

SCIENTIFIC OPINION

The Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety¹

Scientific Opinion of the Scientific Committee

(Question No EFSA-Q-2007-124a)

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SCIENTIFIC COMMITTEE MEMBERS

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SUMMARY

Following a request from the European Commission the European Food Safety Authority (EFSA) was asked to provide a scientific opinion on potential risks arising from nanoscience and nanotechnologies on food and feed safety. In view of the multidisciplinary nature of this subject, the task was assigned to the EFSA Scientific Committee.

This opinion addresses engineered nanomaterials (ENMs). Food and feed are addressed together, since the basic aspects (applications and potential impacts) are expected to be similar. This opinion is generic in nature and is in itself not a risk assessment of nanotechnologies as such or a survey of tentative applications or possible uses thereof or of specific products.

It is claimed that nanotechnologies offer a variety of possibilities for application in the food and feed area – in production/processing technology, to improve food contact materials, to monitor food quality and freshness, improved traceability and product security, modification of taste, texture, sensation, consistency and fat content, and for enhanced nutrient absorption. Food packaging makes up the largest share of current and short-term predicted markets.

Formulation at the nanosize may change the physico-chemical characteristics of materials as compared to the dissolved and micro/macroscale forms of the same substance. Their small size, high surface-to-mass ratio and surface reactivity are important properties, both for new applications and in terms of the associated potential health and environmental risks.

Current uncertainties for risk assessment of ENMs and their possible applications in the food and feed area arise due to presently limited information on several aspects. Specific uncertainties apply to the difficulty to characterize, detect and measure ENMs in food/feed and

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biological matrices and the limited information available in relation to aspects of toxicokinetics and toxicology. There is limited knowledge of current usage levels and (likely) exposure from possible applications and products in the food and feed area.

The risk assessment paradigm (hazard identification, hazard characterization, exposure assessment and risk characterization) is considered applicable for ENMs. However, risk assessment of ENMs in the food and feed area should consider the specific properties of the ENMs in addition to those common to the equivalent non-nanoforms. It is most likely that different types of ENMs vary as to their toxicological properties. The available data on oral exposure to specific ENMs and any consequent toxicity are extremely limited; the majority of the available information on toxicity of ENMs is from *in vitro* studies or *in vivo* studies using other routes of exposure. The risk assessment of ENMs has to be performed on a case-by-case basis.

Current toxicity-testing approaches used for conventional materials are a suitable starting point for risk assessment of ENMs. However, the adequacy of currently existing toxicological tests to detect all aspects of potential toxicity of ENMs has yet to be established. Toxicity-testing methods may need methodological modifications. Specific uncertainties arise due to limited experience of testing ENMs in currently applied standard testing protocols. Additional endpoints presently not routinely addressed may need to be considered in addition to traditional endpoints.

For hazard characterization, the relationship of any toxicity to the various dose metrics that may be used is currently discussed and several dose metrics may need to be explored in addition to mass.

The different physicochemical properties of ENMs compared to conventional dissolved and micro/macroscale chemical counterparts imply that their toxicokinetic and toxicity profiles cannot be fully inferred by extrapolation from data on their equivalent non-nanoforms.

Appropriate data for risk assessment of an ENM in the food and feed area should include comprehensive identification and characterization of the ENM, information on whether it is likely to be ingested in nanoform, and, if absorbed, whether it remains in nanoform at absorption. If it may be ingested in nanoform, then repeated dose toxicity studies are needed together with appropriate *in vitro* studies (e.g. for genotoxicity). Toxicokinetic information will be essential in designing and performing such toxicity studies. For ENMs which are intended to increase the bioavailability of incorporated substances (i.e. ENM carrier systems), the changes in bioavailability should be determined.

Although, case-by-case evaluation of specific ENMs may be currently possible, the Scientific Committee wishes to emphasise that the risk assessment processes are still under development with respect to characterisation and analysis of ENMs in food and feed, optimisation of toxicity testing methods for ENMs and interpretation of the resulting data. Under these circumstances, any individual risk assessment is likely to be subject to a high degree of uncertainty. This situation will remain so until more data on and experience with testing of ENMs become available. The limited database on assessments of ENMs should be considered in the choice of appropriate uncertainty factors.

The Scientific Committee makes a series of recommendations; in particular, actions should be taken to develop methods to detect and measure ENMs in food/feed and biological tissues, to survey the use of ENMs in the food/feed area, to assess the exposure in consumers and livestock, and to generate information on the toxicity of different ENMs.

Key words: Nanotechnologies, Nanotechnology, Nanoscience, Engineered Nanomaterial, ENM, Nano, Food, Feed, Agro-chemical, Food Contact Material, Exposure, Toxicokinetics, Toxicity, Environment, Risk Assessment, Guidance

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BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

The prospects for applications of nanoscience and nanotechnologies to the food chain range from the almost certain (e.g., membranes, antibacterials, filters, packaging) through to the probable (e.g., pathogen and contaminant sensors, environmental monitors, coupled sensing & warning devices, and remote sensing & tracking devices) to the improbable (e.g., “creating unlimited amounts of food by synthesis at the atomic level”). Some market analysts² flag smart packaging, on demand preservatives, and interactive foods as the most promising areas. In addition, all seem to agree that the development of foods with new or modified molecular structures holds promise. Yet, the actual use and potential use of nanoscience and nanotechnologies in the food, feed, and pesticide industry still require clarification. The need for clarification also holds true for the benefits and improvements that these applications should bring about. In the USA, the Food and Drug Administration has approved products containing nanomaterials. FDA-approved products known to date include drugs, medical devices, sunscreen lotions, and pet food supplements.

Various European Commission (EC) initiatives establish a framework for the Health & Consumers Protection Directorate-General action on nanotechnologies. The European Action Plan on “Nanosciences and nanotechnologies: An action plan for Europe 2005-2009” (COM(2005) 243), adopted on 7 June 2005, defines a “safe, integrated, and responsible approach” for nanotechnologies.³ The Commission adopted on 6 September 2007 a report for the European Parliament on the implementation of the Action Plan.⁴ Moreover, the 7th EC Framework Program for Research, Technological Development and Demonstration Activities allocates 3.5 billion euros for nanotechnologies in support to the Action Plan,⁵ part of which will finance research on safety. Recently, the European Group on Ethics produced an opinion on ethical issues in nanomedicine.⁶ The Commission adopted on 17 June 2008 a Communication on the Regulatory Aspects of Nanomaterials⁷, which is a legislative review on the suitability of the existing regulation for nanotechnologies. Finally, the services of the Commission are involved in international activities (OECD⁸, Transatlantic Dialogue, etc.).

The EC’s non-food, Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) first adopted a scientific opinion on “The appropriateness of existing methodologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies” on 10 March 2006 (after public consultation).⁹ It subsequently adopted a scientific opinion on “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” 29 March 2007.¹⁰

These opinions conclude that current risk assessment methodologies for micro/macroscale chemicals require modification in order to deal with the risks associated with nanotechnologies and in particular that existing toxicological and ecotoxicological methods may not be sufficient to address all of the issues arising from nanoparticles as size confers unique properties to nanomaterials. For example, decreased size increases the reactive surface per unit volume for nanoparticles compared to larger particles. Size also potentially reduces the effectiveness of barriers to the penetration of foreign objects into the body and to their movement within it. The

² <http://www.nanoforum.org/dateien/download.php?userid=6385071&dateinr=714&dateiorig=000714.upl&dateiname=nanotechnology+in+agriculture+and+food.pdf&zeitcode=31052007175920>

³ <http://cordis.europa.eu/nanotechnology/actionplan.htm>

⁴ COM(2007) 505 final

⁵ http://cordis.europa.eu/nanotechnology/src/eu_funding.htm

⁶ http://ec.europa.eu/european_group_ethics/activities/docs/opinion_21_nano_en.pdf

⁷ COM(2008) 366 final

⁸ http://www.oecd.org/departement/0,2688,en_2649_37015404_1_1_1_1_1,00.html

⁹ http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_003b.pdf

¹⁰ http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_004c.pdf

opinions also indicate that very little is known about the physiological responses to nanoparticles and that there are major gaps in the knowledge necessary for risk assessment.

The European Commission would like to address the possible safety issues arising from nanoscience and nanotechnologies in a stepwise fashion, thereby facilitating the establishment of a roadmap for future actions in the area of food and feed safety and the environment. As a first step in this exercise, the Commission asks EFSA to prepare a scientific opinion in order to identify the needs for risk assessment, to assess the appropriateness of methods for risk assessment, and to perform an assessment of the potential risks posed by nanoscience and nanotechnologies in the above mentioned areas and assess the appropriateness of current risk assessment methods.

This first opinion will allow the Commission to explore appropriate measures, assess existing legislation and determine the scope of possible further requests for scientific opinions.

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority to produce a scientific opinion on the need for specific risk assessment approaches for technologies/processes and applications of nanoscience and nanotechnologies in the food and feed area. In support of this work, the Authority should, inter alia, take into account existing documents on the risk assessment nanotechnologies that have been prepared by scientific advisory bodies at the European level (such as the SCENIHR, the EC Joint Research Centre, and EU agencies) EU Member States, third countries and international organisations.

The Authority is requested to identify the nature of the possible hazards associated with actual and foreseen applications in the food and feed area and to provide general guidance on data needed for the risk assessment of such technologies and applications.

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ASSESSMENT

1. Introduction to the opinion

This opinion focuses on engineered nanomaterials (ENMs) that are deliberately introduced into the food and feed chain and have the potential to be consumed. Such ENMs range from food contact materials (including those used in production processes), ingredients and additives, to fertilizers and pesticides that are used in the food and feed area. "Natural" nanoscale materials (e.g. micelles) will be considered if they have been deliberately used e.g. to encapsulate bioactive compounds or further engineered to retain their nanoscale properties. "Natural" nanoscale components present as emulsions (e.g. in homogenized milk, mayonnaise, etc.) will not be considered.

Residues in food/feed from the use of nanoscale pesticides and fertilizers and veterinary medicines will be discussed, since they may be present in the food/feed as consumed. However, the opinion will exclude incidental ambient nanostructured material contamination of food/feed, resulting from other anthropogenic and natural sources. ENMs used for waste water (i.e. not intended for consumption) or soil treatments are not considered, nor is the possible impact of ENMs on plant health. These applications of nanotechnologies in the food and feed area are not less important for an assessment, but have not been prioritized in this opinion.

Environmental aspects will be discussed in so far as they may provide a potential for re-introduction of ENMs into the food and feed chain, while effects on the environment itself were considered to be outside the remit.

Workers engaged in manufacture, packaging, transport, use and elimination of nanomaterial and nanotechnology products may be exposed to ENMs. Possible exposure to ENMs in occupational settings is recognised but will not be discussed as it is interpreted to be outside the scope of this opinion.

For the purpose of this opinion, ENMs in feed will be treated in a similar way as those in food, since the impact on animals is likely to be similar to that on humans. A potential route of human exposure to ENMs or their residues is from feed via the animal to animal-derived food products (i.e. "carry over").

This opinion takes account of reports produced by other Scientific Committees, Member States, risk assessment agencies, (inter)national organisations and other bodies (reports are grouped in the reference list). The opinion is based upon published, peer-reviewed scientific papers and other information deemed reliable, although this information may not be directly related to the food and feed area.

EFSA launched a call for data through its Advisory Forum and on its website between 23 January and 28 March 2008 for scientific contributions on this subject from third parties; a list of all documents made available to EFSA can be found at the end of the opinion. A draft of the opinion was published on the EFSA web for public consultation between 17 October and 1 December 2008. All the public comments received that related to the remit of EFSA were assessed and the opinion has been revised taking relevant comments into consideration. The comments received and a report of the outcome of the public consultation has been published on the EFSA website.

2. Introduction to nanotechnologies in the food and feed area

Nanotechnologies are a broad assemblage of processes, materials, and applications that span physical, chemical, biological, engineering and electronic sciences, with the common theme

that they all involve manipulation of substances at sizes in the nanoscale range. Due to the small size of ENMs, unique properties may arise. One important consequence of nanoscaling of materials is increased surface area, which can affect reactivity with other materials and may result in increased ability of ENMs to translocate across biological membranes.

It is claimed that nanotechnologies offer technological advancement in food packaging and storage that enhances shelf-life of fresh foods. Nanotechnologies may also offer more efficient nutrient delivery and formulations with improved bioavailability. Nanotechnology applications for food and food packaging are relatively new, and several of the possible applications have been suggested to belong to the sub-sectors at the intersection between the food, medicines and cosmetics sectors (Chaudhry *et al.*, 2008).

Nanotechnology applications for the food sector have raised a number of safety, environmental, ethical, policy and regulatory issues. The main concerns stem from the lack of knowledge about the potential effects and impacts of nano-sized materials on human health and the environment. Consumer concerns regarding nanotechnology applications in the food sector are mainly related to safety issues and it is recognised that public expectation about the safety of products derived from new technologies may differ from those using established technologies.

Surveys of public opinion in some Member States indicate that consumer opinion is not favourable to the use of nanotechnologies in food or if nanomaterials are used in food or food packaging, these technologies should be independently assessed for safety before they are placed on the market (BFR, 2008; Which?, 2008).

Terms used in the opinion

In relation to risk assessment (RA) of ENMs, the actual characteristics and properties of the ENMs in question are the determining factors, rather than the terms used for its description. This opinion does not intend to provide any definitions. However, to describe ENMs it is important to provide a few terms for a common understanding. In this opinion, the terms and definitions suggested by the SCENIHR are used, as they are considered relevant for RA (SCENIHR, 2007b). For convenience, the most relevant are described below. A glossary of additional terms is given at the end of the opinion. There is also a recent ISO technical specification publication on terminology and definitions (ISO, 2008).

The prefix “nano” specifically means a measure of 1×10^{-9} units, the nature of this unit being determined by the word that follows, e.g. “nanometre” as a measure of dimension. In this opinion, nanoscale refers to a dimension of the order of 100 nm and below. Since the changes in characteristics that are seen on reducing dimensions do not occur exactly at the 100 nm size, it is important that some latitude is allowed in this definition with respect to the meaning of “the order of” and it is recognised that there will be various borderlines. Generally, we are in the order of 100 nm or less, but there are size-related effects that can appear at larger size.

An engineered nanomaterial is any material that is deliberately created such that it is composed of discrete functional and structural parts, either internally or at the surface, many of which will have one or more dimensions of the order of 100 nm or less. In this opinion engineered nanoparticles are included in the general use of the term ENMs. The term “engineered” as used in this opinion is equivalent to the term “manufactured” as used in other reports (e.g. SCENIHR, 2009).

Food and feed may contain components that have internal structures that individually could be present at the nanoscale, e.g. naturally occurring molecules, micelles or crystals. However, as said above, “natural” components are considered within the context of this opinion, only if they have been deliberately used or engineered to have nanoscale properties, or used e.g. to encapsulate bioactive compounds.

Micro/macroscale material (i.e. bulk material) refers to a material predominantly in sizes well beyond the nanoscale, while the dissolved chemical describes a size generally smaller than the nanoscale.

An agglomerate is a group of particles (such as primary ENMs) held together by weak forces, such as Van der Waals forces or electrostatic forces.

An aggregate is a group of particles (such as primary ENMs) held together by strong forces, such as those associated with covalent or metallic bonds.

3. Application of nanotechnologies in the food and feed area

Information in this section is derived from industry, producers, marketing organisations, scientific publications, patent searches, etc. However, in many instances the claimed nanoscale character of the applications cannot be verified, as methods for detection and characterization of ENMs in food and feed are not readily available (see section 4.1). Some, if not many, of the products claimed to have been derived from nanotechnologies may in fact not be so. Conversely, other products may contain a nano-component, whose presence is not declared. In this respect it is acknowledged that the size range of microscale materials may contain a nanoscale fraction.

The following broad categories of nanotechnology applications in the food and feed sector have been described (Chaudhry *et al.*, 2008; Observatory-nano, 2009):

1. Where nanotechnology processes and materials have been employed to develop food contact materials (FCM). This category includes nanomaterial-reinforced materials (also referred to as nanocomposites), active FCM designed to have some sort of interaction with the food or environment surrounding the food, and coatings providing surfaces with nanomaterials or nanostructures.
2. Where food/feed ingredients have been processed or formulated to form nanostructures. This category includes applications that involve processing food ingredients at nanoscale to form nanostructures or enhance taste, texture, and consistency of the foodstuffs.
3. Where nano-sized, nano-encapsulated, or ENM ingredients have been used in food/feed. This category includes nanoscale ingredients, including additives (such as colorants, flavourings, preservatives) and processing aids (including nano-encapsulated enzymes) that can be produced for a variety of uses.
4. Biosensors for monitoring condition of food during storage and transportation. This category includes packaging which include indicators.
5. Other indirect applications of nanotechnologies in the food and feed area, such as the development of nanosized agro-chemicals (including fertilizers, pesticides etc.), or veterinary medicines.

Whilst most nanotechnology applications for food and beverages are currently at R&D or near-market stages, it has been reported that applications for food packaging are rapidly becoming a commercial reality (Chaudhry *et al.*, 2008). Examples of currently available FCM include polyethylene (PET) beer bottles with nano-clay gas-barrier, polypropylene food containers with nano-silver for antimicrobial action and nano-zinc oxide containing films for food wrapping. Market estimates for the current and short-term predicted applications suggest that nanotechnology-derived food packaging materials already make up the largest share of the overall nanofood market (Cientifica, 2006). Another report has estimated that nanotechnology-derived packaging (including food packaging) will make up to 19% of the share of nanotechnology products and applications in the global consumer goods industry by 2015 (Nanoposts, 2008). A contributing factor to the rapid commercial developments in the FCM area may be that there is a possibility to fix or embed such ENMs in plastic polymers, such that they are not likely to provide any significant exposure to the consumer (FSA, 2008; Simon and Joner, 2008a).

There are reports (e.g. PEN; 2006a; FoE, 2008a) and an inventory of claimed nanotechnology applications currently on the global food market and associated areas available on the internet

from the Project on Emerging Nanotechnologies¹¹ (Woodrow Wilson International Center for Scholars and the Pew Charitable Trusts). EFSA is not aware of any database providing information on nanotechnology applications or products on the EU market. However, many nanotechnology-derived consumer products in the food sectors can be obtained via the internet from outside EU. Based on information provided to EFSA “...the food industry is not yet using appropriate applications...” and “...nanomaterials with new functionalities, [are] up to now not used in the food field...” (CIAA, 2008, communication provided to EFSA; BLL, 2008). The current status of FCM or uses of nanotechnology processes (e.g. nanofiltration) are more uncertain, and such applications may be on the EU market. The control of products which are placed on the market are under the competence of the EU Member States and falls outside the remit of EFSA..

4. Prerequisite for risk assessment of ENMs in food and feed

Risk assessment (RA) is a scientifically based process where the probability for the occurrence of harmful effects on human or animal health or the environment is evaluated. The traditional RA paradigm comprises four stages; hazard identification, hazard characterization, exposure assessment and risk characterization (FAO/WHO, 1995, 1997; SSC, 2000; CODEX, 2007). Health risk is defined as the combination of the probability of occurrence of harm to health and the severity of that harm. The traditional RA paradigm is considered an appropriate starting point to address the additional safety concerns that may arise due to the nanocharacteristics of ENMs (SCENIHR, 2006; 2007a; COT, 2005; 2007) and it is the view of the Scientific Committee that this is also appropriate in the food and feed area.

The special characteristics and properties of ENMs, such as the small size, surface reactivity and translocation across biological membranes, are issues that may need special considerations as well as interactions of ENMs with the surrounding matrix and unexpected effects resulting from this. The need for proper identification of any particulate matter (including physico-chemical characterization) used in the food and feed sector is particularly emphasised. Detailed knowledge of a set of representative ENMs with respect to physico-chemical and toxicological properties is essential for future development of predictive models.

4.1. Physico-chemical characterization of ENMs, stability in food and feed matrices, and analytical tools

The physico-chemical properties of ENMs make them different from either the micro/macroscale material or the same material in solution, which besides offering a wide range of novel applications, may also give rise to altered kinetics and toxicity profiles. Several comprehensive publications on the properties of ENMs have been published recently (Balbus *et al.*, 2007; Rose *et al.*, 2007; Simon and Joner, 2008a; ICON 2008; OECD, 2008a, b). In the following sections, characteristics are briefly reviewed with a focus on aspects of specific importance for the RA of ENMs in food and feed.

4.1.1. Characteristics of ENM

The principal physical parameters for the characterization of ENMs are size (including its distribution), shape (including aspect ratios where appropriate) and the morphological sub-structure of the substance (OECD 2008b). Further characteristics are chemical composition, solubility, surface area and particle concentration, surface properties (e.g. composition, charge adsorbed biomolecules) and the presence of impurities such as residual catalyst. For tissue

¹¹ <http://www.nanotechproject.org/inventories/consumer/>

distribution and/or accumulation, the lipophilicity/hydrophilicity is an important trait. Additional characteristics of importance are biodegradability and biopersistence.

In general molecules at the surface of a material are in an energetically unstable state, not having their full quotient of covalent bonds met giving rise to increased surface reactivity. This is what leads to the interesting surface properties that are used in the food industry. Micelles, liposomes, microemulsions, etc. result from surface properties and the tendency of the constituent molecules to lower their surface energy. However, for macroscopic or microscopic materials, the proportion of the molecules in the material that are in this energetically unstable state is very low, with the majority of the molecules being in their lowest free-energy state (in the bulk), and hence it is the properties of this majority of molecules that determine the properties of the material, such as its conductance or strength.

What makes ENMs special is that as the size of the particles decreases, the specific surface area increases indirectly proportional to their size, until the properties of the surface molecules dominate, and so ENMs have novel properties determined by their high surface-to-volume ratios. This leads many ENMs to have altered characteristics, which may be used for a range of applications. The very high surface area of ENMs has several consequences that need to be considered in RA contexts, as it makes them different from their micro/macroscale counterparts: they have increased (surface) reactivity compared to the non-nanoscale material, since many more molecules are located at the surface in energetically unstable states. Many types of ENMs catalyze reactions, mainly oxidation reactions. They may also act as nuclei in heterogeneous nucleation processes during crystallisation and recrystallisation in material sciences (and potentially modifying the secondary or tertiary conformation of proteins).

ENMs undergo dynamic changes in response to their environment. The high surface energy and unstable surface forces may bring about particle interaction. Hence, free ENMs (also referred to as primary ENMs) tend to agglomerate or aggregate, resulting in bigger particles (secondary ENM), which may preserve some of the nanoscale properties, such as high surface area and reactivity. The tendency of ENMs to agglomerate or aggregate can be enhanced or hindered by the modification of the surface, e.g. in the presence of chemical agents (e.g. coatings, surfactants, ions).

4.1.2. Properties of ENMs in food, feed and biological tissues

Although ENMs are likely to be present in food/feed in an agglomerated form, it can not be excluded that agglomerates break up under certain conditions that occur in food, feed, the gastrointestinal tract and biological tissues. ENMs can interact with proteins, lipids, carbohydrates, nucleic acids, ions, minerals and water in food, feed and biological tissues. The interaction with proteins is of particular interest (Lynch and Dawson, 2008). ENMs may be fully surrounded by a dynamic "corona" of proteins and the ENMs may affect the behaviour of the protein, and the protein that of the ENMs. Hence, interaction of ENMs with specific biomolecules in the food/feed matrix and following ingestion can influence their uptake and distribution. The significance of this interaction for the safety and biological impact of ENMs implies that detailed characterization of the ENMs in the relevant biological environment is necessary. However, there are several complicating factors, such as the fact that the biomolecule corona is not a static, but rather a dynamic state, which equilibrates with the surroundings, with high abundance proteins binding initially, but being replaced gradually by lower abundance, higher affinity proteins. However, a considerable portion of the biologically relevant biomolecules (e.g. proteins) will be associated with the nanoparticles for a sufficiently long time that they are not affected by the time frame of the measurement processes, the so-called "hard-corona".

4.1.3. Analytical tools for detection, quantification and characterization of ENMs in food and feed matrix

A number of analytical tools exist for the qualitative and quantitative characterization of ENMs, both the single-particle techniques and the techniques characterizing the ensemble of ENMs (Powers *et al.*, 2006; Hasselov *et al.*, 2008; Luykx *et al.*, 2008; Tiede *et al.*, 2008). Due to the enormous variety of ENMs, there are many different ways to analyse particles and there is no “best” technique for “all” situations and therefore a combination of techniques is usually necessary.

It is important to measure the ENMs in the relevant matrix, as properties of ENMs may depend on the surrounding matrix and be affected by processing. This is usually a much more demanding task than to analyse in simpler or in model matrices.

In the case of nanoscale metal or semiconductors containing ENM, these can be detected even in rather complex matrices like food and feed and biological tissues by means of electron microscopy (EM) coupled with chemical analytical tools. However, detection by EM is only possible if the number of ENM is sufficiently high in the matrix to localize them since high magnification is required due the small size of ENMs. As a result, the investigation of ENMs biodistribution in organs is generally extremely time-consuming. A second complication is the fact that some ENMs cannot be distinguished from naturally occurring variants of the same material; e.g. engineered nanoscale silicon dioxide (SiO₂) or endogenous lipids used in capsule membranes. Detection may also be hindered by interactions with solutes or cell constituents that obscure clear analytical signals.

The current limited number of standardized reference materials for ENMs is another limitation on precise and reproducible detection and quantification of ENMs in food, feed and biological tissues. A quality control material (IRMM-304) of silica nanoparticles has recently been released from the Joint Research Centre, Institute of Reference Materials and Measurements¹² and gold nanoparticles (NIST RM 8011, 8012 and 8013) from the National Institute of Standards and Technology (NIST)¹³.

A lower analytical ambition is to determine the chemical composition of the ENM, without generating information on the physical state of the ENM. Hence, the metal content of ENMs can be quantified by chemical analytical tools, such as inductively-coupled plasma mass-spectrometry (ICP-MS) or by radio-analysis after appropriate neutron irradiation and other tools. The limitations of chemical analysis result from artificial losses during the preparatory steps and the analytical detection limits. In the case of organic ENM, detection or quantification of the chemical may be possible, where a test for the species exists, but a focus on characteristic structures may be needed to determine whether it is still in nanoform.

In summary: On the one hand, there are methods to analyse nanomaterials in food/feed and biological tissues, but because of the background occurrence of nanomaterials, it is usually not possible to establish the presence of ENMs. On the other, there are methods to analyse the chemical in specific ENMs, but most often not to establish its presence in nanoform. Only in exceptional cases is it at present possible both to specifically detect and measure a defined ENM.

¹² <http://www.irmm.jrc.be>

¹³ <http://ts.nist.gov/measurementservices/referencematerials/index.cfm>

4.2. Exposure to ENMs from food and feed

In view of the present difficulties in detection of ENMs in food and feed matrices, knowledge regarding the present use of ENMs relies on information provided by industry itself on the addition of ENMs to their products.

Consumers can be exposed to ENMs from various sources as indicated below. However, due to the current limited knowledge on availability of products with declared (or undeclared) use of nanotechnologies in the food and feed area, the exposure scenarios outlined below are describing presumed (potential) exposures. Information on the absolute and relative importance of different possible sources of exposure to ENMs in food and feed is extremely limited.

There is quite an amount of information on exposure to NPs by inhalation of air pollutants in the general and work environments and the methods of detection and analysis of air levels are more developed than those for food and feed (SCENHIR, 2006, 2007a, 2009).

4.2.1. Sources of exposure

Several examples of FCM with incorporated ENMs have been developed. A major uncertainty is the likelihood and extent of migration of nano-components from FCM into the food. Only a few studies have investigated the possible migration of ENMs from FCM which indicate that some ENMs may migrate while others do not (Avella *et al.*, 2005; EFSA, 2007; FSA, 2008; Simon *et al.*, 2008b). Migration is likely to be dependent on the type of ENM and FCM and no general conclusion can be drawn from the limited information currently available.

There may be release of ENMs (or their residues) into food/feed through wear of food/feed processing equipment with coatings containing ENMs. It is not known if such exposure would be any different from wear from conventional materials used in processing. There is no information on the potential exposure to residues following the use of nanotechnology processes (e.g. nanofiltration etc.) in the manufacturing process of food/feed.

Exposure from applications of nano-sized or nano-encapsulated food/feed ingredients or the incorporation of ENMs due to processing of food/feed ingredients or use in food supplements has not yet been assessed.

Exposure assessment from applications in feed for the target animal (e.g. food-producing species) would follow the same lines as for human exposure assessment. In order to pose a hazard for humans, ENMs in feed need to be transferred to edible tissues. Currently there are no studies available on whether such transfer occurs.

Residues from nano-formulated or nano-particulate agro-chemicals and veterinary products are currently likely to be minimal as no major-use nano-formulated pesticides, fertilizers or veterinary drugs are currently placed on the market in the EU. However, there are some claimed ENM-containing plant care products available. The presence of ENMs in particular individual cases can not be ruled out due to lack of respective information of actual size and size distributions of so called micro- or nano-emulsions. In principle, human exposure is possible by carry over from animals and crops, although there are currently no data from this route of exposure.

Production and widespread use of ENMs in consumer products (e.g., electronics, medicines, packaging materials) will result in environmental release of these particles over the product life-cycle (Nowack and Bucheli, 2007). ENMs may theoretically also reach food crops through contamination of sewage sludge that is applied to agricultural soils. Due to a lack of information at present, the contribution of environmental disposition to oral exposure to ENMs has not been estimated.

In conclusion, widespread consumer and animal exposure to ENMs ingredients in food and feed is currently not likely within the EU, though there may be exposure to nanoscale fractions within existing micro/macroscale food/feed ingredients. However, products which may contain ENMs are also available via the Internet; this contribution to consumer exposure is not quantified.

4.2.2. Estimations of dietary exposure

Exposure assessment is the qualitative and/or quantitative evaluation of the likely exposure to ENMs via food or feed. Basically, the principles of exposure assessment of ENMs (via food and feed) will be the same as in exposure assessment of non-nanoscale materials (Kroes *et al.*, 2002). Issues like food/feed sampling and variability within composite samples and variation in concentrations between samples are not different from the exposure assessment of micro/macroscale or dissolved chemicals. The current food consumption databases can be used to estimate consumption of food/feed products containing ENMs. However, there is limited information on the consumption (amounts and frequency) of food supplements.

A central aspect of exposure assessment is the determination of the amount and characterization of the substance present in the food or feed as consumed. In most cases, the starting point for determining the amount of ENMs currently has to rely on information on the material added (primary/secondary particles etc) or that is in contact with food/feed. The initial characteristics of the added ENMs can be assessed and used as an assumption in the exposure assessment, however, currently it is not possible to routinely determine ENMs *in situ* in the food or feed matrix (see section 4.1) which increases the uncertainty in the exposure assessment.

The exposure assessment of a nanoscale delivery system should in addition to the assessment of the nanocarrier system itself include assessment of the amount of encapsulated bioactive compound as well as the amount present in free form in the food. For this, the analytical isolation, detection and characterization procedures need to be designed to meet these requirements. The same approach is relevant for FCM. In both cases, due to the lack of methods to determine ENM, it might be necessary, when appropriate, to analyse the relevant chemical as such.

The structure of the ENMs may be changed in the food/feed production chain and during processing or storage because of their interactions with proteins, lipids and other substances present in the food/feed matrices (see section 4.1.2). Hence, if ENMs are analysed at an early stage of the food chain, effects of processing and storage should be considered in the exposure assessment. Also, effects of digestion of the matrix on nanoparticle characteristics need to be considered. There is currently no information available on processing effects.

4.3. Toxicokinetics of ENM

Toxicokinetics is the science dealing with absorption, distribution, metabolism (biotransformation) and excretion/elimination (ADME) of substances in the body. This whole cascade of events, which occur following ingestion, determines the internal exposure of organs to potentially toxic substances. Studies on absorption of ENMs (as well as other aspects of ADME) have been made almost only with metals and metal oxides, and in a few cases with polymer ENM.

4.3.1. Absorption

Little is known regarding the behaviour and fate of ENMs in the gastrointestinal (GI) tract. It is possible that they will not remain in a free form in the lumen (and hence not be available for absorption), due to transformations, such as solubilization, agglomeration, aggregation, adsorption or binding with other components of food, reaction with GI tract components (acid and enzymes), etc. (see also Section 4.1.2). Thus, nanoproperties may disappear totally or partially.

Translocation of particles through the intestinal wall is a multi-step process, involving diffusion through the mucus lining the gut wall, contact with enterocytes or M-Cells, cellular or paracellular transport, and post-translocation events (Hoet et al., 2004). Translocation of ENMs through the epithelium depends on their physico-chemical properties, e.g. size, surface charge, lipophilicity/hydrophilicity, presence/absence of a ligand, and physiology of the intestinal tract, e.g. healthy vs. diseased state (Des Rieux et al., 2006). Under normal physiological conditions, para-cellular transport of ENMs would be extremely limited, as pore size at tight junctions is 0.3-1.0 nm (Des Rieux et al., 2006).

Smaller particles are generally absorbed more readily and faster than larger ones. Diffusion across the pre-epithelial mucus gel layer of rat distal colon showed that 14 nm (diameter) latex ENMs cross within 2 minutes and 415 nm within 30 minutes, while 1000 nm did not cross this barrier (Szentkuti, 1997). Oral administration of gold nanoparticles (Au-NP) (58, 28, 10 and 4 nm) to mice, showed increased GI uptake with diminishing size (Hillyer and Albrecht, 2001). The amount of absorption of polystyrene ENMs (50, 100, 200, 300, 1000 and 3000 nm) has been shown to be 34 % in rats at 50 nm, and decreasing to almost zero with increasing ENMs size (Jani *et al.*, 1990). Titanium dioxide (TiO₂) particles as large as 500 nm have been found to be absorbed, with 12 % of the administered dose (where 7 % were present in the GI tract) after repeated oral gavage administration for 10 days to rats (Jani *et al.*, 1994). In contrast for much smaller TiO₂ particles (25, 80 and 155 nm) only minute percentages were reported 14 days after administration of single doses of TiO₂ in mice (Wang *et al.*, 2007). However in this paper the characterisation of the particles was insufficient and the administered dose (5 g/kg/bw) was high.

Particles may pass through the epithelial cells through transcytosis by enterocytes (as in normal digestion), transcytosis by M-Cells in Peyer's patches (PP), or by passive diffusion. The GI uptake rate of ENMs is 2-200 times greater in PP than in enterocytes; however, the PP only represent ~1% of the total intestinal surface area (Des Rieux *et al.*, 2006). Translocation of ENMs (100 nm; average tested size 116±5 nm) is 15-250 times greater than that of microparticles, which are more likely to become lodged within PP (Desai *et al.*, 1996; Des Rieux *et al.*, 2006). The GI absorption of ENMs may be affected by different surface coatings, as shown for detergent coated polymethyl methacrylate (130±30 nm) administered by oral gavage to rats (Araujo *et al.*, 1999) and an effect on degradation in the GI tract of poly(D,L-lactic acid) NPs (95 and 150 nm) coated with albumin or polyvinylalcohol administered by gavage to guinea pigs (Landry *et al.*, 1998).

4.3.2. Distribution

Upon contact with the intestinal sub-mucosal tissue, ENMs can enter the capillaries, which will carry them through the portal circulation to the liver, or they enter the lymphatic system, which via the thoracic duct empties into the systemic blood circulation.

An important property of ENMs is interaction with proteins (Linse *et al.*, 2007; Lynch and Dawson, 2008). Protein adsorption to ENMs may enhance membrane crossing and cellular penetration (John *et al.*, 2001; Pante and Kann, 2002; John *et al.*, 2003). Furthermore,

interaction with ENMs may affect the tertiary structure of a protein, resulting in malfunctioning (Lynch *et al.*, 2006). Such ENM-protein interactions may not be static but change over time (Cedervall *et al.*, 2007a; Cedervall *et al.*, 2007b).

There is limited information on the distribution pattern of ENMs after oral exposure. In a 28-day rat oral study of 60 nm silver nanoparticles (Ag-NP), the highest Ag levels occurred in the stomach, followed by kidney and liver, lungs, testes, brain and blood (Kim *et al.*, 2008). Ag levels in the kidneys were, for all doses, twice as high in female rats as in males. The distribution is dependent upon particle size. With administration of gold nanoparticles (Au-NP) (58, 28, 10 and 4 nm) to mice, smaller particle size resulted in increased distribution to organs (Hillyer and Albrecht, 2001). If surface area is considered instead of mass, the impact of small size is greater. The smallest particles were found in kidney, liver, spleen, lungs and brain, while the biggest ones remained almost solely inside the GI tract. After uptake of polystyrene ENMs (50 nm) as high as about 7 % were found in the total of liver, spleen, blood and bone marrow (Jani *et al.*, 1990).

Preferential retention of large particles in the GI tract was also shown with 500 nm TiO₂ particles, which were present in PP and the mesenteric lymph nodes (Jani *et al.*, 1994). However, there was systemic distribution, and TiO₂ particles were detected in lung and peritoneal tissues, but not in heart or kidney. By chemical analysis Ti could be detected in liver, lungs, spleen, heart and kidney – however, as indicated in section 4.1.3, chemical detection does not provide information on whether it is present in its nanoform.

In the absence of information on distribution after oral exposure, data from other routes may give some knowledge on the fate of ENMs reaching the systemic circulation. After intravenous injection of 2 or 40 nm Au-NP in mice, the ENMs were taken up primarily by Kupffer cells in the liver by transcytosis and secondly by macrophages in the spleen and in other sites (Sadauskas *et al.*, 2007). In the Kupffer cells, Au-NPs were located in the lysosomes. After a single inhalation of 15 and 80 nm iridium nanoparticles (Ir-NP), the majority were found in the lungs of the rats, from which they were predominantly cleared via the mucociliary route into the GI tract and the faeces (Kreyling *et al.*, 2002). Minute translocation (<1%) was observed into liver, spleen, heart and brain. The translocation of the 80 nm particles was about one order of magnitude less than that of the 15 nm ones. Similar results have been reported in inhalation studies with various ENMs in rats (Oberdorster *et al.*, 2002; Takenaka *et al.*, 2006) and in humans (Mills *et al.*, 2006; Wiebert *et al.*, 2006a; Wiebert *et al.*, 2006b; Semmler-Behnke *et al.*, 2007a).

Two studies (Semmler *et al.*, 2004; Semmler-Behnke *et al.*, 2007a) provide the only existing data on long-term ENMs biokinetics in secondary target organs. After a single short-term inhalation of ENMs, only about 1-5 % of the inhaled ENMs crossed the air-blood-barrier and accumulated in secondary target organs (liver, spleen, kidneys, heart, brain, bone and remaining carcass). NP concentrations remained constant over the six months period. Prolonged inhalation exposure to Au-NP (mean diameter 20 nm) in rats over a total of 15 days during 3 weeks resulted in systemic distribution (Yu *et al.*, 2007; Kwon *et al.*, 2008). Similar wide distribution was seen in mice administered (~ 50 nm) fluorescent magnetic NPs (Yu *et al.*, 2007; Kwon *et al.*, 2008).

When rats were intravenously injected with solutions containing various sized Au-NP (10, 50, 100 and 250 nm), the distribution was found to be size-dependent, the smallest particles showing the most widespread distribution, including blood, heart, lungs, liver, spleen, kidney, thymus, brain, and reproductive organs (De Jong *et al.*, 2008). The largest NPs were present mainly in liver and spleen. Other intravenous studies showed similar results (Hillyer and Albrecht, 2001; Niidome *et al.*, 2006; Semmler-Behnke *et al.*, 2007b). Coating of Au-NP with

polyethylene glycol resulted in a prolonged systemic circulation compared to uncoated Au-NP (Niidome *et al.*, 2006). For composite nanodevices (CND, dendrimeric polymers with an inorganic core; 11 and 22 nm), size was also a determining factor for distribution (Balogh *et al.*, 2007). In addition, the positively charged CND of 5 nm showed highest uptake in the kidney, while for negatively charged and neutral CND the highest uptake was in spleen and liver.

C₆₀ fullerene appears to pass through the placental barrier, as shown after intraperitoneal administration of C₆₀ fullerenes, solubilised with polyvinyl pyrrolidone (50 mg/kg; day 18 of gestation), with distribution throughout the embryo (Tsuchiya *et al.*, 1996). However, Au-NP injected intravenously (2 and 40 nm) or intraperitoneally (40 nm), did not seem to penetrate the placental barrier (Sadauskas *et al.*, 2007). In contrast, small fractions of both Au-NP (1.4 and 18 nm) were found in the placenta and in foetuses 24 hours after intratracheal or intravenous administration to pregnant rats in their 3rd trimester (Semmler-Behnke *et al.*, 2007b).

4.3.3. Metabolism (biotransformation)

There is little information regarding biotransformation of ENMs after oral administration. The metabolism of ENMs should depend, among other properties, on their surface chemical composition. Polymeric ENMs can be designed to be biodegradable. For metal and metal oxide ENMs the slow dissolution will be of importance. The importance of the particle surface area on the dissolution kinetics was discussed for micron-sized particles (Kreyling and Scheuch, 2000); there was enhanced dissolution kinetics of metal-containing particles in the acidic milieu of phagolysosomes of macrophages, as compared to that within pH-neutral biofluids.

4.3.4. Excretion/elimination

There is very limited information on the excretion of absorbed ENM. After intravenous administration in mice, 2 nm Au-NPs were partially filtrated into the preurine (Sadauskas *et al.*, 2007). After intravenous administration of Au-composite nanodevices (5 nm) to mice, Au was excreted in both urine and faeces. A positive surface charge (compared to neutral and negative surface charge) was found to increase both urinary and faecal excretion (Balogh *et al.*, 2007). For polymethyl methacrylate NPs (mean size 130 nm) administered orally to rats, 95 % of the total amount absorbed was eliminated after 2 days, and after 8 days less than 0.5 % of the administered dose remained (Nefzger *et al.*, 1984). The absorption of the administered dose was 10-15 %, and within 8 days 5-8 % was excreted via the bile, and 4-6 % via the urine.

There is little information on the rate of ENMs elimination. For intravenously administered TiO₂-NP in rats, the highest levels were found on day 1 in all organs. TiO₂ was retained in the liver for 28 days; there was a slight decrease in TiO₂ levels from day 1 to days 14 and 28 in the spleen, and a return to control levels by day 14 in the lung and kidney (Fabian *et al.*, 2008).

Renal clearance of intravenously injected quantum dots (QD) with a cadmium-selenide (CdSe) core and zincsulfide (ZnS) shell in rats has been described. A surface-modified QD with a neutral coating (cysteine) prevented protein binding and thereby particle aggregation, such that QD with a hydrodynamic diameter less than 5.5 nm were prominently cleared by the kidneys into urine, while larger QD (up to 8.6 nm) were less cleared and accumulated in secondary target organs (Choi *et al.*, 2007).

The clearance of unmodified and surface modified carbon single-walled nanotubes (SWNT) and carbon multi-walled nanotubes (MWNT) injected intravenously into a guinea-pig model was compared (Singh *et al.*, 2006). The surface coating increased the hydrophilicity and the positive charge of the SWNT and MWNT and led to significantly increased dispersability in

blood and to prominent excretion via urine. There is no information on transfer of ENMs into milk.

4.3.5. Conclusion on Toxicokinetics

- There is likely to be large differences in toxicokinetic properties between varying types of ENMs.
- Toxicokinetic studies on ENMs following oral exposure have been performed mainly on metals and metal oxides (i.e. insoluble materials) and some gradually degrading polymers. For other ENMs, there is very little information available at present.
- In most toxicokinetic studies the ENMs were not characterized as administered (e.g. as to formation of agglomerates).
- In the available studies, quantification has almost always been through determination of the element in the ENM, without confirmation that the nanostructure was preserved.
- Formulation at the nanosize may modify the toxicokinetic behaviour of ENMs, as compared to the micro/macroscale form.
- Current data indicate that ENMs dispersed in the food/feed matrix may undergo changes in the food/feed and/or in the GI tract (solubilisation, interaction with food/feed or GI components), which may modify their physico-chemical properties and absorption.
- ENM studied to date are absorbed only to a limited extent from the GI tract. ENMs absorbed through enterocytes will go through the portal circulation to the liver. ENMs can also enter via the lymph system into the thoracic duct, thus bypassing the liver.
- The liver and the spleen are known to be two major organs for systemic distribution of metallic ENM. However, for certain ENMs, all organs may be targets, as in all organs investigated so far, the chemical component of the ENM, or the ENMs themselves, could be detected.
- Smaller-sized ENMs have a more widespread tissue distribution compared to larger ENMs, although data following oral exposure is limited. Surface coating and charge also seem to be of importance, but these have been investigated to a lesser extent. What other properties are important is not known at present.
- There is some information that certain ENMs can pass across the placenta. There is no information on whether ENMs are transferred into milk.
- There are only limited data on potential, long-term accumulation/persistence of ENMs. However the limited data available suggest that insoluble ENMs may be retained for a long time and accumulate.

4.4. Toxicity of ENMs

4.4.1. Acute, subacute and subchronic oral toxicity to ENMs

Only a limited number of oral toxicity studies using ENMs have been published, mostly using insoluble metals and metal oxides.

4.4.1.1. Metals

Single gavage administration to mice of copper NPs (Cu-NP) with average size 23.5 nm was compared to microparticles (MP)-Cu (17 µm) and Cu ions (Chen *et al.*, 2006). The doses were high (up to 1,080 mg/kg bw), which caused agglomeration of particles, with intestinal obstruction. The relative toxicity of ions was higher than for NP which in turn was higher than the MP. Dose-dependent pathology occurred in kidney, liver, spleen and blood (but not lung, heart, brain, testes or ovaries) in animals exposed to NPs (but not in those exposed to MPs).

After single gavage administration of high doses (5 g/kg bw; attempts were made to avoid particle agglomeration) of Zn as NPs (58 nm) and MP (1.08 µm) to mice there was GI inflammation in both groups (Wang *et al.*, 2006). The toxicity patterns were not consistent: in some aspects, the NPs were more toxic (anemia, kidneys, heart) than the MPs, which seemed to be more hepatotoxic. In a later single-dose oral toxicity study of ZnO (1-5 g/kg bw) in mice, two sizes of ENMs (20 and 120 nm) were compared to conventional micro/macroscale material (Wang *et al.*, 2008). The sizes of the ENMs were analysed in the gavage, and were found to average 44.8 and 187.5 nm, respectively. Again, the toxicity pattern was complex: the 120 nm ENMs were most toxic in stomach, liver, heart, spleen, kidneys and blood, while the 20 nm ENMs were similar to the toxicity of the micro/macroscale material (except in pancreas, where they were the most toxic). However, no dose-dependency was observed.

TiO₂ NPs (25, 80 and 155 nm) administered as single high-dose gavage (5 g/kg bw) to mice resulted in frequent oesophagus rupture (Wang *et al.*, 2007). All particles accumulated predominantly in the liver and spleen. Kidney, liver and heart pathology was observed with all sizes, with 80 and 155 nm particles producing the most pronounced effects, while changes in serum biochemical parameters (e.g. increased lactate dehydrogenase and alpha-hydroxybutyrate dehydrogenase levels) were most pronounced for the 80 nm particles. Administration of TiO₂ particles (500 nm) by daily gavage for 10 days (12.5 mg/kg) to rats produced no pathology (Jani *et al.*, 1994).

A 28-day oral toxicity study in rats of Ag-NPs (60 nm in doses 30, 300 and 1000 mg/kg/day) showed minimal dose-dependent biochemical liver toxicity, with no effect at 30 or 300 mg/kg bw/day (Kim *et al.*, 2008).

4.4.1.2. Other ENMs

Only a few studies have been reported on non-metal ENMs.

Several studies report oral toxicity of 20-60 nm selenium nanoparticles (Se-NP) in rats. With single gavage dosing, sodium-selenite ions were more toxic than the Se-NP (Zhang *et al.*, 2001; Zhang *et al.*, 2004). This was confirmed when the Se-NP were administered in feed to rats (2-5 mg/kg bw; appearance in the feed not defined) for 13 weeks (Jia *et al.*, 2005) and to mice (2-6 mg/kg bw/day; 12-15 days; NP-Se 20-60 nm (Zhang *et al.*, 2005)).

In broiler chickens (1-42 days old) fed a diet containing nanoclay (montmorillonite nanocomposite; 10-60 nm) for 42 days, no toxicity was found (Shi *et al.*, 2006). In a small single-dose (2 g/kg bw) rat study of amphiphilic chitosan NPs (~200 nm by scanning, 85 nm by

transmission electron microscopy), no toxic effects were observed (Yoksan and Chirachanchai, 2008). When carbon MWNT (diameter <50 nm, length 450 µm) and nitrogen-doped carbon MWNT (nitrogen atoms embedded in the carbon network) (diameter 20-40 nm, length 100-300 µm), were administered to mice in a single oral dose (1, 2.5 and 5 mg/kg bw), no toxicity was observed (Carrero-Sanchez *et al.*, 2006).

4.4.2. Toxicity from non-oral exposure to ENMs and *in vitro* studies

Data on toxicity are available from studies of inhalation and dermal exposure (SCENIHR, 2007), and some may be useful in indicating effects following oral exposure. Immune and inflammatory effects can be triggered by oxidative stress and/or production of pro-inflammatory cytokines in the lungs, liver, heart and brain (Oberdorster *et al.*, 2005a; Oberdorster *et al.*, 2005b; Borm *et al.*, 2006; Oberdorster *et al.*, 2007). Effects of some inhaled ENMs on the cardiovascular system include heart-rate changes, pro-thrombosis and acute myocardial infarction (Borm *et al.*, 2006).

There is a wealth of *in vitro* studies of ENMs in human or animal cells. A wide range of ENMs (e.g. Ti, Ag, Zn, Mn, Se and Si), concentrations and exposure times have been studied. There are intricacies in testing particulates in *in vitro* systems. Typical problems in such studies have been administration of physiologically non-relevant doses, aggregation of particles, direct exposure of the cells to the ENM, as well as the interpretation of the results.

However, a common finding in the *in vitro* assays, independent of the ENMs studied, seems to be the generation of reactive oxygen species (Donaldson and Borm, 2004; Oberdorster *et al.*, 2005b; Nel *et al.*, 2006; Balbus *et al.*, 2007; Chen *et al.*, 2008; Lewinski *et al.*, 2008). A major consequence of oxidative stress is damage to nucleic acid bases, membrane lipids and proteins (including formation of intranuclear protein aggregates) (Chen *et al.*, 2008). Generally these effects are observed only after exposure to high concentrations of ENMs and it is difficult to know whether the effects are physiologically relevant (Lewinski *et al.*, 2008). Interaction between ENMs and subcellular organelles may lead to cell death by activation of apoptotic and necrotic pathways (Xia *et al.*, 2006; Hong *et al.*, 2006; Di Pasqua *et al.*, 2008; Kagan *et al.*, 2006). Other *in vitro* effects observed are ion channel blockage, pore formation and physical disruption (ICON, 2008).

The genotoxic effects of conventional particles are driven by two mechanisms – direct genotoxicity and indirect (e.g. mediated through oxidative stress or inflammation) genotoxicity (e.g. Schins and Knaapen, 2007). Nanoparticles may act via either of these pathways. Several studies with ENMs have indicated that some ENMs may be genotoxic including iron/platinum, cobalt/chromium (CoCr), ZnO, SiO₂, TiO₂, carbon black (CB), carbon SWNT and carbon MWNT (reviewed by Gonzalez *et al.*, 2008; Landsiedel *et al.*, 2008). The assays used were the Comet assay for DNA damage, the micronucleus assay (*in vitro*) for numerical or structural chromosomal alterations, and gene-mutation assays (e.g. Ames test). For Comet and micronucleus assay systems, the majority of studies were positive. However, the interpretation of the data presented in the reviewed papers was hampered by differences in methodology, sometimes minimal characterization of the ENMs used and the lack of information on possible contaminants. For TiO₂ and CB it was reported that the nanosized (~ 20 nm) particles induced DNA damage, while larger particles (~ 200 nm) did not (Gurr *et al.*, 2005; Mroz *et al.*, 2008; Rahman *et al.*, 2002). Similar observations were reported for Co-NPs (Papageorgiou *et al.*, 2007).

4.4.3. Metrics for dose-response relations of ENM

So far, it has not been possible to establish a single dose-describing parameter that correlates with the possible toxicity of ENM. It is likely that mass concentration alone, which is the only parameter given in most studies, is not a good metric, as it does not incorporate the specific characteristics of ENMs (SCENIHR, 2006; SCENIHR, 2007a). Number of particles and surface area may be more appropriate. It is clearly desirable to characterize ENMs as completely as possible (Thomas and Sayre, 2005; Oberdorster *et al.*, 2005a; Powers *et al.*, 2006); OECD, 2008b). It seems unlikely that one specific dose-metric will be sufficient.

The currently proposed definitions of nanomaterials are solely based on size metric (e.g. in the order of 100 nm or less), which means that larger agglomerates/aggregates of primary nanoparticles are not recognized as belonging to the ENMs. As a metric, the specific surface area is independent of the agglomeration status of the particles. Therefore, a definition including specific surface area seems relevant from a risk assessment point of view (SCENIHR, 2009). As an example, nano scale spheres of 100 nm diameter of unit density provide a specific surface area of 60 m²/g which increases with decreasing diameter.

4.4.4. Additional considerations

Some other aspects increase the uncertainty in assessment of ENM. The presence of ENMs in food might affect normal food components or contaminants. It is known that ENMs can adsorb or bind different compounds and moieties on their surfaces (Simon and Joner, 2008a), including proteins (Lynch and Dawson, 2008). This has raised speculation whether some ENMs can act as carriers of potentially harmful chemicals and foreign substances into the blood, and different tissues and organs of the exposed organism. There is currently no direct evidence for this, but some studies may provide indirect evidence for such a “Trojan horse” effect. For example, iron oxide (magnetite) nanoparticles are known to adsorb and transport different toxic elements, including arsenic (Shipley *et al.*, 2008). Further, immunisation with carbon soot (particle size ~500 nm) has been reported to yield specific antibodies to polycyclic aromatic hydrocarbons (PAHs) (Matschulat *et al.*, 2006), indicating that the particles were probably acting as a carrier of PAHs. Enhanced toxicity of phenanthrene has been shown in the presence of C₆₀ fullerene aggregates (~200 nm) to algae and to daphnids (Baun *et al.*, 2008). In vitro cytotoxic effects of carbon SWNT in cultured human keratinocytes were exacerbated due to traces of iron catalyst that resulted from the manufacturing process. The GI absorption of the drug azidothymidine (AZT) was increased when it was bound to coated hexacyanoacrylate NPs (230±20 nm) (Lobenberg *et al.*, 1997).

The use of nano-carrier systems that are designed to deliver nutrients and supplements in food raises similar concerns that they may carry unintended macromolecules, such as undigested proteins across the GI tract. If particles pass through the epithelial cells via transcytosis by M-Cells this may lead to accumulation within the PP and subsequently a possible immune reaction. This may lead to unexpected allergic effects.

There are preliminary indications of association of GI disorders with absorption of ENM. There are reports of increased uptake of ENMs during GI inflammation, findings of particles in colon tissue in subjects suffering from ulcerative colitis and speculation that ENMs exposure might be associated with Crohn’s disease (McMinn *et al.*, 1996; Lomer *et al.*, 2002; Gatti *et al.*, 2004; Hoet *et al.*, 2004; Buzea *et al.*, 2006).

The surface properties (e.g. coatings) that increase the active uptake of encapsulates might also be a reason for concern. Lectins used for coatings of nano-encapsulates can be cytotoxic or induce inflammatory responses (Govers *et al.*, 1994; Des Rieux *et al.*, 2006).

Recently, specific carbon nanotubes with characteristics similar to asbestos, in terms of fibre length, rigidity and persistence, were shown to induce "asbestos-like" granulomatous inflammation after intraperitoneal administration in mice (Poland et al., 2008; Takagi et al., 2008), which indicates that the morphology of the ENMs affects toxicity.

4.4.5. Conclusion on Toxicity of ENM

- The understanding of the potential toxicity after oral intake of ENMs is in its infancy. Only a very limited number of ENMs have been studied after oral administration, mainly metals and metal oxides. The ENMs used in the toxicity studies were often characterized only to a very limited extent.
- Only a few studies have compared the toxicity of nanoformulated and conventional (dissolved or micro/macroscale) form of the same chemical species. These data are insufficient to draw general conclusions.
- In only one study was the ENM administered via feed, but the ENM was not characterized in this matrix (e.g., as to formation of agglomerates). In all other studies, the ENMs were administered in artificial dispersions (i.e. via gavage).
- Most of the reported oral *in vivo* studies are on acute toxicity of ENMs. Long-term studies have not been conducted.
- It is unlikely that there is a generic toxicity of ENMs that would allow prediction of effects for untested ENMs. There is no adequate information that allows general conclusions on the relationship between physico-chemical properties (size, surface properties, chemical composition, etc.) of ENMs and toxicity *in vivo* or *in vitro*.
- Mass is most likely not a sufficient dose-metric; it is unlikely that one specific metric will be sufficient. Candidates for dose-metrics are mass, number of particles and surface area.
- It is generally not possible to extrapolate the potential toxicity of ENMs from information on dissolved or micro/macroscale chemicals.
- Numerous *in vitro* studies have shown that some ENMs induce oxidative stress at high concentrations. There are some data to indicate possible genotoxic and inflammatory responses *in vitro*.

5. Environmental aspects of nanotechnologies in the food and feed area

It is considered by the Scientific Committee that it is beyond the scope of this opinion to treat environmental impacts of nanotechnology comprehensively. Environmental aspects are covered thoroughly by SCENIHR (2007a, 2009), the UK Royal Commission on Environmental Pollution (2008), and in several recent reviews (Klaine *et al.*, 2008; Handy *et al.*, 2008b; Wiesner and Bottero, 2007; Nowack and Bucheli, 2007). Still, we would like to point out a few environmental issues that are relevant for the possible re-entry of ENMs in the food and feed chain.

In some instances, there is the possibility that certain ENMs enter the food and feed chain as contaminants. Dispersal of ENMs to the environment during production, use and disposal of ENMs is likely also in the food and feed area. Contamination may also arise through traditional processes of waste disposal, e.g. via sewage, from waste incineration or leakage from landfills. Consequent contamination of water and soil may theoretically lead to uptake of ENMs in plants and seafood for human or animal consumption. Uptake of ENMs in plants and aquatic species has been demonstrated (Lin and Xing, 2008; Handy *et al.*, 2008a).

6. Guidance for risk assessment (RA) of ENMs in food and feed area

In this section, generic aspects of the RA of ENMs will be discussed. The intention is not to supply detailed guidance to petitioners but to outline the general data needed for a risk assessment.

The Scientific Committee view is that the RA paradigm (namely hazard identification, characterisation, exposure assessment and risk characterisation) can also be applied to the RA of ENMs in the food and feed area. From the RA perspective ENMs are different from the same chemicals in other forms and existing knowledge on chemicals cannot be fully extrapolated (e.g. SCENIHR, 2007a). A number of national and international advisory committees have recommended strategies for the RA of ENMs (e.g. SCENIHR, 2007a, 2009; SCCP, 2007).

A difficulty at the present time in giving detailed specific RA guidance is the lack of sufficient data and information, which would allow for a comprehensive understanding of potential hazards of ENMs. The conventional toxicological testing methods should be used as a starting point to identify hazards from ENMs. However, additional issues, specific for the properties of ENMs, e.g. toxicokinetics and the possibility of additional toxicological effects, need to be considered. Specific attention should also be paid to exposure assessments. A major difficulty is the lack of routine analytical methods for detection and analysis of ENMs in food and feed. Hence, until a sufficient body of data is developed, RA of ENMs will have to be carried out on a case-by-case basis (which, in general, is not different from RA of chemicals in other forms).

The RA methods will need to be adapted and refined as the knowledge-base develops. The specific RA framework applied to substances in food and feed areas (such as in FCM, pesticides, or in the food/feed additive area) will in general still be applicable but modifications may be necessary to take account of the special properties of the ENMs in these areas. Current guidance documents in the food and feed area do not address ENMs (see recommendations section).

Information to be supplied for the RA should describe the intended use and function of the ENM. The efficiency and reliability of applications which claim antimicrobial activity and of ENMs used as biosensors in food or feed to indicate or imply the safety of the product in terms of biological hazards at the industrial and consumer levels require to be confirmed and documented as a condition for use as this may have important downstream consequences for food safety.

A first step of the RA of ENMs is the proper identification and detailed characterization of the product as used in food/feed. There is ongoing activity within OECD and ISO for the adequate characterization and description of ENMs (OECD 2008a; b; ISO 2008). At present time, the more important characteristics are from a RA perspective the following: size (including distribution), agglomeration/aggregation, mass, surface area, specific surface area, number, shape, density, morphology, porosity, chemical composition (including impurities and processing chemicals), surface properties (e.g. coating, charge) and solubility (including lipophilicity/hydrophilicity). Additional characteristics of importance are biodegradability or biopersistence. For this purpose standard methodologies (including additional reference/benchmark materials) are needed. As indicated earlier, the relationship of any observed toxicity to the various dose metrics that may be used is currently discussed and several dose metrics may need to be explored in addition to mass, e.g. surface area and particle number (See section 4.4.3 and SCENIHR, 2007a, 2009).

It should be emphasised that characterization of the ENM, both as manufactured or added, as well as of the ENMs as present in the food/feed is desirable as it is likely that ENMs will interact with food/feed components. A crucial step is to define (confirm) qualitatively and quantitatively the presence of the ENM in the nanoform in the food/feed. The same applies to FCM in which it is essential to investigate the migration using a suitably sensitive method. This is closely linked to the availability of sufficiently sensitive analytical methods.

As it is generally difficult at present to analyse food and feed for the presence of ENMs, a conservative approach in the RA is to assume that the entire amount of ENMs added to the food/feed or migrating from FCM is present in its nanoform.

If it is properly demonstrated that the product as such does not contain ENM, or that the ENM does not persist in the food/feed, then there is likely no exposure to ENM, and the further RA would not differ from that of a conventional chemical in the dissolved or micro/macroscale form.

Where exposure to ENM with preserved nanoscale structure can not be excluded in animals or humans, a number of points should be addressed. Based on the physico-chemical properties of the ENM, a consideration of the potential fate in the lumen of the GI tract of the ENM following ingestion should be undertaken. Also, testing with relevant biological fluids may be appropriate. If there is evidence that the ENM dissolves in the lumen, this may be sufficient to allow the conclusion that, if absorbed, the ENM would behave the same as the non-nanoform of the chemical, and the RA can be based on this. However, possible local exposure and potential effects should still be considered. If there is no information to prove the disappearance of the nanostructure, it shall be assumed that the nanoform is still present in the GI tract.

If the nanostructure persists in the GI tract, there will be a need for toxicokinetic data. Information on toxicokinetics will have to rely on *in vivo* studies, since proposed *in vitro* systems have not yet been validated for extrapolation to *in vivo* conditions. Because of the current difficulties in analysing ENMs as such in biological tissues, the toxicokinetic studies may have to rely on determination of the chemical constituent of the ENM, without knowledge of whether it is still present in nanoform. In that case, a conservative approach should be applied and it shall be assumed that it still is present in its nanoform. The toxicokinetic studies supply important information for decisions regarding further testing regimes and assessment.

For ENMs which are intended to increase the bioavailability of incorporated substances (i.e. ENM carrier systems), the changes in bioavailability should be determined. A difference in bioavailability of the incorporated substance needs to be considered when using information from the RA of that incorporated substance. In addition, a RA should be performed on the nanoscale carrier.

In general, the toxicological properties of substances, including ENMs, used in the food and feed area need to be assessed by *in vivo* assays. Guidelines for toxicity testing of conventional chemicals are available (e.g. OECD guidelines). These tests should be able to pick up toxic effects of ENMs (OECD 2008b). However, experience in using these guidelines/tests with ENMs is very limited and the adequacy of the endpoints assessed in existing toxicological tests to detect all aspects of potential toxicity of ENMs has yet to be established.

In vivo toxicology studies on food chemicals are normally conducted using admixture into the diet. For ENMs, the way of administration must be considered in the context of the likely interaction of the ENMs with food/feed components. This is an argument for inclusion of the testing material into food/feed for toxicology and exposure assessment. Administration via oral gavage, using a liquid vehicle, into which the ENM is incorporated, might be regarded as a more controllable dose administration method than incorporation into the diet. It may also, as

an initial step, represent a worst case, conservative approach. However, incorporation of ENMs into liquids may alter their physico-chemical characteristics (e.g. with respect to agglomeration/aggregation) in a way that would not necessarily reflect their characteristics in the anticipated food/feed matrix. The choice of administration method should always be justified.

In vitro studies are generally suited for screening purposes and studies on mechanisms of toxicity (COT, 2005; 2007; Oberdorster *et al.*, 2005a; Borm *et al.*, 2006). Concerning *in vitro* tests, the sensitivity and validity of available assays for assessing risks of ENMs exposure is uncertain, as was also concluded by SCENIHR (2007a). For some toxicological endpoints, such as mutagenicity/genotoxicity and oxidative stress, *in vitro* assays are available.

For the risk characterization step, the strategy for ENMs would not, in principle, differ from that followed for soluble chemicals or the micro/macroscale material. The considerations in the use of uncertainty factors for RA of ENMs is not different from other forms of the same chemical. However, the problems in analysis and the limited database on ENMs assessments should be taken into account.

OVERALL CONCLUSIONS AND RECOMMENDATIONS¹⁴

CONCLUSIONS

This opinion is generic in nature and is not in itself, a risk assessment of nanotechnologies as such or of tentative applications or possible uses thereof or of specific products. The possible uses of nanotechnologies and the applications in the food and feed area are varied and developing. The possible uses and applications span all the various steps and processes throughout the food chain, including production processes, agrochemicals, feed and food contact materials, and food/feed ingredients. There is as yet no overview of products that may be present on the EU market. The nanospecific properties and characteristics of ENMs are likely to affect their toxicokinetic behaviour and toxicity profile. The guidance section indicates the general data needs and aspects that will need to be considered when performing a RA of an ENM.

The Scientific Committee specifically concludes that

- Current uncertainties for risk assessment of nanotechnologies and their possible applications in the food and feed area arise due to presently limited information in several areas. Specific uncertainties apply to the difficulty to characterize, detect and measure ENMs in food/feed and biological matrices and the limited information available in relation to aspects of toxicokinetics and toxicology, including optimal methods for testing ENMs. There is limited knowledge of (likely) exposure from possible applications and products in the food and feed area and of environmental impacts of such applications and products. The current usage levels of ENMs in the food and feed area is unknown. The limited database on ENMs assessments should be considered in the choice of appropriate uncertainty factors in the risk characterization step.

Whilst recognising these limitations, the usual risk-assessment paradigm (hazard identification, hazard characterization, exposure assessment and risk characterization) is considered applicable for ENMs.

- Risk assessment of ENMs in the food and feed area should consider the specific properties of ENMs in addition to those common to the equivalent non-nanofoms.

¹⁴ It was not within the scope of this opinion to consider the whole life cycle of nanotechnology products and applications.

- The available data on oral exposure to specific ENMs and any consequent toxicity is extremely limited; the majority of the available information on toxicity of ENMs is from *in vitro* studies or from *in vivo* studies using other routes of exposure.
- Current toxicity testing approaches used for conventional materials are a suitable starting point for case-by-case risk assessment of ENMs. However, the adequacy of currently existing toxicological tests to detect all aspects of potential toxicity of ENMs has yet to be established. Toxicity-testing methods may need methodological modifications (e.g. regarding sample preparation and characterization). Specific uncertainties arise due to limited experience of testing ENMs in currently applied standard testing protocols. There may also be additional toxic effects caused by ENMs that are not readily detectable by current standard protocols. Additional endpoints not routinely addressed may need to be considered in addition to traditional endpoints.
- For hazard characterization, the relationship of any toxicity to the various dose metrics that may be used is currently discussed and several dose metrics may need to be explored in addition to mass e.g. number concentration and total surface area.
- The different physicochemical properties of ENMs compared to conventional soluble and/or micro/macroscale chemical equivalents imply that their toxicokinetic and toxicity profiles cannot be fully inferred by extrapolation from data on their equivalent non-nanoforms. Thus, the risk assessment of ENMs has to be performed on a case-by-case basis.
- Appropriate data for risk assessment of an ENM in the food and feed area should include comprehensive identification and characterization of the ENM, information on whether it is likely to be ingested in nanoform, and, if ingested, whether it remains in nanoform at absorption. If it may be absorbed in nanoform, then repeated-dose toxicity studies are needed together with appropriate *in vitro* studies (e.g. for genotoxicity). Toxicokinetic information will be essential in designing and performing such toxicity studies. For ENMs which are intended to increase the bioavailability of incorporated substances (i.e. ENM carrier systems), the changes in bioavailability should be determined.
- Although, case-by-case evaluation of specific ENMs may be currently possible, the Scientific Committee wishes to emphasise that the risk assessment processes are still under development with respect to characterisation and analysis of ENMs in food and feed, optimisation of toxicity testing methods for ENMs and interpretation of the resulting data. Under these circumstances, any individual risk assessment is likely to be subject to a high degree of uncertainty. This situation will remain so until more data on and experience with testing ENMs become available.

RECOMMENDATIONS

- When RA guidance documents in the food and feed area are revised, nanotechnology aspects shall be considered.

In relation to nanoscale definitions:

- Include into the current definition of nanoscale materials, which is now solely based on size metric, the additional metric of specific surface area.

In relation to applications of nanotechnologies in the food/feed area it is recommended to:

- Monitor current and future commercial applications of ENMs in the food and feed sectors and developments of nanotechnologies, especially since more complex ENMs may be foreseen.

In relation to the physico-chemical characterization of ENM, stability in FCM, food and feed matrices, and analytical tools it is recommended to:

- Determine the effects of size of ENMs on physico-chemical properties, compared to those of the dissolved chemical or micro/macroscopic materials.
- Investigate the interaction and stability of ENMs in the presence of components in food and feed matrices, in the GI tract and biological tissues.
- Develop and validate routine methods to detect, characterize and quantify ENMs in FCM, food and feed matrices and in biological tissues.
- Generate information on the effects of processing on the characteristics of ENMs.

In relation to exposure assessment of ENMs it is recommended to:

- Generate information on the amount and form (dispersed or aggregated) of ENMs content in food and feed, and the bioavailability of the nanoform following ingestion.
- Generate information on consumption of products containing ENM.
- Determine migration of different ENMs from FCM into food and feed.

In relation to toxicokinetics and toxicity of ENMs it is recommended to:

- Generate information on toxicokinetic properties of ENMs after oral exposure. Correlate these data with the physicochemical characteristics to see whether different ENMs can be grouped. Generate information on appropriate dose metrics in relation to toxicity of ENMs.
- Generate information on the bioavailability from food and feed of a range of ENMs and investigate potential accumulation in different organs and transport through the placenta and into milk. Also, biotransformation and excretion should be addressed.
- Generate information on carry-over of ENMs along feed/food chain, e.g. incorporation in edible animal tissues.
- Develop, improve and validate *in silico*, *in vitro* and *in vivo* (in particular oral) test methodologies to assess toxicity of ENMs (including reliability and relevance of the test methods).
- Develop understanding of the toxicity following oral intake of a wide range of ENMs for which there is likely exposure.
- Develop understanding on whether ENMs interact with biomolecules (e.g. enzymes), nutrients and foreign compounds and the significance of such interactions for human and animal health, including potential transport of toxic chemicals, allergens and other substances (“Trojan horse” effect).
- There are substances approved for use in food and feed, which have been claimed to also be available in nanoscale dimensions. In view of the concerns about nanoscale preparations, a re-evaluation of the risk assessment of such substances should be considered.

DOCUMENTATION PROVIDED TO EFSA

EFSA published a call for data on its website between 23 January and 28 March 2008. Information was received from the following organisations:

Bund für Lebensmittelrecht und Lebensmittelkunde e. V. (BLL)

Communication of information, e-mail 31/03/2008.

Sachstands- und Positionpapier Nanotechnologie Stand März-2008. Pages 1-4.

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Dr. Eric Gaffet

Communication of information, e-mail 18/02/2008. Nano and alimentation/Emballage. Power point presentation. Pages 1-71.

Dr. Antonietta Gatti

Communication of information, e-mail 30/01/2008. References to publications. 1 page.

Environmental Defense Fund

Communication of information, e-mail 2/04/2008.

Nano Risk Framework, June 2007. Environmental defense – DuPont. Nano Partnership. Pages 1-104.

Nano Risk Framework, Executive Summary, June 2007. Pages 1-3

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GLOSSARY / ABBREVIATIONS

To assure a consistent use and understanding throughout this opinion, some words of key importance are provided below.

Glossary

Term	Definition as used in the opinion
Agglomerate	A group of particles held together by weak forces such as van der Waals forces, some electrostatic forces and or surface tension.
Aggregate	A group of particles held together by strong forces such as those associated with covalent or metallic bonds.
Aspect ratio	A ratio describing the dimension length over dimension height or width. The higher the aspect ratio, the longer the material is in comparison to its height or width, and approaches a more fibre/tread like appearance. Usually denoted as L/H.
Degradation	A change in the chemical structure, physical properties or appearance of a material
Engineered nanomaterial	Any material that is deliberately created such that it is composed of discrete functional parts, either internally or at the surface, many of which will have one or more dimensions of the order of 100 nm or less.
Nanocarrier	A nanoscale structure whose purpose is to carry a second substance (e.g. a vitamin.)
Nanocomposite	A multi-phase material in which the majority of the dispersed phase components have one or more dimensions of the order of 100 nm or less.
Nanomaterial	Any form of a material that is composed of discrete functional parts, many of which have one or more dimensions of the order of 100 nm or less.
Nanoparticle	A discrete entity which has all three dimensions in the order of 100 nm or less.
Nanoscale	A feature characterised by dimensions in the order of 100 nm or less.
Nanostructure	Any structure that is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less. Often used in a similar manner to nanostructure is the word 'nanomaterial'.
Nanotube	A discrete hollow entity which has two dimensions of the order of 100 nm or less and one long dimension.
Primary particle	A discrete entity that may make up an agglomerate or aggregate
Secondary particle	An agglomerate or aggregate made up of primary particles
Solubilisation	The process of dissolution.

Abbreviations

Abbreviation	Description
ADME	Science dealing with absorption, distribution, metabolism and excretion of substances in the body
CB	Carbon black
ENM	Engineered Nanomaterial
ENMs	Engineered Nanomaterials

FCM	Food Contact Materials
MP	Microparticle
MWNT	Multi walled nanotube
nm	Nanometre, 10^{-9} metre
NP	Nanoparticle
NPs	Nanoparticles
RA	Risk assessment
SWNT	Single walled nano tube
WG	Working Group