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

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Glossary

Updated version

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The Glossary 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). 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.

Content

1. Selected working definitions relevant for "nanomaterials" by different Organisations / Countries

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 matertial 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***
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 nanocrystals.	***CSI 2007 Glossary***
An insoluble or biopersistant and intentionally manufactured material with one or more external dimensions, or an internal structure, in the nanoscale.	***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 intentionally produced material in the nanoscale.	***ACC-The American Chemistry Council***
Industrial materials intentionally produced, manufactured 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 Precautionary Matrix the Swiss Authorities recommend including particulate materials up to 500nm 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***

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***

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,***

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***

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***

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***

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***

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***

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***

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 agglomerates and aggregates 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

COMMISSION RECOMMENDATION of 18 October 2011 on the definition of nanomaterial

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

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

2. Manufactured nanomaterials (MNMs)

2.1 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.

2.2 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 buckminsterfullerine.* *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)**	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.
	Aluminium oxide(Al_2O_3)	
	Cerium dioxide (CeO_2)	
	Zinc oxide (ZnO)	
	Silicon dioxide (SiO_2)	
Metals	**Silver nanoparticles(Ag)**	Nanoparticulate zerovalent metals
	Iron nanoparticles (Fe)	
	Gold nanoparticles (Au)	
	Bimetallic nanoparticles Fe-Pd, Fe-Ni, Fe-Ag	
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*

3. 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

3.1 Aggregation/agglomeration

The terms agglomeration and aggregation are often used interchangeably to describe the attractions that hold together a collection of particles. However has been suggested that it is more appropriate to consider nanoparticle aggregation and agglomeration as distinct phenomena (*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 separated (*Oberdoerster, G. et al., 2007, "Toxicology of nanoparticles: A historical perspective", Nanotoxicol, vol. 1, no.1, pp. 2-25*). Aggregation and agglomeration can occur due to a number of deliberate and accidental mechanisms (*Schneider and Jensen. 2008, Ann. Occ. Hyg. 52*). 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/**	

3.2 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). And Nanomaterials? Are they implicitely included?

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 /** ≈ 0.5nm	
Nuclear magnetic resonance spectroscopy and Pulsed field gradient / **NMR**	
X-ray photoelectron spectroscopy /**XPS/** ≈1 mm	
Auger Electron Spectroscopy /**AES /** ≈1–2nm	
Differential scanning calorimetry /**DSC**	
Thermogravimetric analysis **/TGA**	
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)	

3.3. 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

Electron microscopy/ **SEM/** 1 nm to 1μm	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
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**	

3.4. Crystallite or grain size

Crystallites are small, often microscopic crystals that, held together through highly defective boundaries, constitute a polycrystalline solid. Metallurgists often refer to crystallites 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)**.

3. 5 Octanol-Water Partition Coefficient (K_{OW}) (when relevant)

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 to 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.*

3. 6 Photocatalytic activity and radical formation potential

Photocatalytically active materials are semiconductors in which electron-hole pairs are formed upon exposure to light that generate highly reactive free radicals on the material surface. Titanium dioxide is a semiconductor of this kind. The photocatalytic decomposition of water on the surface of a TiO2 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.

The study of UVA or visible light spectra are specific methods to investigate the photocatalytic activity.

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).

3. 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 (*M. Lundqvist et al., Proc. Natl. Acad. Sci. USA 2008, 105, 14265).*
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 (*G. Oberdörster, Concepts of Nanotoxicology, NANOAGRI Conference 2010, Cairo, Egypt 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, R. Chen, P. C. Ke, J. M. Brown and A. M. Rao, Effects of surface functional groups on the formation of nanoparticle-protein corona, Appl. Phys. Lett. 101, 263701 (2012); http://dx.doi.org/10.1063/1.4772509).* 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. *Shruti R Saptarshi, Albert Duschl, Andreas L Lopata, Interaction of nanoparticles with proteins: relation to bio-reactivity of the nanoparticle, Journal of Nanobiotechnology 2013, 11:26 http://www.jnanobiotechnology.com/content/11/1/26;*
*S. Tenzer et al., Rapid formation of plasma protein corona critically affects nanoparticle pathophysiology, Nature Nanotechnology **8**,772–781(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.

3. 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 et al., 2009).* Standard electrochemical methods, such as **cyclic voltammetry**, may be used to study the redox activity of nanomaterials.

Referring to the redox potential of NM, 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.

3.9 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 can be used as a paradigm to assess nanomaterial toxicity, not all nanomaterials exhibit the electronic configurations or surface properties that allow spontaneous or a cellular 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, Hotze, E.M. et al. (2008), Mechanisms of photochemistry and reactive oxygen production by fullerene suspensions in water. Environ. Sci. Technol. 42, 4175*).

Analytical methods suitable for ROS generation measurements

Name/ Acronym
Electron spin resonance / ESR
XTT (XTT is a tetrazolium derivative) assay
Electron paramagnetic resonance /EPR
Spectrofluorimetry
Singlet Oxygen Sensor Green /SOSG
Dithiothreitol (DTT) assay/DTT
Furfuryl alcohol assay / FFA assay
Nanosensors

3. 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 NANO-MATERIALS: 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□m; Sed FFF: 50nm-1μm	
FFF-ICP-MS **FFF-Confocal Microscopy** **FIFFF-SLS** **SedFFF-DLS**	

3. 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
Differential mobility Analyzer/ **DMA/**3 nm to □m particles	

Field flow fractionation / **FFF/** Flow FFF 1 nm - 1□μm; Sed FFF 50 nm -1□μm	ISO/13320:2009 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□m	
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 - □m	
Cryo transmission electron Microscopy/ **Cryo-TEM**	
Fluorescence correlation spectroscopy (Confocal microscopy)/ **FCS /**~200nm	

3. 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 nanomaterials 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.	
Nanoparticle Surface Area Monitor **NSAM**	The effect of initial aerosol charge, the composition of the material, presence of aggregates and the effect of particle	

Similar method with the **Electrical Aerosol Detector**	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.	

3. 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.

Name/ Acronym / Spatial resolution or LOD	**Referenced documents**
Analytical electron microscopy / **AEM** / **Spatial resolution** ≈ 0.5nm	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 ≈1 μm / Atomic composition of layers from 1–10nm	
Auger Electron Spectroscopy / **AES** / ≈1–2nm	
Secondary ion mass spectrometry / **SIMS /** Atomic composition of layers from 1–3nm	

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

3. 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

[Introduction to modern colloid science Robert J. Hunter, 1993, p.22]
British Standards Institution, PAS (Publicly Available Specification) 71: Vocabulary — Nanoparticles

Analytical methods suitable for surface charge/zeta potential measurements

Name/ Acronym	Referenced documents
Capillary electrophoresis/**CE**	ISO/CD 13099-1 ISO/CD 13099-2 ISO/AWI TR 13014
Zeta potential Indicates the degree of repulsion between adjacent, similarly charged particles in a dispersion	
Zeta potential measurement, combined with Dynamic Light Scattering (**DLS**)	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

3.15. 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, W.J., Reehl, W.F., Rosenblatt, D.H., 1990, Handbook of chemical property estimation mathods--Environmental behavior of organic compounds: Washington, DC, American Chemical Society, 960 p.; - U.S. Environmental Protection Agency, 2009*

- 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 predetermined 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.

4. Exposure to Manufactured Nanomaterials

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

Benchmark Material

Inhalation is the preferred method of exposure of the respiratory tract for hazard identification and to obtain dose response data. 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.

Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy, Günter Oberdörster, Andrew Maynard, Ken Donaldson, Vincent Castranova, Julie Fitzpatrick, Kevin Ausman, Janet Carter, Barbara Karn, Wolfgang Kreyling, David Lai, Stephen Olin, Nancy Monteiro-Riviere, David Warheit, Hong Yang and A report from the ILSI Research Foundation/Risk Science Institute Nanomaterial Toxicity Screening Working Group, Published: 06 October 2005, Particle and Fibre Toxicology 2005, 2:8 doi:10.1186/1743-8977-2-8

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

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

Dose (for chemicals that are not radioactive)

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) 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 eyes, skin, stomach, intestines, or lungs.
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 ab-

sorption (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

Dose - response relationship

The relationship between the amount of exposure [dose] to 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

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

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

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.
Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy, Günter Oberdörster, Andrew Maynard, Ken Donaldson, Vincent Castranova, Julie Fitzpatrick, Kevin Ausman, Janet Carter, Barbara Karn, Wolfgang Kreyling, David Lai, Stephen Olin, Nancy Monteiro-Riviere, David Warheit, Hong Yang and A report from the ILSI Research Foundation/Risk Science Institute Nanomaterial

Toxicity Screening Working Group, Published: 06 October 2005, Particle and Fibre Toxicology 2005, 2:8 doi:10.1186/1743-8977-2-8

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

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.
Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy, Günter Oberdörster, Andrew Maynard, Ken Donaldson, Vincent Castranova, Julie Fitzpatrick, Kevin Ausman, Janet Carter, Barbara Karn, Wolfgang Kreyling, David Lai, Stephen Olin, Nancy Monteiro-Riviere, David Warheit, Hong Yang and A report from the ILSI Research Foundation/Risk Science Institute Nanomaterial Toxicity Screening Working Group, Published: 06 October 2005, Particle and Fibre Toxicology 2005, 2:8 doi:10.1186/1743-8977-2-8

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

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).*

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 expo-

sure (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

Exposure registry

A system of ongoing followup of people who have had documented environmental exposures.
Agency for Toxic Substances and Disease Registry (ATSDR)
http://www.atsdr.cdc.gov/glossary.html

Exposure routes (including occupational, environmental and consumer exposure scenarios)

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 because 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

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 NPs in food, food packaging and oral medicines. The intestinal epithelial layer represents the initial gate that ingested NPs must pass to reach the body. The NPs 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 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)
http://www.nature.com/nnano/journal/v7/n4/pdf/nnano.2012.3.pdf

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 human lung. This is often referred to as size-selective health hazard sampling. The criterion for size-selective sampling depends on the aerosol being sampled. For example, 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. (Oberdorster, G., et al., 2001) After 1, 3, 5,and 7 days exposure to carbon nanaparticles labeled with 13C (CMD = 36 nm; GSD = 1.66) traslocate to cerebrum, cerebellum, olfactory bulbs.

(G. Oberdörster et al. 2004)

Behaviour and translocations of NPs: 100-5nm and 5nm> nanoparticles 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. (ICRP Model, 1994; Nose Breathing). 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 circulation. Nanoparticles phagocytized by interstitial macrophages.It causes delay of clearance.

NSAM-001appnote[TSI 2010].pdf

International Commission of Radiological Protection (1994)

U.S. Environmental Protection Agency (1996a).

Air Quality Criteria for Particulate Matter (Final Report, Oct 2004)
http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=87903#Download

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) www.dguv.de/ifa/en/pub/rep/rep04/biar0703/

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

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

Respiratory Tract

The respiratory tract is an organ system 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

5. Environmental fate and behaviour

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.

Bioaccumulation of nanomaterials

The potential for nanomaterials to bioaccumulate in living organisms and to enhance the bio-accumulation 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.

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

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

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

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

Half-life ($t_{½}$)

The time it takes for half the original amount of a substance to disappear. In the environment, the half-life 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-life 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 ini-

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

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

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

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

Nanopollution

Generic name for all waste generated by nanodevices or during the nanomaterials manufacturing process.

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	Processses 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.

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/environment/chemicals/reach/reach_intro.htm).

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 (*SCENIHR, 2010*).

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.

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***

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***

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.

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.

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

Risk Management Policy

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

ISO 31000 Definitions (ISO/IEC Guide 73)

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)

6. 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.

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

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

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 hasproduced 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

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

7. 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

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

Note: Was 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 com-

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

Other terms used in relation to MNMs health effects:

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

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.

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.

Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy, Günter Oberdörster, Andrew Maynard, Ken Donaldson, Vincent Castranova, Julie Fitzpatrick, Kevin Ausman, Janet Carter, Barbara Karn, Wolfgang Kreyling, David Lai, Stephen Olin, Nancy Monteiro-Riviere, David Warheit, Hong Yang and A report from the ILSI Research Foundation/Risk Science Institute Nanomaterial Toxicity Screening Working Group, Published: 06 October 2005, Particle and Fibre Toxicology 2005, 2:8 doi:10.1186/1743-8977-2-8

Carcinogens

Chemicals that are associated with lung cancer.

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

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))

Exposure of Engineered Nanoparticles to Human Lung Epithelial Cells: Influence of Chemical Composition and Catalytic Activity on Oxidative Stress, L. L i m b a c, H, P. Wick, P. Manser, R. N. Grass, A . Bruinink , W. Stark

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.

Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy, Günter Oberdörster, Andrew Maynard, Ken Donaldson, Vincent Castranova, Julie Fitzpatrick, Kevin Ausman, Janet Carter, Barbara Karn, Wolfgang Kreyling, David Lai, Stephen Olin, Nancy Monteiro-Riviere, David Warheit, Hong Yang and A report from the ILSI Research Foundation/Risk Science Institute Nanomaterial Toxicity Screening Working Group, Published: 06 October 2005, Particle and Fibre Toxicology 2005, 2:8 doi:10.1186/1743-8977-2-8

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.

Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy, Günter Oberdörster, Andrew Maynard, Ken Donaldson, Vincent Castranova, Julie Fitzpatrick, Kevin Ausman, Janet Carter, Barbara Karn, Wolfgang Kreyling, David Lai, Stephen Olin, Nancy Monteiro-Riviere, David Warheit, Hong Yang and A report from the ILSI Research Foundation/Risk Science Institute Nanomaterial Toxicity Screening Working Group, Published: 06 October 2005, Particle and Fibre Toxicology 2005, 2:8 doi:10.1186/1743-8977-2-8

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 encoun-

tered 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.

Genotoxic

Denoting a substance that by damaging DNA may cause mutation or cancer.
Medical Dictionary for the Health Professions and Nursing © Farlex 2012

Histopathology

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 respiratory 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.
Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy, Günter Oberdörster, Andrew Maynard, Ken Donaldson, Vincent Castranova, Julie Fitzpatrick, Kevin Ausman, Janet Carter, Barbara Karn, Wolfgang Kreyling, David Lai, Stephen Olin, Nancy Monteiro-Riviere, David Warheit, Hong Yang and A report from the ILSI Research Foundation/Risk Science Institute Nanomaterial Toxicity Screening Working Group, Published: 06 October 2005, Particle and Fibre Toxicology 2005, 2:8 doi:10.1186/1743-8977-2-8

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.

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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.

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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

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

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

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

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

Mutagen

A substance that causes mutations (genetic damage).

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

Mutagenic

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

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

Oxidative stress markers – ROS/RNS

Reactive oxygen and nitrogen species have been implicated in DNA damage and induction of inflammatory cytokines and growth factors. Acellular 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.

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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