods

Summary

The aim of this chapter is to provide the first results of a large overview of the techniques available to characterize nanoparticles properties, health effects and environmental impacts and to perform a benchmarking of existing methods. Information was collected from scientific papers, peer literature and internal documents available. In addition a survey was launched in order to compare the most useful characterization techniques in the frame of the European projects taking part of the nanosafety cluster (see http://www.nanosafetycluster.eu/) and to validate the current techniques, for which 18 answers were collected. Some preliminary results are given as well.

For measuring in the air
Work on the uncertainties and detection limits is certainly urgently needed to achieve data qualities good enough for the comparison of results coming from different studies. An important drawback of current state of the art measurement devices is their lack of differentiation of background from nanomaterial related particles.

For measuring in liquids
For reliable studies with MNMs the availability of stable suspensions and harmonized dispersion protocols is very important. The development of fast, quantitative, robust, and cost effective methods in order to address the EU definition of nanomaterials is needed.

Characterization methods in the matrices
Research on techniques for collecting, preserving, and storing samples containing MNMs is urgently needed. There is a lack of adequate techniques for the detection and quantification of MNMs at environmentally relevant concentrations in complex media. The detection limits given by ICPMS depend of the type of nanoparticle. More rapid, more sensitive and specific detection of MNMs are needed. To assess exposure, analytical methods will be required to detect MNM in a range of environmental media, in some cases including complex matrices, such as biosolids and biological tissues. In addition, analytical methods are required to evaluate the levels of transformation products present in environmental matrices.

Characterization of MNMs

Introduction

The necessity to characterize MNMs (manufactured nanomaterials or engineered nanomaterials) appear all along their total life cycle, from production to fate and in completely different media, as presented below [Lin, 2010; Som, 2010].

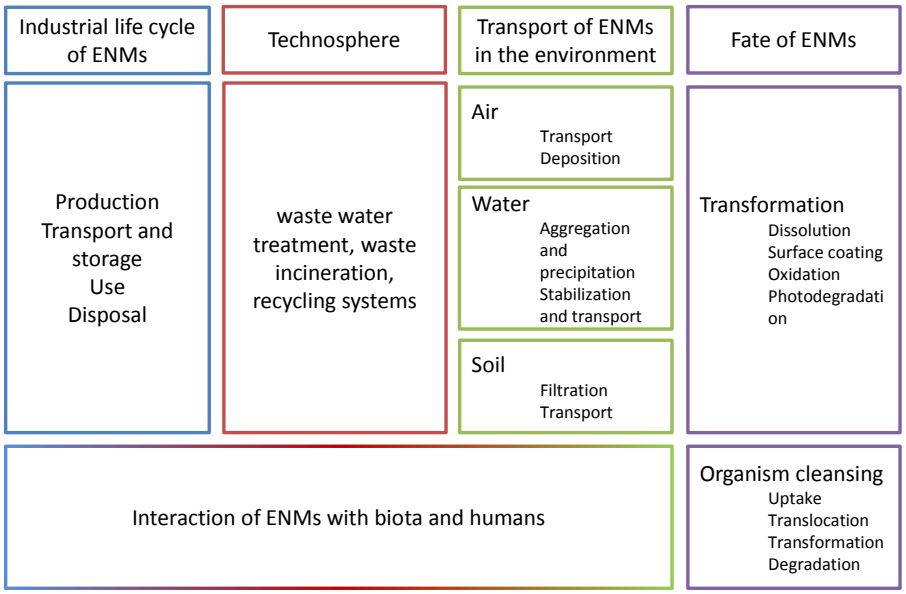

Figure 2: Total life cycle of engineered nanomaterials

At each step, specific methods, appliances and standards will have to be implemented. The community of users will be very different, including non-specialized workers. Today, a lot of techniques and instruments are available; whatever is the stage of the MNMs in their life cycle. This number of available technologies induces a lack of reliability and reproducibility for the measured parameters. In that sense, the improvement of the characterization methods is a real challenge for the future. In the next chapter, a presentation of the most common techniques will be introduced; it will show the profusion of such instruments.

Measurement techniques

The literature presenting the measurement techniques available for the characterisation of MNMs is extremely abundant, and the purpose of this report is not to make an exhaustive analysis of it. The approach chosen here will be to attempt to highlight most relevant parameters allowing a classification.

Table 15 below [ICCR, 2010] gives an approach of some currently available methods and associated measured parameters for nanoparticles and nanomaterials.

Parameter	Method
Chemical composition	Mass spectrometry, EDX, NMR, and other analytical methods
Size and size distribution	Electron microscopy (AFM, TEM, SEM) Chromatography (FFF, hydrodynamic chromatography, size exclusion), Centrifugation (ultracentrifugation), (SPMS, Inductively Coupled Plasma Mass Spectrometry ICP-MS), XRD (crystal size), STXM, CPS, Brookhaven X-Ray Disc centrifuge, Light Scattering, PCCS
Agglomeration/aggregation	Electron microscopy (AFM, TEM, SEM, STEM) Spectroscopy (XRD), BET Light Scattering (Brookhaven X-Ray Disc Centrifuge, PCCS)
Mass concentration	AEM, CFM Gravimetric methods, Centrifugal Sedimentation
Particle number	Particle counters
Shape	Electron Microscopy (AFM, TEM, SEM) Chromatographic (SedFFF-DLS), XRD, STXM
Surface chemistry	AEM, CFM UV/Visible spectrometry, XPS, IR, Raman
Surface charge	Chromatography (e.g. capillary electrophoresis), Zeta potential
Surface area	BET
Solubility/dispersibility	Water solubility, log Kow

Table 15: Overview of critical parameters and examples of available methods for their measurement

In a **more exhaustive way**, Table 16, adapted from Salamon [Salamon, 2010], gives the following correspondence between nanomaterial characteristics and analytical characterization techniques, with their acronyms:

Nanomaterial characteristics	Analytical Technique	Acronym
Concentration	Inductively Coupled Plasma – Mass Spectrometry Field-flow Fractionation + Liquid Chromatography – Mass Spectrometry Optical Spectroscopy – UV/Vis Fluorescence Spectroscopy	ICP-MS FFF-DRI-UV-Vis LC-MS UV/Vis FL
Particle size	Field-flow Fractionation Optical Spectroscopy – UV/Vis Fluorescence Spectroscopy Turbidity Scanning Electron Microscopy Transmission Electron Microscopy (+EDX) Atomic Force Microscopy Confocal Microscopy Field Flow Fractionation-DRI- Dynamic Light Scattering Static Light Scattering Dialysis	FFF UV/Vis FL SEM TEM AFM FFF DLS SLS

	Electrophoresis and Capillary Electrophoresis Ultrafiltration Centrifugation Filtration Nanoparticle Tracking Analysis Size Exclusion Chromatography Selected Area Electron Diffraction Thermogravimetric Analysis Quartz Microbalances Raman Spectroscopy	 NTA SEC SAED TGA
Particle size distribution	Turbidity Scanning Electron Microscopy Transmission Electron Microscopy (+EDX) Atomic Force Microscopy Confocal Microscopy FFF – MALLS / DLS / DRI/UV-Vis Dynamic Light Scattering Electrophoresis and Capillary Electrophoresis Ultrafiltration Centrifugation Filtration Nanoparticle Tracking Analysis Size Exclusion Chromatography Selected Area Electron Diffraction	 SEM TEM AFM FFF – MALLS / DLS / DRI DLS NTA SEC SAED
Surface charge	Atomic Force Microscopy Molecular Gas Adsorption Ultrafiltration Zeta Potential by DLS X-ray Photoelectron Spectroscopy	AFM BET XPS
Surface area	Transmission Electron Microscopy (+EDX) Atomic Force Microscopy Molecular Gas Adsorption X-ray Photoelectron Spectroscopy	TEM AFM BET XPS
Shape	Scanning Electron Microscopy Transmission Electron Microscopy (+EDX) Atomic Force Microscopy Confocal Microscopy	SEM TEM AFM
Agglomeration	Turbidity Scanning Electron Microscopy Transmission Electron Microscopy (+EDX) Atomic Force Microscopy Confocal Microscopy Dynamic Light Scattering Static Light Scattering Centrifuge Nanoparticle Tracking Analysis (for agglomerate formation)	 SEM TEM AFM DLS SLS NTA

Structure	Scanning Electron Microscopy Transmission Electron Microscopy (+EDX) Selected Area Electron Diffraction X-ray Diffraction FT-IR Imaging Raman Spectroscopy	SEM TEM SAED XRD
Composition / Composition and size	Inductively Coupled Plasma – Mass Spectrometry Field-flow Fractionation + ICP-MS Liquid Chromatography – Mass Spectrometry Optical Spectroscopy – UV/Vis Fluorescence Spectroscopy Transmission Electron Microscopy (+EDX) Thermogravimetric Analysis Differential Scanning Calorimetry Dynamic Mechanical Analysis Fourier Transform Infrared Spectroscopy FT-IR Imaging Raman Spectroscopy TGA coupled with Gas Chromatography – Mass Spectrometry	ICP-MS FFF-ICP-MS LC-MS UV/Vis FL TEM TGA DSC DMA FT-IR TGA-GC/MS

Table 16: Correspondence between nanomaterial characteristics and analytical characterization techniques

In addition to the above table, some further information can be obtained:

- shape: some information on the shape can be derived from the comparison of 2 different detection techniques (MALLS that gives the root mean square radius R_g and QELS that gives the hydrodynamic radius R_h), coupled with FFF
- agglomeration: FFF coupled with MALLS does give some information of the agglomeration state of the particles. A steady R_g throughout the peak means rather monodisperse population whereas a large increase at the tail means that agglomeration is occurring.

From the two tables above, it is clear that it is almost impossible to get an exhaustive and hierarchical list, including a wide variety of parameters, such as reliability, easiness, cost, resolution, availability, portability, etc.

In order to simplify, we propose to use Table 17 below, adapted from Stintz [Stintz, 2010]. The nanoparticles are first considered by their constitution, then appearance, and finally their interactions with the outside world. To summarize:

- Constitution: What is it made of?
- Appearance: What does it look like?
- Interactions: Beyond the above, what influences interactions?

Then, these three main characteristics will be further broken down in secondary parameters, which will have in turn adequate instruments and methods to be measured.

Main characteristic	Secondary parameter	Instruments and methods
Constitution	Composition	Tbd subsequently
	Surface chemistry	
Appearance	Particle size/size distribution	
	Agglomeration state/aggregation	
	Shape	
	Surface Area	
Interactions	Surface charge	
	Solubility	
	Dispersibility	

Table 17: How to break down characterisation techniques and measurement

Using this rough classification and data from Salamon [Salamon, 2010], it is possible to number the available methods for the main characteristics and associated parameters. The result is presented in Figure 3.

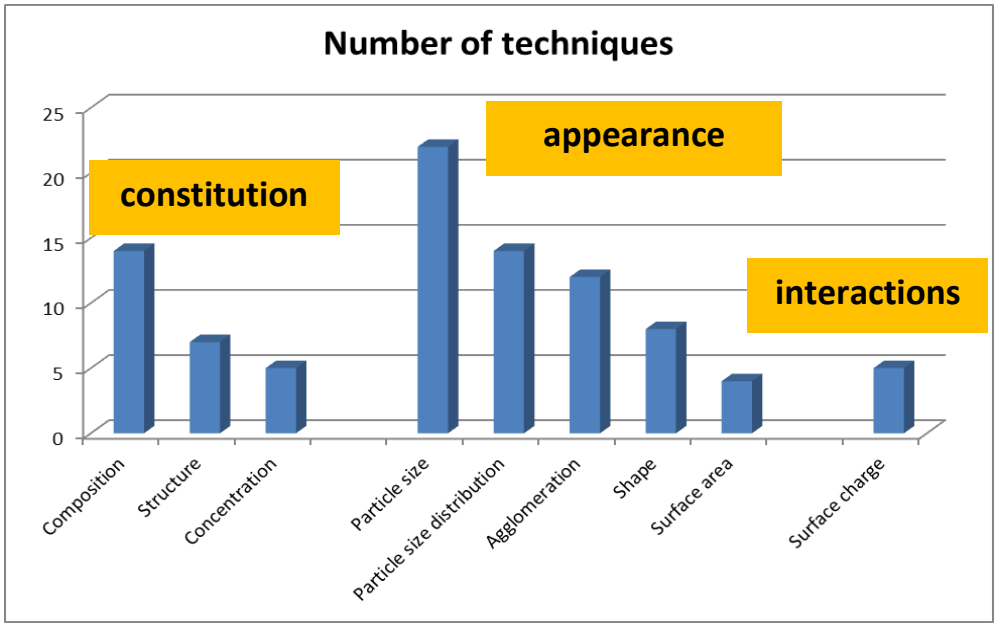

Figure 3: Number of available techniques vs. measured characteristics (from Table 16 and Table 17)

The result is interesting: the “appearance” parameters benefit of the highest number of techniques for their measurements, an especially concerning the size (more than 20 available techniques). At the low end, only 4 techniques are available for the measurement of the surface area: Transmission Electron Microscopy (+EDX), Atomic Force Microscopy, Molecular Gas Adsorption and X-ray Photoelectron Spectroscopy.

The fact has to be emphasized that the MNMs can be characterized by a somewhat limited number of parameters, but that there is a big number of available methods and instruments to do it. Some of these methods are used for liquids, or air, others are more appropriate for solid state environment: the medium is also a parameter to account with.

This leads to a number of difficulties: i) how to make a hierarchy of these methods, ii) how to calibrate the instruments and exchange results, iii) which standards. Moreover, at this point, no classification has been proposed from the field point of view. Those points will be considered in the next chapters.

Benchmarking of characterization methods from the field point of view

We propose a first benchmarking of a number of methods when used in three different media:

- in air,
- in liquids,
- in solid matrices,

which are the conditions found in the field by the experimentalists. The approach cannot be quite exhaustive, since the methods are extremely numerous and sometimes declined as a single unique experiment. We will give here tables collecting instruments, methods, the associated metric, the main characteristics and some comments. The listed instruments correspond to state of the art available technologies which can be found in specialized laboratories. The challenges to cope with in the future are presented for each medium.

a. Characterization methods in air

As already pointed out, the characterization of nanoparticles in air cannot rely on one single technique, due the high dynamics of measurements to proceed with and also to the number of available methods.

i. Available instruments and methods

Some commonly used methods and instruments available are summarized in Table 18.

Instrument	Method	Metric	Main characteristics	Comments
OPC	Light scattering	Concentration # / cm^3	d_p > 80 nm	☺Time of analysis : 1s ☹ limited information about the size in the range below 100nm
CPC	Light scattering	Concentration # / cm^3	d_p > 2.5 nm $C_{max} = 10^7$ # / cm3 C_{min} = 0.01 # /cm3	☺Time of analysis : 1s ☹ No information about the size
SMPS	Electrical classification Light scattering	Size distribution # / cm^3 Electrical mobility diameter	2.5 nm < d_p < 1 µm $C_{max} = 10^7$ # / cm^3 C_{min} = 0,01# /cm^3	☺High resolution (54 channels)) ☹ Time of spectrum analysis : 30 s à 3 min
ELPI	Aerodynamic classification Electric detection	Size distribution # / cm^3 Aerodynamic diameter	7 nm < d_p < 10 µm $C_{max} = 10^7$ # / cm^3 C_{min} = 50 # / cm^3	☺ Collection of particles ☺ Time of analysis : 1s ☹ Needed information : ρ_p ☹ Low resolution (13 plateaux)

FMPS	Electric detection Electric classification	Size distribution # / cm^3 Electrical mobility diameter	6 nm < d_p < 560 nm C_{max} = 10^7 # / cm^3 C_{min} = 10^3 # / cm^3	☺Time of analysis : 0.1 s to 1 s ☺ Average resolution (32 channels) ☹High concentration ☹ Data processing
SEM/TEM	Direct imaging/Electron Microcopy, only after sampling e.g. with ESP or TSP	Structure, Aggregates, Shape, Size distribution	0.5nm-100nm	☺ Very high resolution < 1nm ☹Time consuming for size distribution measurement

Table 18: Methods and instruments for the characterization of nanoparticles in air

Direct imaging: SEM, TEM allow to measure the number based size distribution, structure, aggregates, shape. Using these imaging techniques, it is difficult to have a global statistic measurement (time consuming, lot of images). Thus, the direct imaging techniques are usually used preferentially to validate the others. A common agreement is that there is no universal technique to characterize metrics or physico-chemical properties (eg. size, shape…) of the nanoparticles, as clearly pointed out by Figure 3 (huge number of available methods, > 20).

ii. Common challenges

The harmonization of the measurement strategy for exposure to manufactured nano-objects using the available commercial techniques is a major concern [Brouwer, 2011]: "Key conditions for such intended uses include harmonization of data collection, data analysis and reporting, and data storage". Uncertainty arises from the calibration of the devices which sometimes may differ by up to 30% [Asbach, 2009; Kuhlbusch, 2011]. Work on the uncertainties and detection limits is certainly urgently needed to achieve data qualities good enough for the comparison of results coming from different studies [Brouwer, 2011]. An important drawback of current state of the art measurement devices is their lack of differentiation of background from nanomaterial related particles.

b. Characterization methods in liquids

Clearly, one should not fully rely on one technique only, but rather on multiple measurements. At least two analytical approaches (e.g. techniques) should be employed to characterize MNMs dispersions: Dynamic Light Scattering and Transmission with Scanning Electron Microscopy. To assess the agglomeration state the only method is cryoTEM in which the sample is frozen in liquid helium and observed by TEM in the frozen state.

i. Available instruments and methods

Some common used methods and instruments available are summarized in Table 19.

Instrument	Method	Metric	Main characteristics	Comments
Dynamic light scattering DLS Malvern	Dynamic light scattering (DLS)	$\phi_{hydrodynamic}$ Size distribution	0,3 nm < d_p < 1 µm C_{max} = 400g/l *C_{min} = 0,1 µg/l*	☺Time of analysis : few min ☹ Need to know the viscosity n ☹ Data processing
Nanosight	Nanoparticle Tracking Analysis (NTA)	$\phi_{hydrodynamic}$ Number concentration	15 nm < d_p < 1 µm C_{max} = 10^{10} # / ml C_{min} = 10^{7} # / ml	☺ Time of analysis : few min ☹ Need to know the viscosity n ☹ Data processing

FFF/UV/MALLS/ RI-ICPMS	Separation technique: FFF (field flow fractionation) UV-Vis absorption Multi-Angle Light Scattering Refractive index Inductively Coupled Plasma – Mass Spectrometry	$\phi_{hydrodynamic}$ $\phi_{giration}$ Concentration Molar mass Agglomera-tion state	1 nm < 10 µm C_{max} = 200 mg/l *C_{min} = 0.1µg/l (eg.for Ag)*	☺ Complete information ☹ Need to know dn/dc, η, ε ☹ Data processing ☹ Time of analysis: > 30 min
SEM/TEM/Scan ning probe Microscopy+E DX, EELS	Direct imaging coupled to EELS (electron energy loss spectrosco-py) or EDX (energy X ray Dispersive Spectroscopy) detectors	Structure, Shape, Size distribution Chemical analysis	0.5nm-1000nm	☺ Very high resolution < 1nm ☹ Time consuming for size distribution measurement ☺ TEM : Can be directly coupled to chemical analysis (EELS, EDX) ☹ TEM is a complex equipment Sample preparation

Table 19: Methods and instruments for the characterization of nanoparticles in the liquids

Direct imaging: SEM, TEM allow to measure the number based size distribution, structure, aggregates, shape. These techniques are usually used to validate the other techniques. A common agreement is that no universal technique is available to characterize the physico-chemical properties (eg. size, shape…) metrics of the nanoparticles.

ii. Common challenges

For reliable studies with MNMs the availability of stable suspensions and harmonized dispersion protocols is very important. The following methods are currently widely used for dispersing MNMs:

- Ultrasonication (by use of a ultrasonic bath or tip).
- Shaking and vortexing~
- Various solvents~
- Use of dispersants.

The development of fast, quantitative, robust, and cost effective methods in order to address the EU definition of nanomaterials is needed.

c. Characterization methods in matrices

The analysis of MNM in matrices differs from the traditional chemical analysis of MNMs because the size has to be measured as well. A great need is the development of reliable (robust and validated) methods in order to detect, identify and quantify nano-objects in complex matrices e.g. dispersions, water pastes, creams, polymers/ soil, sediment (solids). The characterizing of MNM after the aging coming out of products could be addressed by using existing techniques, as described by Handy [Handy, 2012]. Many efforts are underway to apply these techniques to MNM in environmental and biological media [Baalousha, 2011]. In cases in which the MNM are composed of common elements (such as Ti, Si, Al, and C), however, it will be more difficult to develop analytical methods capable of detecting the low concentrations of these materials in nanoform relative to background levels [Nowack, 2012].

i. Available instruments and methods

Some common used cmethods and instruments available are summarized in Table 20.

Instrument	Method	Medium	Metric	Main characteris-tics	Comments
Fluorescence spectrometer	Fluorescene X	Solid matrix			Map-ping/Identificatio n
TXRF	Fluorescence X	Solid matrix	Chemical identification		Chemical identification
FFF/UV/MALLS/ RI	Analytical methods :	Solid matrix, Liquids (Eg.soils)	$\phi_{hydrodynamiic}$ $\phi_{giration}$ Concentration Molar mass Agglomeration state	1 nm < d_p < 10 µm Cmax = 200 mg/l C_{min} = *0.1µg/l (eg.for Ag)*	Stable suspensions are needed
UV spectrometer Gamma counting	Fluorescent, Magnetic, Radiactive label-ling/Tracing	Solid matrix (Eg.soils)	Detection and quantification	Depending of the type of nanoparticle ~ 50ppb detection limit could be achieved for SiO_2-FITC@TiO_2	Pristine nanoparticles with a radioactive, magnetic, fluorescent core need to be synthesized. Stable traced NP in time has to be achieved
Cryo TEM	Direct imaging		Structure Size Shape	Very high resolution < 1nm	Detection, characterization Difficult to quantify ☺ TEM : Can be directly coupled to chemical analysis (EELS, EDX) ☹ TEM is a complex equipment Sample preparation

Table 20: Methods and instruments for the characterization of nanoparticles in complex matrices

ii. Common challenges

Research on techniques for collecting, preserving, and storing samples containing MNMs is urgently needed. The simple act of trying to isolate, observe and quantify MNMs may change their physico-chemical properties, making analysis extremely susceptible to artefacts. The development of techniques for extraction, cleanup, separation, and sample storage that introduce minimal artefacts to increase the speed, sensitivity, and specificity of analytical techniques, as well as the development of techniques that can differentiate between abundant, naturally, occurring particles, and manufactured nanoparticles is of paramount importance [Von der Kammer, 2012]. The requirements for the development of these techniques in terms of properties of interest and detection limits need to be guided by well-designed ecotoxicological studies that identify the relevant concentrations and properties of interest so that focused analytical strategies can be developed. There is a lack of adequate techniques for the detection and quantification of MNMs at environmentally relevant concentrations in complex

media. The detection limits given by ICPMS depends of the type of nanoparticle. More rapid, more sensitive and specific detection of MNMs are needed. To assess exposure, analytical methods will be required to detect MNM in a range of environmental media, in some cases including complex matrices, such as biosolids and biological tissues [Baalousha, 2011]. In addition, analytical methods are required to evaluate the levels of transformation products present in environmental matrices. In some cases, these analytical techniques are already available using methods and instrumentation dedicated to metal speciation (for example, analysis of Ag_2S by electrochemical techniques), but, in other cases, novel analytical methods will have to be developed (for example, analysis of hydroxylated transformation products of CNT) [Nowack, 2012].

2.10 Reference materials

Calibration and standards

The landscape of the MNMs characterization is somewhat particular, since it is in its majority still governed by specialists in R&D laboratories. Moreover, for a few number of parameters to be measured, a big number of methods can be used and have been developed. For an extended and accepted use, the MNMs need to be characterized according to standards and using reference materials.

A few numbers of organisations worldwide have yet developed certified reference materials:

In US, NIST has developed certified reference materials for chemical and physical characterization of nanomaterials (http://www.nist.gov/characterization-nanometrology-and-nanoscale-measurements-portal.cfm).

D - Detail C - Certificate I - Report of Investigation M - MSDS R - References F - Data Files Q - Questionnaire

	SRM	Description	Unit Size
D C M R	1963a Now Selling	Nominal 100 nm Diameter Polystyrene Spheres	5 mL
D C M R	1964 Now Selling	Nominal 60 nm Diameter Polystyrene Spheres	5 mL
D C M	2483 Now Selling	Single-Wall Carbon Nanotubes (Raw Soot)	250 mg
D I M R	8011 Now Selling	Gold Nanoparticles, Nominal 10nm Diameter	two 5 mL ampoules
D I M R	8012 Now Selling	Gold Nanoparticles, Nominal 30nm Diameter	two 5 mL ampoules
D I M R	8013 Now Selling	Gold Nanoparticles, Nominal 60nm Diameter	two 5 mL ampoules

Table 21: Nanomaterials (less than or equal to 100 nm) available from NIST

Other nano-objects are under development: single-walled carbon nanotubes (SWCNTs) declined in three types (powder, pellet, liquid), and TiO_2.

In Europe, the JRC has built up a nanomaterials repository and a database of test and research results:

https://ec.europa.eu/jrc/en/scientific-tool/jrc-nanomaterials-repository

A list of the materials available in the JRC Nanomaterials Repository (updated December 2014) can be found here:

https://ec.europa.eu/jrc/sites/default/files/JRC%20Nanomaterials%20Repository%20-%20List%20of%20Representative%20Nanomaterials.pdf

The BAM (German Federal Institute for Materials Research and Testing) is also commercialising a number of Nanoscaled Reference Materials, which are listed in Table 23

(excerpt limited to 100 nm), see http://www.nano-refmat.bam.de/en/category_10_nanoobjects_nanoparticles_nanomaterials.htm. Some of the references are part of the JRC repository. A certain number of references allow calibrating specific surface area (Table 22):

Nano-Alumina Specific Surface Area	445,4 m^2/g	GBW 13901	CRM	106
Standard Reference Materials	359,4 m^2/g	GBW 13906	RM	107

Table 22: Reference materials for specific surface area (BAM)

The reference materials are characterized by a metrological valid procedure for one or more specified properties accompanied by a certificate which provides the value of the specified property, its associated uncertainty and a statement of metrological traceability. These particles should be used mainly for the calibrations of the instruments in the laboratories. Other reference materials with other compositions need to be developed in the future [Stamm, 2009].

Concerning the measurement in complex matrices, a limited availability of the reference nanomaterials could be outlined. Suitable reference nanomaterials for complex matrices need to be developed. Labelled analogue nanoparticles similar to the targeted nanoparticles (size, physical-chemical behavior) need to be developed. Radiolabelling is not suited for the routine food labs. Fluorescent, biomarker labelling are some of the current ideas in order to define nanoparticles of reference for the food application [Weigel, 2011].

The work on standards for the characterization methods is very active in Europe, since year 2004: The International Standards Organization (ISO) Technical Committee (TC) 229, in conjunction with the International Electrotechnical Commission (IEC) TC 113 (and other national standards bodies), has been directing activities on nanotechnologies standards since 2004. European standards activities are coordinated by the European Committee for Standardization, Technical Committee on nanotechnologies (CEN TC352). There is a strong liaison between CEN TC352 and ISO TC229.

Description	Certified value (nm)	RM name	RM type	RM no.
Absolute contamination standards polystyrene latex spheres on Si Wafer, sphere diameter, concentration; 40 nm - ...; used to calibrate instruments which size and detect particles on the surface of bare silicon wafers	40 nm	Absolute Contamination Standard	RM	42
Polystyrene particle size standard sphere diameter	61 nm	GBW 12011	RM	67
Silica nanoparticles; quality control (method development, proficiency tests, control charting)	40 nm	IRMM-304	RM	48
Gold nanoparticles sphere diameter	10 nm	RM8011, RM8012, RM8013	CRM	46
Silica contamination standards silica spheres on Si wafer Used to calibrate instruments which size and detect particles on the surface of bare silicon wafers	100 nm	Silica Contamination Standard	RM	43
Polystyrene spheres sphere diameter	60 nm	SRM 1964	CRM	44
Polystyrene spheres sphere diameter	100 nm	SRM1963a	CRM	47
Size standard particles > 25 products, mean diameter: 29 nm - 814 nm sphere diameter	29 nm	STADEX SC-0030-A, SC-0050D	RM	59
Size standard particles > 25 products, mean diameter: 29 nm - 814 nm sphere diameter	70 nm	STADEX SC-0070-A, SC-0075D	RM	60
nanomag®-D particles 50/100 nm Covalent binding of proteins by cyanogen bromide activation (CNBr-Method)	50 nm	TN 09001	RM	49
nanomag®-D –spio particles 50/100 nm Covalent binding of proteins by carbodiimide activation	50 nm	TN 790201	RM	50
Collodial Silica in water sphere diameter	19 nm	ERM-FD100	CRM	82
Titanium Dioxide (anatase) Part of JRC NM Repository (Oct 2011)	38 nm	NM-101	RM	84
Titanium Dioxide (thermal, hydrophobic, rutil) Part of JRC NM Repository (Oct 2011)	67 nm	NM-104	RM	87
Silicon Dioxide (Synthetic Amorphous Silica PR-A-02), precipitated Part of JRC NM Repository (Oct 2011)	47 nm	NM-200	RM	91
Silicon Dioxide (Synthetic Amorphous Silica PR-B-01), precipitatedPart of JRC NM Repository (Oct 2011)	62 nm	NM-201	RM	92
Silicon Dioxide (Synthetic Amorphous Silica PR-A-05), precipitated Part of JRC NM Repository (Oct 2011)	75 nm	NM-204	RM	95
Cerium Dioxide (Cerium (IV) Oxide precipitated, uncoated -- Part of JRC NM Repository (Oct 2011)	28 nm	NM-212	RM	97
Silver < 20 nm Part of JRC NM Repository (Oct 2011)	15 nm	NM-300K	RM	98
MWCNT Part of JRC NM Repository (Oct 2011)	9,5 nm	NM-400	RM	101
MWCNT Part of JRC NM Repository (Oct 2011)	20 nm	NM-401	RM	102
MWCNT Part of JRC NM Repository (Oct 2011)	11 nm	NM-402	RM	103

Table 23: Reference nanomaterials available from BAM (limited to 100 nm)

2.11 Conclusions

Risk assessment of MNM, as of any potentially hazardous agent, for human health and the environment requires knowledge of exposure and hazard potential. Due to the large diversity of MNM and that specific knowledge regarding general mechanisms for MNM toxicity is lacking, most or possibly all risk assessment up to date has been performed on a case-by- case basis. Following the general trends in risk assessment, it is currently also becoming clear that risk assessment of MNM is exposure driven.

There is a large data base of knowledge available regarding characterization methods for MNM, and regarding human health and acute environmental hazard potential. Much less is known regarding exposure characterization, dose characteristics, life-cycle analysis, modes of action, and long-term effects both for human health and the environment. Improvements in risk assessment must take these aspects into consideration, as well as applying a risk-benefit perspective.

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3 Precautionary measures and Safe-by-Design approach

3.1 The precautionary principle

If a preliminary scientific evaluation emphasizes that there are reasonable grounds for concern that a particular activity might lead to damaging effects on the environment, or on human, animal or plant health, the precautionary principle is triggered [Hansen, 2007; Maynard, 2007; Groso, 2010; Kessler, 2011]. In 1996, the American Public Health Association passed a resolution entitled, “The Precautionary Principle and Chemical Exposure Standards for the Workplace”. This resolution recognized the need for implementing the precautionary approach, where chemicals are considered potentially dangerous, until the extent of its toxicity is sufficiently known, and the establishment of strict, preventive chemical exposure limits. In February 2000, the European Commission published a Commission Communication on the precautionary principle [EU Resolution on the Precautionary Principle, 2000] providing a general framework for its use in EU policy [Andorno, 2004].

The precautionary principle is recognized today as a basic principle of environmental, health protection and consumer protection law in many jurisdictions, including the EU and many other states. Together with the related requirement for impact assessments on new technologies, the precautionary principle goes beyond the conventional approach to averting danger by demanding that risks of new technologies should be avoided or at least minimized. Faced with reasonable suspicion of harm, the precautionary approach urges a full evaluation of available alternatives for the purpose of preventing or minimizing harm [Kessler, 2011].

Within this context, the precautionary principle is directly applicable to emerging nanotechnologies. Currently, a broad range of processes have been influenced by nanotechnology which will pose likely higher exposure potential to workforces in the nanotechnology occupational settings than consumers of final products. Considering inadequate information, until the results from research studies can fully elucidate the characteristics of MNMs that may potentially pose a health risk, precautionary measures are warranted [British Standard Institute (BSI), “Nanotechnologies – Part 2: Guide to safe handling and disposal of manufactured nanomaterials,” PD 6699-2:2007, UK, 2007; E 2535 – “Standard Guide for Handling Unbound Engineered Nanoscale Particles in Occupational Settings,” ASTM, USA, 2007]. Minimizing the risks from known and unknown health, safety and environment hazards of handling, use and disposal of nanoparticles in nanotechnology workplaces (research laboratories and industrial firms) is considered as a priority [Code of Conduct for responsible nanosciences and nanotechnologies research, C(2008)424; Communication Regulatory Aspects of Nanomaterials, COM(2008)366].

The document “Responsible Production and Use of Nanomaterials“ – Cefic, 17th January 2012, brings together best practice on the concrete application of Responsible Care to the development and use of nanomaterials. It focuses on the six Core Principles of the Responsible Care Global Charter – and, using concrete examples from member companies and federations, describes ways in which each of these principles can be applied to nanomaterials.

The 6 Core Principles of the Responsible Care Global Charter defined here are:

1. Continuously improve the environmental, health and safety knowledge and performance of our technologies, processes and products over their life cycles so as to avoid harm to people and the environment
2. Use resources efficiently and minimise waste
3. Report openly on performance, achievements and shortcomings

4. Listen, engage and work with people to understand and address their concerns and expectations
5. Cooperate with governments and organisations in the development and implementation of effective regulations and standards, and to meet or go beyond them
6. Provide help and advice to foster the responsible management of chemicals by all those who manage and use them.

Public and private institutions as well as industries have the duty to adopt preventive and protective measures proportionate to the risk intensity and the desired level of protection. Currently, agencies charged with providing safety guidelines, promote the incorporation of precautionary measures in research, with a view toward minimizing or eliminating exposures to MNMs.

To fulfill this main objective, a lot of Guidelines have been issued to address the potential health, safety and environment hazards of MNMs and available good practices for mitigating the risks. These guidelines are intended to help the decision makers to:

- develop site-specific controls that will protect workers and the environment,
- offer reasonable guidance for managing the uncertainty associated with MNMs whose hazards have not been determined and reducing to an acceptable level the risk of worker injury, worker ill-health and negative environmental impacts and
- promote consistency in policy and procedures between the nanotechnology workplaces.

3.2 Precautionary tools

There are some approaches in use or currently being developed, which can be seen as precautionary tools in the field of nanomaterials. The following list is meant as a non-exhaustive overview of only some of these tools.

Swiss Federal Office of Public Health FOPH: Swiss Precautionary matrix for synthetic nanomaterials [Hoeck, 2013]: The precautionary matrix for synthetic nanomaterials is geared toward industry and trade. It was first published in 2008. The matrix is regularly revised based on experiences and new scientific knowledge.

The precautionary matrix enables the structured assessment of the “nano-specific need for precautions” when handling synthetic nanomaterials. The precautionary matrix is designed to help industry and trade comply with their due diligence and their duty to exercise self-control opposite employees, consumers and the environment.

Thus, potentially risky applications can be identified and precautionary measures initiated to protect people’s health and the environment. The matrix helps ensure safety in connection with the development of new products. It indicates when further clarification is needed.

The precautionary matrix is refined and extended in close cooperation with industry, science and trade as well as consumer and environmental organisations.

More information can be found at:

http://www.bag.admin.ch/nanotechnologie/12171/12174/index.html?lang=en

An online version of the precautionary matrix is accessible here:

http://www.bag.admin.ch/nanotechnologie/12171/12174/12175/index.html?lang=en

Dutch Ministry of Social Affaires and Employment: **Stoffenmanager Nano Version 1.0** (based on Stoffenmanager 4.5 [Schinkel, 2010; Tielemans, 2008]): A Web-Based

Tool for Risk Prioritization of Airborne Manufactured Nano Objects. Stoffenmanager Nano (version 1.0) is a risk-banding tool developed for employers and employees to prioritize health risks occurring as a result of exposure to manufactured nano objects for a broad range of worker scenarios and to assist implementation of control measures to reduce exposure levels. In order to prioritize the health risks, the Stoffenmanager Nano combines the available hazard information of a substance with a qualitative estimate of potential for inhalation exposure. More information can be found at: http://nano.stoffenmanager.nl/

Danish Ministry of the Environment: NanoRiskCat – A Conceptual Decision Support Tool for Nanomaterials [Hansen, 2011]. NanoRiskCat is a screening tool for the evaluation of exposure and hazard of nanomaterials contained in products for professional and private use. The aim is to identify, categorize and rank the possible exposure and hazards associated with a nanomaterial in a product. NanoRiskCat is using a stepwise approach based on existing data on the conventional form of the chemical as well as the data that may exist regarding the nanoscale form. However, the tool still needs to be further validated and tested on a series of various nano products in order to adjust and optimize the concept and thereby to achieve a screening tool as informative and practical as possible. More information can be found at:

http://orbit.dtu.dk/en/publications/nanoriskcat--a-conceptual-decision-support-tool-for-nanomaterials%28e20b1154-8cdc-43ef-a2c0-75ebfb8e0906%29.html

LICARA project and NanoSCAN: LICARA is the acronym for an EU FP7 project named 'Life Cycle Assessment and Risk Assessment of Nanoproducts'. It has developed the LICARA concept [Som, 2014] which helps SMEs to:

- Make decisions about developing and producing safe, sustainable products by gathering relevant information to answer the pertinent questions
- Learn from best practices
- Build a coherent argument about nanoproducts for suppliers, clients, consumer groups, authorities and other stakeholders (a comprehensive guide to the nanoproduct).

The LICARA guidelines help to implement the concept itself and are directed at SMEs that:

- Produce nanoparticles for wide or narrow fields of application
- Produce intermediate products using nanoparticles
- Produce end products with nanoparticles and nanomaterials.

The guidelines are accompanied by a first version of an analytical tool in Excel, the LICARA NanoSCAN that facilitates the implementation of the guidelines themselves.

The guidelines are based on the scientific work of three research institutes – TNO, Empa and RAS – and the experiences of private sector companies (NCB, SNT, Freso, Nanothinx and AGPYME) which were partners in the LICARA project.

For further information as well as access to the NanoSCAN tool check http://www.empa.ch/plugin/template/empa/*/137000/---/l=2.

Collaboration of Environmental Defense Fund and DuPont: Nano Risk Framework. The Framework offers guidance on the key questions an organization should consider in developing applications of such materials, and on the critical information needed to make sound risk evaluations and risk management decisions. The Framework allows users to address areas of incomplete or uncertain information by using reasonable assumptions and appropriate risk management practices. Further, the Framework describes a system to guide information generation and update assumptions, decisions, and practices with new information as it becomes available. And the

Framework offers guidance on how to communicate information and decisions to stakeholders. More information can be found at:

http://www.nanoriskframework.com/

3.3 Control banding

3.3.1 Guidance on developing a control scheme (control banding)

In the absence of occupational exposure limits and definitive knowledge of toxicity, control banding is a qualitative strategy for classifying and handling chemicals and hazards associated with chemical exposures in the workplace, as well as for assessing potential risks for consumers and the environment. Control banding has its origins in the pharmaceutical industry [Naumann, 1996] and it is based on the appropriate control technology recommended to a chemical that falls within a given hazardous group (based on risk phrases from safety data sheets and handling). As the principle of control banding was applied to dangerous chemicals, chemical mixtures, and fumes, the premise was that the greater the potential for harm, the greater the degree of control needed to manage the situation and make the risk "acceptable." Control banding has the potential to be a useful concept for workplaces that handle MNMs [Maynard, 2007; Schulte, 2008]. An overview can be found for instance in: [Brouwer, 2012]

Although there are various approaches, four main control bands have been described for exposure to chemicals by inhalation:

Band 1: Use good industrial hygiene practice and general ventilation.

Band 2: Use an engineering control, typically local exhaust ventilation.

Band 3: Enclose the process.

Band 4: Seek expert advice.

About 50 different Control Guidance Sheets are appreciated as standardized working practices and evaluated in field studies by means of workplace monitoring.

The precautionary tools described in the previous chapter can be seen as control banding tools. Some other tools and guidelines are described in the following.

The **GoodNanoGuide** (www.goodnanoguide.org) is an Internet-based platform for the exchange of ideas on handling nanomaterials, and it recommends a simplified approach to control banding of nanomaterials. With this approach, MNMs are grouped into three hazard groups:

(A) known to be inert,

(B) understand reactivity and function, or

(C) unknown properties.

The exposure duration is described as Short (<4 hours/day, 2 days/week), Medium (4–6 hours/day, 3–5 days/week) or Long (>6 hours/day, 3–5 days/week). The potential for exposure is described through the state of the MNM: bound (nanoparticles in a solid matrix), potential release (nanoparticles in friable matrix), or free/unbound (nanoparticles unbound, not aggregated). These elements are used to determine the recommended control band.

Another tool, the **CB Nanotool**, bases the control band for a particular task on the overall risk level (RL), which is determined by a "severity" score and a

"probability" score. The severity score is determined by the sum of points assigned to the following factors: surface chemistry, particle shape, particle diameter, solubility, carcinogenicity, reproductive toxicity, mutagenicity, dermal toxicity, and hazard potential of the nanomaterial and the macro-parent material. The overall probability score is based on the following elements: estimated amount of nanomaterial used during the task, dustiness or mistiness, number of employees with similar exposures, frequency of operation, and duration of operation [Paik, 2008]. The CB Nanotool is being used at the Lawrence Livermore National Laboratory (LLNL) and can be downloaded at http://controlbanding.net/Home.html.

ISO/TS 12901-2:2014 describes the use of a control banding approach for controlling the risks associated with occupational exposures to nano-objects, and their aggregates and agglomerates greater than 100 nm (NOAA), even if knowledge regarding their toxicity and quantitative exposure estimations is limited or lacking.

Source: ISO/TS 12901-2:2014, Nanotechnologies -- Occupational risk management applied to engineered nanomaterials -- Part 2: Use of the control banding approach
www.iso.org/iso/catalogue_detail.htm?csnumber=53375.

The following list gives a **non-exhaustive overview of selected literature** in the field of control banding for nanomaterials:

- ANSES (2010) Development of a specific control banding tool for nanomaterials
  http://www.etui.org/content/download/3554/40003/file/ANSES_2011_nano.pdf
- BSI (2007) Guide to safe handling and disposal of manufactured nanomaterials
  http://www.safenano.org/knowledgebase/guidance/banding/
- SWA (2010) Engineered nanomaterials: feasibility of establishing exposure standards and using control banding in Australia
  http://www.safeworkaustralia.gov.au/sites/SWA/about/Publications/Documents/546/Engineered_Nanomaterials_feasibility_establishing_exposure_standards_August_2010.pdf
- Development of a Control Banding Tool for Nanomaterials, M. Riediker, C. Ostiguy, J. Triolet, P. Troisfontaine, D. Vernez, G. Bourdel, N. Thieriet, and A. Cadene , Hindawi Publishing Corporation Journal of Nanomaterials Volume 2012, Article ID 879671, 8 pages doi:10.1155/2012/879671
  www.hindawi.com/journals/jnm/2012/879671/
- Nanomaterials Risk Assessment in the Process Industries: Evaluation and Application of Current Control Banding Methods, Dominique Fleury , Guillaume Fayet , Alexis Vignes , François Henry, Emeric Frejafon, CHEMICAL ENGINEERING TRANSACTIONS VOL. 31, 2013 The Italian Association of Chemical Engineering Guest Editors: Eddy De Rademaeker, Bruno Fabiano, Simberto Senni Buratti, ISBN 978-88-95608-22-8; ISSN 1974-9791
  http://www.aidic.it/lp2013/webpapers/172fleury.pdf
- Banding the World Together; The Global Growth of Control Banding and Qualitative Occupational Risk Management David M ZALK and GA Henri HEUSSEN, Safe Health Work 2011;2:375-9
  http://dx.doi.org/10.5491/SHAW.2011.2.4.375

3.3.2 Limitations of control banding

Control banding is not without limitations and still requires professional knowledge and experience to verify that the control measures specified have been properly installed, maintained, and used. Controls should be validated prior to use by either using substance specific industrial hygiene methods or performing surrogate monitoring.

One limitation of control banding tools for MNMs is that there are very few toxicological data on which to recommend control levels, other than the highest two levels, and to evaluate the validity of the tool. As health hazard studies continue to expand, and the understanding of the toxicity of MNMs improves, the severity scores may be adjusted to reflect the new knowledge and thereby affect the severity score to elicit a more defined control band [Zalk, 2009].

The ability to apply a control banding approach, as well as more extensive exposure assessment and risk management, will require some type of science-based classification scheme for engineered nanoparticles. This is likely to involve an evaluation of the nanoparticle hazard, including consideration of the physicochemical properties influencing toxicity and the ability of the material to become airborne or present an exposure hazard by other routes [Schulte 2008].

3.3.3 Further organisations working on this issue

http://www.baua.de/en/Topics-from-A-to-Z/Hazardous-Substances/Workshops/Control-Banding-2011/Control-Banding-2011.html;jsessionid=D2685F705360D9DF525D61FB7649CEA9.2_cid135

1. European perspectives and contributions to the global development strategy 2010 - 2015 of the WHO / ILO ITG Control Banding
   http://www.baua.de/en/Topics-from-A-to-Z/Hazardous-Substances/Workshops/Control-Banding-2011/pdf/Control-Banding-2011-14.pdf?__blob=publicationFile&v=2

2. OCCUPATIONAL RISK MANAGEMENT TOOLBOX GLOBAL DEVELOPMENT STRATEGY Work Plan 2010 - 2015
   http://www.baua.de/en/Topics-from-A-to-Z/Hazardous-Substances/Workshops/Control-Banding-2011/pdf/Control-Banding-2011-15.pdf?__blob=publicationFile&v=2

3. NIOSH (USA)
   CONTROL BANDING
   http://www.cdc.gov/niosh/topics/ctrlbanding/

3.4 Safe-by-Design approach

The Safe by Design (SbD) concepts are creating integrated processes combining product and material innovations with the safe implementation strategy addressing the aspects for humans and the environment. Safe by Design means recognition and avoidance of risks at the earliest time of a material development process combined with the respective product and/or process development.

SbD forms a platform for the early stage application of precautionary measures and tools as well as control banding approaches, as described above.

The following list gives a non-exhaustive overview of projects, programmes, and activities in this field:

- Toward safer and eco-designed innovative nanomaterials "The new generation of materials safer by design" Serenade (ANR) 2012-2021 https://www.eccorev.fr/IMG/pdf/ECCOREV-SERENADE18-01.pdf
- NANOMICEX Approach: Mitigation of risk by means of safe by design approaches and effective engineering controls 2012-2015 (FP7) www.industrialtechnologies2014.eu/presentation/ws20-2/
- Project Nanomile (A strategy for grouping of nanomaterials based on key physico- chemical descriptors as a basis for safer-by-design MNMs) http://nanomile.eu-vri.eu/
- The Sustainable Nanotechnologies (SUN) project (2013-2016) EU FP7 research programme is based on the idea that the current knowledge on environmental and health risks of nanomaterials - while limited - can nevertheless guide nanomanufacturing to avoid liabilities if an integrated approach addressing the complete product lifecycle is applied http://www.sun-fp7.eu/
- The NanoNextNL Program (Innovating with micro and nanotechnology) www.nanonextnl.nl/about-nanonextnl.html
- Guidance document created by the Consultants' Health & Safety Forum: Safe by design-FAQs http://www.hse.gov.uk/construction/cdm/safety-by-design.pdf
- Nanosafety in Europe 2015-2025: Towards Safe and Sustainable Nanomaterials and Nanotechnology Innovations, This publication was carried out during the years 2011-2013 by the request of the European Commission http://www.nanosafetycluster.eu/news/83/66/Nanosafety-in-Europe-2015---2025.html
- Examining the Holy Grail of Nanotechnology: Safe By Design, Dr. Sally Tinkle, Senior Science Advisor, National Institute of Environmental Health Science, National Institutes of Health http://www.azonano.com/article.aspx?ArticleID=2508
- Understanding Nanotechnology, U. Fiedeler et al. (Eds.)© 2010, AKA Verlag Heidelberg, Towards Safety and Sustainability by Design: Nano-sized TiO2 in Sunscreens, Johannes F. JACOBS, Ibo VAN DE POEL and Patricia OSSEWEIJER http://www.thesnet.net/site/wp-content/uploads/2013/09/12_Jacobs-et-al_Towards-Safety-and-Sustainability-by-Design.pdf
- Science-based grouping of nanoparticles for industrial application of safe-by-design, Hans Bouwmeester, Tomasz Puzyn, Robert Landsiedel, Andrea Haase, RIKILT Wageningen UR http://cefic-lri.org/wp-content/uploads/2014/04/N4-Science-based-grouping-of-nanoparticles-for-industrial-application-of-safe-by-design.pdf
- Towards Efficient Designing of Safe Nanomaterials: Innovative Merge of Computational Approaches and Experimental Techniques; Jerzy Leszczynski, Tomasz Puzyn, Royal Society of Chemistry, 2012 - Science - 362 pages http://books.google.ro/books/about/Towards_Efficient_Designing_of_Safe_Nano.html?id=wa9rxwXSVfgC&redir_esc=y

- The next ten years of nano risk research http://2020science.org/2010/10/13/nanotechnology-2-0-the-next-ten-years-of-nano-risk-research/
- Safe Nano Design Workshop http://cnse.albany.edu/Outreach/NIOSHPresentations.aspx

Today, there is no standardised Safe by Design process available. Several approaches are under development. Exemplarily, within the NANoREG project (http://nanoreg.eu/, c.f. chapter 8.6 for more information) a Safe-by-Design concept is currently elaborated. The SbD approach involved aims at reduced uncertainty and managed risks of innovative materials, products and processes at the time of market introduction. The approach will especially consider the characteristics and peculiarities of manufactured nanomaterials (MNMs) or products containing MNMs and related processes.

The conceptual basis for both concepts and thus the approach are the already industrially used stage gate innovation and standard risk management processes:

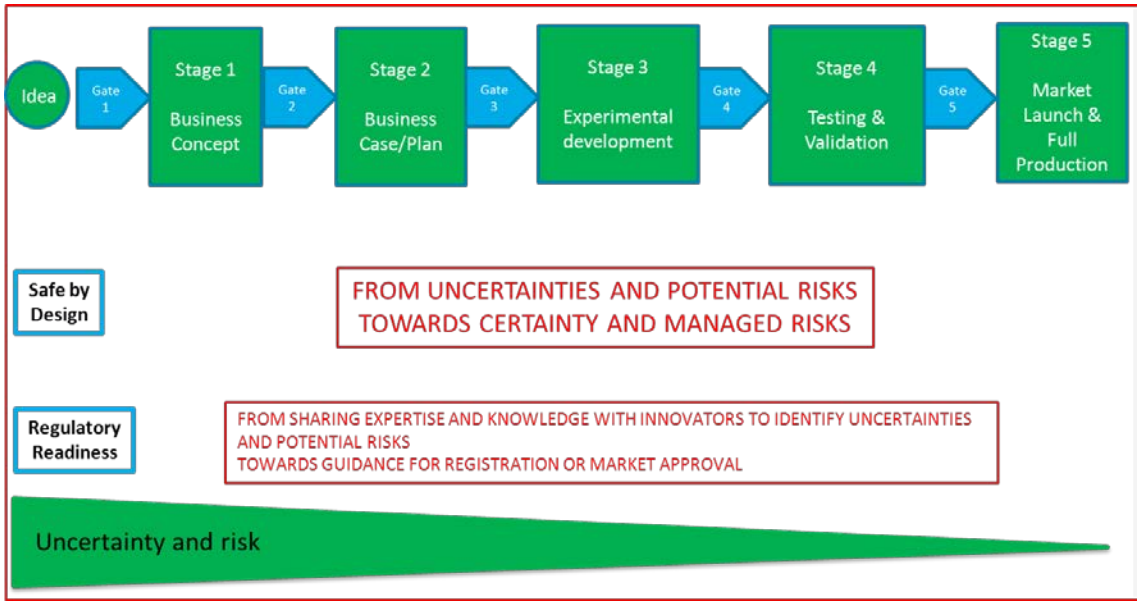

Figure 4: Safe Innovation Approach (courtesy of Adrienne Sips, National Institute for Public Health and the Environment RIVM, and Karl Höhener, TEMAS)

Uncertainties or risks can be reduced by

- altering the way the processes are run for an individual innovation project (i.e. flexible adaption and fit size of processes to extent of risks),
- the right tools for the problem at hand,
- enough reliable data,
- being aware of case specific uncertainties and risks (e.g. the similarities and differences between “normal” i.e. macro- and nano-scalar materials).

Within this NANoREG approach, a harmonised SbD concept based on a common understanding of safety, uncertainty and risk will be developed, and further used in the ProSafe and NANoREG II projects.

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Tielemans, E, Noy, D, Schinkel, J, Heussen, H, van der Schaaf, D, West, J, Fransman, W. (2008) Stoffenmanager exposure model: development of a quantitative algorithm. Ann. Occup. Hyg., 52(6) 443.

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4 Good practices

4.1 Introduction

The current situation with MNM is such that there are no international standards of how to do a complete risk assessment and on the data needed. Risk assessment is possible on a case by case basis, but this is expensive and time consuming.

In such a situation it is recommendable to refer to good practices in the field, offering information on solutions that may or may not work for a given system. This is especially so because, as opposed to "best practice", very often it will be impossible to find one single best solution. Accordingly, this chapter summarizes currently applied good practices solutions in the field of MNM.

4.2 Safety guidelines for handling and working with MNMs

One of the first areas where exposures to manufactured nanoparticles will occur is in the workplace. Organizations involved may fall into multiple categories:

- Research and Development of MNMs;
- Inventory companies;
- Manufacturer of MNMs, such as manufacture of metal oxides, carbon nanotubes, fullerenes or others;
- Manufacturer of materials such as paints, plastics, textiles, and ceramics;
- Manufacturer of consumer products such as cosmetics and appliances;
- Electronics/Information Technology mostly referred to producers of electronic components;
- Chemicals;
- Coatings;
- The developing nanotechnology measurements and standards, manufacturing technologies, environmental remediation and various applications.

The quantities of NPs handled in a research/laboratory setting may be smaller compared to an industrial setting; however, unknown, as a whole, are still prevalent in regards to the occupational safety and health risks of NPs (Nanotechnology safety and health program, NIH, 2012).

Occupational exposure to MNMs can occur:

- during manufacture
- through incorporation in other materials, e.g. polymer composites, medical applications and electronics
- by generating nanoparticles in non-enclosed systems
- during research into their properties and uses
- cleaning of dust collection systems used to capture nanoparticles
- as a result of incorrect disposal
- as a result of accidental spillage

4.2.1 Basic principles

NPs or MNMs will likely be in one of three forms: as powder, in suspension, or in a solid matrix. The form of the NPs or MNMs will play a large role in the exposure potential. Certain basic principles will contribute to minimizing risk.

- Nanomaterials in dry powder form pose the most risk for inhalation exposure and must be handled with care to minimize the generation of airborne dust and to minimize dermal contact. There is also an increasing body of evidence to suggest that CNTs and other nanomaterials with a long, thin and straight shape (referred to as high aspect ratio nanomaterials or HARN) may be particularly hazardous.
- Nanomaterials suspended in a liquid present less risk for inhalation exposure than nanomaterials in dry powder form, but may present more risk from skin contact. Skin up-take of nanomaterials as dry powders and in solvents is poorly understood at this time. It is likely that skin uptake of nanoparticles may be enhanced significantly in compromised skin and/or in the presence of solvents!
- Nanomaterials incorporated into a solid matrix present the least risk for inhalation expo-sure due to their limited mobility. Although poorly understood at this time, there is circumstantial evidence to caution that certain nanomaterials incorporated into bulk solids may still pose some risk through skin contact, especially nanomaterials with immunological properties, which have some solubility in sebaceous fluids.

4.2.2 Recommendations for exposure control

Controls must be assessed on a case by case basis dependent upon the nanomaterial, quantity, sample matrix, and steps of the process. Among the most effective means to prevent occupational injuries and illnesses are: anticipating potential occupational safety and health hazards early in the development of the technology or process and incorporating safe practices into all design, implementation, and operation phases.

Prevention through Design (PtD) is a management tool for protecting workers from potentially unsafe work conditions. It emphasizes the importance of employee health and safety through the design, construction, manufacture, use, maintenance, and ultimate disposal or reuse of tools, equipment, machinery, substances, work processes, and work premises [NIOSH 2010]. PtD addresses occupational safety and health needs by eliminating hazards and minimizing risks to workers throughout the life cycle of the process [Schulte, 2008b]. Many nanotechnology research laboratories recognize PtD as a cost-effective means to enhance occupational safety and health and have incorporated PtD management practices within their facilities [Murashov, 2009].

Prevention through Design strategies follow the standard hierarchy of controlling workplace hazards, which includes

(1) hazard minimization, eliminating, substituting, or modifying the nanomaterials;

(2) engineering the process to minimize or eliminate exposure to the nanomaterials;

(3) implementing administrative controls that limit the quantity or duration of exposure to the nanomaterials; and

(4) providing for use of PPE.

Hazard Minimization, Elimination, and Substitution

Hazard control starts with determining ways to substitute, minimize, or eliminate the more hazardous materials or processes where possible. Some examples include:

- Minimizing the scale of an experiment.
- Eliminating hazardous materials used in a process.
- Substituting process chemicals for less hazardous ones.
- Using processes or techniques that produce lower airborne concentrations and minimize skin contact.

For nanomaterial researchers, it is often not feasible to eliminate or substitute the nanomaterial. It may be possible, however, to change some aspects of the process in a way that reduces release of the MNM. A liquid or solid sample matrix may be used with some MNMs. For example, working with MNMs suspended in a liquid is a significant improvement over working with them in dry powder form, because the potential for airborne release is reduced in most laboratory processes. However, physical agitation of the liquid (e.g., sonication) may aerosolize small droplets containing the nanomaterial [Johnson, 2010]. A liquid matrix such as an organic solvent may enhance in this case dermal absorption.

Opportunities for eliminating the use of hazardous materials or substituting for less hazardous forms do exist in other aspects of MNMs production. Engineered nanoparticle research often requires the use of solvents and other potentially hazardous chemicals. Researchers should always attempt to identify and use chemical processes that utilize nontoxic or less-toxic alternatives whenever possible, in order to minimize worker exposures and environmental releases when the process is scaled up to full production. This control strategy, substituting a less toxic material in production processes, has been the focus of much research during the past 20 years.

It is also possible to substitute a less “energetic” operating condition, and thereby modify a process to make it inherently safer. An example of process modification was demonstrated in a laboratory producing CNTs by chemical vapor deposition. Optimizing the furnace reaction temperature maximized the production of CNTs while minimizing the release of CNTs in the furnace exhaust [Tsai, 2009].

Isolation and Engineering Controls

Isolation includes the physical isolation of a process or piece of equipment either by locating it in an area separate from the worker or by placing it within an enclosure that will contain the MNMs released. Engineering controls include any physical change to the process or workplace that reduces contaminant emissions and subsequent employee exposure. Several factors will influence the selection of exposure controls for MNMs, including quantity of nanomaterial handled or produced, physical form, and task duration. As each one of these variables increases, exposure risk becomes greater, as does the need for more efficient exposure control measures (NIOSH 2009). Operations involving easily dispersed dry MNMs deserve more attention and more stringent controls (e.g., enclosure) than those where the MNMs are suspended in a liquid matrix or imbedded in a solid. Liquid nanoparticle suspensions rarely pose a danger of inhalation exposure during routine operations, but they may represent a significant hazard when aerosolized or in unexpected situations such as a spill. MNMs incorporated into bulk solids may pose some risk if the solid matrix is cut, sawed,

drilled, sanded, or handled in any way that creates a dust or releases the nanomaterial.

Containment refers to the physical isolation of a process or a piece of equipment to prevent the release of the hazardous material into the workplace. An example of process isolation would be the location of a twin-screw extruder used to make CNT composites in a room separated from the rest of the research facility. An example in chemistry labs is the use of specially designed separate storage cabinets for flammables, acids, and bases. Another example of containment would be a glovebox, which is a sealed container with attached gloves that allows the researcher to carry out process or tasks while being physically separated from the hazard.

Ventilation. It is important that any workplace involving MNMs have sufficient general exhaust ventilation (GEV); GEV is typically provided by the building's heating, ventilation, and air conditioning (HVAC) system. They should have non-recirculating ventilation systems (preferably, 100% exhaust air), and lab pressurization should be negative to the hallway. Recommended ventilation rates for general laboratory use range from 4 to 12 air changes per hour, if GEV systems are used as the primary means of exposure control [OSHA, 1990]. Additionally, the air supply and air exhaust should be carefully located so that supplied air passes through the area that is being controlled. The exhaust should be as close as possible to the source of contamination, and the workers should be positioned between the air supply and the source. Exhausted air should be discharged away from windows, other air intakes, or other means of re-entry [ACGIH 2007]. **HEPA-filtration** is recommended for passing the exhaust air [OECD Series on the Safety of Manufactured Nanomaterials No. 28, 2010].

Care must be taken to prevent the migration of MNMs into adjacent rooms or areas through the building's HVAC system, because of area pressurizations and directional airflows, or as a result of equipment and personnel moving from one area to another.

Administrative Controls and Work Practices

Administrative controls contribute to worker exposure reduction, but they do not always reduce the airborne concentration of the contaminant in the workplace. They often include limiting exposure by reducing the time the employee is handling the material, specifying good housekeeping and other good work practices, training employees, and implementing proper labeling and storage of materials. Administrative controls in some research laboratories may include maintaining clean room conditions [Schulte, 2008].

Some administrative controls that should be considered include:

- Providing known information to workers and students on the hazardous properties of the nanomaterial precursors or products;
- Education of workers and students on the safe handling of nanomaterials;
- Restricting access to areas by using signs or placards to identify areas of nanoparticle research;
- Transport dry nanomaterials in closed containers;
- Handle nanoparticles in suspension on disposable bench covers;
- Always perform nanoparticle aerosol generating activities in a fume hood, externally ducted biological safety cabinet, or glove box; and

- Clean the nanomaterial work area daily at a minimum with vacuum cleaners equipped with **HEPA filters** or wet wiping method for any operation involving powdered NPs.

Training is an important component of the administrative control:

- Ensure that employees/researchers/students have both general safety training and lab-specific training relevant to the MNMs and associated hazardous chemicals used in the process/experiment. Some specific Laboratory Chemical Safety Toolkit for guidance on training (SU Toolkit) could be used (http://chemtoolkit.stanford.edu/ChemSafetyTraining)
- Lab-specific training can include a review of this safety fact sheet, the relevant Material Safety Data Sheets (if available), and the lab's Standard Operating Procedure for the experiment.
- It is necessary to inform and involve the employees in the risk assessment process. They should know the significant findings of the risk assessment; the precautions they should take to protect themselves and their fellow employees; the results of any monitoring of exposure, especially if these exceed any workplace exposure limit (WEL); the collective results of any health surveillance. Without the informed and competent participation of employees, any measures identified as necessary in the risk assessment are unlikely to be fully effective.

Work Practices

- **Selection of Nanomaterials:**
  - Whenever possible, handle nanomaterials in solutions or attached to substrates to minimize airborne release.
  - Consult the Material Safety Data Sheet (MSDS), if available, or other appropriate references prior to using a chemical or nanomaterial with which you are unfamiliar. Note: Information contained in some MSDSs may not be fully accurate and/or may be more relevant to the properties of the bulk material rather than the nano-size particles.
- **Safety Equipment:**
  - Know the location and proper use of emergency equipment, such as safety showers, fire extinguishers, and fire alarms.
- **Hygiene:**
  - Do not consume or store food and beverages, or apply cosmetics where chemicals or MNMs are used or stored since this practice increases the likelihood of exposure by ingestion.
  - Do not use mouth suction for pipetting or siphoning.
  - Wash hands frequently to minimize potential chemical or nanoparticle exposure through ingestion and dermal contact.
  - Remove gloves when leaving the laboratory, so as not to contaminate doorknobs, or when handling common use objects such as phones, multiuser computers, etc.

- **Labeling and Signage:**
  - Store in a well-sealed container, preferable one that can be opened with minimal agitation of the contents.
  - Label all chemical containers with the identity of the contents (avoid abbreviations/ acronyms); include term “nano” in descriptor (e.g., “nanozinc oxide particles” rather than just “zinc oxide.” Hazard warning and chemical concentration information should also be included, if known.
  - Use cautious judgment when leaving operations unattended: i) Post signs to communicate appropriate warnings and precautions, ii) Anticipate potential equipment and facility failures, and iii) Provide appropriate containment for accidental release of hazardous chemicals.
- **Cleaning:**
  - Wet wipe and or HEPA-vacuum work surfaces regularly.
- **Transporting:**
  - Use sealed, double-contained container when transporting MNMs inside or outside of the building.
- **Buddy System:**
  - Communicate with others in the building when working alone in the laboratory; let them know when you arrive and leave. Avoid working alone in the laboratory when performing high-risk operations.

Clothing and Personal Protective Equipment

Personal protective equipment (PPE) should be required when engineering and/or administrative controls are not feasible or effective in reducing exposures to acceptable levels and wherever it is necessary because of hazards. Protective equipment must be used and maintained in a sanitary and reliable condition [OSHA, 2008]. Based on the uncertainty of the health risk of nanomaterials, it may be prudent to wear appropriate PPE on a precautionary basis. PPE can include respirators, gloves, clothing, face shields, safety glasses, and other garments designed to protect the wearer.

There are limited referenced guidelines for appropriate PPE (e.g. gloves, clothing) for protection from nanoparticles.

PPE is typically tested at certain particle size ranges. For example, some protective clothing is tested at the 1 µm (1,000 nm) size range for particle penetration. In respirators, the 3 µm (3,000 nm) size range is used in respirator filter testing. The size of the nanoparticle may be a factor in determining appropriate PPE.

Research is ongoing into the appropriate selection of labcoat material as it related to NP penetration.

Standard laboratory PPE (e.g. lab coat, gloves, etc.) should be utilized when working with NPs.

Gloves

Personnel should wear polymer gloves (e.g. nitrile) when handling MNMs. Wearing two layers of gloves may be a best practice until more is known on na-

noparticle penetration through glove materials and skin. Reference the following for general guidelines on glove type selection in reference to the chemical or material being used:

Appendix D of the *NIH Chemical Hygiene Plan*:
http://www.ors.od.nih.gov/sr/dohs/LabServices/Pages/default.aspx

PPE selection guide (link from OSHA website):
http://www.osha.gov/Publications/osha3151.pdf

Respirators

Personnel should utilize appropriate engineering controls in lieu of respirator use. Respirators may be considered for use for certain job task/procedures where engineering controls are not feasible.

Research into the effectiveness of respirators used for protection from nanoparticles is ongoing and incomplete. Some studies indicate that respirators, including N-95 respirators, may provide some protection. The particle size of the nanoparticle should be evaluated in determining the appropriate respirator (penetrating particle size of the respirator).

Respirators, if used, should be in accordance with the Respiratory Protection Program (RPP)
http://medicine.yale.edu/intmed/prep/tools/respiratory.aspx

Dust Masks (and Surgical Masks)

Dust masks (and surgical masks) should not be used for protection from nanoparticles.

Eye protection

Safety glasses are mandatory when manipulating nanoparticles, whether in powdered form or in solution (Guide for the Safe Handling of Nanotechnology-based Products, 2009).

For increased protection, safety goggles with a full seal around eyes should be worn for certain applications (e.g., those involving significant exposure to aerosols). Some universities recommend that a face shield be worn.

(Canadian Centre for Occupational Health and Safety
http://www.ccohs.ca/oshanswers/prevention/ppe/glasses.html)

Prevention of injection

Exposure by accidental injection (skin puncture) is also a potential route of exposure, especially when working with animals or needles. To prevent this, wear gloves and lab coats, and apply the standard practices for working with sharps. Use of safer sharps is strongly recommended. (Guidelines for Nanomaterials, WFUSM General Guidelines for Handling and Working Safely with Nanomaterials, 2009; Nanotechnology: Guidelines for Safe Research Practices, Fact Sheet Environment, Health and Safety Information for the Berkeley Campus No. 73)

Prevention of ingestion

As with any particulate, ingestion can occur if good hygiene practices are not followed. Once ingested, some types of nanoparticles might be absorbed and transported within the body by the circulatory system. To prevent ingestion, eating and drinking are not allowed in laboratories.

4.2.3 Standard Operating Procedures

It is recommended to prepare Standard Operating Procedures (SOP) for all operations involving MNMs:

- The SOP should be tailored to be specific to the proposed experimental procedure
- Consider the hazards of the precursor materials in evaluating the process
- Special consideration should be given to the high reactivity of some nanopowders with regard to potential fire and explosion [Pritchard, 2004].

Within the DaNa project (http://www.nanoobjects.info) the project team has compiled an SOP template to fill in, based on carefully evaluated scientific practice. The "DaNa SOP template" as well as guidelines for its use can be downloaded at the following site: http://nanopartikel.info/en/nanoinfo/methods

4.3 Management of MNMs

General

MNMs must be managed as a hazardous material. The following label should be placed on all containers containing MNMs:

CAUTION
Nanomaterials Sample
Consisting of (Technical Description Here)
Contact: (POC)
at (Contact number)
in Case of Container Breakage.

Management of Nanomaterial-containing Waste Streams

The MNM-bearing waste streams considered here are:

- Pure MNMs (e.g., carbon nanotubes)
- Items contaminated with MNMs (e.g., wipes/PPE)
- Liquid matrices containing MNMs (e.g., hydrochloric acid containing carbon nanotubes)
- Solid matrices with MNMs that are friable or have a nanostructure loosely attached to the surface such that they can reasonably be expected to break free or leach out when in contact with air or water, or when subjected to reasonably foreseeable mechanical forces. The guidance does not apply to MNMs embedded in a solid matrix that cannot reasonably be expected to break free or leach out when they contact air or water.

The following guidance notes are used for the **Waste Streams management**:

- Do not put MNM waste in the regular trash or dump it down the drain.
- All MNM waste, as defined above, should be collected in labeled, enclosed hazardous waste containers. The label should include a description of the waste and the words "*contains nanomaterials*".
- Collect paper, wipes, PPE and other items with loose contamination in a plastic bag or other sealable container and store it in a fume hood until it is full, then double-bag it, label it, and dispose of it according to these procedures.
- MNM hazardous waste containers shall be collected and disposed of as hazardous waste following the standard procedures of your university.

Management of Nanomaterial Spills

Procedures should be developed to protect employees from exposure to MNMs during the cleanup of spills and spill-contaminated surfaces. Inhalation and dermal exposures will likely present the greatest risks. The potential for inhalation exposure during clean-

up will be influenced by the likelihood of MNMs becoming airborne, with powder form presenting a greater inhalation potential than MNMs in solution, and liquids in turn presenting a greater potential risk than encapsulated MNMs.

Until relevant health and workplace exposure information is available, it is prudent to base strategies for dealing with spills and contaminated surfaces on the use of current good practices such as dust control and suppression. Standard approaches for cleaning powder spills can be used for cleaning surfaces contaminated with dry powder nanomaterials. These include access control, using HEPA-filtered vacuum cleaners, wiping up dry powders with damp cloths, or wetting the powder before wiping. Liquid spills containing nanomaterials can typically be cleaned by applying absorbent materials/liquid traps. If vacuum cleaning is employed, HEPA-filtered vacuums should be used, and care should be taken that HEPA filters are installed properly and that vacuum bags are changed according to the manufacturer's recommendations. Dry sweeping or air hoses should not be used to clean work areas. As in the case of any material spills or cleaning of contaminated surfaces, the handling and disposal should follow all applicable state, federal, and local regulations.

Equipment to contain and clean a MNM spill should be readily available in or near each laboratory working with such materials. A MNM spill kit for a laboratory environment may contain the following:

- Barricade tape.
- Nitrile or other chemically impervious gloves.
- Elastomeric respirator with appropriate filters.
- Adsorbent material.
- Wipes.
- Sealable plastic bags.
- Walk-off mat (e.g., Tacki-Mat®).
- HEPA-filtered vacuum.
- Spray bottle with deionized water or other appropriate liquid.

Fire and Explosion

Because of their size, NPs may pose a greater fire and explosion risk than those same particles that are larger in size. A general guideline in the fire hazard of airborne particles: As the particle size decreases, and those particles are dispersed into the atmosphere, the fire hazard can increase.

Personnel working with NPs shall identify from the manufacturer or distributor whether or not the nanoparticle or material is flammable and/or combustible.

Both carbon-containing and metal dusts can explode if they are aerosolized at a high enough concentration and if oxygen and an ignition source are present. Because the surface-to-volume ratio increases as a particle becomes smaller, NPs may be more prone to explosion than an equivalent mass concentration of larger particles. In general, the potential and severity of MNMs explosions increase proportionally to the quantity of combustible MNMs being used. Thus, bench-scale research should present fewer explosion risks than work in pilot plants or full-scale manufacturing facilities. Nonetheless, all researchers should avoid creating large, highly concentrated aerosols of combustible MNMs.

Lately, two studies have been conducted in Switzerland with relevance for this topic [FOEN, 2010, 2013].

4.4 Medical surveillance

The need of medical surveillance for employees involved in working with NPs is still emergent [*Nanotechnology Safety and Health Program*, National Institutes of Health Office of Research Services, Division of Occupational Health and Safety, Technical Assistance Branch, 2012].

Occupational health surveillance involves the ongoing systematic collection, analysis, and dissemination of exposure and health data on groups of workers for the purpose of preventing illness and injury [NIOSH 2009]. Occupational health surveillance, which includes hazard and medical surveillance, is an essential component of an effective occupational safety and health program [Harber, 2003; Baker, 2005; NIOSH 2006; Wagner, 2008]. NIOSH continues to recommend occupational health surveillance as an important part of an effective risk management program for nanomaterial workers.

Medical screening in the workplace focuses on the early detection of health outcomes for individual workers and may involve an occupational history, medical examination, and application of specific medical tests to detect the presence of toxicants or early pathologic changes before the worker would normally seek clinical care for symptomatic presentations. Medical screening and resulting interventions represent secondary prevention and should not replace primary prevention efforts to minimize employee exposures to MNMs. Medical surveillance involves the ongoing evaluation of the health status of a group of workers through the collection and aggregate analysis of health data for the purpose of preventing disease and evaluating the effectiveness of intervention programs.

Specific guidance for workers exposed to Carbon Nanotubes or Nanofibers is described in the NIOSH *Current Intelligence Bulletin: Occupational Exposure to Carbon Nanotubes or Nanofibers* [NIOSH 2010]. NIOSH has developed interim guidance relevant to medical screening (one component of an occupational health surveillance program) for nanotechnology workers (see NIOSH *Current Intelligence Bulletin: Interim Guidance on Medical Screening of Workers Potentially Exposed to Engineered Nanoparticles* [http://www.cdc.gov/niosh/docs/2009-116]).

If medical screening recommendations exist for chemical or bulk materials of which nanomaterials are composed, they would apply to nanomaterials as well. A basic medical surveillance program should contain the following elements [Trout, 2010]:

- An initial medical evaluation performed by a qualified health professional and other examinations or medical tests deemed necessary by the health professional.
- Periodic evaluations including symptoms surveys, physical exams, or specific medical tests based on data gathered in the initial evaluation.
- Post-incident evaluations.
- Employee training.
- Periodic analysis of the medical screening data to identify trends or patterns.

4.5 Conclusions: needs and challenges for addressing the recommended practices

In order to address the recommended practices, and based on the findings of this report, several needs and challenges are still to be addressed. The following listing gives a short summary of these items.

- According to GAARN (Group Assessing Already Registered Nanomaterials) [GAARN 2013] special attention should be given to endpoints for which no classification is derived based on hazard data (e.g. mutagenicity, soil and sediment ecotoxicity), but where available data show such hazardous effects. Moreover, it is

important not to overlook a potential hazard because of (technical) difficulties encountered (e.g. when applying or adapting the current standardised test guidelines for NMs as well as other forms or when implementing sample preparation considerations in OECD TGs). When registering NMs and bulk substances under the same technical dossier, specific exposure scenarios for NMs (or other forms) should be included in the registration dossiers if these differ from the ones for the bulk materials. It is important that the exposure scenarios describe:

- how the substance is produced;
- its life-cycle uses; and
- how the manufacturer or importer controls the exposure for humans and the environment.

- Reference: GAARN, The third meeting of the Group Assessing Already Registered Nanomaterials (GAARN) from Helsinki, 30 September 2013.Labeling - There are no requirements to list nanoparticle content for the end user either on the label or on the Material Safety Data Sheets (MSDS):
  a. MSDSs are only required to list known hazards. There is no requirement to identify nanomaterials.
  b. Before a label would help, awareness needs to be raised in consumers so they can understand the information on nanoparticles in general as well as the specific ones added to the product.
  c. No official nomenclature currently exists for MNMs. This is misleading to the extent that the nanoscale form of a substance is subsumed under its macroscale form. This has implications for example when substances are included in positive lists or products are labelled with the substances they contain.
- Given the fact that the health impacts of a MNM can change based on its chemical environment, it is not clear who is responsible for developing toxicity information (for instance in the case of cosmetics: the cosmetics company or the nanoparticle manufacturer?)
- End of lifecycle analysis is not often included in product development especially for consumer. When and how will longer term lifecycle issues be addressed?
- There is little consensus even on how to destroy some NPs – incineration is basically how they are made so it cannot be relied on to degrade them and the metals remain regardless.
- Management of substances hazardous to waters:
  a. It should be ensured that MNMs are separately classified according to the various water hazard classes. Classifying MNMs in this way will trigger the application of appropriate requirements for industrial facilities. If a MNM cannot be classified with certainty, any facilities manufacturing or processing it must come under the strictest requirements.
  b. The use of the product involves environmental exposure (surface waters, etc.) and washing it into wastewater plants. Any claimed disposal method that doesn't take this into account will make the message incomplete or inconsistent.
  c. What are the environmental fate, potential bioaccumulation, and effects on water treatment plants?
- Unknowns include: bioavailability in the waste stream under various conditions, its effect on a treatment plant, and particles' behavior. How might this issue relate to the current hot topic of drugs in wastewater?

- Testing requirements: Key factors with regard to testing requirements are the solubility of MNMs, their distribution in biota and the environment, and chronic toxicity. Data on these aspects should be presented and the test design tailored to the special characteristics of MNMs.
- The lack of good toxicological information for the vast number of NPs with any number of different functional groups attached, each of which may contribute to different health outcomes. Should there be a difference in handling functionalized CNTs and does this require different guidelines for each?
- The lack of good measurement information. We don't know which parameters are most important in terms of possible biological effects. There are also issues in terms of the cost of advanced monitoring equipment, which would make it difficult for smaller employers to self-monitor.
- There is a need to consider the human factor. Even with training, engineering controls, and personal protective equipment, how do you ensure that employees will actually work in an appropriate manner?
- Can the nanoparticles captured in HEPA filters be released?
- How will the waste handler actually treat the waste?
- Medical monitoring: is baseline testing and periodic retesting for lung capacity a help or a potential liability? Is it necessary? If desired, what should the metrics be?
- Regulating the manufacture and use of MNMs in industrial facilities. MNMs are already produced in industrial facilities, in some cases in large quantities. However there are no statistics on the numbers of such facilities involved. Requirements for plant construction and operation are decisive to the safety of the environment and of the population in the surrounding area. The manufacture and use of MNMs in industrial facilities should therefore be subject to official monitoring where necessary.
- Minimising environmental releases of synthetic MNMs. Substances can never fully be prevented from entering the environment. To ensure the best possible level of environmental protection despite this, prohibitions and quality standards are supplemented with emission limits that are generally based on currently available knowledge. Too little is so far known both about the release of MNMs and about their behaviour in the environment.
- How can small companies afford to do monitoring and measurement of NPs?
- There are no worker exposure limits (Recommended Exposure Limits, Permissible Exposure Limits or Threshold Limit Values) specific to NPs.
- Incomplete information on protective measures to some MNMs. It is a challenge the evaluating the effectiveness of current personal protective equipment and safety devices, such respirators, filters and ventilation systems, to see if they are sufficient for protection from MNM exposure. There is a need for an interdisciplinary approach (fundamental material studies, instrumentation, risk evaluation, implementation of standards and protocols) to tackle the complex problem of understanding the environmental and health impacts of MNMs.
- Need for rethinking hazards. Previously, "less is better," has been guidance for reducing exposure. Now, although the total mass quantity of material is reduced, the hazard may be greater.
- With regard to clothing and Personal Protective Equipment PPE: this should be tested at 300 nm, because particles in this size range have been found to be the most penetrating ones.

4.6 References for Chapter 4

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5 Standardisation and assessment recommendations

5.1 Introduction

Standards and assessment recommendations play a critical role in:

- ensuring the safety, quality and reliability of products, processes and services;
- efficient production;
- cost reduction through competition;
- supporting regulation.

By providing a bridge that connects research to industry, standards and assessment recommendations by acknowledged institutions are equally valuable as a tool for promoting innovation and commercialization through:

- dissemination of new ideas and good practice;
- validation of new measurement tools and methods;
- implementation of new processes and procedures

5.2 ISO

ISO standards are part of the SIINN liaisons framework.

The following ISO Technical Committees are the main ones in addressing the nano dimension (source: nanoSTAIR project, http://nanostair.eu-vri.eu/home.aspx?lan=230&tab=2383&itm=2384&pag=1426):

- ISO/TC 229 Nanotechnologies
- ISO/TC 194: Biological evaluation of medical devices - Part 22: Guidance on nanomaterials, (ISO/NP TR 10993-22)
- ISO/TC 24: SC 4 Particle Characterization
- ISO/TC 35 Paints and varnishes
- ISO/TC 44 Welding and allied processes
- ISO/TC 45 Rubber and rubber products
- ISO/TC 45/SC 3 Raw materials (including latex) for use in the rubber Industry
- ISO/TC 48 Laboratory equipment
- ISO/TC 61 Plastics
- ISO/TC 94 Personal safety - Protective clothing and equipment
- ISO/TC 142 Cleaning equipment for air and other gases
- ISO/TC 146/SC 2 Air Quality – Workplace Atmospheres
- ISO/TC 150 Implants for surgery
- ISO/TC 150/SC2 Cardiovascular implants and extracorporeal systems
- ISO/TC184/SC4 Industrial automation systems and integration – Industrial data
- ISO/TC 201 Surface chemical analysis
- ISO/TC 201/SC 9 Scanning probe microscopy

- ISO/TC 202 Microbeam analysis
- ISO/TC 206 Fine ceramics
- ISO/TC 207 Environmental Management
- ISO/TC 207/SC 1 Environmental Management Systems
- ISO/TC 209 Cleanrooms and associated controlled environments
- ISO/TC 213 Dimensional and geometrical product specifications and verification
- ISO/TC 215 Health informatics
- ISO/TC 217 Cosmetics
- ISO/TC 229 Nanotechnologies
- ISO/TC 256 Pigments, dyestuffs and extenders

Further references to ISO can be found in the Glossary and under: http://www.iso.org.

The main part of work on nanotechnologies in the ISO is done within TC 229. This TC is subdivided as follows (http://www.iso.org/iso/iso_technical_committee?commid=381983):

- ISO/TC 229/CAG Chairman Advisory Group
- ISO/TC 229/JWG 1 Terminology and nomenclature
- ISO/TC 229/JWG 2 Measurement and characterization
- ISO/TC 229/TG 2 Consumer and societal dimensions of nanotechnologies
- ISO/TC 229/TG 3 Nanotechnologies and sustainability
- ISO/TC 229/WG 3 Health, Safety and Environmental Aspects of Nanotechnologies
- ISO/TC 229/WG 4 Material specifications

The following list gives an overview of documents published by ISO/TC 229, including their date of publication:

- ISO/TS 10797:2012 'Nanotechnologies -- Characterization of single-wall carbon nanotubes using transmission electron microscopy'
- ISO/TS 10798:2011 'Nanotechnologies – Charaterization of single-wall carbon nanotubes using scanning electron microscopy and energy dispersive X-ray spectrometry analysis'
- ISO 10801:2010 'Nanotechnologies – Generation of metal nanoparticles for inhalation toxicity testing using the evaporation/condensation method'
- ISO 10808:2010 'Nanotechnologies – Characterization of nanoparticles in inhalation exposure chambers for inhalation toxicity testing'
- 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/TR 10929:2012 'Nanotechnologies -- Characterization of multiwall carbon nanotube (MWCNT) samples

- 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'
- ISO/TR 11360:2010 'Nanotechnologies – Methodology for the classification and categorization of nanomaterials'
- ISO/TR 11811:2012 'Nanotechnologies -- Guidance on methods for nano- and microtribology measurements'
- ISO/TS 11888:2011 'Nanotechnologies -- Characterization of multiwall carbon nanotubes -- Mesoscopic shape factors'
- ISO/TS 11931:2012 ' Nanotechnologies -- Nanoscale calcium carbonate in powder form -- Characteristics and measurement'
- ISO/TS 11937:2012 ' Nanotechnologies -- Nanoscale titanium dioxide in powder form -- Characteristics and measurement'
- ISO/TS 12025:2012 'Nanomaterials -- Quantification of nano-object release from powders by generation of aerosols'
- ISO/TR 12802:2010 'Nanotechnologies – Model taxonomic framework for use in developing vocabularies – Core concepts'
- ISO/TS 12805:2011 ' Nanotechnologies -- Materials specifications -- Guidance on specifying nano-objects'
- ISO/TR 12885: 2008 ‘Nanotechnologies – Health and safety practices in occupational settings relevant to nanotechnologies’ (No SR - unlimited life)
- ISO/TS 12901-1:2012 'Nanotechnologies -- Occupational risk management applied to engineered nanomaterials -- Part 1: Principles and approaches'
- ISO/TS 12901-2:2014 'Nanotechnologies -- Occupational risk management applied to engineered nanomaterials -- Part 2: Use of the control banding approach'
- ISO/TR 13014:2012 'Nanotechnologies - Guidance on physico-chemical characterization of engineered nanoscale materials for toxicologic assessment'
- ISO/TR 13014:2012/Cor 1:2012
- ISO/TR 13121:2011 'Nanotechnologies -- Nanomaterial risk evaluation'
- ISO/TS 13278:2011 'Nanotechnologies -- Determination of elemental impurities in samples of carbon nanotubes using inductively coupled plasma mass spectrometry'
- ISO/TR 13329:2012 'Nanomaterials -- Preparation of material safety data sheet (MSDS)' ISO/TS 13830:2013 'Nanotechnologies -- Guidance on voluntary labelling for consumer products containing manufactured nano-objects'
- ISO/TS 14101:2012 'Surface characterization of gold nanoparticles for nanomaterial specific toxicity screening: FT-IR method'
- ISO/TR 14786:2014 'Nanotechnologies -- Considerations for the development of chemical nomenclature for selected nano-objects'
- ISO/TS 16195:2013 Nanotechnologies – Guidance for developing representative test materials consisting of nano-objects in dry powder form

- ISO/TR 16197:2014 Nanotechnologies – Compilation and description of toxicological and ecotoxicological screening methods for engineered and manufactured Photonics components and systems nanomaterials
- ISO/TS 16550:2014 Nanoparticles - Determination of muramic acid as a biomarker for silver nanoparticles activity
- ISO/TS 17200:2013 Nanotechnologies – Nanoparticles in powder form – Characteristics and measurements
- X ISO/TS 27687: 2008 'Nanotechnologies – Terminology and definitions for nano-objects – Nanoparticle, nanofibre and nanoplate' (SR in 2011)
- ISO 29701:2010 'Nanotechnologies – Endotoxin test on nanomaterial samples for in vitro systems – Limulus amebocyte lysate (LAL) test'

Dr. Peter Hatto, former Chairman of ISO/TC 229, and the WG Standardization in NANOfutures have produced two guidelines:

- For a better understanding of the standardisation process, please consult the "Standards and Standardisation Handbook"
- For a detailed description of the main steps of the standardisation process of research results, please consult the "standards and standardisation practical guide for researchers"

Both documents are available from DG Research and Innovation
http://ec.europa.eu/research/industrial_technologies/standardisation_en.html

The following Figure gives an overview of the different stages of a nano product development value chain, and the possible respective interfaces for standardisation:

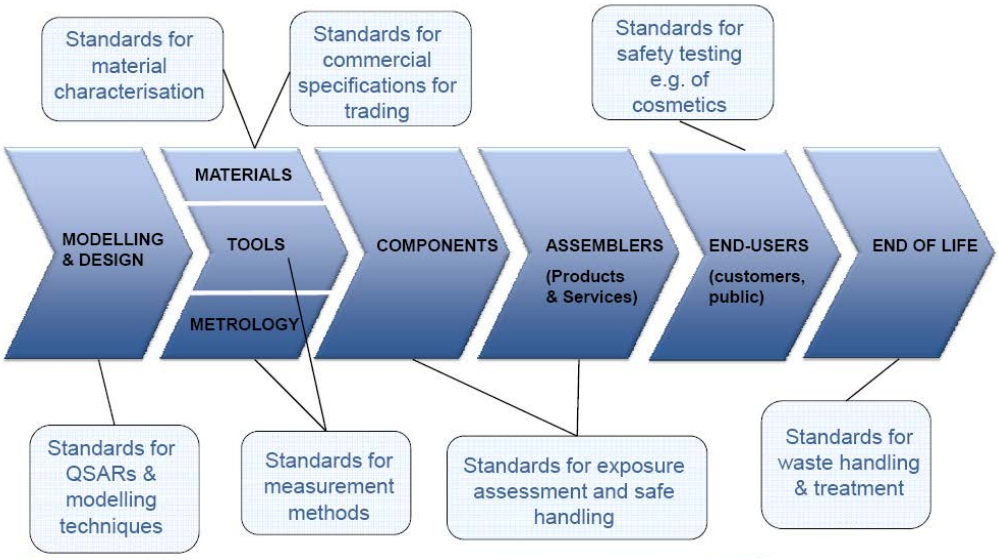

Figure 5: Standardisation along the Value Chain (*Courtesy of Dr. Rob Aitken, IOM and NANOfutures)*

In order to evaluate current research and development with respect to the potentials for developing new standards based on these research and development results, a decision tree has been developed, as depicted in the following Figure 6.

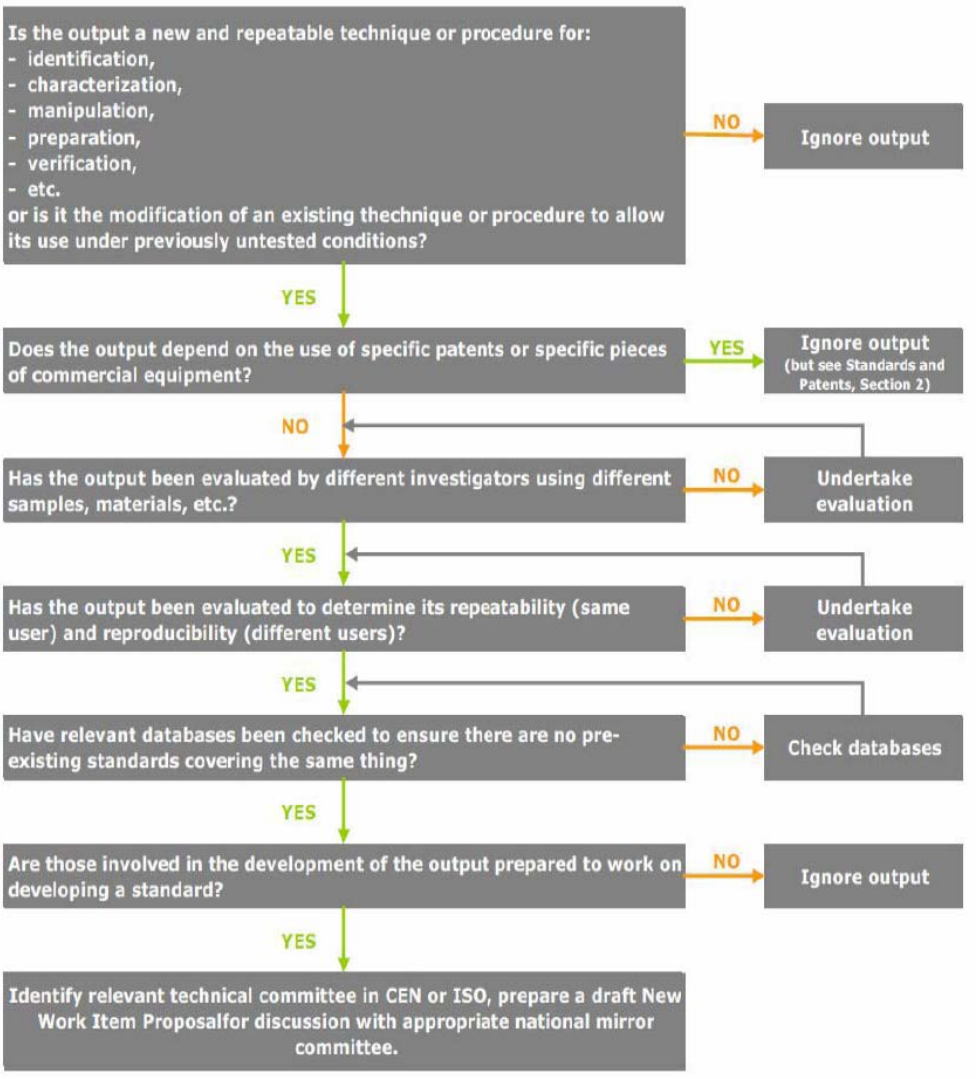

Figure 6: Decision tree identifying potentials for Standards from RTD project results (courtesy of Dr. M. Stintz, TU Dresden and NANOfutures WG)

5.3 CEN

CEN (http://www.cen.eu/about/Pages/default.aspx) is a business facilitator in Europe, removing trade barriers for European industry and consumers. Its mission is to foster the European economy in global trading, the welfare of European citizens and the environment. Through its services it provides a platform for the development of European Standards and other technical specifications.

CEN is a major provider of European Standards and technical specifications. It is the only recognized European organization according to Directive 98/34/EC for the planning, drafting and adoption of European Standards in all areas of economic activity with the exception of electrotechnology (CENELEC) and telecommunication (ETSI).

The following TCs are addressing nanotechnologies (source: nanoSTAIR project, http://nanostair.eu-vri.eu/home.aspx?lan=230&tab=2383&itm=2384&pag=1426):

- CEN/TC 137 Assessment of workplace exposure to chemical and biological agents
- CEN/TC 138 Non-destructive testing

- CEN/TC 162 Protective clothing including hand and arm protection and lifejackets
- CEN/TC 195 Air filters for general air cleaning
- CEN/TC 230 Water analysis
- CEN/TC 352 Nanotechnologies
- CEN/TC 392 Cosmetics

The main TC coping with nanotechnologies is TC 352, with the following subdivisions:

- CEN/TC 352/WG 1 Measurement, characterization and performance evaluation
- CEN/TC 352/WG 2 Commercial and other stakeholder aspects
- CEN/TC 352/WG 3 Health, safety and environmental aspects

The European Commission has given a mandate to CEN/TC 352, the European Mandate M/461 "Standardization activities regarding nanotechnologies and nanomaterials", focusing specifically on issues of characterization and exposure but also health, safety and the environment:

http://ec.europa.eu/growth/tools-databases/mandates/index.cfm?fuseaction=search.detail&id=443

CEN/TC 352 plays the coordination role between the various European and international Technical Committees - also including the electronics sector.

Useful readings: Guide for FP7 project proposers (available for download on the CEN CENELEC website):
ftp://ftp.cencenelec.eu/PUB/Publications/Brochures/LinkingResearch.pdf

5.4 OECD

An international collaboration on Environment and Health Safety aspects (EHS) of nanotechnology is organized by the Organisation for Economic Cooperation and Development (OECD).

A “Working Party on Safety of Manufactured Nanomaterials” (WPMN) was established in 2006 that consists of OECD member countries and organisations. It focuses on safety aspects relating to human health and the environment over the whole lifecycle.

More information about the work of the WPMN, as well as publications and updates on efforts of governments and other stakeholders to address safety issues of nanomaterials is available at http://www.oecd.org/env/nanosafety.

The OECD working party creates a very important platform for the diverse tasks and activities relating to the assessment of nanomaterials at international level. The results of this ongoing activity are presented at the OECD website (http://www.oecd.org/env/ehs/organisationoftheenvironmenthealthandsafetyprogramme.htm).

The OECD Harmonised Templates (http://www.oecd.org/ehs/templates/introductiontooecdharmonisedtemplates.htm) are standard data formats for reporting studies done on chemicals to determine their properties or effects on human health and the environment (e.g. hydrolysis, skin irritation, repeated dose toxicity, etc.). They are no data entry screens, but guides for structuring data entry/database management systems so they are developed. All of the data elements are listed which could be relevant for a summary of a study as well as the format in which the information should be entered and stored electronically, together with field-specific help texts intended to guide end users. Harmonisation in this context means

that these templates can be used as models for reporting studies and other information on any type of a chemical (e.g. pesticides, biocides, industrial chemicals). Templates 101-113 are specific for nanomaterials and can be found under the following link: http://www.oecd.org/ehs/templates/templates.htm.

The OECD also issues a newsletter on environment, health and safety news (http://www.oecd.org/chemicalsafety/environmenthealthandsafetynews.htm). In the issue No. 32 a section on safety of manufactured nanomaterials is included.

5.5 ECHA

The European Chemicals Agency, ECHA (http://echa.europa.eu/about-us), is the driving force among regulatory authorities in implementing the EU's chemicals legislation for the benefit of human health and the environment as well as for innovation and competitiveness. ECHA helps companies to comply with the legislation, advances the safe use of chemicals, provides information on chemicals and addresses chemicals of concern.

ECHA is continuously publishing up-dates for the Guidance on Information Requirements and Chemical Safety Assessments (ID &CSA). Recently published information on nanomaterials as examples:

- Guidance on information requirements and chemical safety assessment: Appendix R7-2 Recommendations for nanomaterials applicable to Chapter R7c Endpoint specific guidance (http://echa.europa.eu/documents/10162/13632/appendix_r7c_nanomaterials_en.pdf)
- Guidance on information requirements and chemical safety assessment: Appendix R8-15 Recommendations for nanomaterials applicable to: Chapter R8 Characterization of dose [concentration]-response for human health (http://echa.europa.eu/documents/10162/13643/appendix_to_r8_clean_en.pdf)

Under the following link, further information on ECHA and nanomaterials can be found: http://echa.europa.eu/regulations/nanomaterials

At this site information on related documents can also be found.

6 Regulatory aspects of nanomaterials

6.1 Introduction

The field of regulation of nanomaterials is an ever evolving one [Eisenberger, 2010; RIVM, 2015], starting in the early 1990s [Fiedler, 1994]. The process of risk assessment and adapting existing legislation and filling gaps with respect to regulatory requirements is on-going [Studer, 2015], possibly still intensifying over the next few years. There are a number of reasons why this field is so complex:

- based on their many different forms and types, and therefore broad applicability in many different sectors and markets, nanomaterials need to be treated in the corresponding variety of legislation regimes
- properties of nanomaterials can differ from the ones of their bulk forms, existing legislation may therefore not be entirely applicable for their regulation: current legislation refers to substance identities, not to particle properties
- for regulating nanomaterials in the respective legislation regimes there are still gaps with respect to data requirements for registration and authorisation
- the landscape of nanomaterials and derived products is developing fast, making it difficult to keep up with sufficiently adapted regulatory regimes
- research in the field of risks of nanomaterials, especially in nanotoxicology, during the last 20 years has only led to preliminary tools which are mostly not applicable for regulatory decision making [Hristozov, 2012]
- standardisation and harmonisation of test systems is not yet completed (c.f. chapter 5 of this document), leading to the necessity of case-by-case risk assessment
- there are still various definitions of the term nanomaterial, due to the different stakeholder perspectives (c.f. chapter 1 of this document). These definitions are still subject of discussions.

6.2 History of nano regulation in Europe

Activities of the European Union with regard to nano-regulation aspects started in 2004, and have subsequently steadily intensified [Eisenberger, 2010]. The following listing gives an overview of the most important milestones:

- 2004 European Strategy Communication (COM (2004) 338.): this document gave a first definition of the goals of the European policy on nanotechnology
- 2005 Action Plan (COM (2005) 243.): this communication, the "Action Plan for Europe 2005-2009", further specified the goals defined in the 2004 strategy paper
- 2007 First Implementation Report (COM (2007) 505.): in this communication the Commission identified public health, safety, environmental and consumer protection as the main regulatory goals - lack of data on human and environmental risks was acknowledged
- 2008 Communication on Regulatory Aspects of Nanomaterials (COM (2008) 366.): in this report the Commission stated that it found existing legislation sufficient
- 2008 Code of Conduct (COM (2008) 424.): the "Commission Recommendation on a code of conduct for responsible nanosciences and nanotechnologies re-

search" contains a set of guidelines for integrated, safe, and responsible research

- 2009 European Parliament Resolution (P6_TA (2009) 0328.): this resolution is a turning point in the European debate on nanoregulation. In this document the European Parliament states doubts regarding the Commission's view that existing legal provisions are sufficient. In this paper drafting of new regulations is recommended, the idea of "no data, no market" is first mentioned
- 2009 Second Implementation Report (COM (2009) 607.): this second report on the Action Plan states that in some areas legislation needs adoption, including chemicals, novel food, food additives, and cosmetics
- 2011 Regulation on plastic materials and articles intended to come into contact with food (EU No 10/2011)
- 2011 EU definition of nanomaterials (currently under review)
- 2011 regulation on the provision of food information to consumers (EU No 1169/2011)
- 2011 Commissions FP7 Call NMP.2012.1.3-3 Regulatory testing of nanomaterials
- 2012 regulation on biocidal products (EU No 528/2012): evaluation and authorisation of nanoscaled active compounds
- The 2012 Communication on the Second Regulatory Review on Nanomaterials (COM (2012) 0572.): describes the Commission's plans to improve EU law and its application to ensure their safe use. It is accompanied by a Staff Working Paper on nanomaterial types and uses, including safety aspects, which gives a detailed overview of available information on nanomaterials on the market, including their benefits and risks. The Communication was presented at a workshop on 30 January 2013
- 2013 Start of the project NANoREG (http://nanoreg.eu/), as a result of the 2011 FP7 Call
- 2014 the EU Commission comes forward with suggestions for the revisions of the annexes of REACH, to adapt to the specificities of nanomaterials
- 2014 Public consultation on a European nanomaterials registry: In mid-May, the European Commission set going a public consultation on possible measures to increase the transparency of nanomaterials on the European market.

6.3 Regulation of nanomaterials under existing regulatory regimes

Due to the wide range of available nanomaterials with numerous different properties and functionalities, they are and will be applied in a number of different sectors. Depending on their field of application, they are regulated under different regulatory regimes [Studer, 2015]:

	Authorisation	Registration	Labelling
Food additives; Regulation of food additives (EC) No 1333/2008	Nanoscale additives and ingredients require an evaluation and authorisation	-	

Materials and articles made from plastic which come into contact with foodstuffs; Regulation on plastic materials and articles intended to come into contact with food (EU) No 10/2011	Nanoscale additives and ingredients require an evaluation and authorisation	-	
Biocidal products; Regulation on biocidal products (EU) No 528/2012	Nanoscale active compounds require an evaluation and authorisation	-	Information labelling (Nano) and hazard classification, and labelling according to CLP ((EC) No 1272/2008)
Cosmetics; Regulation on cosmetic products (EC) No 1223/2009	-	Nano cosmetics: specification of the nanoscale ingredient and safety information	Infolabel (Nano)
Chemicals; REACH, guidance document: CA/59/2008	-	Registration of nanomaterial as new substance or form of the bulk substance	Hazard classification and labelling according to CLP
Information on food packaging for consumers; Regulation on the provision of food information to consumers (EU) No 1169/2011	-	-	Information labelling (Nano)

Table 24: Nano-specific regulations in the EU, adapted from [Studer, 2015]

In order for regulators to support the safety of humans and the environment, a number of regulatory tools are available and applicable as well [Studer, 2015]:

- chemicals leading to exposure situations with adverse outcome can be prohibited or restricted
- for biologically active ingredients authorisation procedures and lists of approved / forbidden substances can be applied
- even if the burden of proof for the safety of chemicals rests with the producer or importer, the authorities can check the underlying data and risk evaluations
- classification schemes and labelling can be applied. Currently, there is a review under way to check whether classification schemes for chemicals are applicable also for nanomaterials [UNECE, 2014]

6.4 Adaptation of existing regulation

Prerequisites and gaps for regulation

As depicted above, nanomaterials are already regulated today within the frame of existing legislation. However, there are gaps with respect to nanospecific data requirements in the existing registration and authorisation schemes [Studer, 2015]. Before exact dossier requirements can be standardised, there is a need for further knowledge and consensus with respect to the following prerequisites:

- Identity: it is difficult to distinguish between different nanomaterials and classify them either as an individual substance, a form of a bulk substance, or as a mixture. Rules on the identity of nanomaterials would clarify when a registration, including testing is necessary. In this context a grouping of nanomaterials according to toxicological and at least physico-chemical properties would be beneficial
- Characterisation: there is still no harmonised set of required physico-chemical properties available to characterise nanomaterials. This should include required parameters and standardised measurements for physico-chemical properties
- Analytics: methods are needed for the measurement of nanomaterials in complex media and products, as well as at the workplace, in the human body and the environment. These methods need to be robust and cost-efficient
- Toxicology and ecotoxicology: The approaches for the testing and assessment of traditional chemicals are in general appropriate for assessing the safety of nanomaterials, but may have to be adapted to the specificities of nanomaterials [OECD, 2013]. Moreover, relevant endpoints for in vitro tests need to be defined and standards developed, including standard media, applied dose and cell types. The use of in vitro tests may reduce costs and the number of animal tests. Reproducible methods can then become part of an integrated test strategy as a supporting tool for regulators. An important part of such a strategy would also be the elucidation of modes of action responsible for long-term effects as well as read-across and quantitative structure-activity relationship (QSAR) approaches
- Exposure models and bioaccumulation: existing exposure models for conventional chemicals are of limited value for nanomaterials [ECHA, 2002]. Homo-agglomeration, hetero-agglomeration and sedimentation are important parameters that should be addressed. In addition, estimation of bioaccumulation and biomagnification of nanomaterials in the food chain need further improvement.

Initiatives and projects

Several European funded projects with a reference to regulation of nanomaterials are currently running or starting, the most prominent ones being:

- NANoREG, A common European approach to the regulatory testing of Manufactured Nanomaterials (http://nanoreg.eu/): 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, Aus-tralia, Japan, and Russia. Within the NANoREG project, an overall framework is being developed applicable for most types of legislations. The framework will deliver a concept on how to address safety of nanomaterials, including, as appropriate, legislation and sector specific issues

- ProSafe, (no project website online yet, project has only started in February 2015): a Coordination and Support Action of EU's Horizon 2020 programme coordinates a number of EU funded initiatives such as NANoREG, NANoREG II, and SIINN. The project goal requires the active involvement of policy makers, regulation authorities, and innovation supporting agencies (all ministerial organisations) who can be seen as representatives of national governments, and who are key players in nano safety research, nano regulation and risk reduction from all Member States and Associate States as well as interested Third Countries and opinion leaders from industry.
- NANoREG 2, Development and implementation of Grouping and Safe-by-Design approaches within regulatory frameworks (no project website online yet, the project will start in autumn 2015): The project, built around the challenge of coupling Safe by Design to the regulatory process, will demonstrate and establish new principles and ideas based on data from industrial value chain implementation studies to establish Safe by Design as a fundamental pillar in the discovery, screening and commercialisation of novel nanomaterials.

6.5 References for Chapter 6

European Chemicals Agency (ECHA) (2002). Guidance on information requirements and chemical assessment, Chapter R. 16: Environmental Exposure Estimation Version 2.1.

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Fiedler, F.A., Reynolds, G.H. 1994 "Legal problems of nanotechnology: an overview", S. Cal. Interdisc. L. J., 593-629.

Hristozov, D.R., Gottardo, S., Critto, A., Marcomini, A. 2012, "Risk assessment of engineered nanomaterials: a review of available data and approaches from a regulatory perspective", Nanotoxicology 6 (8), 880-898.

National Institute for Public Health and the Environment (RIVM), the Netherlands, RIVM Report 2014-0157: Bleeker, E.A.J., Evertz, S., Geertsma, R.E., Peijnenburg, W.J.G.M., Westra, J., Wijnhofen, S.W.P. 2015 "Assessing health and environmental risks of nanparticles. Current state of affairs in policy, science and areas of application", chapter 2 Policy and Legislation, p. 33-44.

OECD (2013). Recommendation of the Council on the Safety Testing and Assessment of Manufactured Nanomaterials C (2013) 107.

Studer, C., Aicher, L., Gasic, B., von Goetz, N., Hoet, P., Huwyler, J., Kägi, R., Kase, R., Kobe, A., Nowack, B., Rothen-Rutishauser, B., Schirmer, K., Schneider, G., Vermeissen, E., Wick, P., Walser, T. 2015 "Scientific basis for regulatory decision-making of nanomaterials", CHIMIA International Journal for Chemistry, Volume 69, Number 1, 52-56.

UNECE GHS Sub Committee (2014). Review of the applicability of GHS to nanomaterials ST/SG/AC.10C.4/2014/9.

7 Data sources

7.1 Introduction

An overview of the field of nanosafety is always only covering a temporary situation and also not including all available studies or data, because those are already too abundant. Also, this consolidated framework does not aim to be complete, and it is recognized that there is a continuous production of new studies and other data in this area.

Therefore, this chapter gives an overview of accessible and restricted data sources, which are regularly updated or otherwise of high general importance.

7.2 Sources of information

7.2.1 Useful entry points

For a general overview of the field with respect to different aspects of safe nanotechnologies, the following entry points seem useful to the authors of this document:

- DaNa Knowledge base
  http://www.nanoobjects.info/cms/lang/en/page3.html;jsessionid=D24C8289F8E022C378861E9FA6FBA208
- EU NanoSafety Cluster: a DG RTD NMP initiative to maximise the synergies between the existing FP6 and FP7 (and newly evolving H2020) projects addressing all aspects of nanosafety including toxicology, ecotoxicology, exposure assessment, mechanisms of interaction, risk assessment and standardisation. on the website information on all projects can be found
  http://www.nanosafetycluster.eu/
- OECD Science and Technology Policy: Nanotechnology
  http://www.oecd.org/sti/nano/
- ECHA database on registered Substances
  http://echa.europa.eu/information-on-chemicals/registered-substances
- GoodNanoGuide - Beta Version
  http://goodnanoguide.org/tiki-index.php?page=HomePage
  - The GoodNanoGuide provides both environmental, health and safety ("EHS") Protocols and an EHS Reference Manual
  - The EHS Reference Manual outlines the approaches taken by professionals using research about nanomaterials and other precedents to develop appropriate protocols and guidelines.
    https://nanohub.org/groups/gng/ohs_reference_manual
- Nanowerk (private news letter)
  http://www.nanorisk.org/
- InfoNano: the Swiss central federal information platform for nanotechnology. The Federal Offices of Public Health, for the Environment and for Agriculture, the Commission for Technology and Innovation, Swissmedic and the State Secretariats for Economic Affairs as well as for Education and Research are involved in the website
  http://www.bag.admin.ch/nanotechnologie/index.html?lang=en

- Institute of Technology Assessment of the Austrian Academy of Sciences (2007): http://www.oeaw.ac.at/ita/projekte/nanotrust/ueberblick
- Nanotechnology Industries Association (NIA): http://www.nanotechia.org/nia-activities
- Safety of Nanoparticles Interdisciplinary Research Centre (SnIRC, 2004): http://www.safenano.org/
- Woodrow Wilson Inventories (no longer maintained, already outdated, but good overview): http://www.nanotechproject.org/
- EU-OSHA – European Agency for Safety and Health at Work, https://osha.europa.eu/en
- National Institute for Occupational Safety and Health (NIOSH) http://www.cdc.gov/niosh/topics/nanotech/
- US-EPA, Environmental Protection Agency, http://www.epa.gov/oppt/nano/
- CEFIC, The European Chemical Industry Council http://www.cefic.org/Policy-Centre/Environment--health/Nanomaterials/

7.2.2 Data bases and data collections

Organisation for Economic Cooperation and Development (OECD), web-sites and specific OECD documents:

- For all OECD publications: http://www.oecd.org/document/53/0,3343,en_2649_37015404_37760309_1_1_1_1,00.htm
- Safety of Manufactured Nanomaterials http://www.oecd.org/department/0,3355,en_2649_37015404_1_1_1_1_1,00.html
- Publications in the Series on the Safety of Manufactured Nanomaterials (for all OECD reports) http://www.oecd.org/document/53/0,3343,en_2649_37015404_37760309_1_1_1_1,00.html
- OECD Road Map 2009 – 2010 http://www.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono%282009%2934&doclanguage=en
- ENV/JM/MONO(2009)20/REV. OECD. 2010. Guidance manual for the testing of manufactured nanomaterials: OECD's sponsorship programme; first revision. No. 25, Paris: OECD
- Organisation for Economic Co-Operation and Development, ´Tour de Table at the 3rd Meeting of the Working Party on Manufactured Nanomaterials´, Current Developments/Activities on the Safety of Manufactured Nanomaterials/ Nanotechnologies, Paris, France, 28-30 November 2007
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- Organisation for Economic Co-operation and Development. 2010. Current developments/activities on the safety of manufactured nanomaterials. OECD Environment, Health and Safety Publications Series on the Safety of Manufactured Nanomaterials. No. 26. ENV/JM/ MONO (2010) 42, Paris, France
- OECD 301 A-F OECD (OECD 105, OECD 107/117/123, OECD 111, OECD 106/121; OECD 305; OECD 315, 317) GUIDELINE FOR TESTING OF CHEMICALS, ENV/JM/TG(2005)5/REV1, 2005
- OECD Environment, Health and Safety Publications, Environment directorate joint meeting of the chemicals committee and the working party on chemicals, pesticides and biotechnology, Series on the Safety of Manufactured Nanomaterials No. 28, Compilation of nanomaterial exposure mitigation guidelines relating to laboratories, 01-Dec-2010
- ENV/JM/MONO(2009)17**,** Environment directorate joint meeting of the chemicals committee and the working party on chemicals, pesticides and biotechnology, Series on the Safety of Manufactured Nanomaterials Number 12, Comparison of guidance on selection of skin protective equipment and respirators for use in the workplace: manufactured nanomaterials, 19-june-2009.

World Health Organization (WHO)

- World Health Organization, Summary listing of projects within six Activity Areas. Work plan 2006-2010 of the WHO Global Network of Collaborating Centres, accessed on 23 April 2008. http://www.who.int/occupational_health/network/summary_listing_projects_apr08.pdf
- World Health Organization, Work Plan 2006-2010 of the WHO Global Network of Collaborating Centres, accessed on 23 April 2008. http://www.who.int/occupational_health/network/compendium_apr08.pdf
- WHO projects connected with nanomaterials
    - AA6:NM 1 Dialogue on Nanoparticles Federal Institute of Occupational Safety & Health – BAuA, Germany
    - AA6:NM 2 How to assess the adequacy of safety measures for manufactured nanoparticles, Institute for Work and Health, Lausanne, Switzerland
    - AA6:NM 3 Best practices globally for working with nanomaterials NIOSH – National Institute for Occupational Safety and Health, USA
    - AA6:NM 4 NANO-Comms: A Technical observatory for the dissemination of information regarding nanoparticles health and safety issues HSL, - Health and Safety Laboratory, UK

  - AA6:NM 5 Assessing the Hazard of Nanoparticles Institute of Occupational Medicine – IOM, UK

- WORLD HEALTH ORGANIZATION INTERNATIONAL AGENCY FOR RESEARCH ON CANCER, *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans,* VOLUME 93, Carbon Black, Titanium Dioxide, and Talc, LYON, FRANCE 2010, This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon.

SCENIHR

Scientific Committee on Emerging and Newly Identified Health Risks, http://ec.europa.eu/health/scientific_committees/emerging/index_en.htm

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- SCENIHR 2010, "Scientific basis for the definition of the term nanomaterial”, The SCENIHR approved this opinion by written procedure on 8 December 2010 http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_032.pdf
- SCENIHR 2014, "Opinion on Nanosilver: safety, health and environmental effects and role in antimicrobial resistance" http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_039.pdf
- SCENIHR 2015, "Opinion on the guidance on the determination of potential health effects of nanomaterials used in medical devices" http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_045.pdf.

EU-US Community of Research (CoR)

For more information on the EU-US Communities of Research: http://us-eu.org

EU-US CoR "Databases and Ontologies": The EU-US Community of Research "Databases and Ontologies" is setting up an inventory of nanotechnology information resources (contribution of new information is possible via the online form: http://j.mp/TZweax).

Scope: Interconnected, freely communicating and agreed information systems are urgently needed for collating

- nanoscale material descriptions;
- their intrinsic and context-dependent properties and their effects, including environmental and health-related; and
- their interactions with biological entities.

The goal of the US-EU Nanotechnology Databases and Ontology Community of Research (CoR) is to enable the sharing, searching, and analysis of nanoscale material characterization data across a wide range of active and archived experimental sources and to give advice on how to structure these data to enable their widest possible use.

7.2.3 Action plans

The following list gives a non-exhaustive overview of some nano-related action plans:

- Action Plan Nanotechnology 2015 of the Federal Government of Germany, BMBF (2011)
  http://www.nanopartikel.info/files/downloads/Brosch%C3%BCre-BMBF-aktionsplan-nanotechnologie-2015.pdf
- NanoSafety Cluster Action Plan
  http://www.nanosafetycluster.eu/working-groups/2-hazard-wg/immunosafety/action-plan.html
- Nano Action Plan of the Austrian Ministry on Traffic, Innovation, and Technology (BMVIT)
  http://www.lebensministerium.at/publikationen/umwelt/gefaehrliche_stoffe/austrian_nanotechnology_action_plan.html
- Action Plan of Switzerland on Nanomaterials (2012)
  http://www.bag.admin.ch/nanotechnologie/12167/index.html?lang=en
- Swiss action plan for synthetic nanomaterials, Federal Office of Public Health, FOPH (2008)
  http://www.bafu.admin.ch/publikationen/publikation/00574/index.html?lang=en
- Nanotechnology and the Nanotechnology Action Plan (EU, 2004) (Archived 01.01.2012)
  http://cordis.europa.eu/nanotechnology/actionplan.htm
- National Nanotechnology Initiative (NNI, USA), founded in 2001
  http://www.nano.gov/
  http://www.cdc.gov/niosh/topics/
- NanoQebéc,
  http://www.nanoquebec.ca/en/28.php

7.2.4 Other relevant sources of information

Existing standards and standards under development as well as an inventory on national activities and documents with a national focus on MNM and their EHS implication can be found in the Annex to this report.

7.3 SIINN data platform

A dedicated SIINN data platform has been installed, based on NANOhub as the portal (http://www.nanohub.eu/), for which the Joint Research Centre (JRC) of the European Commission is the provider. The SIINN NANOhub portal is the backbone for the structured data storage and data retrieval within SIINN.

NANOhub is based on an older IUCLID (http://iuclid.eu/) version 5.1, complemented with nanospecific endpoints. Now, a new IUCLID version 6 is developed and equipped with such endpoints, so NANOhub will become increasingly obsolete, a convergence between the two systems is therefore sought. JRC is currently developing a migration tool to transfer data from NANOhub to the new IUCLID version. As soon as this tool is developed and tested (in the frame of the NANoREG project), also the SIINN data will be transferred. Important points in this respect:

- NANOhub will not be developed further
- new IUCLID will be adapted to all JAVA versions
- the portal at JRC will be the same, including the same tools
- a downloadable IUCLID version will be available
- JRC will take care of the transfer from NANOhub to IUCLID
- IUCLID will have sophisticated query functions
- user groups with specific access rights will be available

The integration of the SIINN data platform contents into follow-up projects like ProSafe or NANoREG II is currently being discussed.

The SIINN NANOhub database is only accessible to SIINN partners and members of project consortia resulting from the SIINN calls. For stakeholders interested in the data base an access can be granted in consultation with the SIINN consortium or the consortium of the follow-up projects, respectively. Requests can be addressed to the authors of this document.

So far, the first validated data included in the SIINN data platform are referring to the substance TiO_2 in the forms of Rutile, Anatase and Brookite. Taking into account the great amount of data existing in the field, the use of existing data for this activity has been examined and the following aspects have been pursued:

- including of data of several studies with the same nanomaterial (e.g. TiO_2 anatase), but with different biological material and with different end-points
- including of data referring to different TiO_2 polymorph forms, but with the same biological material and with specific endpoints
- including of data highlighting the effect of MNM concentration, as well as the influence of the time for interaction with a biological material
- including of data regarding TiO_2 degraded surface-treated nanoparticles
- including information about the effect of translocated nanoparticulate TiO_2
- including new specific endpoints by taking into consideration some data obtained in the Laboratory of Chemical Thermodynamics (from the Institute of Physical Chemistry of the Romanian Academy) as concerns not only physico-chemical, but also thermochemical properties of TiO_2 polymorphs synthesized in the laboratory and by comparing with commercial samples.

As data will become available from the projects resulting from the three SIINN calls (c.f. chapter 8.5 of this document), these data will be integrated in the database as well.

8 SIINN roadmap

8.1 Introduction

Within the SIINN project, one task has been the setting up of “roadmaps for the safe handling of nano-objects, safe processes (incl. “end-of–pipe” processes and safe operational procedures), safe products and safe transportation of nano products addressing identified gaps”. In the context of the SIINN work, a roadmap is an extended look at the future of a chosen field of inquiry composed from the collective knowledge and imagination of the brightest drivers of change in that field [Galvin, 1998, 2004].

The roadmap addresses the following areas:

- Safe handling of nano objects, based on the state-of-the-art and the identified gaps
- Safe processes, products and transport, based on the state-of-the-art and the identified gaps
- Safe end-of-pipe processes (recycling and waste disposal), based on the state-of-the-art and the identified gaps
- Standard operational procedures, based on the state-of-the-art and the identified gaps.

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 environment by investigating the toxicological behaviour of MNMs. Besides available information about the important criteria for MNM toxicology, (concerning environment, health and safety EHS) is examined in order to identify main knowledge gaps. In order to find and address these gaps, the described roadmapping methodology has been used. Based on the roadmapping results, three joint transnational calls have been organised during the lifetime of SIINN.

The roadmap represents an analysis and compilation of previous strategic documents’ outcomes regarding nanosafety, including on-going and future research based on a mapping of the EU project portfolio as well as input from within the SIINN project. The roadmap recommends **priority actions** either at **short, medium and long term** for each **axis of interest**.

The purpose of the roadmap is thus to provide inputs for transnational RTD programmes in the field covered by SIINN, i.e. the “Safe Implementation of Innovative Nanoscience and Nanotechnology”.

8.2 Methodology

8.2.1 Overview

The roadmapping methodology consists of different steps: data mining, filtering, processing and ordering according to the mapping axes, and subsequent definition of the final roadmap:

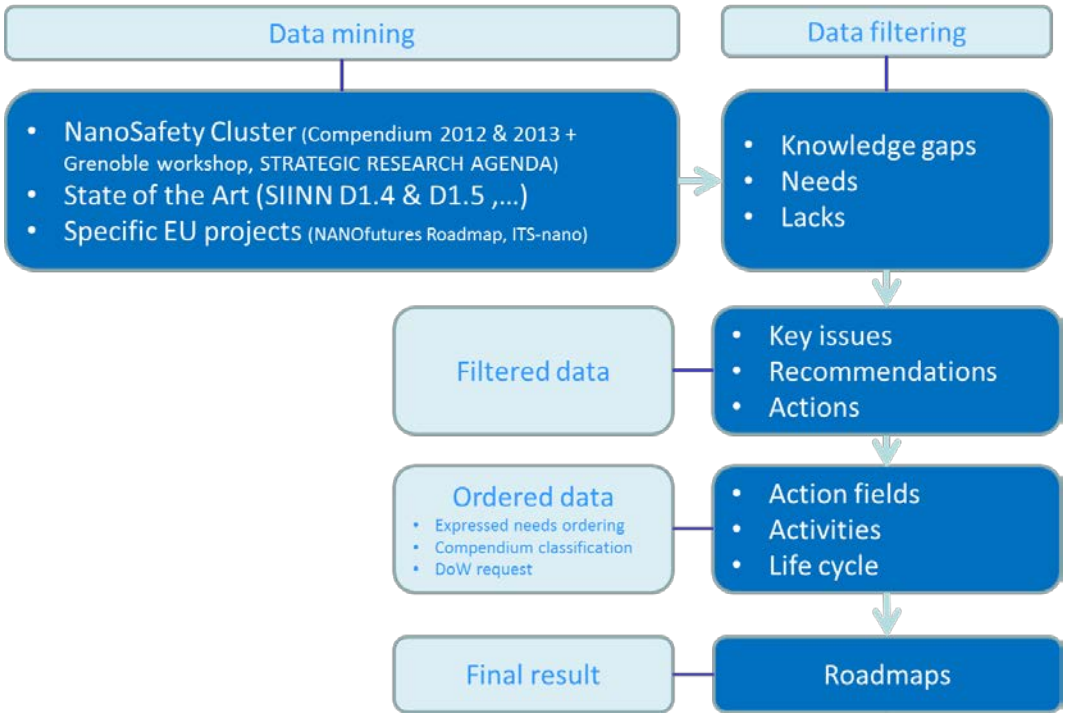

Figure 7: Roadmapping methodology

8.2.2 Data mining

As shown by Figure 8 different sources are used, chosen for their relevance and relationships with the SIINN project:

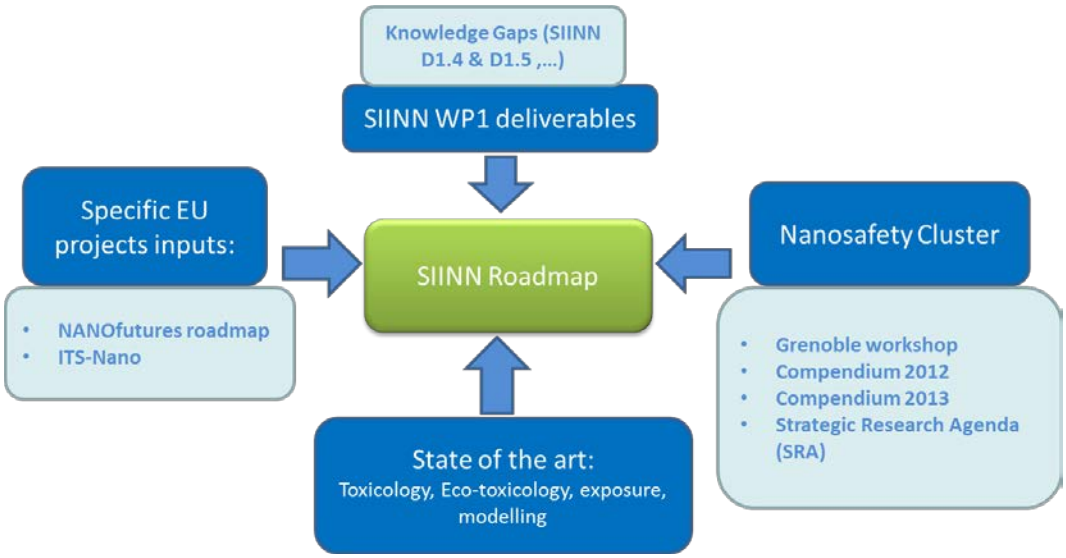

Figure 8: Final roadmap data collection

8.2.3 Mapping axes

Within SIINN, three main mapping axes have been considered. They correspond to relevant segmentations which are commonly used to classify the data: the nano product life cycle, the thematic activities which are handled in the examined projects, and finally the action fields:

- **The life-cycle** of manufactured Nano Materials (MNM) corresponds to the domains safe handling, standard operational procedures, safe processes, products and transport, safe end-of-pipe processes, as shown by Figure 9.

Figure 9: The Life Cycle of MNM

- **The thematic activities**:
  This segmentation corresponds to the one used in the NanoSafety Cluster compendium which was also used in the Grenoble workshop [Minatec 2012] where the EU projects taking part in the NanoSafety Cluster have been positioned, according to their main thematic activities: i) Characterisation and measurement, ii) Interaction with organisms, iii) Interaction with environment, iv) Control, handling, manufacturing. Even though some of the projects cover a broader scope, the workshop outputs have been carefully classified according to these activities during the discussion sessions.

- **The action fields**:
  The notion of action fields has been introduced for two main reasons: i) the necessity to precise the data in a dimension suitable for suggesting new calls, ii) the comparison with other roadmaps.
  The chosen action fields are, respectively: Materials, Exposure/Hazard, Methods, Modelling, Data management, and as a transverse one: Life cycle considerations. The action fields correspond to the major different skills to address when a key action is identified after the expression of needs during the roadmapping process. They appeared during the expression of needs and were extracted using a “heat map“-process.

The results are ideally mapped in 3D (see Figure 10) but, for convenient reasons are 2D projected upon the axes chosen for demonstrations or comparisons.

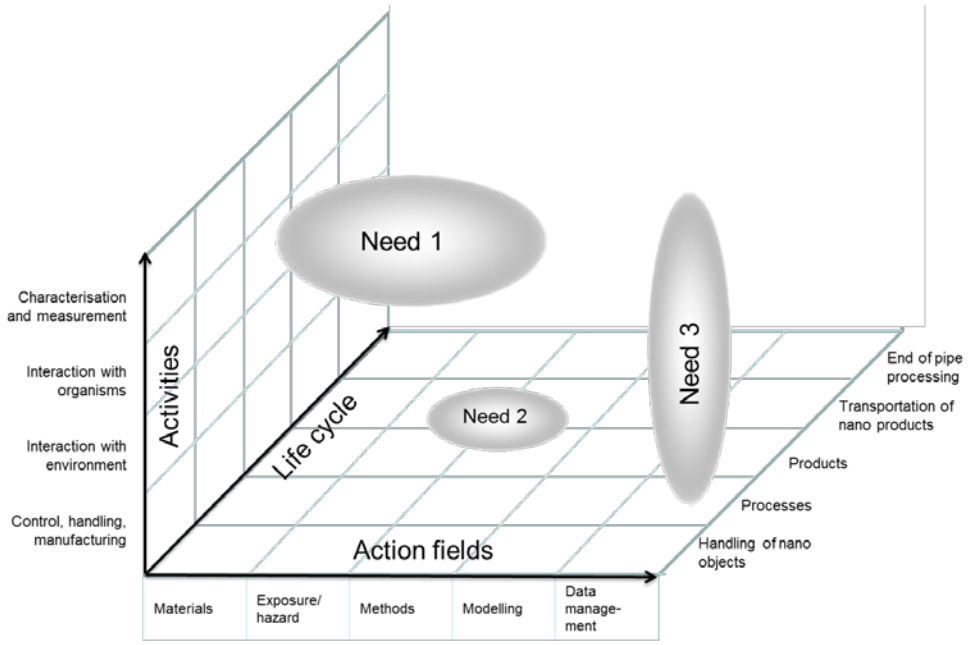

Figure 10: 3D roadmap representation

A matching exercise is done to order each outcome from the consulted sources into the mapping axes used in the SIINN roadmap (Figure 11):

- Thematic activities: correspond to the EU projects from the NanoSafety Cluster Compendium [Katalagarianakis, Riediker, 2012; Riediker, 2013] and the actions proposed by ITS-NANO and Nano*futures* projects.
- Action fields: are nurtured by the inputs from Grenoble workshop, knowledge gaps from the SIINN Deliverables D1.4 and D 1.5, and the mile-stones established by SRA.

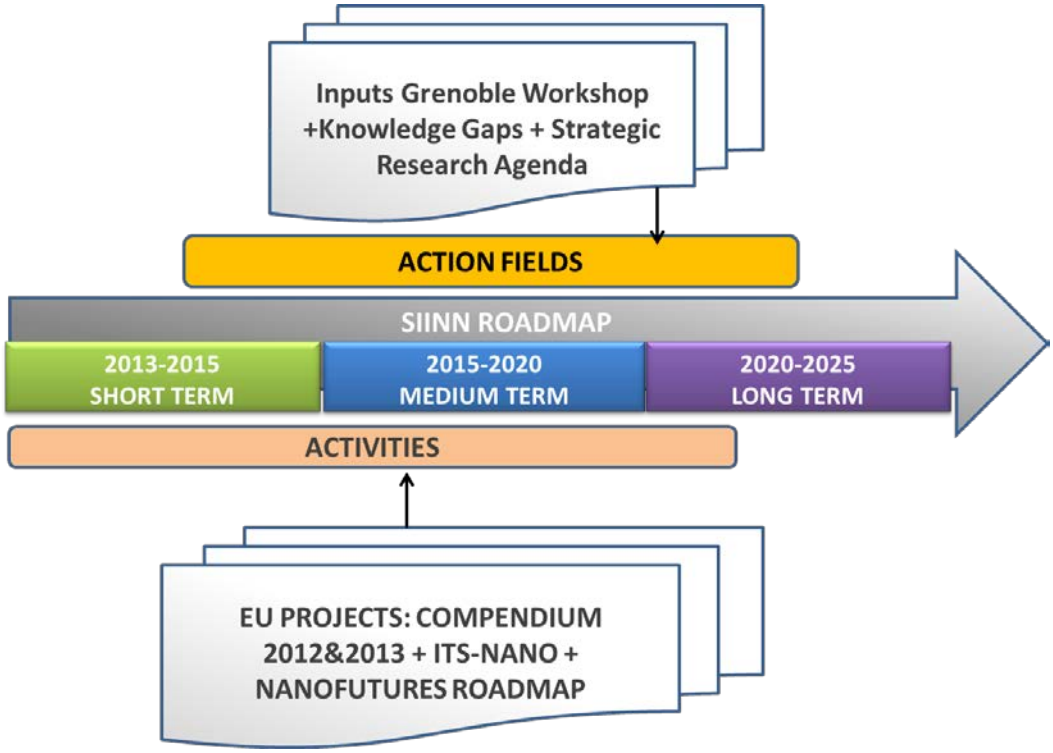

Figure 11: Origin of information to be ordered into the mapping axes action fields and activities

8.3 Analysis of data input

8.3.1 State-of-the-art

An analysis of the state-of-the-art with respect to toxicology, ecotoxicology, exposure, and modelling issues has been carried out. Building on this analysis, the following key issues have been defined:

Toxicology

- To focus on all body systems potential targets for MNM for a proper assessment of MNM hazard.
- Lung, cardio-vascular system, liver, kidney, developmental effects.

Eco-Toxicology

- To synthesise concepts on the current knowledge of bio-nano interactions: nanoparticles, living systems and surrounding environment.
- To connect physico-chemical properties with biological identity in situ, and behaviour and functional impacts of EMN at system and cellular level.

Exposure

- Key issues: discrimination from background nanoparticles, measurement of size distribution, particle size, aspect ratio, application of exposure models and choice of metric, and instrument and measurement strategy.
- Identification of aggregates and agglomerates.
- Comparative strategies between workplace air concentrations and personal exposure.
- Metric for risk assessment of MNM should be based in number, mass and surface area.

Modelling

- To establish a Quantitative Structure Activity Relationship (QSAR) model to: understand which properties have a large influence on the biological activity; predict the activity of previously untested structures/compounds.

8.3.2 SIINN activities

In order to set up the roadmap, the knowledge gaps identified by the SIINN project have been reformulated and connected to the expressed action needs. Building on this, a ranking for the number of occurrences of expressed needs versus the identified knowledge gaps was achieved. This procedure delivers a graphic representation of the emerging priorities to be undertaken for future calls (research projects).

Synthesis and conclusions

A synthesis effort has been made in the following Table 25 to bring together and compare the knowledge gaps and the condensed needs. This table provides a number of occurrences ranking for the condensed needs with regard to knowledge gaps that allows a graphical representation afterwards.

Knowledge gaps elaborated in SIINN	Actions (condensed needs)
MNMs' functionality and structure	Standards and ref. materials (for methods validation)
	Labelled MNM as standards
Toxicity and MNMs' surroundings	Quality of data in a database/common database
	Tools to discriminate particles from env. background
	Methods for detection of MNM in complex matrices
	Occupational exposure
Toxicity and form of MNMs	Tools to discriminate particles from env. background
	Methods standardisation, including sample preparation
	Improvement of analytical instruments
Dose metric and toxicity evaluation	Risk assessment through the life cycle
	Improvement of analytical instruments
	Tools to discriminate particles from env. background
	Occupational exposure
Quantification, tracing of MNMs and the current available techniques and Methodologies	Improvement of analytical instruments
	Screening tools
	Occupational exposure
	Standards and reference materials
	Methodological innovation
Prediction models and the influence of MNMs' concentration	Modelling
	Methods standardization, including sample preparation
	Methods for detection of MNM in complex matrices
Environmental disposal and Release Dynamics	Basic Research
	Improvement of analytical instruments
	Methodological innovation
Bioavailability and the uptake mechanisms of MNMs	Occupational exposure
	Consumer exposure
	Methods standardization, including sample preparation
	Quality of data in a database/ common databases
Lack of data regarding the other possible pathways on which MNMs can enter into the human body except inhalation	More basic research
	Chronic studies
	In vivo studies (and correlation in vivo/in vitro)
	En of life
Distribution of MNMs in the human body	Screening tools
	Improvement of analytical instruments
	Modelling
	Risk assessment through the life cycle
Lack of in-situ measurements	Methods for the detection of MNM in complex matrices
	Methods standardization, including sample preparation
	In vivo studies (and correlation in vivo/in vitro)
	Standards and ref materials (for methods validation…)

Knowledge gaps elaborated in SIINN	Actions (condensed needs)
Lack of reliable MNMs dispersion models.	Modelling
	Improved LCA and LCIA models
	Risk assessment through the life cycle
	Tools to discriminate particles from env. background
Epidemiological time-series studies	Modelling
	Risk assessment through the life cycle
	In vivo studies (and correlation in vivo/in vitro)
The size of particles	Standards and ref. materials (for methods validation, …)
	Basic research
	Improvement of analytical instruments
Measurement of MNMs exposure	Tools to discriminate particles from env. background
	Improvement of analytical instruments
	Methodological innovation
Measurements of CNT airborne	Risk assessment through the life cycle
	Basic research
	Occupational exposure
Biomarker studies for MNM toxicity are currently at their early stage	Basic Research
	Consumer exposure
	Occupational exposure
Controlling effectively airborne exposures to MNMs in the workplace is difficult in the absence of OELs (occupational exposure limits).	Occupational exposure
	Consumer exposure
	Tools to discriminate particles from env. background
Lack of registries and medical surveillance of MNMs workers	Quality of data in database/common databases
Need for research on what factors and parameters influence the effectiveness of engineering controls and personal protective equipment	More basic research
	Quality of data in database/common database

Table 25: Knowledge gaps and associated actions (heat mapping process)

Comparison between expressed needs and knowledge gaps:

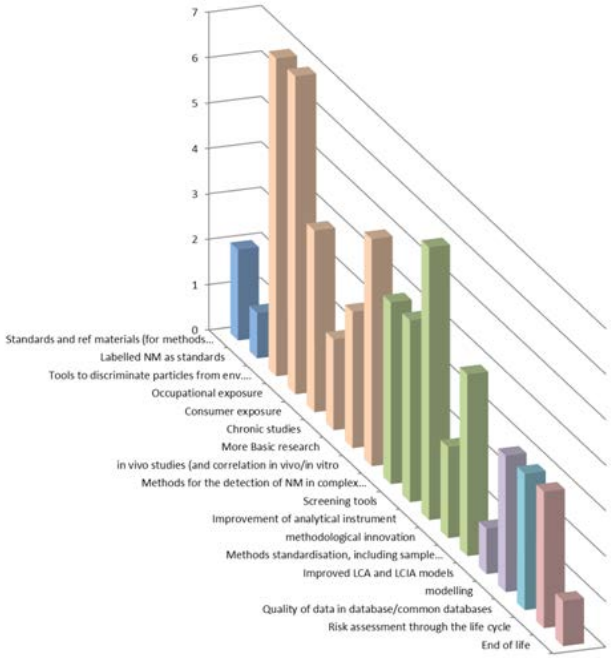

Figure 12: Condensed needs for all knowledge gaps

Semi-quantitative representation of Table 25 is possible considering the occurrence of the Actions (condensed needs) regarding with the knowledge gaps. As represented in Figure 12, several actions appear to be critical to solve the knowledge gaps from SIINN WP 1.

- Developing new Tools to discriminate particles from environmental Background,
- Better achievement of Occupational exposure and related data
- Improvement of analytical instrument

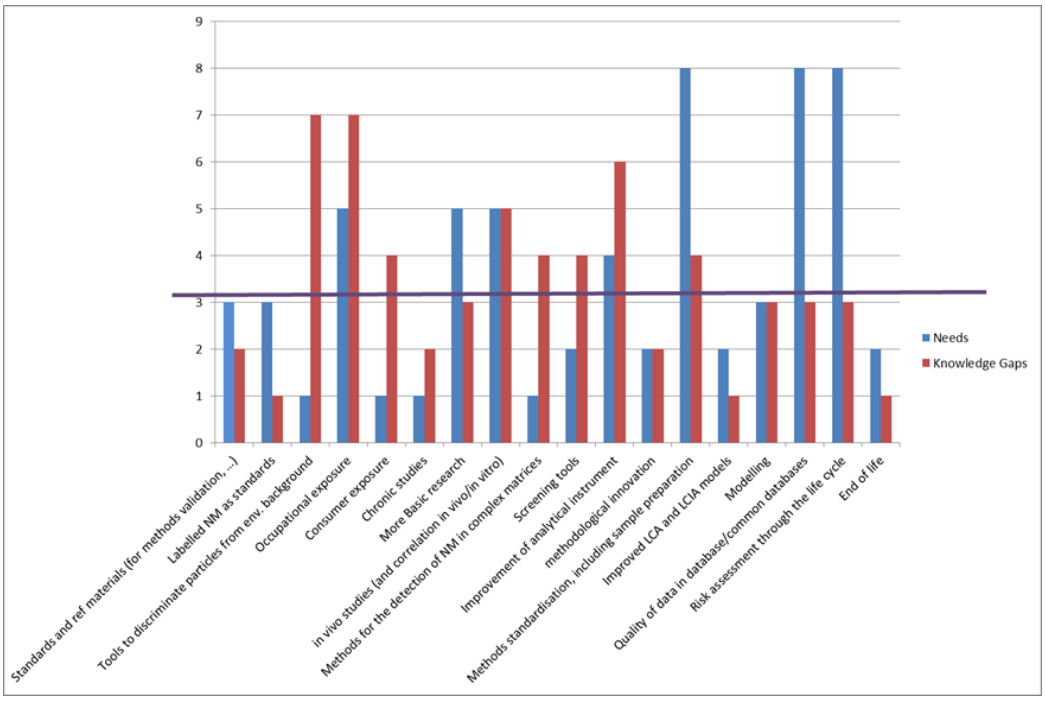

Figure 13: Comparison between expressed needs and knowledge gaps

The considered main emerging priorities are those with occurrence higher than 3. They are listed in the following Table 26 and correspond mainly to the action fields Exposure/Hazard and Methods development and validation. Action Fields as Data management and Life cycle are also represented. Any condensed need with a frequency of occurrence lower than 3 is not a topic considered as critical for futures activities (e.g.: Materials or Modelling).

These topics can be considered as **main actions to launch in future nanosafety calls** but not only, main emerging priorities can be pulled out also after plotting an arbitrary threshold (e.g.: 3) on Figure 13, and choosing those items that overtake the threshold, **either as expressed needs or knowledge gaps.**

Action Fields	Condensed needs
Materials	--
Exposure/Hazard	**Tools to discriminate particles from env. background**
	Occupational exposure
	Consumer exposure
	More Basic research
	In vivo studies (correlation in vivo/in vitro)
Methods development and validation	**Methods for the detection of MNM in complex matrices**
	Screening tools
	Improvement of analytical instruments
	Methods standardisation, including sample preparation
Modelling	--
Data management	**Quality of data in database/common databases**
Life cycle	**Risk assessment through the life cycle**

Table 26: Condensed needs as emerging priorities for future calls

Main outputs from the needs and knowledge gaps analyses

Subjects related to occupational exposure, in vivo studies, improvement of analytical studies and methods standardization have a higher frequency of occurrence for both expressed needs and knowledge gaps found in SIINN Deliverables.

There is an existing lack of projects in end of pipe processing, safe processes, products and transportation, interaction with organisms, Safe handling of nano-objects and processes, Interaction with environment, that should be covered in future transnational RTD calls for “Safe Implementation of Innovative Nanoscience and Nanotechnology”.

8.3.3 NanoSafety Cluster

Evolution of the EU projects portfolio

The EU project portfolio can be represented by mapping the projects on a 2D matrix, by positioning the projects according to their main thematic activity (vertical axis) and their positioning in the life cycle (horizontal axis). The colour code

represents the status of the project: red for projects ending during 2014, orange for projects ending during 2015 and green for projects which will end after 2015.

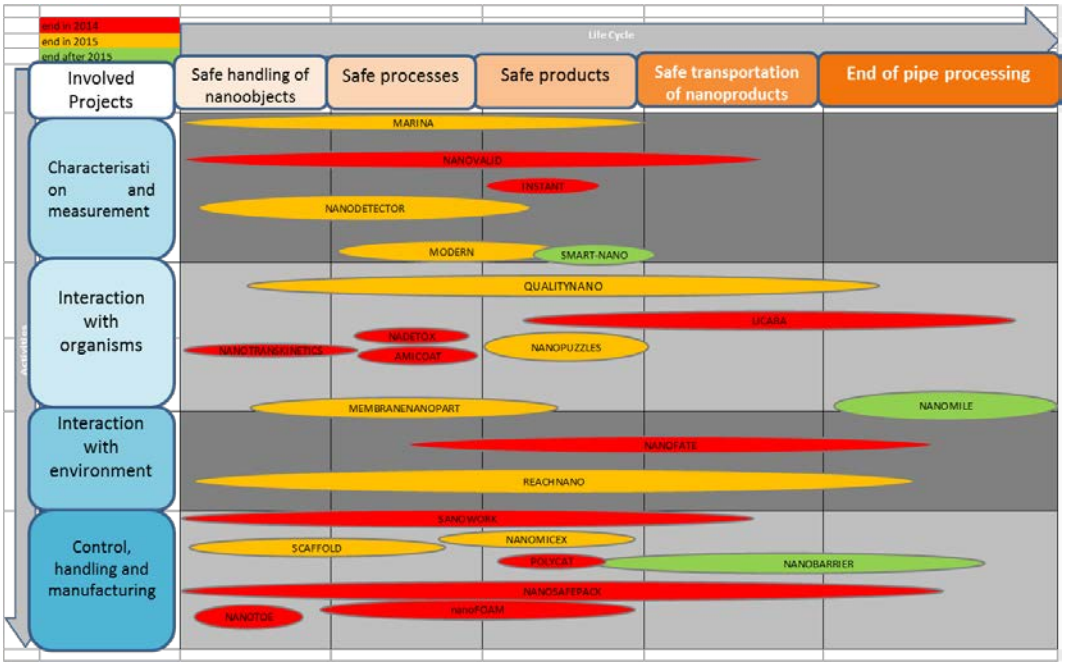

Figure 14: EU portfolio projects on nanosafety.

At a glance, the positioning of the projects in the life cycle shows where the main activities take place (and where they lack). More important is the fact that the landscape shows a neat evolution at mid-term, as shown by Figure 15. This approach is an excellent tool to identify the relevant nanosafety topics where funded research is weakened in the near future:

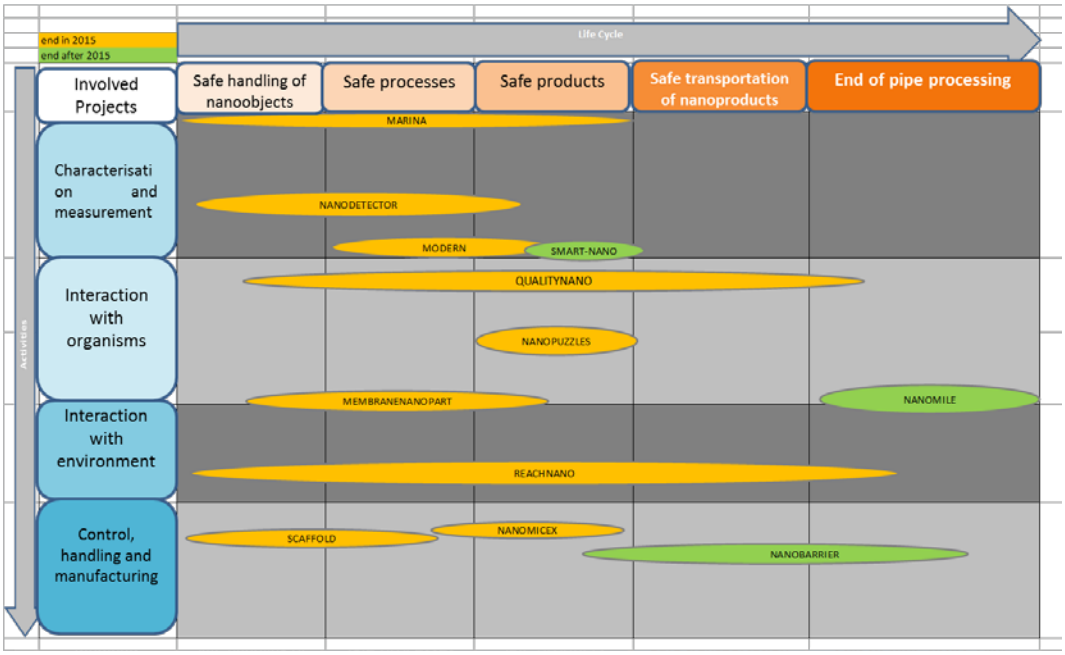

Figure 15: Mid-term evolution of the EU project portfolio

Main outputs from the evolution of the EU projects portfolio (Compendium 2013)

At short term, the end-of-pipe processing will be covered by two projects (LICARA and NANOFATE), both end in 2014. At medium term three more projects that will end in 2015 and beyond are related to end point of the value chain: NANOMILE, NANOBARRIER and REACHNANO.

At short and medium term the works related to the interaction with organisms, which are probably one of the major concerns in the field of nanosafety, will benefit from the outcomes of 5 projects: Short-term (NANOTRANSKINETICS, NADETOX AND AMICOAT) at the low end of the life cycle, and LICARA at the upper end of the life cycle. At Mid-term only NANOMILE project will deal the MNMs interaction with organism at the end-of-pipe processing.

Nevertheless, in general end-of-life cycle processes are not well covered neither by the on-going nor the future research. Future transnational activities should be implemented therefore in these identified priority areas: Characterisation and measurement at the end of pipe, interaction with environment and control, handling and manufacturing also at the end-of-pipe.

Finally, in Figure 16 all the action needs are plotted where there is a lack of projects. This figure can be considered as a tool for identifying future relevant topics for nanosafety.

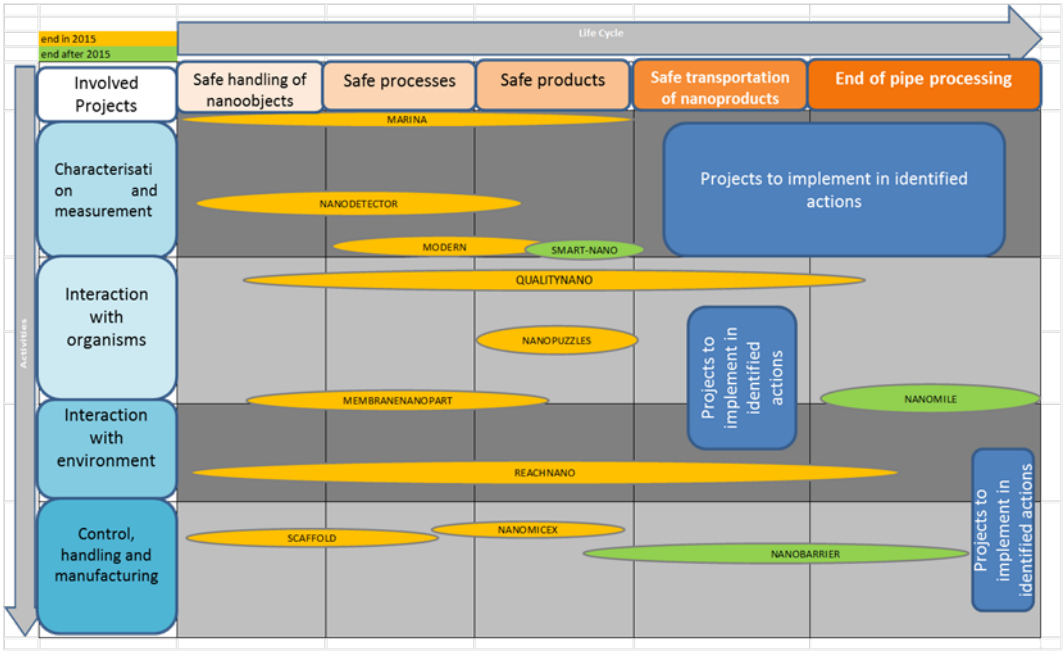

Figure 16: Identified “holes” and lacks of on-going projects

Priority needs for all the action fields:

During the sessions in Grenoble workshop, each project was asked to give its view concerning the further research needs. At the end of each session, a discussion with the audience was used to consolidate these outputs, which were classified in short term needs (pre 2015) and long term needs (post 2015).

The needs expressed during these sessions have been condensed to a list of 18 actions; then these actions have been regrouped within five so-called “action fields”: Materials, Exposure/hazard, Methods, Modelling, Data management and life-cycle (which is transverse)

Main outputs from the needs versus action fields analysis

The results of the heat maps for all action fields have been gathered to assess the future research interest, by means of the expressed needs during the Grenoble workshop. The analysed results show how several needs are identified as critical for a relevant number of projects. Methods standardisation, including sample preparation, Quality of data in database and Risk assessment through the life cycle, are the more frequent actions expressed as priority needs for future research, followed by Occupational exposure, More basic research and In vivo studies.

Sustainable nanomaterials and nanotechnology innovations (Strategic Research Agenda)

The Nanosafety Cluster Strategic Research Agenda (SRA) [European NanoSafety Cluster, 2013] describes the current level of knowledge of the safety of nanomaterials and nanotechnologies in order to identify knowledge gaps and to set out concrete goals for research on safety of MNMs within foreseeable future, thus providing an overview of the nanosafety landscape.

SRA identifies the research needs and priorities for the coming 10 years in four main thematic areas:

a. nanomaterial identification and classification;

b. nanomaterial exposure and transformation;

c. hazard mechanisms related to effects on human health and the environment; and

d. tools for the predictive risk assessment and management including databases and ontologies.

SRA's roadmap milestones have been positioned into a 2D matrix with the SIINN mapping axes MNM life cycle and thematic activities, in order to be expressed as actions to be achieved at short, medium and long term (Figure 17). The mapping illustrates the lack of proposed actions at the end of pipe processing for all thematic activities either at short, medium and long term:

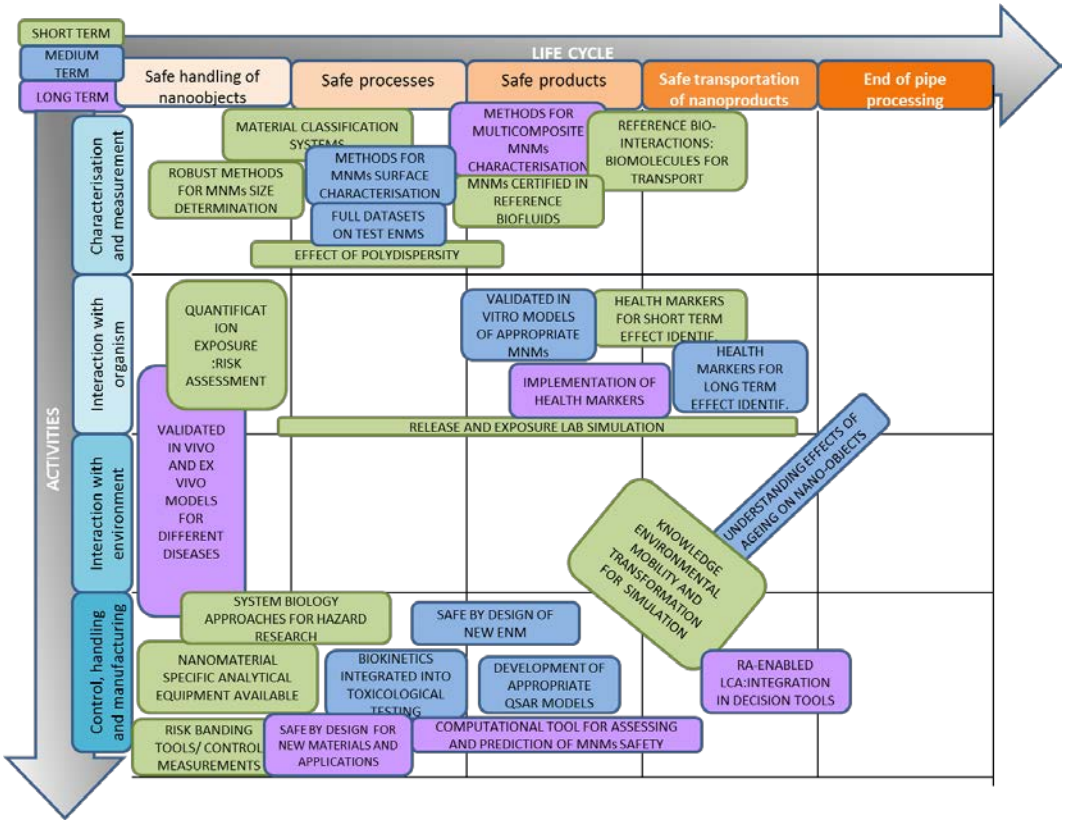

Figure 17: Safety short-term, medium-term and long-terms actions identified in Strategic Research Agenda (NanoSafety Cluster).

Main outputs from SRA's Roadmap

Lack of actions at the end-of-pipe processing for all thematic activities at short, medium and long term.

Several priority needs are identified as critical at short term:

- Standards and reference materials (for methods validation, …),
- Labelled MNM as standards, Tools to discriminate particles from environmental background,
- More Basic research in exposure/hazard and
- Quality of data in database/common databases

At medium term only the actions for understanding the effects of ageing on nano-objects are suggested at the end of pipe processing. The priority needs are identified in Labelled MNM as standards and Quality of data in database/common databases.

Actions to establish "safe by design" operation for new materials in particular in the MNMs life cycle phases comprising safe handling of nano-objects and safe processes have been identified at long term. The main priority needs are associated to risk assessment through the life cycle.

8.3.4 Main outputs from Nano*futures* Roadmap

At short term there is an absence of actions proposed for characterisation and measurement activities during the safe production, transportation and the end of pipe processing of MNMs.

Control potential release in different scenario (environment, occupational, consumer…) seems to be actions which should not be dismissed at short term. The same approach shall be encompassed for actions linked to understand fate and behaviour of MNMs in environment and adopting safe by design strategies.

At medium term, only regulation and education actions are suggested for activities related to control, handling and manufacturing for the safe processes, products, transportation and end-of pipe of MNMs.

The Technology Readiness Level (TRL) scale was used for technical actions, for safety only non-technical actions are proposed by Nano*futures* roadmap, thus TRL level is not applicable.

8.3.5 Main outputs from ITS-Nano Research Strategy

The analysis reveals the actions proposed for end-of-pipe processing at short-term. The life-cycle approach had been highlighted on ITS-NANO Strategy as a cross-cutting priority in the development of exposure themes. This implies also the development of new instruments and tools, in particular concerning the physicochemical methods to discriminate MNMs from environmental background particles.

New actions related to dose-response understanding, including hazard identification methods for *in-vivo/in-vitro* dose-response studies are advised at short term, as well as developing models to predict real exposure scenarios from measured concentrations, in human and environment.

At long term end-of-pipe actions as simulation of release scenarios, recycling, incinerating or disposal into land fields have become priorities.

8.4 The SIINN roadmap

8.4.1 Research priorities

The SIINN ERA-NET roadmap proposes an extended view to the nanosafety and nanomaterials EHS field to yield the future objectives by their chronology:

- **SIINN short-term (2013-2015) priorities** to support next transnational calls. The coming research activities where further transnational nanosafety calls can be launched should be focused on priorities related to:

Priorities	Actions
Safe handling of nano-objects and processes, Characterisation and measurement/ Control handling, manufacturing	Tools to discriminate particles from background Methods standardisation, including sample preparation Apply standards/SOPs for safe handling and disposal in entire life cycle Improving nanomaterial specific analytical equipment available (including portable devices)
Safe processes, products and transportation, interaction with	Consumer exposure Occupational exposure, including

Priorities	Actions
organisms	exposure during recycling or disposal Risk assessment through the life cycle
Safe processes, products and transportation, interaction with environment	Risk assessment through the life cycle Exposure scenarios and models to predict real exposure from measured concentrations in environment and human, including control potential release
End of pipe processing	Tools to discriminate particles from env. background In vivo studies (correlation in vivo/in vitro) Consumer exposure Occupational exposure, including exposure during recycling or disposal Risk assessment through life cycle

Table 27: Identified priorities and actions at short term.

- **Medium-term (2015-2020) research priorities to future H2020 calls.** The acknowledged future research activities are associated to Safe handling of nano-objects and Safe processes, products and transportation, characterisation and measurement, interaction with organisms, interaction with environment, processing, control, handling, manufacturing and end of pipe processing, addressing topics related to:
  - Methods for MNMs surface characterisation
  - Validated in vitro models of MNMs
  - Understanding effects of ageing on nano-objects
  - Biokinetics integrated into toxicological testing
  - Safe by design on new MNMs
  - Health markers for long-term effect identification
  - Full datasets on test MNMs
  - QSAR models
  - Regulation to address EHS issues
- **Long-term (2020-2025) research priorities.** A glimpse beyond the 2020 Horizon allowed the identification of future research needs in activities linked to Safe products, processes and safe transportation, Safe handling of nano-objects, interaction with environment, characterisation and measurement, interaction with organisms, processing control, handling, manufacturing and end-of-pipe processing, addressing the following topics:
  - Methods for multicomposite MNMs characterization
  - Key metrics for harmful impact
  - Validated in vivo and ex-vivo models
  - Safe by design for new material and applications

- Clarifying release scenario at the end of life during incineration, re-cycling or disposal into land fields
- Computational tool for assessing and prediction of MNMs safety
- Standards protocols of simulation of release scenarios at end of life

End-of-life cycle processes are not well covered neither by the on-going nor the future research.

8.4.2 Summary

A roadmap diagram of the particular actions to be undertaken to reach the MNMs research priorities for every time line (short, medium and long term) as a structured combination of each document studied:

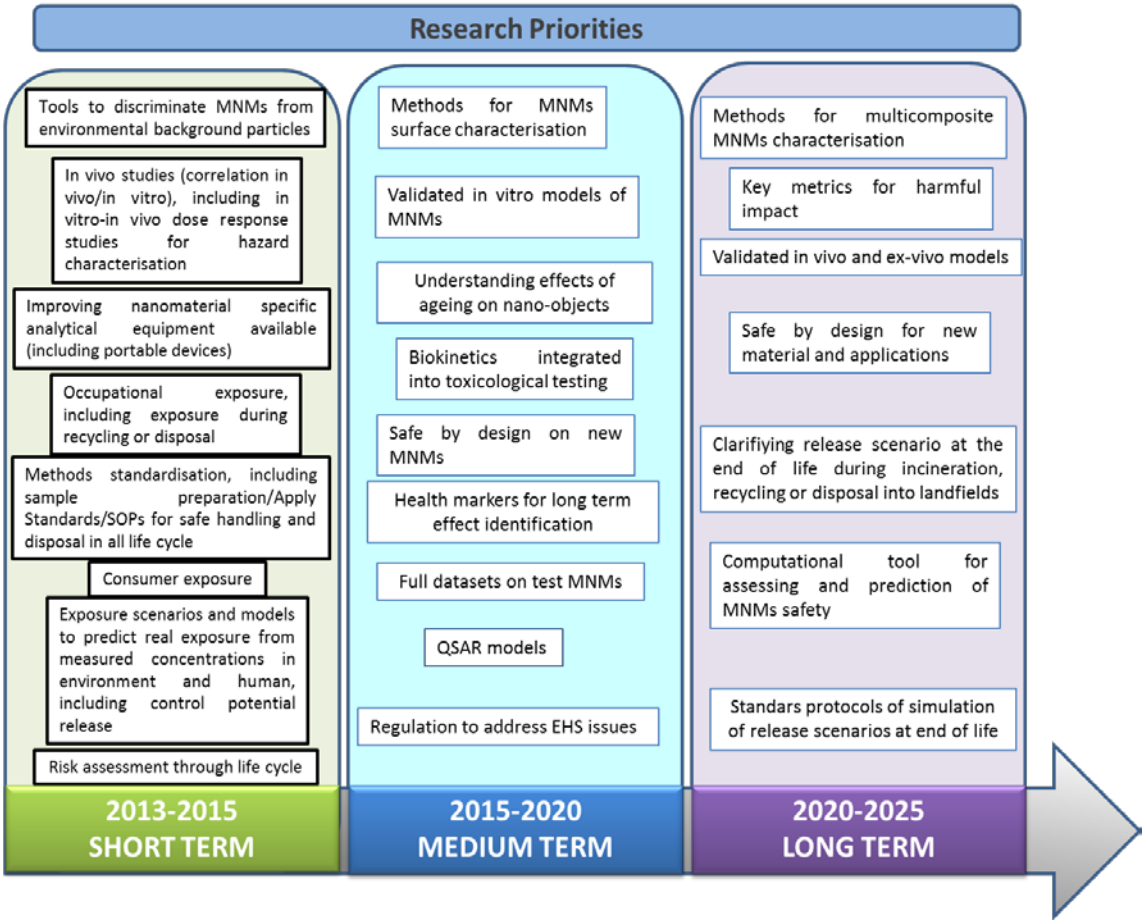

Figure 18: Nanosafety short-term, medium-term and long-term research priorities proposed in SIINN ERA-NET roadmap.

8.5 SIINN transnational calls for proposals

During the lifetime of the SIINN project, the following transnational calls have been launched (for details check http://www.siinn.eu/en/joint-calls/2014-third-siinn-call/,161):

2012 First SIINN Call

The First SIINN Call for innovative transnational research proposals was published in March 2012, and addressed the following four overall topics:

- Establishment and development of models and methods for analytical tools, theoretical prediction, and characterization
- Exposure assessment
- Studies on Impacts of MNMs on environment
- Studies on properties and effects of MNMs on human health.

The following three projects were selected for funding:

NanoIndEx (http://www.nanoindex.eu/): Assessment of Individual Exposure to manufactured nanomaterials by means of personal monitors and samplers

Nanoheter (http://nanoheter.cerege.fr/): Fate of engineered nanoparticles in the water column under natural conditions. Role of the heteroaggregation with naturally occurring suspended matter

NanOxiMet (http://www.nanoximet.eu/): Assessment of the use of particle reactivity metrics as an indicator for pathogenic properties and predictor of potential toxicological hazard.

2013 Second SIINN Call

The Second Call for proposals in SIINN was published in June 2013, with the main topics to be addressed:

- Over-arching aspects of nanosafety research: methods for the understanding and prediction of the importance of intrinsic and extrinsic properties of MNMs for their interaction with surrounding matrices
- Exposure assessment
- Toxicity mechanisms
- Environmental impacts of MNMs
- Effects of MNMs on human health.

The following five projects were selected for funding:

FENOMENO (no web-link available yet): Fate and effect of wastewater-borne manufactured nanomaterials in aquatic ecosystems

NanoToxClass (no web-link available yet): Establishing nanomaterial grouping / classification strategies according to toxicity and biological effects for supporting risk assessment

NanoSafeLeather (no web-link available yet): The effect on human health of Ag/TiO_2 nanomaterial-treated leathers for footwear industry

NANOGECO (no web-link available yet): Nanoparticle generation by atomization processes in spray coating

PLATOX (no web-link available yet): In vitro and in vivo investigations to generate validated toxicity data of graphene nanoplatelets vs a carbon black reference.

2014 Third SIINN Call

The Third SIINN Call was launched in October 2014, with topics in the field of:

- Exposure assessment
- Toxicity mechanisms
- Effects of MNMs on human health
- Environmental impacts of MNMs

Within this call, international funding initiatives enabling researchers to pool know-how, data and resources are called for, and collaborative efforts with U.S. research partners will contribute to strengthening the international dimension of the European Research Area. Three US funding organisations are enabling this type of collaboration by joining the call:

- National Science Foundation (NSF)
- Consumer Product Safety Commission (CPSC)
- National Institute of Environmental Health Sciences (NIEHS).

8.6 Relation to activities within other European projects

NANoREG – A common European approach to the regulatory testing of Manufactured Nanomaterials (http://nanoreg.eu/):

Based on questions and requirements supplied by regulators and legislators, NANoREG will:

- provide answers and solutions from existing data, complemented with new knowledge,
- provide a tool box of relevant instruments for risk assessment, characterisation, toxicity testing and exposure measurements of MNMs,
- develop, for the long term, new testing strategies adapted to innovation requirements,
- 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.

Within the NANoREG project, an overall framework is being developed applicable for most types of legislations. The framework will deliver a concept on how to address safety of nanomaterials, including, as appropriate, legislation and sector specific issues (although the primary focus will be on REACH, other regulations covering Cosmetics, Biocidal Products, Food and Feed, as well as Food and Feed Contact Materials, etc. will be looked at). The framework development will be based on the results collected from the NANoREG project and the input from the regulators and stakeholders. It will be iteratively developed by a dedicated working group. Specific answers and tools resulting from NANoREG will be mapped against this framework.

Via personal interactions between SIINN and NANoREG, knowledge created within the SIINN roadmapping activities, and the Consolidated Framework in general, will be transferred to the NANoREG project's framework activities.

ProSafe (no project website online yet, project has only started in February 2015):

ProSafe, a Coordination and Support Action of EU's Horizon 2020 programme coordinates a number of EU funded initiatives such as NANoREG, NANoREG II, and SIINN. The project goal requires the active involvement of policy makers, regulation authorities, and innovation supporting agencies (all ministerial organisations) who can be seen as representatives of national governments, and who are key players in nano safety research, nano regulation and risk reduction from all Member States and Associate States as well as interested Third Countries and opinion leaders from industry.

As a dedicated instrument for the pooling and networking of the respective Stakeholders, a Strategic Policy Development Group SPDG is being installed within ProSafe. The SPDG stakeholders play a central and important role in their countries for the development and implementation of a novel safety culture by facilitating the integration of Safe-by-Design and Safe Innovation in industry's innovation processes, in research and regulation as well as in safety and regulation policy.

Within ProSafe, the definition and implementation of common calls on nano safety research, innovation on nano materials and nano inspired products is foreseen, building on knowledge gathered in the frame of the SIINN calls.

8.7 References for Chapter 8

Galvin, R., Science Roadmaps, Science, 280 (1998) 803.

Galvin R, Roadmapping – a practitioner's update, Technol. Forecast. Soc. Change 71 (2004) 101-103.

Compendium of Projects in the European NanoSafety Cluster, 2012 Edition, edited by M. Riediker and G. Katalagarianakis.

Compendium of Projects in the European NanoSafety Cluster, 2013 Edition, edited by Michael Riediker.

Nanosafety in Europe 2015-2025: Towards Safe and Sustainable Nanomaterial and Nanotechnology Innovations, Strategic Research Agenda, European NanoSafety Cluster, 2013.

Safe implementation of nanotechnologies: Common challenges, Minatec, Grenoble, 29-31 May 2012.

9 ANNEX

9.1 Standards and standards under development by the referenced committees

Committee	Reference	Title	Current state	ICS/DAV codes
ASTM International	ASTM E2490-09.	Standard Guide for Measurement of Particle Size Distribution of Nanomaterials in Suspension by Photon Correlation Spectroscopy (PCS) http://www.astm.org/Standards/E2490.htm	April 2009 published	ICS: 71.100.01 (Products of the chemical industry in general)
ASTM International	ASTM E2456 - 06	Standard Terminology Relating to Nanotechnology/ http://www.astm.org/Standards/E2456.htm	DOI: 10.1520/E2456-06	ICS: 01.040.71 (Chemical technology (Vocabularies)); 71.100.01 (Products of the chemical industry in general)
ASTM International	ASTM E2524 – 08	Standard test method for Analysis of Hemolytic Properties of Nanoparticles http://www.astm.org/Standards/E2524.htm	DOI: 10.1520/E2524-08	ICS: 11.040.20 (Transfusion, Infusion); 71.100.01 (Products of the chemical industry in general)
ASTM International	ASTM E2525 - 08	Standard Test Method for Evaluation of the Effect of Nanoparticulate Materials on the formation of Mouse Granulocyte-Macrophage Colonies http://www.astm.org/Standards/E2525.htm	DOI: 10.1520/E2525-08	ICS: 07.100.10 (Medical microbiology)
ASTM International	ASTM E2526 -08	Standard Test Method for Evaluation of Cytotoxicity of Nanoparticulate Materials in Porcine Kidney Cells and Human Hepatocarcinoma Cells http://www.astm.org/Standards/E2526.htm	DOI: 10.1520/E2526-08	ICS Number Code 07.100.10 (Medical microbiology)
ASTM International	ASTM E2578 -07	Standard Practice for Calculation of Mean Sizes/Diameters and Standard Deviations of Particle Size Distributions http://www.astm.org/Standards/E2578.htm	DOI: 10.1520/E2589-11	ICS Number Code 19.120 (Particle size analysis. Sieving)

Committee	Reference	Title	Current state	ICS/DAV codes
ASTM International	ASTM E2535 -07	Standard Guide for Handling Unbournd Engineered Nanoscale Particles in Occupational Settings http://www.astm.org/Standards/E2535.htm	DOI: 10.1520/E2535-07	ICS: 71.100.01 (Products of the chemical industry in general)
ASTM International	ASTM E729 – 96	Standard guide for conducting acute toxicity tests on test materials with fishes, macroinver-tebrates, and amphibians. http://www.astm.org/Standards/E729.htm	2007 published	ICS Number Code 07.080 (Biology, Botany, Zoology)
ASTM International	ASTM F1671 - 07	Standard Test Method for Resistance of Materials Used in Protective Clothing to Penetration by Blood-Borne Pathogens Using Phi-X174 Bacteriophage Penetration as a Test System	2007 published	ICS Number Code 11.140 (Hospital equipment); 13.340.10 (Protective clothing)
ASTM International	E 2019-99	Standard Test Method for Minimum Ignition Energy of a Dust Cloud in Air	2007 published	ICS Number Code 13.230 (Explosion protection)
Australian Standard (AS)	AS 3544—1988	Industrial vacuum cleaners for particulates hazardous to health	1988 Published by Standards Australia	ISBN 0 7262 5148 8
Australian Standard (AS)	AS 4260—1997	High efficiency particulate air (HEPA) filters —Classification, construction and performance	1997 Published by Standards Australia	ISBN 0 7337 1060 3
BSI - British Standards Institution	BS EN 13925-1:2003	Non-destructive testing. X-ray diffraction from polycrystalline and amorphous materials. General principles http://shop.bsigroup.com/ProductDe-tail/?pid=000000000030027544	March 2003 Published	ICS Number Code 19.100 (Non-destructive testing)
BSI - British Standards Institution	BS EN 13925-3:2005	Non-destructive testing. X-ray diffraction from polycrystalline and amorphous materials. – Part 3: Instruments http://shop.bsigroup.com/ProductDe-tail/?pid=000000000030071999	July 2005 Published	19.100 (Non-destructive testing)
European Committee for Standardiza-tion (CEN)	CEN/TC 137	Assessment of workplace exposure to chemical and biological agents		
European Committee for Standardiza-tion (CEN)	CEN Nanotech-nologies	http://www.cen.eu/work/areas/Nanotech/Pages/default.aspx		

Committee	Reference	Title	Current state	ICS/DAV codes
European Committee for Standardiza-tion (CEN)	CEN/TC 352	'Nanotechnologies' work programme http://standards.cen.eu/dyn/www/f?p=204:22:0::::FSP_ORG_ID,FSP_LANG_ID:508478,25&cs=18E152154F73BA190A16C4D279047F5FD		
European Committee for Standardiza-tion (CEN)	CEN/TC 352	Published standards http://standards.cen.eu/dyn/www/f?p=204:32:0::::FSP_ORG_ID,FSP_LANG_ID:508478,25&cs=18E152154F73BA190A16C4D279047F5FD		
European Committee for Standardiza-tion (CEN)	CEN/TC 352 2013	Standards under development: Guidance on voluntary labelling for consumer products containing manufactured nano-objects (ISO/TS 13830:2013) http://standards.cen.eu/dyn/www/f?p=204:110:0::::FSP_PROJECT:31997&cs=119A0A09FD39AC1C4354FB93F5D0C3E19	2013 Published	2013-12-18
European Committee for Standardiza-tion (CEN)	EN 943-1:2002	Protective clothing against liquid and gaseous chemicals, including liquid aerosols and solid particles. Performance requirements for ventilated and non-ventilated gas-tight (Type 1) and non-gas-tight (Type 2) chemical protective suits	2002 *Published*	
European Committee for Standardiza-tion (CEN)	*CEN/TC 137* EN ISO 28439	Workplace atmospheres. Characterization of ultrafine aerosols/nanoaerosols. Determining the size distribution and number concentration using mobility particle sizers/differential mobility analysers.	April 2011 *Published*	13.040.30 (Workplace atmosphere)
International Standards Organisation (ISO)	ISO/TR 12885:2008.	ISO/TR 12885:2008, Nanotech-nologies -- Health and safety practices in occupational settings relevant to nanotechnologies http://www.iso.org/iso/catalogue_detail?csnumber=52093	TC 229 Published	ICS: 13.100; 07.030 Stage: 60.60 (2008-09-30)
International Standards Organisation (ISO)	ISO/TS 80004-1	ISO/TS 80004-1:2010, Nano-technologies -- Vocabulary -- Part 1: Core terms http://www.iso.org/iso/catalogue_detail.htm?csnumber=51240	TC 229 Published	ICS: 01.040.07; 07.030 Stage: 60.60 (2010-10-06)
International Standards Organisation (ISO)	ISO/13321:1996	ISO 13321:1996, Particle size analysis -- Photon correlation spectroscopy http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=21707	TC 24/SC 4 Published	ICS: 19.120 Stage: 90.93 (2007-05-02)

Committee	Reference	Title	Current state	ICS/DAV codes
International Standards Organisation (ISO)	ISO/13318-1:2001	ISO 13318-1:2001, Determination of particle size distribution by centrifugal liquid sedimentation methods -- Part 1: General principles and guidelines http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=21704	TC 24/SC 4 Published	ICS: 19.120 Stage: 90.92 (2006-05-30)
International Standards Organisation (ISO)	ISO/TS 13762:2001	ISO/TS 13762:2001, Particle size analysis -- Small angle X-ray scattering method http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=22376	TC 24/SC 4 Withdrawn	ICS: 19.120 Stage: 95.99 (2011-11-04)
International Standards Organisation (ISO)	ISO/13322-1:2014.	ISO 13322-1:2014, Particle size analysis -- Image analysis methods -- Part 1: Static image analysis methods http://www.iso.org/iso/home/store/catalogue_ics/catalogue_detail_ics.htm?csnumber=51257	TC 24/SC 4 Published	ICS: 19.120 Stage: 60.60 (2014-05-12)
International Standards Organisation (ISO)	ISO/TC 146/SC-2	ISO/TC 146/SC-2 “Air Quality, Workplace Atmospheres”, working in the field of assessment of workplace exposure to different agents. http://www.iso.org/iso/iso_technical_committee.html?commid=52736	Published	
International Standards Organisation (ISO)	ISO Concept Database	The address (URL) of the ISO/Online Browsing Platform (OBP) is: http://cdb.iso.org	Launched on 2009	
International Standards Organisation (ISO)	TC 229 Nano-technologies	http://www.iso.org/iso/standards_development/technical_committees/list_of_iso_technical_committees/iso_technical_committee.htm?commid=381983		
International Standards Organisation (ISO)	BUSINESS PLAN ISO/TC 229 Nanotechnologies 2011	Glossary of terms and abbreviations used in ISO/TC Business Plans http://isotc.iso.org/livelink/livelink/fetch/2000/2122/687806/ISO_TC_229__Nanotechnologies_.pdf?nodeid=6507632&vernum=-2	2011	
International Standards Organisation (ISO)	ISO/TC 229	Standards catalogue http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_tc_browse.htm?commid=381983&development=on		
International Standards Organisation (ISO)	ISO/TC 229	Working area on ISOTC http://isotc.iso.org/livelink/livelink/open/tc229		

Committee	Reference	Title	Current state	ICS/DAV codes
International Standards Organisation (ISO)	ISO/TC 229	Objectives TC 229 (WG1 Roadmap, Outline Strategy for ISO TC 229 WG2, WG3 Roadmap, WG4 Roadmap) http://isotc.iso.org/livelink/livelink/fetch/6261792/641932/JWG2_N092b_Strategy_Paper_Rev_8.0.pdf?nodeid=7279299&vernum=-2	April 2008	
International Standards Organisation (ISO)	ISO 9276-1	Representation of results of particle size analysis -- Part 1: Graphical representation	TC 24/SC 4 1998	ICS: 19.120 Stage: 90.93 (2009-10-22)
International Standards Organisation (ISO)	ISO 9276-2	Representation of results of particle size analysis -- Part 2: Calculation of average particle sizes/diameters and moments from particle size distributions	TC 24/SC 4	ICS: 19.120 Stage: 90.92 (2010-10-22)
International Standards Organisation (ISO)	ISO 9276-3	Representation of results of particle size analysis -- Part 3: Adjustment of an experimental curve to a reference model	TC 24/SC 4 2008	ICS: 19.120 Stage: 90.93 (2011-11-24)
International Standards Organisation (ISO)	ISO 9276-4	Representation of results of particle size analysis -- Part 4: Characterization of a classification process	TC 24/SC 4	ICS: 19.120 Stage: 90.20 (2012-04-15)
International Standards Organisation (ISO)	ISO 9276-5	Representation of results of particle size analysis -- Part 5: Methods of calculation relating to particle size analyses using logarithmic normal probability distribution	TC 24/SC 4	ICS: 19.120 Stage: 90.93 (2009-10-22)
International Standards Organisation (ISO)	ISO 9276-6	Representation of results of particle size analysis -- Part 6: Descriptive and quantitative representation of particle shape and morphology	TC 24/SC 4	ICS: 19.120 Stage: 90.93 (2011-12-28)
International Standards Organisation (ISO)	ISO 9277	Determination of the specific surface area of solids by gas adsorption using the BET method	TC 24/SC 4 1995	ICS: 19.120 Stage: 95.99 (2010-08-20)
International Standards Organisation (ISO)	ISO 13099-1:2012	Colloidal systems -- Methods for zeta-potential determination -- Part 1: Electroacoustic and electrokinetic phenomena	TC 24/SC 4	ICS: 19.120 Stage: 60.60 (2012-06-18)
International Standards Organisation (ISO)	ISO 13099-2:2012	Colloidal systems -- Methods for zeta-potential determination -- Part 2: Optical methods	TC 24/SC 4	ICS: 19.120 Stage: 60.60 (2012-06-18)
International Standards Organisation (ISO)	ISO/NP 13099-3	Methods for zeta potential determination -- Part 3: Acoustic methods	TC 24/SC 4	ICS: 19.120 Stage: 10.99 (2010-10-04)
International Standards Organisation (ISO)	ISO/DIS 26824	Particle characterization of particulate systems -- Vocabulary	TC 24/SC 4	ICS: 01.040.19; 19.120 Stage: 40.60 (2012-07-08)

Committee	Reference	Title	Current state	ICS/DAV codes
International Standards Organisation (ISO)	ISO/NP 27891	Aerosol particle number concentration -- Calibration of condensation particle number counters	TC 24/SC 4	ICS: 19.120 Stage: 10.99 (2010-11-08)
International Standards Organisation (ISO)	ISO/NP 12187	Particle size analysis – Dispersed stability characterization in liquids	Under development	Current Stage: 00.20
EC: M/461 EN	Mandate addressed to CEN, CENELEC and ETSI for standardization activities regarding nanotechnologies and nanomaterials	ftp://ftp.cenorm.be/CENELEC/EuropeanMandates/M_461.pdf	2010	
International Standards Organisation (ISO)	BS EN ISO 18757:2005	BS EN ISO 18757:2005, Fine ceramics (advanced ceramics, advanced technical ceramics). Determination of specific surface area of ceramic powders by gas adsorption using the BET method http://shop.bsigroup.com/ProductDetail/?pid=000000000030117333	International Equivalent: EN ISO 18757:2005, ISO 18757:2003 Dec 2006 Published	BSi code: BS EN ISO 18757:2005 Product code: 30117333
International Standards Organisation (ISO)	ISO/20998-1:2006	ISO 20998-1:2006, Measurement and characterization of particles by acoustic methods -- Part 1: Concepts and procedures in ultrasonic attenuation spectroscopy http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=39869	TC 24/SC 4 Published	ICS: 19.120 Stage: 90.93 (2011-02-13)
International Standards Organisation (ISO)	ISO/21501-2:2007	ISO 21501-2:2007, Determination of particle size distribution -- Single particle light interaction methods -- Part 2: Light scattering liquid-borne particle counter http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=40275	TC 24/SC 4 Published	ICS: 19.120 Stage: 90.92 (2010-10-25)
International Standards Organisation (ISO)	ISO/TR 27628:2007	ISO/TR 27628:2007, Workplace atmospheres -- Ultrafine, nanoparticle and nano-structured aerosols -- Inhalation exposure characterization and assessment http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=44243	TC 146/SC 2 Published	ICS: 13.040.30 Stage: 90.93 (2010-07-14)
International Standards Organisation (ISO)	ISO/22412:2008	ISO 22412:2008, Particle size analysis -- Dynamic light scattering (DLS) http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=40942	TC 24/SC 4 Published	ICS: 19.120 Stage: 90.93 (2011-11-24)

Committee	Reference	Title	Current state	ICS/DAV codes
International Standards Organisation (ISO)	ISO/13320:2009	ISO 13320:2009, Particle size analysis -- Laser diffraction methods http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=44929	TC 24/SC 4 Published	ICS: 19.120 Stage: 60.60 (2009-09-18)
International Standards Organisation (ISO)	ISO/15900:2009.	ISO 15900:2009, Determination of particle size distribution -- Differential electrical mobility analysis for aerosol particles http://www.iso.org/iso/catalogue_detail.htm?csnumber=39573	TC 24/SC 4 Published	ICS: 19.120 Stage: 60.60 (2009-05-12)
International Standards Organisation (ISO)	ISO/21501-1:2009	ISO 21501-1:2009, Determination of particle size distribution -- Single particle light interaction methods -- Part 1: Light scattering aerosol spectrometer http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=42728	TC 24/SC 4 Published	ICS: 19.120 Stage: 60.60 (2009-05-22)
International Standards Organisation (ISO)	ISO 9277:2010.	ISO 9277:2010, Determination of the specific surface area of solids by gas adsorption -- BET method http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=44941	TC 24/SC 4 Published	ICS: 19.120 Stage: 60.60 (2010-08-20)
International Standards Organisation (ISO)	ISO 28439:2011	ISO 28439:2011, Workplace atmospheres -- Characterization of ultrafine aerosols/nanoaerosols -- Determination of the size distribution and number concentration using differential electrical mobility analysing systems http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=44697	TC 146/SC 2 Published	ICS: 13.040.30 Stage: 60.60 (2011-03-18)
International Standards Organisation (ISO)	ISO/ TS 10797:2012	ISO/ TS 10797:2012, Nanotechnologies – Characterization of single-wall carbon nanotubes using transmission electron microscopy http://www.iso.org/iso/catalogue_detail?csnumber=46127	TC 229 Published	ICS: 07.030 Stage: 60.60 (2012-05-29)
International Standards Organisation (ISO)	ISO/AWI TS 10798.	ISO/TS 10798:2011, Nanotechnologies – Charaterization of single-wall carbon nanotubes using scanning electron microscopy and energy dispersive X-ray spectrometry analysis http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=46128	TC 229 Published	ICS: 07.030 Stage: 60.60 (2011-07-14)
International Standards Organisation (ISO)	ISO/TR 10929:2012	ISO/ TR 10929:2012, Nanotechnologies -- Characterization of multiwall carbon nanotube samples http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=46424	TC 229 Published	ICS: 07.030 Stage: 60.60 (2012-01-20)

Committee	Reference	Title	Current state	ICS/DAV codes
International Standards Organisation (ISO)	ISO/TS 12025:2012	ISO/TS 12025:2012, Nano-materials -- Quantification of nano-object release from powders by generation of aerosols http://www.iso.org/iso/home/store/cata-logue_tc/catalogue_detail.htm?csnumber=62368	TC 229 Published	ICS: 07.030 Stage: 60.60 (2012-10-29)
International Standards Organisation (ISO)	ISO 13099-1:2012	ISO 13099-1:2012, Colloidal systems -- Methods for zeta-potential determination -- Part 1: Electroacoustic and electrokinetic phenomena http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=52807	TC 24/SC 4 Published	ICS: 19.120 Stage: 60.60 (2012-06-18)
International Standards Organisation (ISO)	ISO 13099-2:2012	ISO 13099-2:2012, Colloidal systems -- Methods for zeta-potential determination -- Part 2: Optical methods http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=52832	TC 24/SC 4 Published	ICS: 19.120 Stage: 60.60 (2012-06-18)
International Standards Organisation (ISO)	ISO/TS 10868:2011	ISO/TS 10868:2011, Nanotech-nologies -- Characterization of single-wall carbon nanotubes using ultraviolet-visible-near infrared (UV-Vis-NIR) absorption spectroscopy http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=46247	ISO/TC229 Published	ICS: 07.030 Stage: 90.20 (2014-07-15)
International Standards Organisation (ISO)	ISO/ TR 13014:2012.	ISO/TR 13014:2012, Nanotech-nologies - Guidance on physico-chemical characterization of engineered nanoscale materials for toxicologic assessment http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=52334	ISO/TC 229 Published	ICS: 07.030 Stage: 60.60 (2012-05-08)
International Standards Organisation (ISO)	ISO/TS 11888:2011	ISO/TS 11888:2011, Nanotech-nologies -- Characterization of multiwall carbon nanotubes -- Mesoscopic shape factors http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=50969	ISO/TC 229 Published	ICS: 07.030 Stage: 90.20 (2014-10-15)
International Standards Organisation (ISO)	ISO 6341:1996	ISO 6341:1996, Water quality-Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea). In Acute Toxicity Test, 3rd ed., Geneva, Switzerland. http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=21923	TC 147/SC 5 Withdrawn	ICS: 13.060.70 Stage 95.99 (2012-10-15)

Committee	Reference	Title	Current state	ICS/DAV codes
International Standards Organisation (ISO)	ISO/TS 27687:2008	ISO/TS 27687:2008, Nanotechnologies -- Terminology and definitions for nano-objects -- Nanoparticle, nanofibre and nanoplate http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=44278	TC 229 *Published*	ICS: 01.040.07; 07.030 Stage: 90.92 (2012-01-26)
International Standards Organisation (ISO)	ISO 13982-1: 2004	ISO 13982-1:2004, Protective clothing for use against solid particulates -- Part 1: Performance requirements for chemical protective clothing providing protection to the full body against airborne solid particulates (type 5 clothing) http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=40198	TC 94/SC 13 *Published*	ICS:13.340.10 Stage: 90.20 (2014-07-15)
International Standards Organisation (ISO)	ISO 16604:2004	ISO 16604:2004, Clothing for protection against contact with blood and body fluids -- Determination of resistance of protective clothing materials to penetration by blood-borne pathogens -- Test method using Phi-X 174 bacteriophage http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=32248	TC 94/SC 13 *Published*	ICS:13.340.10 Stage:90.93 (2014-07-31)
National Institute of Standards and Technology NIST	Special Publication 960-3, Vincent A. Hackley Materials Science and Engineering Laboratory Chiara F. Ferraris Building & Fire Research Laboratory	The Use of Nomenclature in Dispersion Science and Technology http://fire.nist.gov/bfrlpubs/build01/art108.html	August 2001 Published	
OSHA Washington, DC: Occupational Safety and Health Administration Occupational Safety and Health Standards	29 CFR 1910.1200.	Hazard communication, toxic and hazardous substances.	Issued in 2012	

Table 28: Standards and standards under development by the referenced committees

9.2 Inventory on national activities and documents with a national focus on MNM and their EHS implication

- AEN/GET 15 "Nanotecnologías", AENOR, Javier García Díaz, ongoing.
- ANR, (Agence Nationale de la Recherche), Biology – Health department, Annual-based program, http://www.agence-nationale-recherche.fr/
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Some of the FP7 projects related to nanosafety:

- NanoImpactNet (http://nanoimpactnet.eu) - The European network on the health and environmental impact of nanomaterials.
- NanoTEST - Development of methodology for alternative testing strategies for the assessment of the toxicological profile of nanoparticles used in medical diagnosis.
- 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.
- Nanoimmune - Comprehensive assessment of hazardous effects of engineered nanomaterials on immune system.
- NanoReTox (proposal under negotiation) - The reactivity and toxicity of engineered nanoparticles, risks to the environment and human health.
- Neuronano (proposal under negotiation) - The brain will be explored as NP target organ.
- NanoSustain, http://www.nanosustain.eu
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- Multi-level protection of materials for vehicles by “Smart” nanocontainers (SEVENTH FRAMEWORK PROGRAMME THEME [NMP-2007-4.0-3], Multifunctional materials for future vehicles, Contract N° NMP3-LA-2008-214261, N.C.S.R. 'Demokritos', G. Kordas, The project MUST aims at providing new technologies based on active multi-level protective systems for future vehicle materials.
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9.3 List of captions

9.3.1 Figures

9.3.2 Tables