Scientific Committee on Emerging and Newly Identified Health Risks

SCENIHR

Preliminary Opinion

Guidance on the Determination of Potential Health Effects of Nanomaterials Used in Medical Devices

SCENIHR adopted this preliminary opinion by written procedure on 17 July 2014

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ABSTRACT

This Guidance addresses the use of nanomaterials in medical devices and provides information for risk assessors regarding specific aspects that need to be considered in the safety evaluation of nanomaterials. According to the EU Recommendation for the definition of a nanomaterial (Commission Recommendation 2011/969/EU, EC 2011) any particulate substance with at least one dimension in the size range between 1 and 100 nm is considered a nanomaterial. These particles (nanoparticles) exhibit specific characteristics that differ from the characteristics of larger sized particles with the same chemical composition.

The use of nanomaterials in medical devices poses a challenge for the safety evaluation and risk assessment of these medical devices as the specific character of the nanomaterial used should be taken into consideration. The various aspects of safety evaluation and risk assessment of medical devices containing nanomaterials are addressed in this Guidance. The use of nanomaterials in medical devices can vary considerably. Examples are the use of free nanomaterials being a medical device and administered to the patient as such (e.g. iron oxide or gold nanomaterials for heat therapy against cancer), free nanomaterials in a paste-like formulation (e.g dental filling composites), free nanomaterials added to a medical device (e.g nanosilver as antibacterial agent in wound dressings), fixed nanomaterials forming a coating on implants to increase biocompatibility (e.g. nano-hydroxyapatite) or to prevent infection (e.g. nano-silver), or embedded nanomaterials to strengthen biomaterials (e.g. carbon nanotubes in a catheter wall). In all these cases, the potential exposure to the nanomaterials should be considered. It is additionally recognised that wear and tear of medical devices may result in the generation of nano-sized particles even when the medical device itself does not contain nanomaterials.

Guidance is provided on physico-chemical characterisation of nanomaterials, the determination of hazards associated with the use of nanomaterials, and risk assessment for the use of nanomaterials in medical devices. The safety evaluation of the nanomaterials used in medical devices is discussed in the context of the general framework for biological evaluation of medical devices as described in the ISO 10993-1:2009 standard. Therefore, the risk assessment is performed taking into consideration type of device, type of tissue contact, and the duration of contact, thus identifying the specific exposure scenario.

This Guidance is aimed at providing information to help with safety evaluation and risk assessment on the use of nanomaterials in medical devices that should be considered in conjunction with the ISO 10993-1:2009 standard. The Guidance highlights the need for special considerations in relation to the safety evaluation of nanomaterials, in view of the possible distinct properties, interactions, and/or effects that may differ from conventional forms of the same materials.

For the risk evaluation of the use of nanomaterials in medical devices, a phased approach is recommended based on potential release and characteristics of the nanomaterials.

Keywords:

Medical devices, nanomaterials, risk evaluation, SCENIHR, Scientific Committee on Emerging and Newly Identified Health Risks.

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1. BACKGROUND

Today, a more widespread application of nanotechnologies and nanomaterials is imminent or already occurring in many areas, including health care. For nanomedicine, the three largest areas of application are diagnostics, drug delivery and regenerative medicine (ETP Nanomedicine 2009). In addition, there are applications in surgery and thermotherapy (Vauthier et al. 2011).

In the field of medical devices, the following cases of alleged use of nanomaterials have been identified by Notified Bodies:

- Carbon nanotubes in bone cements;
- Nanopaste hydroyapatite powder for bone void filling;
- Polymer setting material with nanoparticles in dental cements;
- Polycrystalline nanoceramics in dental restorative materials;
- Nanosilver or other nanomaterials used as coatings on implants and catheters;
- Nanosilver used as an antibacterial agent, for example in wound dressings (see also Wijnhoven et al. 2009).

Furthermore, there are reports on iron-oxide nanoparticles injected into tumour cells to be heated-up by radiation or an external magnetic field[1]**.** This type of use has not yet been clearly attributed to the legislation on medicines or to the legislation on medical devices. On one hand, the immediate effect is mechanical as the tumour cells burst. On the other hand, one might regard the legislation on medicines applicable as the burst cells are metabolised at a later time.

Although the general risk assessment requirements applicable for materials used in medical devices and previous scientific opinions on risk assessment of nanomaterials (see e.g. SCENIHR 2006, 2007 and 2009) are useful when assessing nanomaterials for medical applications, there is a need for further clarification in the risk assessment of such products. Especially for medical devices, there is such a need in view of the decentralised regulatory system ("New Approach"). The risk assessor, be it the manufacturer, the Notified Body or the authority, should be aware of the specific characteristics of nanomaterials before conducting a risk assessment of the application of nanomaterials in a medical technology.

The European Commission has published two proposals for revision of the medical devices legislation: a Proposal on medical devices (COM(2012)542) and a Proposal on *in vitro* diagnostic medical devices (COM(2012)541). These proposals include a definition of nanomaterial taken from Commission Recommendation 2011/969/EU on the definition of nanomaterial and provisions on the risk classification, the labeling and the instructions for use of medical devices containing nanomaterial. In addition, the general safety and performance requirements now contain a specific requirement to design and manufacture medical devices in such a way as to reduce to a minimum the risks linked to the size and the properties of particles used. Special care shall be applied when devices contain or consist of nanomaterial that can be released into the patient's or user's body. The risk classification influences the stringency of the applicable conformity assessment procedure.

[1] See as an example for the latter the product description of MagForce at: http://www.magforce.de/en/home.html

2. TERMS OF REFERENCE

In light of the expected increase in the application of nanotechnologies to medical devices, the SCENIHR is requested to provide a guidance on the risk assessment of medical devices containing nanomaterials. This guidance should enable the classification of different categories of medical devices containing nanomaterials according to their level of risk.

This guidance shall take into account different categories of medical devices such as:

a. Non-invasive medical devices, e.g. devices coming into contact with the intact skin,

b. Invasive devices (surgical or not), e.g.:

- wound care materials,
- implantable medical devices,
- dental and bone fillings and cements,
- injectable nanomaterials.

In this assessment, where relevant, the SCENIHR is invited to differentiate between free, fixed, and embedded nanomaterials.

The guidance should also differentiate the cases where the nanomaterial can be released into the patient's or user's body and the cases where the nanomaterial is deliberately intended to be released into the human body.

Deadline: December 2013

Supporting documents:

Afssaps (Agence française de sécurité sanitaire des produits de santé), Biological assessment of medical devices containing nanomaterials – Scientific Report (19.8.2011).[2]

ETP Nanomedicine (2009). Roadmaps in nanomedicine towards 2020. Downloadable from http://www.etp-nanomedicine.eu/public/press-documents/publications/etpn-publications

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SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks), Risk assessment of products of nanotechnologies, 19 January 2009.

SCENIHR (Scientific Committee on Emerging and Newly-Identified Health Risks), The appropriateness of the risk assessment methodology in accordance with the Technical Guidance Documents for new and existing substances for assessing the risks of nanomaterials, 21-22 June 2007.

[2] http://www.afssaps.fr/Activites/Surveillance-du-marche-des-dispositifs-medicaux-et-dispositifs-medicaux-de-diagnostic-in-vitro-DM-DMDIV/Dispositifs-medicaux-Operations-d-evaluation-et-de-controle-du-marche/Dispositifs-medicaux-Operations-d-evaluation-et-de-controle/Evaluation-biologique-des-dispositifs-medicaux-contenant-des-nanomateriaux

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3. GUIDANCE ON SAFETY EVALUATION OF NANOMATERIALS USED IN MEDICAL DEVICES

3.1. Introduction

Nanomedicine is one of the most promising fields of application of nanotechnologies. It uses new physical, chemical and biological properties related to nanoscale structures in medicinal products and medical devices. Those properties provide opportunities but may also be associated with risks.

This Guidance focuses specifically on medical devices. The directive 93/42/EEC as amended by Directive 2007/47/EC defines a medical device as "any instrument, apparatus, appliance, software, material or other article, whether used alone or in combination, including the software intended by its manufacturer to be used specifically for diagnostic and/or therapeutic purposes, and necessary for its proper application, intended by the manufacturer to be used for human beings for the purpose of:

- diagnosis, prevention, monitoring, treatment or alleviation of disease,

- diagnosis, monitoring, treatment, alleviation of or compensation for an injury or handicap,

- investigation, replacement or modification of the anatomy or of a physiological process,

- control of conception,

and which does not achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means, but which may be assisted in its function by such means."

This definition is slightly amended in the proposal for a new Medical Device Regulation currently (2014) under negotiations (EC 2012). The proposed changes in the definition do not impact this Guidance.

The proposal for a new Medical Device Regulation includes a definition of nanomaterials and provisions on the risk classification, the labeling and the instructions for use of medical devices containing a nanomaterial. In addition, the general safety and performance requirements in the proposal contain a specific requirement to design and manufacture medical devices to minimize the risks linked to the size and the properties of particles used, whereby special care shall be applied when devices contain or consist of a nanomaterial that can be released into the patient's or user's body. The proposal designates medical devices containing nanomaterials in the highest risk class (class III), because of the uncertainties still associated with the potential risks of nanomaterials.

The use of nanomaterials in medical devices varies considerably. Examples are the use of free nanomaterials as a type of medical device itself and administered to the patient as such (e.g. iron oxide or gold nanomaterials for heat therapy against cancer), free nanomaterials in a paste-like formulation (e.g. dental filling composites), free nanomaterials added to a medical device (e.g nanosilver as antibacterial agent in wound dressings), fixed nanomaterials forming a coating on implants to increase biocompatibility (e.g. nano-hydroxyapatite) or to prevent infection (e.g. nano-silver), or embedded nanomaterials to strengthen biomaterials (e.g. carbon nanotubes in a catheter wall). In addition, nanomaterials may be generated as wear nanoparticles from orthopedic implants (Gill et al., 2012).

In the harmonised European standard ISO 10993-1 "Biological evaluation of medical devices, Part 1, Evaluation and testing within a risk management process", general considerations are included on how to perform the biological safety evaluation of medical devices depending on the application and use of a medical device. The following aspects are considered:

- category of device: surface device, external communicating device, implant device,
- location of tissue contact: skin, mucosal membrane, breached or compromised surface, blood, tissue, bone, dentin,
- contact time: defined as, limited ≤24 hours, prolonged > 24 hours to 30 days, permanent >30 days.

Depending on the use of the medical device, a range of tests has to be considered for the biological safety evaluation (ISO 10993-1:2009). Subsequent parts of the ISO 10993 series describe more specific aspects and test methods. A guidance on nanomaterials is currently under development (ISO/TR 10993-22 Biological evaluation of medical devices - Part 22: Guidance on nanomaterials).

The nano-related risk of medical devices containing nanomaterials is mainly associated with the possibility of the release of free nanoparticles from the device, and their potential toxic effects. However, toxic effects of fixed nanomaterials due to their chemical composition and/or enhanced reactivity should be included. For this purpose, a detailed characterisation and identification of the nanomaterials is essential.

Although much knowledge has been gained, the safety evaluation and risk assessment of nanomaterials differ from those pertaining to conventional substances and still pose substantial challenges (SCENIHR 2006, 2007). This Guidance provides information on how to perform risk assessment of medical devices containing nanomaterials. This Guidance does not address the risk assessment of particular individual medical devices containing nanomaterials. This should be performed on a case-by-case basis, for each specific medical device containing a nanomaterial. In this context, it is important to recognise that extrapolation from one nanomaterial to another is not possible. For example, nanosilver has intrinsic properties that differ from gold nanoparticles. Additionally, the properties of 20 nm nanosilver differ from those of 100 nm nanosilver particles (Park et al., 2011).

This Guidance is limited to the use of nanomaterials in medical devices and the risks for patients treated with medical devices containing nanomaterials, and users of these devices, i.e. health care professionals. In addition, it does not address:

- the broader topic of application of nanotechnologies in medical devices including for example, nano-electronics and lab-on-a-chip technologies. Nanotechnologies are enabling technologies with very broad application. Importantly, there are great differences in risk profile between applications using e.g. nanoelectronics – even if they are applied in implants - and applications using nanomaterials.
- *in vitro* diagnostic (IVD) medical devices. Due to the nature of these products, exposure to nanomaterials from IVDs is highly unlikely.
- medical imaging technologies using contrast agents. While medical imaging equipment is classified as medical devices, contrast agents, which may include or consist of nanomaterials, are medicinal products.
- occupational and environmental risks during the production of nanomaterials. In addition, occupational and environmental risks in the manufacturing and disposal of a medical device containing nanomaterials are not included in this Guidance.

Medical devices not containing nanomaterials can generate nanoparticles as a result of wear-and-tear. The approaches indicated in this Guidance may also be applicable for such wear-and-tear generated nanoparticles.

The Guidance addresses the use of nanomaterials as defined in the recommendation of the European Commission of October 2011 (Commission Recommendation 2011/696/EU) (EC 2011), which is also used in the proposed regulation on Medical Devices. Chapter I Scope and Definitions of the proposed Regulation on Medical devices contains Article 2 (15) defining a nanomaterial as follows:

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

Fullerenes, graphene flakes and single-wall carbon nanotubes with one or more external dimensions below 1 nm shall be considered as nanomaterials.

For the purposes of the definition of nanomaterial, 'particle', 'agglomerate' and 'aggregate' are defined as follows:

– *'particle' means a minute piece of matter with defined physical boundaries;*

– *'agglomerate' means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components;*

– *'aggregate' means a particle comprising of strongly bound or fused particles".*

Although this Guidance specifically addresses the use of nanomaterials in medical devices and the generation of nano-sized wear and tear particles, this Guidance may also be applicable for the evaluation of medical devices containing or generating particles, which are not covered by the above definition of nanomaterial (see figure 1). In addition, by analogy, parts of this Guidance may also be useful for the evaluation of nanomaterials when used in medicinal products including tissue engineered medical products."

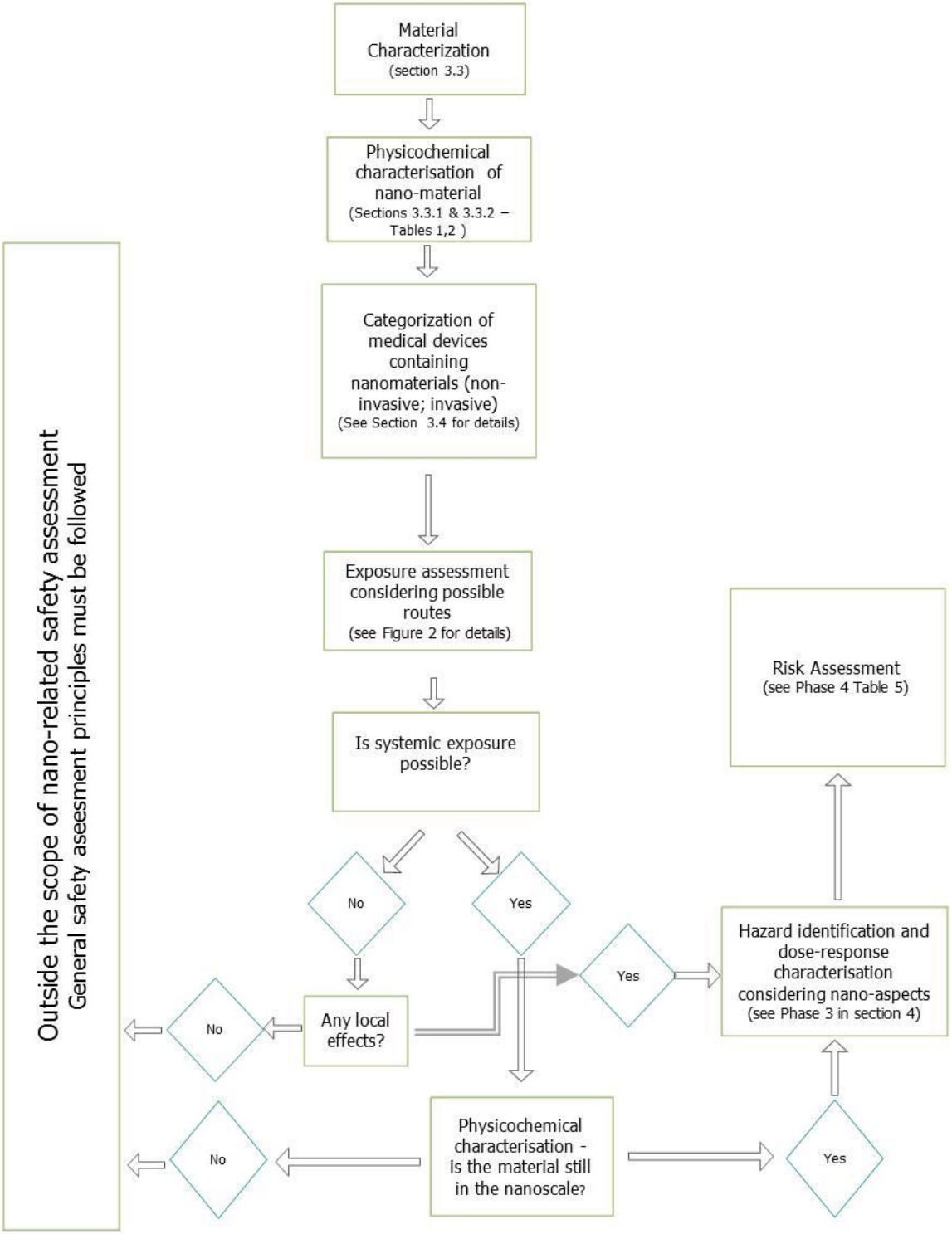

Figure 1: Schematic outline for safety assessment of nanomaterials used in medical devices (the figure is an adapted version of the Fig.13 in the SCCS opinion on Nanomaterials in Cosmetics)

3.2. Methodology

To prepare this Guidance, SCENIHR reviewed recent scientific evidence to identify the state-of-the-art approaches to perform the safety evaluation and risk assessment of the use of nanomaterials in medical devices.

The SCENIHR has considered evidence derived from a wide variety of sources, including peer-reviewed scientific and medical literature and published reports of institutional, professional, governmental and non-governmental organisations. In common with the usual practice of SCENIHR Working Groups, no reliance was made on unpublished work or publicly available opinions that were not scientifically based (SCENIHR 2012).

3.3. Characterisation of nanomaterials used in medical devices

Material characterisation of medical devices regarding chemical, physical, morphological and topographical characteristics is defined in ISO 10993-18: 2005, and ISO 10993-19: 2006.

Nanomaterials exhibit unique properties dependent on their size, shape and surface morphology, which are frequently time-dependent. That is why nanomaterials need characterisation and identification at all stages of design, development, and final production of medical devices containing nanomaterials (SCENIHR 2010, Afssaps 2011, EFSA 2011, SCCS 2012). This information is also essential for risk assessment, both for the identification of the chemical species under evaluation and for exposure identification.

3.3.1. Physicochemical characterisation of nanomaterials

The first step in assessing the risks posed by medical devices containing nanomaterials is to chemically identify and characterise the nanomaterials. It is essential to provide evidence that the characterisation data relate to the same nanomaterial that is used in the final product. If the data relate to a different nanomaterial, or a different form of the same nanomaterial, justification should be provided to show that there is sufficient physicochemical similarity between the nanomaterials to consider the data for risk assessment.

The most important parameters of the nanomaterials intended for use in medical devices are presented in Table 1 together with suitable characterisation and measurement methods. Importantly, nanomaterials may change their surface chemistry during processing, e.g. by acquiring new or additional surface molecules that may act like coatings. Especially in biological test systems, various proteins are known to adhere to the nanomaterial forming the so-called "protein corona". (Maiorano et al., 2010, Lesniak et al., 2012). The protein corona is not static, the composition of the proteins adhering to the surface on the nanomaterial changes during contact. In addition, ISO/TR 13014:2012 provides information and guidance on the characterisation of nanomaterials for toxicological screening.

In view of these potential surface changes, it is important that the physicochemical status of a nanomaterial is determined at different stages of testing and/or usage, (EFSA 2011, SCCS 2012). As the list in the Table 1 is not exhaustive, the manufacturers should be ready to provide additional information on other parameters, if necessary, for the risk assessment.

Table 1: Parameters for characterisation and identification of nanomaterials (NM) intended for use in medical devices.

Parameter	Description	Methods*
Chemical composition/ identity	Information on chemical composition of the NM – including purity, nature of any impurities, coatings or surface moieties, encapsulating materials, processing chemicals, dispersing agents and/or other formulants e.g. stabilizers; information on structural formula(e)/ molecular structure(s) of the constituents of nanomaterial must be provided.	MS, AAS, ICP-MS, FTIR, NMR UVVis, HPLC, GC/LC-MS, XRD Raman spectroscopy
Particle size (Primary/Secondary)	Information on primary particle size, size range and number size distribution (indicating batch to batch variation – if any). The same information would be needed for secondary particles (e.g. agglomerates and aggregates) if present. At least two methods, one being electron microscopy, should be used.	FFF, HDC, HPLC, AUC, CLS disc centrifugation, TEM, SEM, STEM, AFM, DLS, DMA
Physical form and morphology	Information on the physical form and crystalline phase/shape. The information should indicate whether the NM is present in a particle-, spherical-, flake-, tube-, rod-, or fibre- shape, the aspect ratio, crystal or amorphous form, and whether it is in free particulate form or in an agglomerated/ aggregated state as well as whether the preparation is in the form of a powder, solution, suspension or dispersion.	AFM, TEM, SEM, STEM, NMR, XRD
Particle and mass concentration	Information on concentration in terms of particle number and particle mass per volume when in dispersion and per mass when as dry powder.	A wide range of analytical methods, including UV-Vis, HPLC, GC/LC-MS, AAS, ICP-MS
Specific surface area	Information on specific surface area of the NM. At the moment this is only applicable for dry powders.	BET
Surface chemistry	Information on NM surface – including any chemical/ biochemical modifications that could modify the surface reactivity, or add a new functionality.	LDE, SPM, XPS, MS, RS, FTIR, NMR, AUC (for surface composition), GE, SPM, LDE, Nano SIMS, SERS
Surface charge	Information on zeta potential of the NM.	PALS (for zeta potential)
Redox potential	Information on redox potential, especially for inorganic NMs. Conditions under which redox potential was measured need to be documented.	Potentiometric methods, X-ray absorption spectroscopy
Solubility and partition properties [a]	Information on solubility of the NM in relevant solvents and their partitioning between aqueous and organic phase (e.g. as log K_{ow} if appropriate).	Solubility/ dissolution rate in water and other solvents
pH	pH of aqueous suspension.	pH in aqueous media
Viscosity	Information on viscosity of liquid dispersions.	OECD 114
Density and pore density	For granular materials, information on density/porosity of unformulated NM and pore density.	DIN ISO 697, EN/ISO 60
Dustiness	Information on dustiness of dry powder products – such as cements and alginates.	EN 15051:2006, DIN 33897-2.
Chemical reactivity/ catalytic activity[b]	Information on relevant chemical reactivity or catalytic activity of the NM and of any surface coating of the NM.	Kinetic measurements of chemical, biochemical and/or catalysed reactions
Photocatalytic activity	Information on photocatalytic activity of relevant materials used (e.g. coatings, dental materials).	TEM, UV, X-ray topography

* See section 6 Abbreviations and glossary of terms.

a) Dispersion, solution, dissolved: An insoluble NM introduced to a liquid forms a 'dispersion', where the liquid and the NM coexist. In a true solution, the NM is dissolved (and thus, not present) (see OECD 2012 (ENV/JM/MONO(2012)40).

b) If a NM has catalytic properties, it may catalyse a redox or other reaction that may

perpetuate resulting in a much larger biological response even with small amounts of the catalytically active NM. Thus, compared to a conventional biochemical reaction that uses up the substrate, NM reaction centres may perpetuate catalytic reactions.

3.3.2. Methods for characterisation

There are internationally accepted standards for identification and measurements of materials (substances) in their bulk form. Some of these methods can also be used (or adapted) for detection and characterisation of nanomaterials, as shown in the Table 1.

In the last decades, various techniques for measuring at the nanoscale were developed, most of them based on some physical phenomena observed on particle interactions or forces at the nanoscale. Some of the most commonly used techniques are Atomic Force Microscopy (AFM), X-Ray Diffraction, Small-Angle X-ray Scattering (SAXS), dynamic light scattering (DLS), and various electron microscopy techniques (TEM, SEM, STEM, HRTEM). These methods for characterisation were considered in detail in SCENIHR 2010, and additional details were provided in the recent ICCR WG report (ICCR 2011), EFSA Guidance (EFSA 2011), and SCCS Guidance (SCCS 2012). The most important conclusion is that sizing a particulate material needs to be done using different techniques depending on whether the nanoparticles occur as a powder, are dispersed in a liquid, are coated or are embedded in a solid material. Not all methods measure the same size, e.g. TEM and AFM measure the size without any organic coatings, while the size determined by DLS includes the organic coating in the measurement. Each method has its specific limitations and optimal size range. Nanometrology can be defined as the science of measurements at the nanoscale and provides calibration measurements (Proykova et al 2011).

Relevant methods for nanomaterial characterisation can also include size separation and extraction (e.g. ultra- centrifugation, FFF, HDC), and chemical analysis/ detection by spectroscopic or mass spectrometric techniques (e.g. ICP-MS, UV spectroscopy, AAS), surface area determination (BET), and their different variants and combinations. Methods for *in situ* imaging of nanomaterials, e.g. magnetic particle imaging (MPI) and positron emission tomography (PET), are currently under development.

Similarly, antibody binding protein, and enzyme based methods are also under development for determination of organic or coated-inorganic nanomaterials. Some nanomaterials fall in the class of *metamaterials* for which it is not the composition, but the structure, which determines their physico-chemical properties (Engheta and Ziokowski. 2006).

Electron microscopy is perhaps the most generally applicable method used for nanomaterial characterisation. Size and morphology are readily characterised in the Field Emission Scanning Electron Microscopy (FESEM), FEG-SEM, TEM, STEM and FIB/SEM (see Table 2). HRTEM allows structural information on particles and atomic clusters to sub-0.2 nm resolution, while EELS and EDX analysis in the STEM allow the chemical analysis of particles down to nanometre diameters. Combining several methods makes it possible to simultaneously investigate particle size, shape, structure, composition, and surface properties.

Table 2: Examples of methods for size determination

Method	Limitations in range measurements	Phase (liquid, solid, gas) and sensitivity	Particle Distribution
SEM (STEM)	Up to 50-100 nm	Res. 0.4 nm	no
TEM	Few nm	Res. 0.05 nm	yes
STM		Res. 0.01 nm to 0.1 nm	
HRTEM	Below 0.2 nm		yes
AFM	Scanned area is limited	Atomic resolution but sensitivity decreases in time	
SAXS	5-25 nm		
DLS & NTA	(1-2000 nm) & (10-15000 nm)		yes

More information about various characterisation techniques is provided in the Annex.

Each method for size determination, as indicated in Table 2 has its specific limitations. Pitfalls in size measuring techniques are indicated in Linsinger et al., (2012). An excellent illustration of both the target and the AFM tip change in the course of measurement based on quantum phenomenon is available at http://www.loc.ethz.ch/research/grpYamakoshi_EN.

However, characterisation and application of nanomaterials in medical devices is not an easy task. For example, the time required to characterise nanomedicines from their development through the *in vivo* application phase is approximately one year. The success rate of Phase 2 human trials (efficacy trials) is 18% in 2008-2010. (Nanotechnology Characterization Laboratory (NCL), US http://ncl.cancer.gov/). During the workshop "Lessons learned" held in 2011, the NCL presented negative results, "What doesn't work", (Crist et al., 2013). Progress in development and characterisation of nanomaterials used in medicine was the focus of the European CLINAM & ETPN Summit, June 23-26, 2013 (Löffler 2013).

No single method was found that could cover the size range from lower than 1 nm to above 100 nm for all materials. This is one of the reasons that both EFSA and SCCS, in their guidance, require at least two methods for size determination, one of them being an electron microscopy method (EFSA, 2011; SCCS, 2012). Following this principle, the same is considered to apply to the characterisation of nanomaterials used in medical devices.

3.4. Uses of nanomaterials in medical devices

The applications mentioned below are examples of current and possible future use of nanomaterials in medical devices, excluding the larger range of nanotechnologies in medical devices including, for example, nano-electronics and lab-on-a-chip technologies. The following are examples of applications of nanomaterials in medical devices that are currently available (Roszek et al., 2005; Geertsma et al., 2009, ETP 2009, Afssaps 2011).

Examples of devices in current clinical practice:

Non-invasive surface contacting medical devices

These are medical devices which come into contact only with the intact skin. Examples are operating gowns and textile to cover patients in the operating theatre furnished with antibacterial properties, using silver nanoparticles.

Invasive surface contacting medical devices

These are medical devices which come into contact with breached or otherwise compromised skin. Examples are wound treatment products (wound dressings) containing nano-sized silver particles or metal oxide particles which are used for improved antibacterial and anti-fungal activity (Vasilev et al., 2009, Chaloupka et al., 2010).

Invasive external communicating medical devices

These are medical devices which come into contact with the blood path, either indirectly or with circulating blood, and devices in contact with tissue/bone/dentin. Examples include:

- catheters with a nanosilver coating for bladder drainage, haemodialysis and local administering of anaesthesia
- polymer-based dental composite filler materials and dental cements containing nanoparticles (Ferracane 2011).
- surgical and dental instruments with nanostructures used to enhance the cutting behaviour and wear resistance of cutting instruments, e.g. scalpels, needles, catheters, burs for cutting bone or teeth
- instruments with nanostructures used to create non-sticky surfaces to facilitate handling and placement of materials. "Nano-diamond" coatings can be used for this purpose (Dearnaley and Arps 2005).

Invasive implantable medical devices

These are medical devices for introduction into the body in toto, or used to replace the surface of the eye or an epithelial surface by surgical intervention and they remain in place after the procedure. Examples include:

- Carbon nanotubes in bone cements for fixation of implanted prostheses (Van Der Zande et al., 2010)
- Bone fillers with hydroxyapatite and tricalcium phosphate nanoparticles which facilitate rapid integration with the bone of the patient
- Endovascular stents and stent grafts
- Implants for joint replacement (arthroplasties) and implants for fracture repair
- Sutures (Ho et al., 2013)
- Surface coatings: The surface of implants may be modified with the aid of nanotechnologies to enable them to integrate better in the body (improved biocompatibility) (Mercanzini et al., 2010, Thalhammer et al., 2010). In addition, coatings may be used for their antibacterial activity
- Joint prosthetics (hip, knee) with nanohydroxyapatite coating
- Coronary stents with a diamond-like nano composite coating made of ultra-thin polymer

Specific types of medical devices

A special category of nano medical devices are the injectable medical devices. Examples include iron-oxide nanoparticles injected into tumour cells which are then heated-up by radiation or an external magnetic field (Vauthier et al., 2011; Dutz and Hergt 2013; Torres-Lugo and Rinaldi 2013). A more detailed description of one type of this product can be found at: http://www.magforce.de/en/home.html (Magforce Ag, Berlin, Germany). Nanoparticles are additionally being investigated for use in diagnostic imaging (Skotland et al., 2010).

Various manufacturers are developing more products along the lines of the examples mentioned above. Examples of applications that are under development are presented below.

Examples of applications under development:

Non-invasive surface contacting medical devices

No examples identified.

Invasive surface contacting medical devices

Silver nanocoatings for various catheters, contact lenses, and endotracheal tubes

Invasive external communicating medical devices

- Catheters strengthened with carbon nanotubes for minimally invasive surgery
- Electrodes with laminin nanocoating through layer-on-layer self-assembly to improve electrode-tissue interface
- Surface modification of neural micro-electrodes with polymer nanotubes for a low impedance electrode-tissue interface
- Nanoporous micro-electrodes for a brain-machine interface

Invasive implantable medical devices

- Bone cement/ bone replacement products containing nanosilver as an antimicrobial additive
- Coronary stents with nanocoatings of aluminium oxide, glycoproteins, hydroxyaptite, platinum or titanium dioxide (Puranik et al., 2013)
- Silver nanocoatings for various orthopaedic implants and mesh implants
- Orthopaedic implants with nanocrystalline metallo-ceramic coatings
- Modification of the surface roughness of an implant which influences the function of bone-forming and bone-degenerating cells

  Carrier material ('scaffold') for *tissue engineering products* with a nanoporous structure and surface properties which facilitate the growth of living cells and enable the transport of nutrients, signalling molecules, and waste products The purpose of these types of products is to replace, repair or regenerate tissues and ultimately, even organs

Specific types of medical devices

- More injectable nanomaterials for introduction into tumours which may then be radiated externally, including:
  - Heat therapy with super paramagnetic iron oxide nanoparticles

- Heat ablation with gold nanoparticles
- Light therapy
- Boron neutron capture therapy

- Theranostics (therapy combined with diagnostics), i.e. combination of diagnostics and heat therapy with the aid of super paramagnetic iron oxide nanoparticles

3.5. Exposure to nanomaterials from medical devices

Humans may be exposed to nanomaterials from medical devices through various routes. Depending on the relevant exposure route based on the use of a specific medical device, nanomaterials will encounter various barriers before they are taken up by the body.

Two types of people may be exposed: patients and users (health care professionals), although the potential of exposure of patients and/or users will differ depending on the particular device and the way it is used. In general, the highest potential for exposure is associated with devices that consist of "free" nanomaterials or the release/loosening of nanomaterials present as coatings on the surface of medical devices. In addition, exposure to nanomaterials from medical devices may also result from degradation or wear processes, when nanomaterials are fixed on the surface (e.g. as coating on implants) or are embedded within the material of the medical device.

A great variety of nanomaterials is used in nanomedicine, including structures based on lipids, proteins, DNA/RNA or other naturally occurring materials and substances. Furthermore, many different nanomaterials based on polymers, both degradable and non-degradable are applied. The various known forms of carbon like carbon nanotubes (CNT), diamond, carbon black, carbonfibres, and carbonwires are also frequently used. Furthermore, many different sorts of metals and metal oxides are used, as well as silica, quantum dots and a number of specific types that do not fit easily in a larger category.

The intended use of therapeutic devices, sensors/diagnostics for *in vivo* use, regenerative medicine, and implants. inherently implies high exposure potential for patients. For professional users, exposure potential is generally low. When the nanomaterial is used in an unbound (free) state, it can potentially spread throughout the body.

In the ISO 10993 series, the following standards are dealing with characterisation of medical devices and their degradation products. Although nanomaterials are not addressed in these standards, they provide information on the general characterisation of the various components used in medical devices.

ISO 10993-9:2009. Biological evaluation of medical devices – Part 9: Framework for identification and quantification of potential degradation products.

ISO 10993-13:2010. Biological evaluation of medical devices – Part 13: Identification and quantification of degradation products from polymeric medical devices.

ISO 10993-14:2001. Biological evaluation of medical devices – Part 14: Identification and quantification of degradation products from ceramics.

ISO 10993-15:2000. Biological evaluation of medical devices – Part 15: Identification and quantification of degradation products from metals and alloys.

ISO 10993-18:2005. Biological evaluation of medical devices – Part 18: Chemical characterization of materials.

ISO 10993-19:2006. Biological evaluation of medical devices – Part 19: Physico-chemical, morphological and topographical characterisation of materials.

3.5.1. Release of nanomaterials from medical devices

In general, the highest potential for release of nanomaterials from medical devices is associated with devices

- in which the nanomaterial is intended to be released,
- composed of free nanomaterials (e.g. ironoxide nanoparticles for heat therapy) and/or
- containing free nanomaterials (e.g. nanosilver as used in wound dressing, nanomaterials present in bone fillers).

The second possibility for release of nanomaterials from medical devices is associated with release/loosening of nanomaterials present as coatings on medical devices.

The third possibility is associated with medical devices containing nanomaterials, which are released, through chemical breakdown or wear-and-tear processes due to (bio)degradation.

Chemical breakdown occurs as nanomaterials are released from a medical device with a degradable matrix embedding a nano-sized component or released from the surface when applied as a coating. During the degradation, a nano-sized component is released. In the second category, the material may be a composite in which nanoparticles are released as the material is exposed to mechanical and chemical wear-and-tear.

Nanoscale particles may even be generated as a consequence of the degradation of medical devices that do not contain nanomaterials. Solid and porous bulk materials may degrade due to hydrolysis or catalysis. Eventually, the degradation may lead to the production of nano-sized particles. For materials that intentionally or unintentionally degrade upon tissue contact, particles will ultimately be formed as a result of mechanical collapse, which may cause nanoparticles to be generated from either the bulk material or nano-sized components.

Nanoparticles may be generated through abrasive wear or grinding of a material. An example of this includes resin-based composites used in restorative and aesthetic dentistry. These types of composites with nano-sized fillers of various sizes and shapes have in recent years become increasingly popular due to superior aesthetic and mechanical properties. Particles in the nano-size range have been detected in debris after grinding or polishing dental composites on a laboratory surface as well as in the aerosol after polishing of nano-composite restorations in the front teeth Van Landuyt et al., 2012, 2014; Kostoryz et al., 2007). Because there are no occupational exposure limits for nanoparticles, it is not possible to speculate on relative health-associated risks from nanoparticles released when grinding or polishing dental composites. There is also a lack of information to establish such limits.

Joint articulations using metal-on-metal as well as metal-on-polyethylene sliding surfaces produce wear particles most frequently during revision surgery (SCENIHR, 2014). For metal-on-metal joints, metal debris particle size was less than 1 µm for most (>90 %) of the particles, while for metal-on-polyethylene joints, most particles were above 1 µm (Lee et al., 1992). Notably, several reports on the distribution of particles show that the largest number of particles was detected among the smallest analysed category, indicating that nano-size particles are most likely to be present. A generic all-encompassing term "adverse reactions to metal debris" (ARMD), was introduced that summarises the histopathology associated with metal-on-metal hip prostheses including aseptic lymphocytic vasculitis associated lesions, lymphoid neogenesis, granulomatous inflammation and metallosis (Natu et al., 2012).

3.5.2. Exposure of patients to nanomaterials released from medical devices

For patients, the following exposure routes may be applicable:

- inhalation exposure (e.g. related to intubation, dental procedures)
- dermal exposure
- mucosal exposure (via various mucosal tissues, e.g. in the mouth, vagina/penis)
- oral exposure
- parenteral exposure (introduced into the body by a means other than through the gastro-intestinal tract, e.g., by injection into the bloodstream (intravenous) or a muscle (intramuscular), surgical procedures using medical devices or from implanting devices in any tissue)
- ocular exposure

3.5.2.1 Non-invasive medical devices

These are devices in contact with intact skin. Released nano-sized components have a low potential to penetrate through the skin (Labouta and Schneider 2013) (see section 3.6.3).

Note: Under the medical devices regulations, contact lenses worn on the surface of the eye are considered to be invasive medical devices.

3.5.2.2 Invasive medical devices

All classes of invasive devices may potentially generate nano-particles. For invasive devices, the released nanoparticles have a direct port of entry in the body depending on the localisation of the device used.

Products consisting of free nanomaterials always lead to high potential for systemic exposure, i.e. to the entire body, regardless of the administration route (oral, dermal, parenteral or intravenous). Whether or not a high systemic exposure occurs depends on the actual use of the medical device and the route of exposure (i.e the location of the use of the medical device).

Nanomaterials in products used in surgery are generally embedded inside or coated on larger products. The duration of contact with the patient is relatively short. Local exposure to the bound nanomaterials at the site of treatment will therefore be high in all cases, whereas systemic exposure potential to free nanomaterials is likely to be very low. Additionally, for implants, nanomaterials are usually embedded or fixed on the surface and the duration of contact is long-term. Local exposure to fixed nanomaterials at the site of treatment will therefore be high in all cases, whereas systemic exposure potential to free nanomaterials may be considered low, provided there is only slow generation of wear particles. Exposure may also occur during the treatment procedures with dental composite materials cured *in situ*, and with bone and tissue fillers containing nanomaterials. In particular for dental fillings, exposure may also occur during polishing. (Van Landuyt et al., 2014).

3.5.3. Exposure of professional users to nanomaterials released from medical devices

For professional users (e.g. dentists and dental technicians), the potential exposure is highest when free nanomaterials are present in the medical device, e.g. in certain dental composite materials and bone fillers. Exposure may occur especially during polishing of dental fillings (Van Landuyt et al., 2014).

For these professional users, the following exposure routes may be applicable:

- inhalation exposure (e.g. related to dental procedures)
- dermal exposure
- mucosal exposure (via various mucosal tissues, e.g. in the mouth)
- oral exposure
- ocular exposure

3.5.4. Estimation of exposure for risk assessment

Based on the potential exposure to nanomaterials in medical devices, an estimation can be made of the exposure using the exposure times and the exposure categories used in the risk assessment and risk management of medical devices as indicated in ISO 10993-1:2009 (Table 3).

Three exposure categories of devices are considered based on the application site of a medical device:

- surface contacting device
- external communicating device
- implant device

The type of tissue contact considered in the risk assessment includes categories like:

- skin
- mucosal membrane
- breached or compromised surface
- blood
- tissue
- bone
- dentin

The contact time must also be considered:

- limited contact (≤24 hours)
- prolonged contact (> 24 hours to 30 days)
- permanent >30 days

In addition to the potential (bio)degradable property of a material, the "quality" of the material used to manufacture a medical device should be considered in terms of possible wear and tear.

Importantly, measuring of the release of nanomaterials from a medical device may pose analytical challenges. Currently, a robust methodology especially for the measurements of low level release of nanomaterials is lacking. For metal and metal oxide nanomaterials, elemental analysis may be used as a surrogate for nanoparticle release.

Table 3: An estimation of potential external and internal exposure as starting point for a risk evaluation for medical devices containing nanomaterials

			Type of application of nanomaterials External exposure/internal exposure				
			Free	Fixed (coating)	Fixed (coating)	Embedded	Embedded
Type of device	Type of contact	Duration of contact		Weak (physisorb)	Strong (chemisorb)	In degradable materials*	In non-degradable materials
Surface device	Intact skin	≤ 24 h	H/N	M/N	M/N	L/N	N/N
		>24 h to 30 d	H/N	M/N	M/N	M/N	N/N
		>30 d	H/N	M/N	M/N	H/N	N/N
	Intact mucosal membrane	≤ 24 h	H/L	M/L	M/N	L/L	N/N
		>24 h to 30 d	H/M	M/M	M/L	M/M	N/N
		>30 d	H/M	M/M	M/L	H/M	N/N
	Breached or compromised surface	≤ 24 h	H/H	M/M	M/L	L/M	N/N
		24 h to 30 d	H/H	M/M	M/L	M/M	N/N
		30 d	H/H	M/M	M/L	H/M	N/N
External communicating device	Blood path, indirect **	≤ 24 h	na	M/M	M/L	L/L	N/N
		>24 h to 30 d	na	M/M	M/L	M/M	N/N
		>30 d	na	M/M	M/L	H/M	N/N
	Tissue/bone/dentin	≤ 24 h	H/H	M/M	M/L	L/L	N/N
		>24 h to 30 d	H/H	M/M	M/L	M/M	N/N
		>30 d	H/H	M/M	M/L	H/H	N/N
	Circulating blood***	≤ 24 h	na	H/H	H/H	L/L	N/N
		>24 h to 30 d	na	H/H	H/H	M/M	N/N
		>30 d	na	H/H	H/H	H/H	N/N
Implant device	Tissue/bone	≤ 24 h	H/H	H/H	H/L	L/L	N/N
		>24 h to 30 d	H/H	H/H	H/L	M/M	N/N
		>30 d	H/H	H/H	H/L	H/H	N/N
	Blood	≤ 24 h	H/H	H/H	H/L	L/L	N/N
		>24 h to 30 d	H/H	H/H	H/L	M/M	N/N
		>30 d	H/H	H/H	H/L	H/H	N/N

H=high, M=medium, L=low, N=negligible, na= not applicable

H/L means high potential contact and/or external exposure to the nanomaterial / low potential for internal systemic exposure of all organ systems

* the exposure will depend on the degradation time of the medical device

** contacting the blood path at one point. Examples of these types of devices are solution administration sets, transfer sets and blood administration sets (ISO 10993-4:2002)

*** Examples of these types of devices are: intravascular catheters, extracorporeal oxygenating tubing and dialysers (ISO 10993-4:2002).

3.6. Toxicokinetics

3.6.1. Introduction

Toxicokinetic testing provides information on the fate and behaviour of the substances under evaluation and insight in potential target organs and organ burden that may ultimately result in toxicity.

The toxicokinetic properties of nanomaterials, like other substances, can be described by four processes: absorption, distribution, metabolism and excretion (ADME) the study of which is essential for the safety evaluation of engineered nanomaterials. The nature of nanomaterials may result in altered and specific toxicokinetics and tissue distribution when compared to non-nanoforms (EFSA 2011, SCCS 2012). For subgroups of certain solid nanomaterials, it is doubtful whether metabolism (M) really occurs. Tissue distribution, accumulation and elimination from tissues are considered more relevant than blood plasma levels. It is particularly important to evaluate any nanomaterial presence in organs shown to be typical distribution organs (and thus potential targets for toxicity) and that have an increased capacity for uptake of particles (e.g. liver, spleen, and lungs) (EFSA 2011). In addition, the kidney is an important organ, because of possible excretion of the nanomaterials.

The route of entry is important, because it may affect the kinetics of nanomaterials/nanoparticles: For example, Au nanoparticles (1.4 nm) showed a higher uptake in the kidney compared to the liver after intratracheal administration. In contrast, the liver was the predominant target organ after intravenous administration, suggesting the alteration of the nanoparticles during passage through the air/blood barrier in the lung (Oberdörster 2010, Semmler-Behnke et al., 2008). Depending on the site of application, further kinetics of a released nanomaterial may be affected by adherence of molecules to the surface of a nanomaterial. In this respect, the formation of a serum protein "corona" that is thought to enhance recognition and uptake by cells of the mononuclear phagocyte system (MPS) is well known (Lynch et al., 2009, Lynch and Dawson 2008, Nel et al., 2009). The MPS cells are primarily monocytes and macrophages present found in spleen, lymph nodes and bone marrow as well as Langerhans cells in the skin, Kupffer cells in the liver and alveolar macrophages in the lung. There is a rapid clearance of the nanoparticles from the blood mainly into the liver and spleen (De Jong et al., 2008, Demoy et al., 1997, Gibaud et al., 1996, Lenaerts et al., 1984, Sadauskas et al., 2007, Lipka et al., 2010, Lankveld et al., 2010, 2011).

Locally released nanoparticles in tissues may migrate or be transported into the systemic circulation. The primary transportation system is lymphatic, which allows for transportation of free particles and particles phagocytised by tissue macrophages and/or other inflammatory cells. Although these particles accumulate mainly in regional lymph nodes, depending on the primary localisation, and enter into the blood circulation, the nanoparticles may also accumulate in spleen and liver. Nanomaterials released from a medical device can translocate from their site of origin into the body.

The route of exposure to nanoparticles depends on the medical device. Potentially all routes of exposure are possible. Independent of the route of exposure for medical devices, the absorption and bioavailability of potentially released nanomaterials from a medical device, or the generation of nanoparticles via wear and tear (Polyzois et al., 2012), are the starting points for the evaluation of the toxicokinetics of nanomaterials.

3.6.2. Methods to evaluate toxicokinetics of nanomaterials

The design and performance of toxicokinetic studies for chemicals, degradation products and leachable from medical devices is described in ISO 10993-16:2010. Although degradation products are considered, nanoparticles are not mentioned. The OECD 417 test guideline describes the toxicokinetic studies necessary for chemical substances and explicitly states that it is not intended for the toxicokinetic testing of nanomaterials. Analogously, both the *in vivo* and *in vitro* OECD Guidelines (427 and 428, respectively) for dermal penetration were developed for chemicals and not proven to be valid for nanoparticles. Therefore, the use of such methodologies should be evaluated on a case-by-case basis.

For a dissolved chemical, the tissue uptake and release is generally dependent on the blood concentration (when excluding specific active transport, the first-pass effect in the liver and highly bio accumulating chemicals in the adipose tissue) and an equilibrium between blood and organ concentration is generally obtained. This is because nanoparticle uptake in organs occurs rapidly and a repeated administration results in an increase of nanomaterials, predominantly in the liver and spleen after intravenous administration (Lankveld et al., 2010). There is no equilibrium concentration between tissue and blood. Uptake in organs can occur independent of the blood concentration i.e. even with a low blood concentration and high organ concentration, organ uptake can occur. This results in persistence of nanomaterials in organs for long periods: silver could be detected in various organs at day 17 after intravenous administration of silver nanoparticles in rats (Fabian et al., 2008; Pauluhn 2009; Lankveld et al., 2010). Titanium nanoparticles were detected up to 90 days after a single and repeated intravenous administration (Nanogenotox 2013). Therefore, to identify tissue distribution and the potential for tissue accumulation and persistence of a nanomaterial, it is necessary to design single and repeated kinetic studies, with a representative follow-up period of time for adequate extrapolation of the half-life. In OECD 417 on toxicokinetic testing, the follow up period is typically up to 7 days, which may be too short for nanomaterials in view of their potential persistence in organs.

Release/elimination from an organ seems to be associated with a possible dissolution or degradation of the nanomaterials. Potential persistence occurs especially for non-degradable solid nanomaterials.

If a known test usually used for chemical or bulk forms is adapted to conduct (toxico)kinetic studies with nanomaterials, it is critical to have a reliable measurement system for the detection of the nanomaterials. However, the detection of nanoparticles in tissues/organs is complex. Electron microscopy is neither applicable for quantitative measurements nor for all nanomaterials. To date, most studies on toxicokinetics of nanomaterials have used elemental analysis of the components of the nanomaterials e.g. Zn for ZnO, Ti for TiO2, Ag for Ag nanoparticles. Analysis could be performed by using inductively-coupled plasma mass spectroscopy (ICP-MS) or atomic absorption mass spectroscopy (AA-MS). Although this provides a good indication of the possible tissue distribution, the limitation is that the nanoparticles themselves are not detected or measured. In combination with separation techniques like field flow fractionation (FFF), it is possible to evaluate the presence of particles using the so-called single particle ICP-MS (Van Der Zande et al., 2012).

Specific labelling of nanomaterials to follow their fate *in vivo* can be done by using radioactive isotopes as radiolabel or fluorescent dyes. A disadvantage of these forms of labelling is that the label can detach from the nanomaterial (Geiser and Kreyling 2010). A measurement or imaging will then identify the label, but not the distribution of the nanoparticle. Alternatively, radioactive isotopes may be used that are isotopes of a metal being part of the nanomaterial (e.g. gold or silver). With this approach, there is some certainty that the nanoparticles themselves are detected. However, for silver nanoparticles, there is still uncertainty regarding the release of silver ions. In addition,

natural stable isotopes like ^{68}Zn may be used to demonstrate uptake from the application site (Gulson et al. 2010).

There is uncertainty whether the nanomaterial or the released ions are detected especially when a nanomaterial can release ions (e.g. silver or zinc oxide). After skin application of sunscreens containing ^{68}Zn isotope enriched ZnO nanoparticles, the ^{68}Zn was detected in the blood of humans and in internal organs (e.g. liver) in mice, but skin penetration of the ZnO nanoparticles themselves was not detected (Gulson et al., 2010, Osmond-McLeod et al., 2013).

Surface treatments may have a tremendous effect on the toxicokinetics of nanomaterials. The PEGylation (coating a nanomaterial with polyethyleneglycol) decreased the blood clearance of intravenously administered gold nanorods (Niidome et al., 2006, Lankveld et al., 2011). Additionally, specific targeting to organs may be achieved by the coating of nanomaterials.

3.6.3. Toxicokinetics of nanomaterials present in non-invasive medical devices

Uptake after dermal exposure

Dermal penetration can be assessed by using *in vitro* systems, for which the skin of many mammalian species, including humans, may be used as indicated in OECD 428, or *in vivo* according to OECD 427. But these means of assessment were not designed for nanoparticles and the problems mentioned above, related to nanoparticle quantitation, still remain.

However, dermal penetration of nanoparticles is generally considered to be low or absent (Butz et al., 2007, Monteiro-Riviere and Riviere 2009b, Sadrieh et al., 2010, Monteiro-Riviere and Larese Filon 2012). In general, nanoparticle penetration of the skin is limited to the first cell layers of the stratum corneum (Butz et al., 2007). However, for some nanomaterials, limited uptake was suggested. For example, when ZnO nanomaterial was applied on the skin in a sunscreen formulation, the presence of Zn in the blood originating from the ZnO in the sunscreen was observed (Gulson et al., 2010).

Silver (Ag) nanoparticles are widely used as antimicrobial agents, for example, in wound dressings (Rai et al., 2009, 2014). In an *in vitro* system using human skin exposed to Ag nanoparticles, a low translocation into the receptor fluid was found which was increased 5-fold in damaged skin (Larese Filon et al., 2009). However, it could not be clearly demonstrated that nanoparticles were translocated, because the presence of elemental Ag was determined with electrothermal atomic absorption spectroscopy (ETAAS) which cannot discriminate between silver ions and silver particles. Treatment of burn patients with wound dressings containing nanocrystalline silver resulted in an increase in blood silver serum levels, although these levels were considered to be non-toxic to the patients (Vlachou et al., 2007).

When studying skin penetration and absorption, the condition of the skin must be taken into account. Skin that has been damaged through abrasion, over-exposure to UVB (sunburn), exposure to mechanical stressors (skin flexing) or the effects of solvents and other will not react the same as healthy, undamaged skin (Monteiro-Riviere and Larese Filon 2012).

3.6.4. Invasive medical devices

Uptake after ocular exposure (via the eye)

Nanomaterials could be used in contact lenses. However, there are no data available regarding the release and kinetics of such nanomaterials. In general, for the eye, the use of various nanomaterials is aimed at enhancing the uptake and targeting drugs. In a recent review, the therapeutic efficacy of drugs in ocular diseases was enhanced by the use of nanoparticles such as liposomes, micro/nanospheres, microemulsions, and dendrimers (Honda et al., 2013). For chitin containing nanogels, penetration into the deeper sections of the porcine cornea was observed without signs of destruction or inflammation to corneal cells (Mohammed et al., 2013).

Uptake after inhalation exposure (e.g. related to dental procedures)

After exposure via the inhalation route, by either inhalation or instillation, a small but significant fraction of the dose of nanoparticles may be demonstrated systemically, although the majority of the nanoparticles remains in the lung (Kreyling et al., 2002, Semmler-Behnke et al., 2008, Sung et al., 2011, Abid et al., 2013). The elimination half time from the lung for fine and ultrafine (nano)particles in rats was approximately 65 days (Pauluhn 2009, 2011). Due to the mucociliary cascade that removes inhaled particles from the lung, a portion of the inhaled/instilled nanomaterials ends up in the gastrointestinal tract and is excreted via the faeces (Abid et al., 2013). In addition, inhaled nanomaterials may migrate into the brain via the olfactory nerve (Oberdörster et al., 2004, Balasubramanian et al., 2013). The primary particle size of the nanoparticles was important because smaller (7nm versus 20 nm) nanoparticles had a higher uptake from the lung (Balasubramanian et al., 2013). In this study, macrophage-mediated mucociliary escalation followed by faecal excretion was the major pathway of clearing the inhaled nanoparticles in the lungs.

Uptake after oral exposure

Uptake form the gastrointestinal tract (GI-tract) was demonstrated for several nanomaterials (Jani et al., 1990, 1994, Wang et al., 2007, Kim et al., 2008, Park et al., 2010 a, b), but the lack of uptake of nanoparticles was also observed (Yang et al., 2012). In general, smaller particles have a higher uptake (Jani et al. 1990, Park et al., 2010a). However, large titanium particles with a size of 500nm were also absorbed via the gastrointestinal tract (Jani et al., 1994).

Uptake after transdermal exposure (implants)

When present on or in medical devices that penetrate the skin, the local release of coatings consisting of nanomaterials may be possible. In practice, transdermal and other implants will most likely generate only a minor amount of locally released nanoparticles, an exception being wear and tear occurring after arthroplasties. Thus, the subcutaneous administration of nanomaterials may be an alternative for studying particle distribution. Following subcutaneous injection, the largest particle agglomerates were found mainly in draining inguinal lymph nodes, and to a lesser extent, the liver, spleen and lungs (Umbreit et al., 2011).

3.6.5. Conclusions on toxicokinetics of nanomaterials

The performance of toxicokinetic studies to evaluate tissue distribution and kinetics of nanomaterials are indicated when there is the possibility for the release of free (nano)particles from a medical device. Although methods used for chemicals in bulk form can be adapted, specific attention should be given to the detection method. Blood clearance generally appears quite quickly thus, blood levels are less important than the ultimate tissue and organ levels. In addition, consideration should be given to the potential for tissue accumulation and persistence of a nanomaterial (e.g. dissolution/degradation of the nanomaterial), for which repeated exposure and prolonged follow-up time may be necessary.

3.7. Toxicological evaluation

3.7.1. Introduction

The toxicity testing strategy of an individual medical device containing nanomaterials is determined by its potential of external and internal exposure. Therefore, hazard evaluation must be performed on a case-by-case basis, through a series of studies including literature review, *in silico*, *in vitro* and *in vivo* studies. For medical devices, selection of any *in vitro* or *in vivo* tests shall be based on end-use applications. All tests shall be conducted according to recognised current/valid best laboratory/quality practices, for example, Good Laboratory Practice (GLP) or ISO/IEC 17025, where applicable, and data shall be evaluated by competent informed professionals (ISO 10993-1). The required toxicity studies should be performed in accordance with the International Standards ISO 10993 series (ISO 10993 – 1, 3-6, 10-12, 17, 19). However, it should be emphasised that none of currently available test methods, *in vitro* and *in vivo*, have been validated specifically for nanomaterials. Materials in nanoform pose many challenges when tested; unlike solubilised chemicals, nanomaterials generally exist as a suspension/dispersion of insoluble or partially-soluble nanoparticles and/or larger agglomerates and aggregates, which may affect the test system.

The toxicity of nanomaterials is a response to the size and additional specific characteristics, most of them listed in Table 1. Therefore, it is essential that tests are conducted using the same nanomaterial with the same chemical composition, size and size distribution, surface properties and purity/impurity profile as the substance present in the medical device, and should, therefore, be characterized before testing. Thus, the information on the nature and stability of the test substance under experimental conditions is of prime importance for the interpretation of any test results. If a comparable/similar (nano)material is used this should be justified and documented.

There are ongoing developments in *in vitro* methods, but currently there are no validated *in vitro* methods for hazard assessment of nanomaterials (Park et al., 2009, Cockburn et al., 2012, Doak et al., 2012, Nel et al., 2013a). However, *in vitro* tests may be useful for screening purposes, and to elucidate possible mode of action (Basketter et al., 2013, Nel et al., 2013b), but their use should be evaluated on a case-by-case basis.A catalogue of all currently validated *in vitro* methods is published on:

http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/validation-regulatory-acceptance/,

Whilst *in silico* modelling approaches are advancing for conventional chemicals, a relationship between the various physicochemical properties and toxicological effects of nanomaterials has not yet been established/investigated to allow development of reliable models for nanomaterials. As a result, only a few rudimentary *in silico* models are currently available for nanomaterials (Toropov and Leszczynski 2007, Toropov et al.,

2007, Puzyn et al., 2009, 2011, Sayes and Ivanov, 2010; Burello and Worth, 2011, Wang et al., 2014). However, they are unlikely to be useful in the foreseeable future for the assessment of relevant toxicological endpoints that are needed for risk assessment.

3.7.2. Potential pitfalls in toxicity testing of nanomaterials

Following the ISO 10993-1:2009 standard regarding the evaluation and testing of medical devices within a risk management process, the toxicity testing strategy for each device should be considered case-by-case based on the type of medical device, type of contact and duration of exposure. Most of the toxicity assays as described in the various parts of the EN-ISO 10993 series are developed specifically for medical devices, and are based on the OECD Guidelines for the testing of chemicals.

Testing of insoluble or partially-soluble nanoparticles using *in vivo* or *in vitro* methods must also take into account that they will be present in a dosing or test medium as a nano-dispersion rather than in solution. Therefore, any toxicity testing using *in vivo and in vitro* methods should pay special attention to the agglomeration/aggregation behaviour, and the insoluble/ partially-soluble nature of nanomaterials (SCENIHR, 2009; Kreyling et al., 2010, EFSA 2011, SCCS 2012). Possibilities for disagglomeration of nanomaterial should also be considered. During toxicological evaluations, some properties of nanomaterials may change due to interaction with the surrounding media.

Special care is, therefore, needed in regard to the applied doses, which can be affected by the above-mentioned phenomena. In addition, the concentration of a nanomaterial may decrease during a test due to sedimentation, binding with other moieties in the test medium, or adhesion to glass/plastic ware. It is therefore important to ascertain the stability and uniformity of the nanomaterial in a test medium to ensure that the applied concentration/dose is maintained for the intended period during the test.

It is important to consider if vehicle and/or the test or cell culture medium does not modify the physicochemical properties (including adsorption of biomolecules on the surface) of the nanomaterial tested because it may influence general toxicity. It is therefore important to ascertain the stability and uniformity of the nanomaterial in a test medium to ensure that the applied concentration/dose of nanomaterial is as assumed (Allouni et al., 2009).

Since endotoxin may interfere with the test system and may lead to false negative or positive results depending on the test system, it should be excluded before testing nano materials."Endotoxin may interfere with the test system and may lead to false negative or positive results depending on the test system.

Importantly, there may be an interaction between test reagents and the nanomaterials especially in colorimetric assays (such as sulforhodamine B dye, or MTT used in the viability assays). Moreover, some nanomaterials may themselves disperse/ absorb light and therefore, interfere with the measurements in colorimetric assays. These aspects need to be considered when using colorimetric methods. Produced proteins/biological mediators (e.g. cytokines) may also bind/adsorb on nanomaterial surfaces and may lead to low responses or even false negative results (Worle-Knirsch et al., 2006, Monteiro-Riviere et al., 2009a; Wilhelmi et al., 2012).

Some metals (silver) or metal oxides (ZnO) undergo (slow) dissolution in media, therefore, part (or all) of the activity measured might be due to the dissolved ions. It might be warranted for those types of nanomaterials to determine the solubilised fraction before and during testing.. In some assays, adding a suitable control in the ionic form should be considered.

The harmfulness of nanomaterials may arise from their size-related ability to readily enter biological systems and modify the structure of proteins through formation of new

protein complexes or enhanced protein degradation (Lovric et al., 2005, Aggarwal et al., 2009, Mailander and Landfester 2009).

Nano-sized particles are likely to be phagocytised by inflammatory cells, especially macrophages and polymorphonuclear neutrophils. Whether the particles are in aggregated or non-aggregated suspension is critical for absorption and notably these aggregates and agglomerates may be larger than the nano-size range (i.e. 100 nm), although aggregates/agglomerates smaller than 100 nm may also be present. Affinity for and subsequent adsorption of proteins and peptides may change the biological significance and enhance triggering of inflammatory humoral and cellular processes. Endocytosis of spherical NPs is easier and faster compared to rod-shaped or fiber-like nanomaterials (Champion and Mitragotri 2006). Rod-shaped or needle-like NPs may have a larger contact area with the cell membrane receptors than spherical NPs when the longitudinal axis of the rods interacts with the receptors. Hence, rod or needle ends with high curvature at the half-cup stage of endocytosis are very likely to produce higher energy at the membrane surface, causing a large distorting force that exceeds the maximum force provided by the actin polymerisation. This effect stalls the growing ends of the phagocytic cup and results in impaired phagocytosis and the macrophage spreading onto the material rather than internalizing it (Lu et al., 2010).

The metrics used for toxicity assessments are normally measured and expressed in weight or volume units (such as mg/Kg, or mg/L) for conventional chemicals. However, such metric expressions may not be appropriate for nanomaterials, because of the large surface areas per particle mass or volume. For nanomaterials, surface area or number of particles might give a better description of a possible dose-response effect relationship. Nanoparticle shape can modify activity as well. Until suitable parameters are identified, it is important that different dose-describing metrics, such as weight/volume concentration, particle number concentration, surface area etc. are available, which provide sufficient information to converse doses based on mass into other parameters (Donaldson et al., 2013b).

Sample preparation and possible reference materials for the safety evaluation of medical devices are described in ISO 10993-12:2012). Although this standard does not specifically addresses nanomaterials, it provides general information on sample preparation from solid materials. In addition, also the nanomaterials themselves, e.g. when provided as powder of in liquid dispersion, may be used in the assays for safety evaluation.

3.7.3 Toxicity testing methods

Cytotoxicity

The ISO 10993 – 5:2009 describes test methods to assess the *in vitro* cytotoxicity of medical devices. In addition, currently a standard is under preparation for an *in vitro* cytotoxicity assay specifically dedicated to nanomaterials (ISO/AWI 19007 Modified MTS assay for measuring the effect of nanoparticles on cell viability, ISO, Geneva, Switzerland).

Driven by European politics on animal welfare, there are continuous efforts to find *in vitro* alternative methods to *in vivo* testing on animals. However, at the moment, there are no validated *in vitro* methods for hazard assessment of both chemicals and nanomaterials, *In vitro* tests may be useful for screening purposes, for indicating potential toxicity of a nanomaterial and to elucidate possible mode of action (Nel et al., 2013b), providing pointers for further toxicological investigations. For example, *in vitro* tests may indicate the likelihood of generation of reactive oxygen species (Xia et al., 2008), which may provide an alert for potential toxic effects via the induction of oxidative stress and activation of inflammatory and proliferative pathways (Unfried et al., 2008, Donaldson et al., 2010, 2013b).

Considering acute toxicity testing for appropriate classification, a number of cytotoxicity assays have been proposed. Recently, a major effort has been undertaken (AcuteTox project - www.acutox.org) to create an integrated testing strategy to replace the animal testing for predicting human acute oral systemic toxicity which is based exclusively on *in vitro* and *in silico* methods. The 3T3/NRU assay was indicated as a first step in a tiered testing strategy being suitable to identify unclassified substances (LD50 > 2000 mg/kg). However, nanomaterials were not included into the selected tested substances and therefore, the use of such test should be evaluated on a case-by-case basis.

Acute toxicity

Acute toxicity testing for medical devices is part of the ISO 10993-11: 2006 standard dealing with determination of systemic toxicity of medical devices (ISO 2006). An acute toxicity study might be an initial step in establishing a dosage regimen in subacute/subchronic and other studies and may provide information on the mode of toxic action of a substance by the intended clinical exposure route. For medical devices, the test sample preparation, consisting in general of both a hydrophilic and a lipophilic extract of the material or the medical device, is presented in ISO 10993-12: 2012 (ISO 2012). However, to obtain an indication of the toxicity of a nanomaterial, a dispersion of the nanomaterial itself as it is used in the medical device may also be considered. Other information on the performance of an acute toxicity test in addition, to ISO 10993-11:2006 can be found as follows: for acute oral toxicity testing using the fixed dose method [EC B.1 bis, OECD 420], the acute toxic class method [EC B.1 tris, OECD 423], or the up-and-down procedure [OECD 425]. The acute toxic class method by the inhalation route is described in OECD 403 and 436, and the *in vivo* acute dermal toxicity assay is described in EC B.3 and OECD 402 and 404.

Irritation activity

The ISO – 10993-10:2010 describes test methods to assess the potential to produce irritation (and delayed-type hypersensitivity) of medical devices and their components after topical skin application. In addition, ISO 10993-10:2010 describes intradermal irritation tests for medical devices used as implants or transdermally. Specific irritation tests are described in Annex B including eye irritation tests, oral mucosal irritation tests, and rectal, penile and vaginal irritation tests.

For chemicals the determination of irritation/corrosivity an *in vivo* method is used based on the Draize test as described in EC B.5, and OECD 405.

The following five validated *in vitro* alternatives are available (Regulation (EC) No 440/2008) for skin corrosion assessment of chemicals:

a) TER test (rat skin transcutaneous electrical resistance test) [EC B.40, OECD 430]

b) EpiSkin™, EpiDerm™, SkinEthic™, EST-1000 [EC B.40bis, OECD 431]

Three of them, namely EpiSkin™, Modified Epiderm™Skin Irritation Test (SIT) and SkinEthic™ Reconstructed Human Epidermis (RHE) are validated for skin irritation assessment for chemicals (OECD 439).

No specific validation of the *in vitro* alternative tests has been performed for medical devices and/or nanomaterials, although there is no clear scientific basis against the use of these methods for nanomaterials.

The assessment *in vivo* of eye irritancy or corrosivity on substances is based on the result of the classic Draize *in vivo* eye irritation test on rabbits according to EC B.5, and OECD 405 test guideline. There are alternative methods available replacing this test: the Bovine Cornea Opacity Permeability (BCOP) [OECD 437], the Isolated Chicken Eye (ICE) [OECD 438], and an *in vitro* cell assay (OECD 460). These assays, although using animal eyes, are considered alternatives, since they are obtained from animal slaughterhouses. They are able to discriminate corrosive and severe eye irritants, but fail to distinguish mild from non-irritants

There is no indication about the possibility of using these tests (*in vivo* or *in vitro*) for the testing of different forms of nanomaterials as such and/or extracts from medical devices. The ICE test is not suitable for solid samples. The assays can probably also be used for nanomaterials, but validation has not yet been performed and would potentially provide supporting evidence.

It is possible that some insoluble particulate materials can induce eye irritation not only chemically, but also mechanically by interfering with the eye tissue or the cell.

Delayed-type hypersensitivity

ISO – 10993-10:2010 describes test methods to assess the potential to induce delayed-type hypersensitivity for medical devices and their components. Three *in vivo* methods are described, two using guinea pigs and one using mice, for assessing skin sensitisation potential. The murine local lymph node assay (LLNA) in its three versions is the preferred method in view of animal welfare (OECD 429, 442A and 442B). The two guinea pig assays are the Magnusson Kligman Guinea Pig Maximisation Test (GPMT) as described in EC B.6, OECD 406, and ISO 10993-10:2010., and the Buehler test (EC B.6, OECD 406, ISO 10993-10:2010).

Due to the larger surface area of particles, nanomaterials may be regarded as potential allergic chemicals through their adjuvant capacity and complex formation with cell proteins (Larsen et al., 2010, Lee et al., 2011).

The above described standard tests for skin sensitisation have not been specifically evaluated for testing of nanomaterials. A significant difference exists between the LLNA and the Buehler test that both involve application of the test compounds (e.g. nanomaterials) on the surface of the skin, and the GPMT that involves intradermal application. The LLNA has been used to verify sensitisation of nanomaterials, but no positive response has been found (Lee et al., 2011). In addition, the LLNA has been used to verify whether nanomaterials can potentiate the level of sensitisation of known sensitisers (Lee et al., 2011). The value of both tests in Lee et al (2011) was challenged, because dermal penetration was not assessed. Currently, no experimental data is available on nanomaterials tested using GPMT. However, negative results were reported

for ZnO using a modified GPMT with topical application on a FCA treated skin (Jang et al., 2012, Park et al., 2013).

Based on the current knowledge, it is not possible to rely on the use of one specific test method for nanomaterials. The use of LLNA and/or Buehler test will probably not result in sensitisation due to possible low skin penetration of nanomaterials. In view of the intradermal application, the GPMT is currently probably the most relevant test for detecting sensitisation activity of nanomaterials, although the intradermal induction phase is followed by a topical induction phase and topical challenge in the intact skin.

Importantly, these tests only identify the hazard for delayed type hypersensitivity; for acute hypersensitivity mediated by immunoglobulin-E, currently no assays are available.

Genotoxicity

ISO 10993-1 indicates considerations for identifying when the potential for genotoxicity is a relevant hazard. In general, the testing for genotoxicity is not necessary for medical devices, and components thereof, made only from non-genotoxic materials. This rule might also apply for nanomaterials. ISO 10993–3:2003 describes tests for genotoxicity (carcinogenicity and reproductive toxicology).

***In vitro* genotoxicity testing**

In selecting a suitable battery of *in vitro* genotoxicity tests, the three critical genotoxicity endpoints (gene mutation, structural and numerical chromosome aberrations) should also be considered.

Although a bacterial reverse mutation assay (Ames test, OECD 471) is a reliable genotoxicity screen for the analysis of chemicals, it does not appear to be suitable for the assessment of nanomaterials. This might be related to the degree of uptake by the bacterial cells, which is likely to be less than in human cells for two reasons. Firstly, prokaryotes cannot perform endocytosis and secondly, their cell wall forms a barrier against simple diffusion of nanomaterials (particularly those in agglomerated form) into the bacterial cell – this lack of uptake could potentially lead to false negative results. Therefore, the Ames test is unlikely to be a suitable general *in vitro* genotoxicity screening test for nanomaterials, although recently also uptake of nanomaterials was observed in the Ames test (Clift et al., 2013). Additionally, modifications to the technique may need to be considered to promote uptake of nanomaterials into the Ames test bacteria to reduce the potential for false negative results (Landsiedel et al., 2009, Doak et al. 2012, Magdalenova et al, 2012, 2014).

The following *in vitro* tests are recommended for testing of nanomaterials:

1. A test for induction of gene mutations in mammalian cells (preferably the mouse lymphoma *tk* assay with colony sizing) (OECD 476)
2. An *in vitro* micronucleus assay (OECD 487) or a chromosome aberration test (OECD 473)

There may be circumstances under which it may be justified to deviate from the above-mentioned core set (e.g. when there is a need to test the nanomaterial in a matrix that cannot be added *in vitro*). In such cases, a scientific justification should be provided and additional types of considerations or *in vivo* studies may be needed. In certain instances, (e.g. soluble, very small, inducing reactive oxygen species nanomaterials) a bacterial reverse mutation test might still be informative.

For all *in vitro* tests, uptake of the nanomaterial in either bacteria or cells should be demonstrated in order to indicate potential DNA exposure to the nanomaterial under investigation.

In vivo genotoxicity testing

Unless it can be adequately demonstrated that positive *in vitro* findings are not relevant for the *in vivo* situation or if it is impossible to test the nanomaterial, *in vitro* and *in vivo* testing is necessary (Eastmond et al., 2009). Before embarking on any necessary follow-up, other relevant data on the substance, such as information about chemical reactivity (which might predispose the site of contact effects), bioavailability, metabolism, toxicokinetics, and any target organ specificity should be considered.

In vivo genotoxicity tests should relate to the genotoxic endpoint(s) identified as positive *in vitro* and to appropriate target organs or tissues. Evidence, either from the test itself or from other toxicokinetic or repeated-dose toxicological studies, that the target tissue(s) have been exposed to the test substance and/or its metabolites is essential for interpretation of negative results. The choice of the appropriate *in vivo* genotoxicity test(s) requires expert judgement based on all available information, to be applied case-by-case. Any of the following *in vivo* tests may be suitable

- an *in vivo* micronucleus test (OECD 474)
- an *in vivo* mammalian bone marrow chromosome aberration test (OECD 475)
- an *in vivo* mammalian spermatogonial chromosome aberration test (OECD 483)
- a transgenic rodent gene mutation assay (OECD 488)

an *in vivo* Comet assay (no OECD test guideline at present; internationally agreed protocols available, e.g. see hptt://cometassay.com)

However, these guidelines have been developed for testing chemicals, and their suitability for nanomaterials testing should not be taken for granted, because of their distinct physicochemical properties can seriously influence their interactions with DNA (Dusinska et al. 2009; Warheit and Donner 2010, Magdalenova et al, 2012, 2014).

Caution is needed with the micronucleus test when nanomaterials are tested. Cytochalasin B, which is often used to inhibit cytokinesis, may also inhibit endocytosis, and hence, has been suggested to lead to false negative outcomes with nanoparticles (Landsiedel et al., 2009), especially when Cytochalasin B and the nanomaterials are added to the test system simultaneously at the start of the experiment. This might be avoided by adding the Cytochalasin B after the start of the incubation (e.g. at 6 hours after adding the nanomaterials to the cells).

Moreover, for several types of nanoparticles (e.g. titanium dioxide, multi-walled carbon nanotubes), the microscopic evaluation of cytokinesis-block proliferation index and micronucleus identification was found to be rather difficult at high testing concentrations due to the abundant presence of nanomaterials in the cells (Corradi et al., 2012). This problem might be (partly) solved by for example histological staining with fluorescent-labelled DNA probes that reduces the risk of falsely identifying nanoparticle aggregates as micronuclei fragments in the micronucleus test (Magdolenova et al., 2014). In the comet assay, it was shown that nanomaterials tested did not interact with endonucleases used for detection of DNA breaks (Magdolenova et al., 2012).

Haemocompatibility

The ISO 10993-4:2002 (and its amendment 10993-4:2002/Amd 1:2006) standard is applicable to devices that contact the circulating blood and serve as a conduit into the vascular system. Medical devices that need to be evaluated for their blood compatibility include external communicating devices that have an indirect blood contact, external communicating devices directly in contact with circulating blood, and implant devices that are placed largely or entirely within the vascular system.

Most tests for haemocompatibility according to ISO 10993-4:2002 are based on direct contact between a surface and whole blood or components of blood. Thus materials with

nano-structures on their surface can be directly evaluated using the same methods described in the 10993-4 standard on selection of tests for interactions with blood.

For nanomaterials in general or in particular form there are no established tests available today. One of the tests in the 10993-4 standard, the haemolysis test, is based on testing of extracts and a suspension of nano-particles could thus be used for testing.

When contact with blood is possible, especially for free nanomaterials/nanoparticles a potential interaction with phagocytic cells, e.g. polymorphonuclear cells and monocytes, has to be carefully considered. The nanoparticles may be presented with different surface properties and in different aggregate forms depending on which medium they are suspended in. These factors are critical for the interaction with phagocytic cells.

No standards are currently available for the evaluation of particle and especially nanoparticle interaction with phagocytic cells. Although in many *in vitro* tests, phagocytic macrophages are used as target cell. One possible way to indirectly evaluate the haemocompatibility of particulate nano-material is to inject a suspension of nano-particles into the vasculature and evaluate the distribution as well as any local and systemic signs of adverse events like vascular damage, activation of complement, activation of the coagulation cascade or activation of platelets. Methods for testing the activation of complement, coagulation cascade and activation of platelets are described in the 10993-4 standard.

Repeated- dose toxicity

The ISO 10993-11:2006 describes specifically for medical devices tests for repeated dose toxicity appropriate for the route and duration of exposure. Repeated dose toxicity testing for chemicals is described in various OECD test guidelines (407, 408, 409, 411, 412, 413, 415, 416, 422, 443, 451, 452, 453).

ISO 10993-11:2006 addresses the evaluation of generalised systemic toxicity, not specific target organ or organ system toxicity, even though these effects may result from the systemic absorption and distribution of substances released from medical devices. Because of the broad range of substances used for the production of medical devices and intended uses, this part of ISO 10993 is not overly prescriptive. Whilst it addresses specific methodological aspects to be considered in the design of systemic toxicity tests, proper study design for the evaluation of nanomaterials must be uniquely tailored to the nature of the nanomaterials present in a medical device and its intended clinical application or use.

Whenever possible, the nanomaterials in medical device shall be tested in a form representative of its "ready to use" state and applied under most adequate conditions in which it is to be used. Testing shall be performed on nanomaterials obtained from the final product and/or representative component samples of the final product.

Preferably, the repeated dose toxicity studies should be performed based on the location of the potential exposure, i.e. the site of the use of the medical device, and the knowledge regarding the toxicokinetics of the released nanomaterials. However, due to practical reasons, most of the repeated-dose toxicity testing is performed using oral route. The administration of test material in the *in vivo* oral toxicity studies could be done by adding the nanomaterial to the animal feed, to the drinking water, or by gavage. In this case, information should be available on the occurrence of potential differences in the bioavailability of the nanomaterial depending on the route of exposure as was demonstrated for Au nanoparticles for intratracheal and intravenous administration (Oberdörster 2010, Semmler-Behnke et al., 2008).

For administration the nanomaterial should ideally be homogeneously blended into the feed matrix or stably and uniformly dispersed in the drinking water or gavage vehicle. The stability and physico-chemical characteristics of the nanomaterial in the vehicle

should be determined. Possible interactions with the administration vehicle should be also determined in advance, before choosing the way of exposure to nanomaterials.

There may be limitations on the amounts of nanomaterial that can be administered, because it may agglomerate in the drinking water or gavage vehicle, or they may already be blended as agglomerated powder into the feed, which in addition, may not be uniformly mixed within the food matrix. The administration of the test material requires careful control and dynamic characterisation of tested nanomaterial in either the liquid or the feed matrix. For example, a nanomaterial in liquid may adsorb to the walls of the drinking vessel and therefore becomes no longer available (i.e. there will be no exposure).

To overcome some of the obstacles mentioned above, a nanomaterial can be applied by gavage, aiming for the nanomaterial to be dispersed, characterised and administered under well-defined conditions. However, application by gavage is not likely to be representative of the lower concentrations delivered over time from nanomaterial administered via feed. Gavage provides a bolus of the material at a given time that may or may not mix with the gastrointestinal fluids, which might result in a higher local concentration and increased quantity of absorbed material due to the nanomaterial being in the form of a single, large dose and the lack of co-ingestion of dietary components to which nanomaterial can easily bind.

In any of the oral administrations mentioned above, one has to consider that the passage through the acid environment of the stomach and mixing with the chyme in the gut may affect the nanomaterial. Consideration of the potential for time dependent dissolution/ degradation is essential, as is the consideration of physico-chemical nanomaterial modifications such as agglomeration and surface modifications by proteins and biomolecules.

However, the systemic availability of nanomaterials after oral administration may be limited (see 3.6.4). Initial toxicokinetic studies might indicate whether oral administration is a proper method for identifying potential systemic toxicity. Other routes for evaluating systemic toxicity may also need to be considered (e.g. intravenous, subcutaneous administration) depending on the use of the medical device.

Implantation

At present, there are no accepted or validated methods for biological evaluation of implanted nanomaterials. However, some guidance can be found in ISO 19003-6:2007. The test methods apply to a wide range of materials such as solid and non-absorbable, absorbable, non-solid (such as porous materials), liquids, gels, pastes and particulates.

The test methods may also be applied to medical devices that are intended to be used topically in clinical indications when the surface or lining may have been breached, in order to evaluate local tissue responses.
The local effects are evaluated by a comparison between the tissue response caused by a test specimen to the tissue response caused by control materials used in medical devices of which the clinical acceptability and biocompatibility characteristics have been established. The objective of the test methods is to characterise the history and evolution of the tissue response after implantation of a medical device/biomaterial including final integration or absorption of the material. In particular, for absorbable materials the degradation characteristics of the material and the resulting tissue response should be determined. All materials will provoke an inflammatory response when implanted. It is the extent and seriousness of this local inflammatory reaction that indicates whether this reaction should be considered adverse. For non-degradable materials, a steady state on the tissue response is generally obtained after 12 weeks, while for absorbable materials this depends on the rate of absorption that may be shorter or much longer than 12 weeks.

ISO 10993-6:2007 on implantation testing does not deal with systemic toxicity, carcinogenicity, teratogenicity or mutagenicity. However, the long-term implantation studies intended for evaluation of local biological effects may provide insight into some of these properties. Systemic toxicity studies conducted by implantation (ISO 10993-11:2006) may satisfy the requirements of this part of ISO 10993-6. When conducting combined studies for evaluating local effects and systemic effects, the requirements of both standards needs to be fulfilled.

It can be reasonably anticipated that the tissue response to absorbable implant materials will be different from the tissue response found in non-absorbable (durable) implants. The assumption should be one of continuous interaction of the degrading material with the surrounding tissue, accompanied with an ongoing presence of a degradation-rate-dependent tissue response. Such a response may vary over time and may (or may not) be histologically detectable dependent upon the composition and manufacturing of the materials, the rate of degradation, the time post-implantation, and the tissue within which the implant resides. This tissue response should resolve and normal morphology restored as the degrading material is absorbed into the surrounding tissue.

To properly evaluate an absorbable implant and its degradation products, local tissue response may need to be assessed at more and different study intervals than those typical for non-absorbable materials. The provisions in ISO 10993-6 (Annex A, General considerations regarding implantation periods and tissue responses to absorbable materials) are also applicable to the evaluation of the local effects of absorbable materials used as carriers for drug release, scaffolds for tissue-engineered medical products, or surface coatings for non-absorbable implants.

The particles may have a local effect at the site of the implant but may also show migration, for example, to the draining lymph nodes. Of course, the local effects are limited to the site of the implantation (or use of the medical device) and depend on that localisation. An example of such a local effect is wear of joint prostheses leading to particle accumulation in synovial fluid and synovial tissues. Biological effects are greatly influenced whether the particles are deposited in subcutaneous tissue, intra-peritoneally or into the blood.

Chronic toxicity/carcinogenicity

ISO 10993–3:2003 describes tests for genotoxicity, carcinogenicity and reproductive toxicity. The decision to perform a carcinogenicity test that usually lasts for 2 years, shall be justified on the basis of the potential exposure arising from the use of the medical device, nanomaterials and or their extracts. However, in practice, it is rarely considered applicable to investigate carcinogenicity because of the already existing knowledge about the material used for a medical device. The most common *in vivo* tests to assess the carcinogenic potential of chemicals are:

a) Carcinogenicity test [EC B.32, OECD 451]
b) Combined chronic toxicity/ carcinogenicity test [EC B.33, OECD 453]

But no indication about their suitability for nanoparticles has been provided so far. Therefore, the use of such tests should be evaluated on a case-by-case basis.

Reproductive and developmental toxicity

Before a decision to perform reproductive and developmental toxicity tests is made, ISO 10993-1:2009 and ISO 10993-3:2003 should be taken into consideration.

There is no need for reproductive toxicity testing of resorbable medical devices or medical devices containing leachable nanomaterials/nanoparticles if there are adequate and reassuring data from absorption, distribution, metabolism and excretion (ADME) studies indicating that the test item (nor its metabolites) is not distributed and does not

reach the reproductive organs/targets or on lack of the reproductive toxicity of all components in extracts of medical devices.

In the absence of evidence to rule out reproductive/developmental risks, testing should be considered. This may include tests on the following medical devices containing nanomaterials:

prolonged or permanent-contact medical devices likely to come into direct contact with reproductive tissues, embryos or foetus;

energy-depositing medical devices;

resorbable or containing leachable nanomaterials/nanoparticles.

If testing is required, this shall start with OECD 421 (Reproduction/Developmental Toxicity Screening Test) in order to provide initial information on possible effects on reproduction and/or development. Positive results with tests are useful for initial hazard assessment and contribute to decisions with respect to the necessity for timing of additional tests. If additional tests are considered necessary, they shall be performed in accordance with OECD 414 (Prenatal Developmental Toxicity Study), OECD 415 (One-Generation Reproduction Toxicity Study), OECD 416 (Two-Generation Reproduction Toxicity Study) or OECD 422 (Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test), as appropriate. No indication is available on the suitability of these tests designed for chemicals to assess the reproductive toxicity potential of nanoparticles. Therefore, the use of such methodologies should be evaluated on a case-by-case basis.

More recently test guideline OECD 443 was published on the so-called extended one generation reproductive toxicity study which combines several endpoints including reproductive/developmental endpoints, neurodevelopmental and immune developmental endpoints.

Methods for embryotoxicity testing are likely to be applicable to nanomaterials, provided that typical nanomaterial related issues such as dispersion/ aggregation, adsorption, stability and distribution into the tissue are taken into consideration. In an *in vitro* embryonal stem cell assay, which was used for research purposes only, effects on cardiomyocyte development were observed for silica nanoparticles (Park et al. 2009).

Assessment of effects on the first generation (F1) or even second generation (F2) shall be made in accordance with OECD 414, OECD 415, OECD 416, OECD 421, OECD 422 or OECDE 443. As the OECD guidelines were not intended for nanomaterials/nanoparticles in medical devices, the following modifications shall be considered: dose (in the case of energy-depositing devices), route of application (implant, parenteral, other), extraction media (aqueous and non aqueous extracts) or exposure time.

It is not recommended to use methods of exposure that for some reason could affect prenatal development. For example, intraperitoneal administration may cause the tested nanomaterials/nanoparticles to be directly injected in the uterus itself or pass through the wall of the uterus and directly affect the developing embryos/fetuses. Inhalation exposure "nose only" does not seem to be appropriate for pregnant females due to the fact that the animal being tested is kept under forced, stressful conditions and tightly restrained for about 6h/day without access to feed and water.

In the developmental toxicity study one should be aware of possible exposure to offspring via breast milk (Melnik et al., 2013) The presence and concentration of nanomaterials/nanoparticles in the milk of lactating animals should be measured.

3.8. Evaluation of nanomaterials used in medical devices

The evaluation of the risk of chemicals leaching from a medical device is described in EN ISO 10993-17:2002. The methodology for the evaluation of allowable limits for chemicals may also be applied to nanomaterials. In this standard, the estimated exposure needs to be compared with the toxicity information. In addition, the benefit for the patient needs also to be considered in the evaluation of medical devices.

In addition to the selection of safety evaluation assays as presented for medical devices in ISO 10993-1:2009, specific testing for the nanomaterials used in a medical device may be necessary. The testing to be performed is determined similarly to ISO 10993-1 but now based on the potential for release of the nanomaterials from the device and the duration of exposure. According to ISO 10993-1 the need for testing for hazard identification is based on the type of medical device, type of contact and duration of exposure. A schedule is proposed in Table 4.

Table 4: Framework for specific nanomaterial toxicity testing based on potential release (exposure) of nanomaterials from medical devices.

Testing proposed	**Non invasive short term use**	**Non invasive long term use**	**Invasive short term use**	**Invasive long term use**
Low exposure	Phys: chem data	Phys: chem data	Phys: chem data	Phys: chem data
	Cytotoxicity *in vitro*	Cytotoxicity *in vitro*	Cytotoxicity *in vitro*	Cytotoxicity *in vitro*
	Irritancy *in vitro*	Irritancy *in vitro*	Irritancy *in vitro*	Irritancy *in vitro*
	Hypersensitivity	Hypersensitivity	Hypersensitivity	Hypersensitivity
		Genotoxicity *in vitro*		Genotoxicity *in vitro*
				General Immuno toxicity testing
Medium exposure Additional tests		Genotoxicity *in vivo*	Other *in vitro* plus *in silico* testing*	28/90 day *in vivo* toxicity test
		Immuno toxicity at location site	Genotoxicity *in vitro* and *in vivo*	Full genotoxicity testing
		Persistence /accumulation studies at location site only		ADME including persistence /accumulation studies
High exposure Additional tests	Selected *in vivo* acute toxicity tests focussed on location site(s)	Selected *in vivo* chronic toxicity tests focussed on location site(s)	*In vivo* acute toxicity tests	*In vivo* chronic toxicity tests may include reprotox depending on patient group.

*See also ECVAM database (http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/validation-regulatory-acceptance/)

3.8.1. Non-invasive surface contacting medical devices

This category applies to devices that contact intact skin and breached or compromised surface (ISO 10993-1:2009)

Surface contacting medical devices will interact locally as long as the skin has not been breached. There is little or no evidence that nanoparticles will penetrate the natural skin. Therefore, potential for internal systemic exposure is low or negligible regardless the type of application (Table 1). The local effects should be evaluated (e.g. cytotoxicity and irritation) using the same principles as medical devices without nanomaterial components. However, established methods for the evaluation of sensitisation potency of nanomaterials are at the moment not available.

3.8.2. Invasive surface contacting medical devices

If there is a concern that the barrier properties of the skin are changed by a wound or an inflammatory process, the possibility that nanoparticles may penetrate and become deposited locally and migrate to other localisations should be considered. This may be done by investigating the actual penetration through compromised skin in an animal experimental model. Such experimental models are difficult to establish and validate, and a better approach may be to investigate the effects of intra-dermally or subcutaneously introduced particles. This may follow the protocol of the already established intracutaneous irritation test as described in ISO 10993-10:2010, but extended to include histological evaluation of draining lymph nodes.

When a nanomaterial containing medical device is in contact with breached or compromised skin and nanomaterials are released additional testing for systemic toxicity should be considered including genotoxicity testing independent of the contact duration time. A suitable battery of *in vitro* genotoxicity tests addressing three critical genotoxicity endpoints (gene mutation, structural and numerical chromosome aberrations) should be considered.

3.8.3. Invasive external communicating medical devices

This category applies to devices that can contact circulating blood at one point and serve as a conduit for entry into the vascular system (indirectly), circulating blood directly and making contact with tissue, bone, pulp/dentin (ISO 10993-1:2009). Invasive external communicating medical devices may contain nanomaterials that maybe released after material degradation or be present as nano-size structures on its surface. Dialysis and oxygenating equipment are also included in this category.

It is of vital importance to consider the type of tissue that may be exposed. The effect on draining lymph nodes or other organs that may be reached after particle migration should be investigated. The presence of nanoparticles in tissue should be investigated using the appropriate identification techniques (e.g. ICP-MS, electron microscopy, fluorescent dye labelling) and, whenever possible, a quantitation should be performed.

3.8.4. Invasive implantable medical devices

An invasive medical device is defined as a device that, in whole or in part, penetrates inside the body, either through a body orifice or through the surface of the body (Directive 93/42/EEC). This includes implant devices that contact principally blood, tissue and bone (ISO 10993-1:2009). Medical devices applied through body orifices coming into contact with mucosal membranes are also considered invasive medical devices. In general testing of this type of medical devices is performed according to ISO 10993-1 thus depending on the type of tissue contact and the duration of the contact. For nanomaterials used in medical devices a similar approach needs to be considered although special emphasis should be the potential release of the nanomaterials from the devices. Similarly to invasive external communicating devices local particle release needs to be considered and possible effects on draining lymph nodes. Supporting data, if available, on the toxicological evaluation of nanomaterial ingredients may be used in the safety evaluation of medical devices. Depending on the release, the safety evaluation of the nanomaterial itself might be considered, taking into account the intended use of the medical device in which the nanomaterial is used.

3.8.5. Specific types of medical devices

For **wound care materials,** specific considerations apply. In some wound dressings, nanosilver is used for its antibacterial activity (Wijnhoven et al., 2009, SCENIHR 2013). They are also used on breached and compromised skin. Therefore, direct contact with subcutaneous tissues including blood is possible. When there is a considerable release of the nanomaterials used in wound dressings, systemic exposure may also be possible. Hence, a more extensive risk evaluation of the nanomaterial component needs to be considered (SCENIHR 2013).

Dental and bone fillers and cements may contain and even consist of free nanoparticles. Mostly cements and dental fillers are cured *in situ* resulting in a solid mass of (bio)material. During the application of dental materials and also during polishing nanoparticle exposure may occur. Depending on the application site (dental use in the oral cavity or orthopaedic use of bone cement), internal exposure to nanoparticles is possible. For dental materials, lung exposure should also be considered (see below).This specific potential internal exposure needs to be considered in the risk evaluation of such materials.

For **injectable nanomaterials,** the potential of internal exposure is obvious and can be rather high depending on the dose administered. For these applications, extensive distribution studies are warranted. The extent of the systemic exposure is dependent on the injection site. For subcutaneous injection, the distribution via the local draining lymph nodes needs to be evaluated. However, further distribution should also be investigated as it cannot be assumed that further distribution beyond the local lymph node does not occur. For other injections, systemic exposure is likely, or certain (e.g. after intravenous administration) and extensive toxicokinetic and systemic toxicity studies are warranted.

Medical devices resulting in respiratory tract exposure. When nanomaterials are used in medical devices applied in the respiratory tract, the possibility for lung exposure exists. The handling of dental materials may also result in respiratory tract exposure to particles (Van Oberdörster et al. 2014). Inhalation of various particles was shown to consistently induce local adverse effects in the lung. Inhaled particles reach different target compartments of the lung tissues depending on their size e.g. particles about below 50 nm in diameter seem to be most effective in reaching the pulmonary alveoli (ICRP 1994, Cassee et al., 2002). In addition, for lung exposure of nanomaterials effects on the cardiovascular system should also be considered (Donaldson et al. 2013a).

3.8.6. Conclusions

Non-invasive medical devices containing nanomaterials, with the exception of local reactions at the site of contact, do not in most instances pose an additional risk compared to non-invasive medical devices not containing nanomaterials and may be evaluated using the same methodology.

For invasive medical devices containing nanomaterials, including surface contacting devices in contact with breached skin or mucosa, the same principles for toxicity testing apply as for medical devices not containing nanomaterials. However, the biological effects of nanoparticles that are introduced or formed should be investigated both for local effects at the site of application and at possible distribution organs after migration, especially draining lymph nodes. In the safety evaluation, itself the potential release, accumulation, and persistence of the nanomaterials in the tissues is of utmost importance for the need of further testing. In this context, the possible dissolution/degradation of the nanomaterials also needs to be considered.

All safety evaluations should consider the potential specific physical-chemical properties of these nanomaterials, especially those medical devices that consist of free nanomaterials. The biological effects of nano-particles that are introduced should be investigated both at the site of deposition and at possible target organs for migration, especially draining lymph nodes.

In addition, the potential generation of nano-sized particles due to wear and tear needs to be considered for all implant medical devices.

4. RISK EVALUATION

An estimation of the potential risk can be made based on the information obtained on nanomaterial characteristics, use as or in a medical device. The exposure can be considered as the outcome of the potential release from the medical device in the actual use conditions (exposure scenario) and the toxicokinetics of the nanomaterial (giving indication of the possible internal exposure). The risk can be estimated based on the potential exposure and the outcome of the safety testing according to ISO 10993-1:2009. Of major importance for the risk assessment is the possibility for release of the nanomaterial from the medical device. If particle release is not present, it is assumed that material and surface properties that may result in local reactions like inflammation and/or induction of allergy, and which may be related to particle reactivity, are adequately covered by the existing testing regimen as presented in ISO 10993-1:2009. Analogously, in the absence of any absorption, no systemic toxicity testing needs to be carried out.

A phased approach to the risk assessment related to particle release is proposed below and is illustrated in the Figure 2.

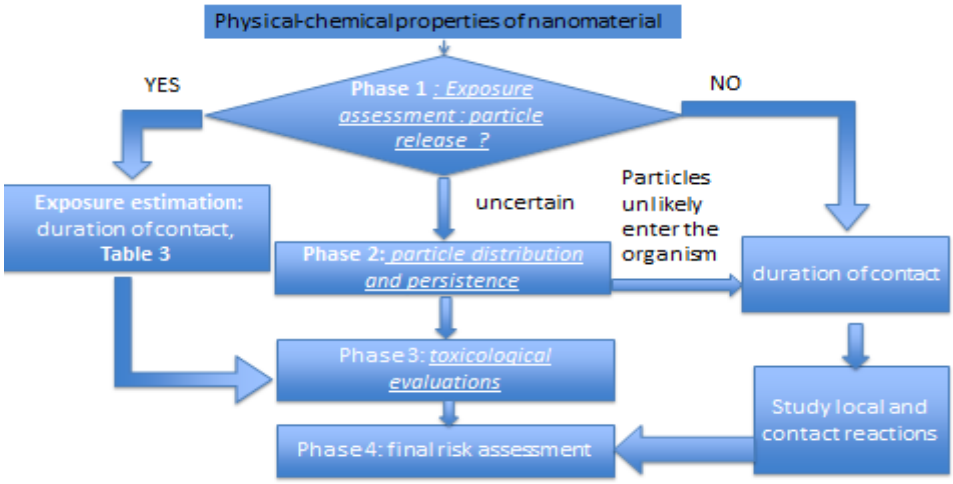

Figure 2: Risk assessment of nanomaterials used in invasive medical devises: a phase approach

Phase 1 Exposure assessment: particle release.

The purpose of this first phase is to consider the likelihood that nanoparticles will be released to estimate potential exposure, either as an intrinsic property of the device or due to wear once implanted. If there is reliable evidence that the nanomaterials are embodied in the device or so well fixed that they will be retained in the device during insertion, period of use and removal then, provided particles are not released as a consequence of wear, no further specific risk assessment regarding the nanoparticle component is required. It is important, however, that the relevant data on non-release are obtained under realistic worst case conditions.

For the exposure assessment, the information as presented in section 3.5.4 Table 3 can be used.

If even a small release of particles is considered possible, then evaluation of the physicochemical properties of the released particles is necessary. It is essential that the particles studied in assays for the risk assessment are equivalent, in terms of both physical and chemical properties, as those that may be released *in situ*.

Physical-chemical properties that need to be considered include:

- Solubility in water. If solubility in water is prompt then no further consideration is needed in regard to the particulate nature of the released material though of course the potential adverse effects of the solubilised material will need to be considered further.
- Particle size distribution and shape. The mobility of particles and the effectiveness of the biological defence mechanisms to deal with them is affected by both the size and shape of the particles.
- Ability to agglomerate and dis-agglomerate. The ability for particles to combine and dissociate is also a factor that affects particle size. The larger the particle size in biological media the less the retention of the surface active properties that are associated with nanoparticles.
- Other characteristics dependent on the nanomaterial used (see also ISO TR 13014:2012)

The realistic worst case conditions for identifying the amount, estimated rate and number of released nanomaterials need to take into account the potential duration of contact of the medical device with the body. When significant exposure is expected due to nanoparticle release further investigation is necessary. The definition of what is considered to be significant is dependent on the particular type of nanomaterial.

If particle release does not occur, the further evaluation may be limited mainly to investigating local reactions. If there is significant uncertainty regarding potential release of (nano)particles then a phase 2 assessment should be embarked upon.

Phase 2 Exposure assessment: particle distribution and persistence

i) particle distribution

The primary purpose of this phase is to identify the kinetic of the particles to address the toxicity testing needed in Phase 3 (below) based on potential exposure scenarios indicated above. It is self-evident that the absorption of particles released from non-invasive medical devices into the systemic circulation and/or location of the invasive device on/in the body and contact duration will have a major influence on the potential for distribution of the particles to other organs. A further consideration is the persistence/stability of the particles in the biological media into which they are released.

a) Non-invasive (skin)

The key issue is to identify the likelihood of significant penetration of the skin barrier. If insignificant/negligible, then only the potential for effects at the topical site of application need to be examined in phase 3.

b) Invasive

Significant uptake of particles from the lung into the systemic circulation is more likely than from other external location sites. Therefore, the potential for released particles to reach the deep lung (alveolar region) and cross into the systemic circulation must be estimated. In addition, local effects in the lung itself need to be considered as most if not all particles induce lung inflammation.

Additionally, for other invasive devices the distribution of released particles needs to be estimated, in particular, whether they remain at the site of the application of the device (if this can be demonstrated then the potential for accumulation needs to be given

specific attention, but in principle only local toxic reactions need to be considered in the next phase). If a more general distribution may be possible, or if this is uncertain, a more in-depth evaluation of the toxicokinetics is needed.

For external communicating devices, e.g. dialysis equipment, the release of particles entering the systemic circulation has to be followed by appropriate toxicokinetic studies.

ii) Particle persistence

Both the number and the duration of particle presence in a specific tissue are important considerations affecting the likelihood of adverse effects occurring. Prolonged exposure of a tissue to released particles may arise for two reasons:

- Continuous release from the device
- Stability of the particles and their entrapment in a tissue or failure of clearance mechanisms.

The release due to the use of the device can be estimated based on short-term physicochemical studies as can the likely stability of the particles. Where significant release appears likely *in vivo* animal studies may be necessary to achieve adequate characterisation of the internal exposure.

Phase 3 Hazard assessment (toxicological evaluations).

If particle release is not identified in phase 1 and/or phase 2, local effects of medical devices are assumed to be adequately covered by the existing testing regimen as presented in ISO 10993-1:2009.

Additional studies are necessary, if there is a significant release of particles. In deciding on the testing strategy, the likely location (as identified in phase 2) is crucial information. If it is estimated from phase 2 that it is unlikely that particles that are released will enter the systemic circulation, then only tests to establish local effects are required. It is vital in such studies that the form of the nanoparticles used in the various studies is equal to that which is actually used and present (either released or created) in biological systems

a) Characterisation of local effects

Of particular interest are the potential for:

- Irritation
- Immune reaction
- Cytotoxicity
- Genotoxicity
- Promotion of cell division

In principle, some of these effects (e.g. genotoxicity) may be assessed initially in *in vitro* systems as described in section 3.7.3, provided such test systems allow the penetration of the nanoparticles into the cell systems.

b) Characterisation of systemic effects

When there is exposure to a significant level of particles in one or more tissues, a case-by-case approach needs to be adopted for which the approach of Table 4 in section 3.8 can be of help. Standard toxicity tests (see section 3.7.3) are suitable to assess the hazard although particular attention should be the ability of the particles to concentrate in the draining lymph nodes and other organs of the mononuclear phagocyte system. This may require some adaptation of traditional toxicity assessment protocols.

For acute exposures, only the scope of testing would be limited to acute studies unless there is a likelihood that a similar device is likely to be used in the same patient on a number of occasions.

Phase 4 Risk characterisation/risk assessment.

Based on the possibility for exposure, the following categorisation of the necessary risk assessment can be made (Table 5).

Table 5: Framework for risk assessment of nanomaterials used in medical devices

Release of nanoparticles	**Non invasive**		**Invasive Lung**		**Invasive Other**	
	Short exposure	Long exposure	Short exposure	Long exposure	Short exposure	Long exposure
Low/insignificant	N/VL*	L/F**	L	F	L	F
Medium	L/F	L/F	L/F	F	L/F	F
High	L/F	L/F	F	F	F	F

F=full assessment L=limited assessment VL =very limited or N= no further assessment
**=limited assessment if it can be shown that penetration/distribution is very limited.*

*** Full assessment when absorption is indicated in toxicokinetic studies*

In cases where significant toxicity is found to be induced by the nanomaterial used, particular attention must be given to the dose response relationship. The findings should be compared against the levels of particles found in the target organs (internal exposure) in order to evaluate the risk. The estimated risk may be compared to the risk from the use of comparable devices not incorporating nanomaterials, and assessed according to ISO 14971. In addition to the estimated potential risk, ultimately also the potential benefit for the patient should be considered in the final benefit risk evaluation.

5. SUMMARY AND CONCLUSIONS

In the light of current knowledge, a case-by-case approach is necessary for risk evaluation of medical devices containing nanomaterials. A phased approach is proposed to avoid unnecessary testing.

In phase 1 an evaluation is needed of the potential for the device to release nanoparticles either directly or due to wear of the device during use. If the nanomaterial is fully embedded in the device, only the consideration of potential wear resulting in the release of particles will probably be necessary. In addition, potential local effects of the device incorporating nanomaterials need to be considered. For other devices containing nanoparticles, both release and wear considerations are necessary. If release of particles during the use of the medical device is deemed to be realistic, physicochemical tests are likely to be required to establish the nature of the released particles, the rate of release and factors likely to influence this. If as a result of these studies, it is concluded that even under realistic worst-case use conditions particle release will be very low, no further consideration of the risk should be required. Further considerations are needed when a substantial release is noted.

In phase 2, the aim is to determine the distribution of the particles released and also their persistence potential. In the case of non-invasive devices, the potential of particles to enter the systemic circulation and thereby, be distributed to various tissues is the prime consideration. If it is concluded that it is unlikely that the particles could enter the systemic circulation even under realistic worst-case conditions of use, then only a very limited toxicity testing protocol is needed, which would be generally limited to local effects at the contact site.

For invasive devices, a more detailed study of the potential of the particles to access and remain in specific tissues is required by toxicokinetic studies. The findings from these studies will influence the choice of further toxicity testing methods.

In phase 3, the hazard is assessed by selecting toxicity tests that are relevant based on the nature of the observed exposure and potential persistent in specific organs.

In the future, as our knowledge of the properties of nanomaterials improves, it may be possible to predict the nature, distribution, tissue levels and potential persistence of the particles, but this is unlikely to be possible in the near future.

The information gathered will give input for the final risk characterisation (phase 4). The estimated risk needs to be compared to the risk from the use of comparable devices not incorporating nanomaterials in judging the acceptability of the risk. In addition to the estimated potential risk, ultimately also the potential benefit for the patient should be considered in the final risk assessment.

In conclusion, the potential risk due to the use of nanomaterials in medical devices is mainly associated with the possibility for release of free nanoparticles from the device and the duration of exposure. The potential release is dependent on the method of use of the nanomaterials, either as free nanomaterial, nanomaterials fixed on surfaces or nanomaterials embedded in a matrix. In addition to particle release and potential effects of these particles, possible local effects at the site of application should also be considered. It should be realised that wear and tear of a medical device may also result in the generation of nano-sized particles, even when the medical device itself does not contain nanomaterials.

6. MINORITY OPINION

None

7. ABBREVIATIONS AND GLOSSARY OF TERMS

Term	Explanation
AAMI	Association for the Advancement of Medical Instrumentation
AAS	Atomic Absorption Spectroscopy
ABPM	Ambulatory Blood Pressure Measurements
Absorption of energy	The way by which the energy of a photon, which is the quantum of the electromagnetic field, is taken up by matter, typically the electrons of an atom.
ADME	Absorption, Distribution, Metabolism, and Excretion (toxicokinetics)
AIMD	Active implantable medical device
AFM	Atomic-force microscopy
AUC	Analytical Ultracentrifugation
ARMD	Adverse Reaction to Metal Debris, overall description of local reactions (observed by histopathology) near metal on metal hip prostheses due to release of metal particles
BET	Specific surface area measurements; Brunauer–Emmett–Teller (BET) theory aims to explain the physical adsorption of gas molecules on a solid surface
CLS	Centrifugal liquid sedimentation
DLS	Dynamic Light Scattering is a method for measuring the particle size distribution in an ensemble
DMA	Differential mobility analysis
EDX	Energy dispersive X-ray allows analysis of particles down to nanometre diameters
EELS	Electron energy loss spectroscopy allows analysis of particles down to nanometre diameters
EFSA	European Food Safety Authority
EN 15051:2006	Procedure for determination of inhalable dustiness (dustiness values, stated as the ratio of the weight of the amount of released dust to the amount of material charged). The standard describes two methods that are based on a British method (MDHS 81) and a presently withdrawn German method (DIN 33897:2). The two methods represent different systems for supplying the mechanical energy.
FESEM	Field Emission Scanning Electron Microscopy
FEGSEM	Variant of FESEM, with a gun emitter
FFF	Field-flow fractioning

FTIR	Fourier Transform Infrared Spectroscopy
Free nanomaterial	Nanomaterials that are not encapsulated or connected in some way to prevent them from being released in the organs, tissues or cells of the user
GC-MS	Gas chromatography–mass spectrometry: the sample is usually ionized directly or indirectly by an electron beam. The high-energy electrons cause the formation of free radical ions.
HDC	Hydrodynamic chromatography
HPLC	High-performance liquid chromatography
HRTEM	High Resolution Transmission Electron Microscopy
ICP-MS	Inductively Coupled Plasma - Mass Spectrometertry
LC-MS	Liquid chromatography – mass spectrometry
LDE	Laser Doppler Electrophoresis
LLNA	Local Lymph Node Assay, murine assay to evaluate potential of chemicals for induction of delayed type hypersensitivity.
MPI	Magnetic Particle Imaging
MS	Mass spectrometry
Nano-object	A material with one, two or three external dimensions on a nanoscale. Nano-objects with two external dimensions on the nanoscale and a larger third dimension include nanofibres, nanotubes, nanofilaments or nanorods.
Nano-particle	A nano-object with three external dimensions on a nanoscale
Nano-reinforced materials	Nano-objects included in their matrices to introduce a new function or to alter physical and mechanical properties. Nanocomposites are a typical case.
Nanoscale	Dimensions between 1 and 100 nanometers
Nano-structured material	A material with a surface or internal structure on a nanoscale and possessing one or more new physical, chemical and biological properties specific to the nanoscale.
NMR	Nuclear Magnetic Resonance
OECD	Organisation for Economic Co-operation, Paris, France
PALS	Phase analysis light scattering (PALS configuration has been shown to be able to measure mobility at least two orders of magnitudes lower than conventional LDE)
PET	Positron emission tomography
PTA	Particle-tracking analysis is a counting method that study particle by particle
Redox potential	A measure of the tendency of a chemical species to acquire electrons and thereby be reduced

SAR	Structure Activity Relationship
SAXS	Small-Angle X-ray Scattering reports on intensity-weighted particle size; it is in the same class of methods as DLS
SCCS	Scientific Committee on Consumer Safety
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCHER	Scientific Committee on Health and Environmental Risks
SEC	Size-exclusion chromatography
SEM	Scanning Electron Microscopy
SERS	Surface enhanced Raman Spectroscopy
SIMS	Secondary Ion Mass Spectrometry
SMPS	Scanning Mobility Particle Size
SP-ICP-MS	Single particle inductively coupled plasma mass spectrometer
SPM	Suspended particulate matter
SPM	Scanning Probe Microscopy
STEM	Scanning Transmission Electron Microscopy. Offers an alternative configuration of TEM and an extended range of analytical methods. In the STEM, as in the SEM, a finely focused electron beam is scanned across a raster on the specimen.
STM	Scanning Tunnelling Microscopy
TDI	Tolerable Daily Intake
TEGDMA	Triethylene glycol dimethacrylate
TEM	Transmission Electron Microscopy
TNF	Tumour Necrosis Factor
US-EPA	United States Environmental Protection Agency
UV spectroscopy	Ultra-violet spectroscopy intended for chemical analysis
UVVis	Ultra-violet visible spectroscopy
XPS	X-ray photoelectron spectroscopy, also known as ESCA
X-ray absorption spectroscopy	A technique for determining the local geometric and/or electronic structure of matter
XRD	X-Ray Diffraction is a method for measurement of an average size value without giving information about the size distribution

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Annex

Performance of some Characterisation Methods

Several methods have been identified and each method has its own performance in terms of possibilities and limitations.

Electron microscopy techniques

Electron microscopy is perhaps the most generally applicable method. Scanning Electron Microscopy (SEM) and transmission electron microscopy (TEM) are two types of electron microscopes and are tools to view and examine small samples. Both instruments use electrons or electron beams. The images produced in both tools are highly magnified and offer high resolution. SEM measures the shape and size of the particles, topography of the surface and determines the composition of elements and compounds the sample is composed of. In SEM the specimen surface is scanned with a high-energy electron beam and scattered electrons are measured while the TEM is based on transmitted electron measurements TEM seeks to see what is inside or beyond the surface. SEM also shows the sample bit by bit while TEM shows the sample as a whole. In terms of magnification and resolution, TEM has an advantage compared to SEM. TEM has up to a 50 million magnification level while SEM only offers 2 million as a maximum level of magnification. The resolution of TEM is 0.5 angstroms while SEM has 0.4 nanometers. However, SEM images have a better depth of field compared to TEM produced images. In SEM, the sample is prepared on specialised aluminium stubs and placed on the bottom of the chamber of the instrument. The image of the sample is projected onto the CRT or television-like screen. On the other hand, TEM requires the sample to be prepared in a TEM grid and placed in the middle of the specialised chamber of the microscope. The microscope via fluorescent screens produces the image. Another feature of SEM is that the area where the sample is placed can be rotated in different angles.

The scanning transmission electron microscope (STEM) offers an alternative configuration of transmission electron microscopy, and with it an extended range of analytical methods. In the STEM, as in the SEM, a finely focused electron beam is scanned across a raster on the specimen. Resultant signals used to image the specimen include the intensity of the transmitted beam, secondary electron emissions and elastically scattered electrons.

TEMs are usually configurable as STEMs, although there is inevitably a degree of compromise with the electron optics, resulting in marginally reduced imaging and analysis capabilities. Spatial resolution in a dedicated STEM is typically better than 1 nm, and may approach *ca*. 0.3 nm in a high-resolution system.

Size and morphology are readily characterised in the FEGSEM, TEM and STEM. HRTEM allows structural information on particles and atomic clusters to sub-0.2 nm resolution, while EELS and EDX analysis in the STEM allow the chemical analysis of particles down to nanometre diameters. By combining analysis methods, investigation of particle size, shape, structure, composition and surface properties is in principle possible.

However, the analysis environment is harsh, and only suited to robust particles with low volatility. Analysis in the ESEM overcomes some of the analysis environment restrictions and allows in principle the characterisation of particles with a significant volatile component, although its application is currently restricted to particles larger than *ca*. 100 nm.

The use of X-ray emissions within the electron microscope is perhaps the most widely applied form of analytical electron microscopy within aerosol. Electrons interacting with

the specimen excite inner shell atomic electrons, and the decay of these excited states leads to the emission of X-rays with energies characteristic of the element.

Energy dispersive X-ray analysis (EDX) allows the quantification of elemental species of atomic number 6 (carbon) and above in the SEM, ESEM, TEM and STEM, although many detectors using a thin silicon protective window are limited to the detection of elements of atomic number 14 (silicon) and above. Analysis in the SEM is not ideal for ultrafine particles, as X-ray emissions from the holding substrate rapidly obscure those from particles under analysis.

For the same reason, spatial resolution within the SEM is relatively low (of the order of 0.5 -1 micrometer). Spatial resolution in the STEM and TEM approaches the electron beam width when using thin substrates or arranging for samples to be over a hole on the substrate. Sensitivity to high Z elements is sufficient for the identification of major elemental species in nanometer-diameter particles.

The sensitivity of EDX analysis in the TEM and STEM is limited by the relatively low detection efficiency for X-ray emissions. However, each core electron excitation within the specimen results in a corresponding energy loss within the electron beam.

By extracting energy loss information from the beam using an energy-dispersive spectrometer, increased sensitivity to core electron excitations is achievable. Electron energy loss spectroscopy (EELS) within the STEM (and TEM in some configurations) is perhaps the most powerful analysis technique available for analysing single particles within the electron microscope.

By recording and analysing the electron energy loss spectrum, details of specific inelastic interactions, and thus sample composition and structure, can be investigated. Energy losses below 50 - 100 eV are dominated by bulk electron excitations (plasmons) within the sample. At higher-energy losses, energy loss is characterised by atomic core electron excitations, appearing as `edges' on a decreasing background. The position, amplitude and shape of each edge contain information on atomic core electron excitations, and the chemical environment surrounding the atom. The energy loss at which the edge occurs is related to the atomic electron transition, allowing identification of elemental components

Scanning probe microscopy (SPM) and Scanning Tunneling Microscopy (STM)

The development of SPM methods has led to further techniques for imaging nanometer-sized particles. All methods are typified by a fine probe that is scanned in a raster across a surface. Probe position above (or on) the surface is controlled by a range of feedback signals which are also used to provide image contrast on the associated display raster.

Initial SPM development used the electron tunneling current between a conducting specimen and probe suspended a few angstroms above its surface to map topographic features at angstrom resolution (scanning tunneling microscopy (STM).

Atomic Force Microscopy

Later developments led to the use of Van der Waals forces between the specimen and the probe (atomic force microscopy (AFM)), allowing imaging of non-conducting specimens. While a gap of *ca*. 1nm is maintained between the probe and specimen in STM, AFM may be carried out with the probe in contact with the specimen, or separated by up to several tens of angstroms. AFM can measure topology, grain size, frictional characteristics and different forces. It consists of a silicon cantilever with a sharp tip with a radius of curvature of a few nanometers. The tip is used as a probe on the specimen to be measured. The forces acting at the atomic level between the tip and the surface of the specimen cause the tip to deflect and this deflection is detected using a laser spot which

is reflected to an array of photodiodes. AFM has several advantages over the scanning electron microscope (SEM). Unlike the electron microscope, which provides a two-dimensional projection or a two-dimensional image of a sample, the AFM provides a three-dimensional surface profile. Additionally, samples viewed by AFM do not require any special treatments (such as metal/carbon coatings) that would irreversibly change or damage the sample, and does not typically suffer from charging artifacts in the final image. While an electron microscope needs an expensive vacuum environment for proper operation, most AFM modes can work perfectly well in ambient air or even a liquid environment. This makes it possible to study biological macromolecules and even living organisms. In principle, AFM can provide higher resolution than SEM. It has been shown to give true atomic resolution in ultra-high vacuum (UHV) and, more recently, in liquid environments. High resolution AFM is comparable in resolution to scanning tunneling microscopy and transmission electron microscopy. AFM can also be combined with a variety of optical microscopy techniques, further expanding its applicability. Combined AFM-optical instruments have been applied primarily in the biological sciences but have also found a niche in some materials applications, especially those involving photovoltaics research {Ref. Geisse, Nicholas A. (July–August 2009). "AFM and Combined Optical Techniques". Materials Today 12 (7-8): 40–45. doi:10.1016/S1369-7021(09)70201-9}. A disadvantage of AFM compared with the scanning electron microscope (SEM) is the single scan image size. In one pass, the SEM can image an area on the order of square millimeters with a depth of field on the order of millimeters, whereas the AFM can only image a maximum height on the order of 10-20 micrometers and a maximum scanning area of about 150×150 micrometers. The scanned area size for AFM can be improved by using parallel probes in a fashion similar to that of millipede data storage {Ref. R. V. Lapshin (2007). "Automatic drift elimination in probe microscope images based on techniques of counter-scanning and topography feature recognition" (PDF). Measurement Science and Technology (UK: IOP) 18 (3): 907–927. Bibcode 2007MeScT..18..907L. doi:10.1088/0957-0233/18/3/046. ISSN 0957-0233}.

The scanning speed of an AFM is also a limitation. Traditionally, an AFM cannot scan images as fast as a SEM, requiring several minutes for a typical scan, while a SEM is capable of scanning at near real-time, although at relatively low quality. The relatively slow rate of scanning during AFM imaging often leads to thermal drift in the image

Other Scanning Probe Microscopy (SPM) techniques

The use of further feedback mechanisms has led to a number of SPM imaging methods, including magnetic force microscopy, lateral force microscopy, shear force microscopy and near field scanning optical microscopy. All methods can be operated in a range of environments, including atmospheric conditions, liquid immersion and vacuum Scanning Tunneling Microscopy (STM), which measures the 3-D topology of the specimen, is based on the concept of quantum tunneling. Electrons from the specimen can tunnel through the vacuum between the conducting tip and the surface in interest due to voltage difference between the tip and the surface. Monitoring the current as the tip's position scans across the surface, which can then be used to display an image, makes measurements.

SPM offers the possibility of analysing nanometre-diameter particles under ambient conditions, thus getting away from some of the constraints imposed by electron microscopy. Imaging methods such as AFM and NSOM offer novel and exciting possibilities for the characterisation of specific aerosols. For instance, the use of NSOM to identify, size and count fluorescently tagged ultrafine particles would seem applicable to identifying particle transport and deposition characteristics within biological systems. While SPM is currently limited in the information that can be obtained from ultrafine aerosol samples, the uniqueness of the information available should allow it to be developed as a complementary tool to electron microscopy.

While electron microscopy and SPM are confined to the analysis of collected samples and are constrained by the limitations of the collection and preparation systems used, developments in aerosol mass spectrometry are providing the means for chemically characterizing size-segregated ultrafine particles on-line.

Current technology allows the speciation of individual particles *ca*. 10 nm in diameter, and as this is reduced still further, the resulting methods should provide invaluable complementary data to off-line methods.
By adopting technologies developed within complementary disciplines, together with the development of aerosol-specific methods, it is possible to develop a basis for characterizing single sub-100 nm particles and features in terms of size, morphology topology, composition, structure and physicochemical properties.

The available methods provide complementary means to characterise single ambient particles in depth. Currently, with few exceptions, they are complex, time-consuming to use, and in many cases still at a developmental stage. As such they are not ideally suited to the routine analysis of aerosols. However, by adopting a multi-disciplinary approach, the potential is there to develop complementary tools that will provide routine and detailed information on the particles that influence the environment we live and work in.

Small-angle X-ray scattering (SAXS)

Small-angle X-ray scattering (SAXS) is a small-angle scattering (SAS) technique where the elastic scattering of X-rays (wavelength 0.1 ... 0.2 nm) by a sample which has inhomogeneities in the nm-range, is recorded at very low angles (typically 0.1 - 10°). This angular range contains information about the shape and size of macromolecules, characteristic distances of partially ordered materials, pore sizes, and other data. SAXS is capable of delivering structural information of macromolecules between 5 and 25 nm, of repeat distances in partially ordered systems of up to 150 nm {Ref. Glatter O, Kratky O, ed. (1982). Small Angle X-ray Scattering. Academic Press. ISBN 0-12-286280-5. } USAXS (ultra-small angle X-ray scattering) can resolve even larger dimensions. SAXS and USAXS belong to a family of X-ray scattering techniques that are used in the characterisation of materials. In the case of biological macromolecules such as proteins, the advantage of SAXS over crystallography is that a crystalline sample is not needed. Nuclear magnetic resonance spectroscopy methods encounter problems with macromolecules of higher molecular mass (> 30-40 kDa). However, owing to the random orientation of dissolved or partially ordered molecules, the spatial averaging leads to a loss of information in SAXS compared to crystallography. The P(r) function or pair-distance distribution function describes the paired-set of all distances between points within an object. In SAXS, the P(r) function is used to describe the paired-set of distances between all of the electrons within the macromolecular structure and is a useful tool for visibly detecting conformational changes within a macromolecule. Since the function describes the set of all paired-distances within a structure, small changes in the relative positions of a few residues can result in detectable changes in a P(r) distribution.

Light scattering techniques

Dynamic light scattering (DLS) (also known as photon correlation spectroscopy or quasi-elastic light scattering) is a technique in physics that can be used to determine the size distribution profile of small particles in suspension or polymers in solution {Ref. It can also be used to probe the behavior of complex fluids such as concentrated polymer solutions.

NanoSight have developed a unique instrument, which allows the tracking of the Brownian motion of nanoparticles in liquid suspension on a particle-by-particle basis. Subsequent application of the Stokes-Einstein equation allows the determination of particle size. Particle count is also available. This technique presents a powerful

alternative to more typical light scattering techniques such as DLS for the analysis of complex and polydisperse sample types of varying composition. Both DLS and nanoparticle tracking analysis (NTA) measure the Brownian motion of nanoparticles whose speed of motion, or diffusion coefficient, is related to particle size through the Stokes-Einstein equation. NTA provides linear size axes, a high-resolution scale compared to wide logarithmic scale in DLS, particle concentration information on the vertical axis. Standard polystyrene beads of sizes ranging from 60 to 1,000 nm and physical mixtures thereof were analyzed with NTA and DLS. The influence of different ratios of particle populations was tested. Drug delivery nanoparticles and protein aggregates were analyzed by NTA and DLS. Also live monitoring of heat-induced protein aggregation was performed with NTA.