DRAFT SCIENTIFIC OPINION

Draft Opinion of the Scientific Committee on the Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety

(Question No EFSA-Q-2007-124)

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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 (ENM). 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 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, 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 changes the physico-chemical characteristics of materials as compared to the dissolved and 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 nanotechnologies and its 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 ENM in food/feed and biological matrices and the limited information available in relation to aspects of toxicokinetics and toxicology. There is limited knowledge of (likely) exposure from possible applications and products in the food and feed area or of environmental impacts of such applications and products. The current usage levels of ENM in the food and feed area is unknown. The limited database on ENM assessments should be considered in the choice of appropriate uncertainty factors in the risk characterization step.

Whilst recognising these limitations, the currently used risk assessment paradigm (hazard identification, hazard characterization, exposure assessment and risk characterization) is considered applicable for ENM.

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

The available data on oral exposure to specific ENM and any consequent toxicity is extremely limited; the majority of the available information on toxicity of ENM is from *in vitro* studies or *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 ENM has yet to be established. Toxicity-testing methods may need methodological modifications. Specific uncertainties arise due to limited experience of testing ENM in currently applied standard testing protocols. There may also be additional toxic effects caused by ENM that are not readily detectable by current standard protocols. Additional endpoints not routinely addressed and pharmacological endpoints 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 ENM compared to conventional dissolved and macroscale chemical counterparts 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 ENM 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 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.

Recommendations are given at the end of the opinion.

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, flavours, filters, food supplements, stabilizers) 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 analysts[1] 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.[2] The Commission adopted on 6 September 2007 a report for the European Parliament on the implementation of the Action Plan.[3] 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,[4] part of which will finance research on safety. Recently, the European Group on Ethics produced an opinion on ethical issues in nanomedicine.[5] The Commission adopted on 17 June 2008 a Communication on the Regulatory Aspects of Nanomaterials[6], 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 (OECD[7], 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).[8] 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.[9]

These opinions conclude that current risk assessment methodologies for bulk 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 opinions also

[1] http://www.nanoforum.org/dateien/download.php?userid=6385071&dateinr=714&dateiorig=000714.upl&dateiname=nanotechnology+in+agriculture+and+food.pdf&zeitcode=31052007175920
[2] http://cordis.europa.eu/nanotechnology/actionplan.htm
[3] COM(2007) 505 final
[4] http://cordis.europa.eu/nanotechnology/src/eu_funding.htm
[5] http://ec.europa.eu/european_group_ethics/activities/docs/opinion_21_nano_en.pdf
[6] COM(2008) 366 final
[7] http://www.oecd.org/department/0,2688,en_2649_37015404_1_1_1_1_1,00.html
[8] http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_003b.pdf
[9] http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_004c.pdf

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 focus on engineered nanomaterials (ENM) that are deliberately introduced into the food chain. Such ENM range from food contact material, 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 or engineered to have nanoscale properties, or used e.g. to encapsulate bioactive compounds.

The opinion will exclude incidental ambient nanostructured material contamination of food/feed, resulting from anthropogenic and natural sources. ENM used for waste water or soil treatment are not considered nor is the possible impact of ENM on plant health.

For the purpose of this opinion, ENM 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. ENM pesticides and fertilizers will be considered since they may be present as residues in food/feed plants. A second potential route of human exposure is the carry over of ENM or their residues from feed to human food.

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). In addition, the opinion is based upon published, peer-reviewed scientific papers and other information deemed reliable. EFSA launched a call for data through its Advisory Forum and on its website 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.

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 a size range in the (lower) nanoscale. Due to the small size of ENM, new unique properties arise. Examples of such properties of ENM are increased surface area, which can affect reactivity with other materials and increased translocation 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 a range of opportunities to improve resource utilization by providing means of 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 (e.g. BFR, 2008) or if nanomaterials are used

in food or food packaging, these technologies should be independently assessed for safety before they are placed the market (Which?, 2008).

Terms used in the opinion

In relation to risk assessment (RA) of ENM, the actual characteristics and properties of the ENM in question are the determining factors, rather than the terms used for its description. However, to describe ENM 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 publication on terminology and definitions (ISO, 2008).

The prefix "nano" specifically means a measure of 10^{-9} units, the nature of this unit being determined by the word that follows, e.g. "nanometre" as a measure of dimension. It is, however, unrealistic, for practical purposes, to consider the prefix "nano" to solely and precisely refer to 10^{-9} metres, just as it is not considered that "micro" specifically and solely concerns something with a dimension of precisely 10^{-6} metres.

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 uniquely 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.

Engineered nanomaterial (ENM) 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 nanoparticle (NP) is included in the use of the term ENM.

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 as ENM 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.

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 held together by weak forces, such as Van der Waals forces, electrostatic forces and/or surface tension. An agglomerate will normally retain a high surface-to-volume ratio.

An aggregate is a group of particles held together by strong forces, such as those associated with covalent or metallic bonds. It should be noted that an aggregate may retain a high surface to volume ratio.

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 ENM 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 five broad categories of nanotechnology applications in the food and feed sector have been described (Chaudhry *et al.*, 2008):

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 nano-textures to 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, pesticides, 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 food contact materials include 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 appears to be the expectation that, due to the fixed or embedded nature of ENM in plastic polymers, they are not likely to provide any significant exposure to the consumer.

An inventory of nanotechnology applications currently on the global food market and associated areas is available on the internet from the Project on Emerging Nanotechnologies[10]. EFSA is not aware of any database providing information on nanotechnology applications or products placed 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 from EU food industry organisations, there is currently no food ready for marketing, which is produced with the use of nanotechnologies or from ENM (CIAA, 2008 (communication provided to EFSA); BLL, 2008). The current status of FCM or uses of nanotechnology processes are more uncertain and such applications may be available on the EU market.

[10] http://www.nanotechproject.org/inventories/consumer/

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

Risk assessment (RA) is the evaluation of the potential for the occurrence of harmful effects on human or animal health or the environment. 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 ENM (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 ENM, such as the small size, surface reactivity and translocation across biological membranes, are issues that may need special considerations as well as interactions of ENM 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.

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

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

4.1.1. Characteristics of ENM

The principal physical parameters for the characterization of ENM are size (including its distribution), shape (including aspect ratios where appropriate) and the morphological sub-structure of the substance. 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 nanoencapsulates and for assessing the sites of distribution and/or accumulation, the lipophilicity/hydrophobicity (solubility) is an important trait.

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 ENM special is that as the size of the particles decreases, the surface area increases dramatically, until the amount of surface molecules is such that their properties dominate, and so ENM have novel properties determined by their high surface-to-volume ratios. This leads many ENM to have altered characteristics, which may be used for a range of

applications. The very high surface area of ENM has several consequences that need to be considered in risk-assessment contexts, as it makes them different from their macroscale counterparts: they have increased (surface) reactivity compared to the non-nanoscale material, since many more molecules are located at the NM surface in energetically unstable states. Almost all types of ENM catalyze reactions, mainly oxidation reactions. They may also act as nuclei in heterogeneous nucleation processes during crystallisation and recrystallisation in material sciences (and potentially with proteins).

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

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

It can be assumed that ENM agglomerates break up under certain conditions that occur in food, feed, the gastro intestinal tract and biological tissues. ENM can react 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). ENM may be fully surrounded by a dynamic "corona" of proteins and the ENM may affect the behaviour of the protein, and the protein that of the ENM. Hence, coating of ENM with specific proteins can influence their uptake and distribution and direct them to specific locations. The significance of this interaction for the safety and biological impact of ENM implies that detailed characterization of the ENM 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 time frame of the measurement processes, the so-called “hard-corona”.

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

A number of analytical tools exist for the qualitative and quantitative characterization of pristine ENM, both the single-particle techniques and the techniques characterizing the ensemble of ENM (Powers *et al.*, 2006; Hassellov *et al.*, 2008; Luykx *et al.*, 2008; Tiede *et al.*, 2008). Due to the enormous variety of ENM, 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 ENM in the matrix, as properties of ENM may depend on the surrounding matrix. This is a much more demanding task than to analyse in simpler 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 to find a detectable number of ENM in the matrix since high magnification is required due the small size of ENM. As a result, the investigation of ENM biodistribution in organs is generally extremely time-consuming, and to date has been possible only in selected cases of radio-labelled particles. A second complication

is the fact that some ENM cannot be distinguished from naturally occurring variants of the same material; one such example is engineered nanoscale SiO_2. Detection may also by hindered by interactions with solutes or cell constituents that obscure clear analytical signals.

The current limited number of standardized reference materials for ENM is another limitation on precise and reproducible detection and quantification of ENM 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 Measurements[11].

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 ENM can be quantified by chemical analytical tools, such as inductively-coupled 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. If ENM contain metals which also are endogenous, or are taken up with natural food (such as SiO_2), it will be impossible to quantify the amount of ENM. In the case of organic ENM, detection or quantification of the chemical may be possible, where a test for the species exists, but still it will be unclear whether it is in nanoform.

In summary, there are methods available to detect and analyse a number of ENM under certain conditions, but there are however no routine methods available for analysing ENM in the food and feed area.

4.2. Exposure to ENM from food and feed

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

Consumers can be exposed to ENM from various sources as indicated below. However, due to the current limited availability of products with declared use of nanotechnology 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 ENM in food and feed is extremely limited.

4.2.1. Sources of exposure

Several examples of FCM with incorporated ENM 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 ENM from FCM which indicate that some ENM may migrate while others do not (Avella *et al.*, 2005; FSA, 2008; EFSA, 2007). 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 ENM (or their residues) into food/feed through wear of food/feed processing machines with coatings containing ENM. There is no information on the potential exposure to residues following the use of nanotechnology devices (filters, etc.) in the manufacturing process of food/feed.

[11] http://www.irmm.jrc.be

Exposure from applications of nano-sized or nano-encapsulated food/feed ingredients or the incorporation of ENM 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, ENM in feed need to be transferred to edible tissues. Currently there are no studies available on whether such transfer occurs.

Residues of nano-formulated or nano particulate agro-chemicals and veterinary products are currently not likely as no nano-formulated pesticides or, fertilizers and veterinary drugs are currently commercially available in the EU. 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 ENM in consumer products (e.g., electronics, medicines, packaging materials) will inevitably result in environmental release of these particles over the product life-cycle (Nowack and Bucheli, 2007). ENM 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 ENM has not been estimated.

In conclusion, significant consumer and animal exposure to ENM ingredients in food and feed is currently not likely within EU, though there may be exposure to nanoscale fractions within other materials. However, products are 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 ENM via food or feed. Basically, the principles of exposure assessment of ENM (via food and feed) will be the same as in exposure assessment of non-nanoscale substances (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 macroscale or dissolved chemicals. The current food consumption databases can be used. 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 ENM 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 ENM can be assessed and used as an assumption in the exposure assessment, however, currently it is not possible to routinely determine ENM *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 ENM 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 ENM 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.

4.3.1. Absorption

Little is known regarding the behaviour and fate of ENM in the gastro intestinal (GI) tract. It is possible that they will not remain in a free form in the lumen (and hence not be available for translocation), due to transformations such as agglomeration, aggregation, adsorption or binding with other components of food, reaction with acid and digestive enzymes, etc. (see also Section 4.1.2). Adsorption studies have mostly been performed on metal and plastic ENM.

Translocation of particles through the intestinal wall is a several 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 ENM through the epithelium is depending 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 ENM would be extremely limited, as pore size at tight junctions is between 3 and 10 Å (0.3-1.0 nm) (Des Rieux et al., 2006).

Smaller particles are absorbed more readily and faster than larger ones. Absorption across the pre-epithelial mucus gel layer of rat distal colon showed that 14 nm (diameter) latex ENM cross within 2 minutes, 415 nm within 30 minutes, and 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 gastrointestinal uptake with diminishing size (Hillyer and Albrecht, 2001). The amount of absorption of polystyrene ENM (50 nm) has been shown to be 34 % in rats (Jani *et al.*, 1990). Titanium dioxide (TiO_2) particles as large as 500 nm have been found to be absorbed (Jani *et al.*, 1994).

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 gastrointestinal uptake rate of ENM 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 ENM (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).

4.3.2. Distribution

Upon contact with the intestinal sub-mucosal tissue, ENM 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 ENM is interaction with proteins (Linse *et al.*, 2007; Lynch and Dawson, 2008). Protein adsorption to ENM may enhance membrane crossing and cellular penetration (John *et al.*, 2001; Pante and Kann, 2002; John *et al.*, 2003). Furthermore,

interaction with ENM may affect the tertiary structure of a protein (or enzyme), 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 ENM after oral exposure. In a 28-day 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 Au-NP (58, 28, 10 and 4 nm) to mice, smaller particle size resulted in increased distribution to organs (Hillyer and Albrecht, 2001). The smallest particles were found in kidney, liver, spleen, lungs and brain, while the biggest particles remained almost solely inside the GI tract. After uptake of polystyrene ENM (50 nm) about 6 % were found in the 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 TiO_2 particles, which were present in PPs and the mesenteric lymph nodes (Jani *et al.*, 1994). However, there was systemic distribution and TiO_2 particles were detected in lung and peritoneal tissues, but not in heart or kidney. By chemical analysis titanium could be detected in liver, lungs, spleen, heart and kidney – however, as highlighted 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 ENM reaching the systemic circulation. 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 ENM in rat (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 ENM biokinetics in secondary target organs over six months after a single short-term nanoparticle inhalation. Only about 1-5 % of the inhaled nanoparticles crossed the air-blood-barrier and accumulated in secondary target organs (liver, spleen, kidneys, heart and brain and the soft tissue and bone and remaining carcass). Nanoparticle 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 nanoparticles (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 ENM 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 is 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.

C_{60} fullerene appears to pass through the placental barrier, as shown after intraperitoneal administration of C_{60} 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, Semmler-Behnke and coworkers (2007b) found small fractions of both Au-NP (1.4 and 18 nm size) in the placenta and in foetuses 24 hours after administration to pregnant rats in their 3rd trimester.

4.3.3. Metabolism (biotransformation)

There is no information regarding biotransformation of ENM after oral administration. The metabolism of ENM depends, among other properties, on their surface chemical composition. Polymeric ENM can be designed to be biodegradable, whereas for metal and metal oxide ENM the (partial) solubility 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 the enhanced dissolution kinetics of metal containing particles in the acidic milieu of phagolysosomes of macrophages was reviewed 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 of gold-composite nanodevices (5 nm) to mice, gold 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).

There is little information on the rate of ENM elimination. For intravenously administered TiO_2 NP in rats, the highest levels were found on day 1 in all organs. TiO_2 was retained in the liver for 28 days; there was a slight decrease in TiO_2 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) in rats has been described. Surface-modified QD with a neutral coating prevented protein binding and thereby particle aggregation such that QD less than 4.5 nm size were prominently cleared by the kidneys into urine while larger QD accumulated in secondary target organs (Choi *et al.*, 2007).

The clearance of pristine 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 latter 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.

4.3.5. Conclusion on Toxicokinetics

- Toxicokinetic studies on ENM following oral exposure have been performed mainly on metals and metal oxides (i.e. insoluble materials). For other ENM, there is very little information available at present.

- 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 ENM, as compared to the macroscale form or the dissolved chemical.

- Current data indicate that ENM dispersed in the food/feed matrix may undergo changes in the food/feed and/or in the GI tract, which may modify their physico-chemical properties and absorption.

- ENM studied to date are absorbed to a limited extent from the GI tract. Absorption through enterocytes will go through the portal circulation to the liver. ENM 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 ENM, all organs may be targets, as in all organs investigated so far, the chemical component of the ENM, or the ENM themselves, could be detected.

- Smaller-sized ENM have a more widespread tissue distribution compared to larger ENM, 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. Which other properties are important is not known at present.

- There is some information that certain ENM can pass across the placenta. There is no information on whether ENM are transferred into milk.

- There are only limited data on potential, long-term accumulation/persistence of ENM. However the limited data available suggest that insoluble ENM may be retained for a long time and accumulate.

4.4. Toxicity of ENM

4.4.1. Acute, subacute and subchronic oral toxicity to ENM

In the sections below, the most important facts are summarized. Only a limited number of oral toxicity studies using ENM have been published, mostly using metals and metal oxides.

Potential intracellular targets of ENM toxicity are e.g. plasma membranes, mitochondria and nucleus. The general mechanisms of injury have been shown to include e.g. lipid peroxidation, ion channel blockage, pore formation, physical disruption, oxidative stress, protein aggregation and DNA damage (ICON, 2008). There are preliminary indications of association of GI disorders with absorption of ENM. There are reports of increased uptake of ENM during GI inflammation, findings of particles in colon tissue in subjects suffering from ulcerative colitis and speculations that ENM 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).

4.4.1.1. Metals

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; appearance in the feed not defined) for 13 weeks (Jia *et al.*, 2005).

Single gavage administration to mice of copper nanoparticles (Cu-NP) with average size 23.5 nm was compared to microparticle (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 was ions > NP > MP. Dose-dependent pathology occurred in kidney, liver, spleen and blood (but not lung, heart, brain, testes or ovaries) in animals exposed to nanoparticles (but not in those exposed to microparticles).

After single gavage administration of high doses (5 g/kg bw) of zinc as nanoparticles (58 nm) and MP (1.08 µm) to mice there was GI inflammation in both groups, in spite of attempts to avoid particle agglomeration (Wang *et al.*, 2006). The toxicity patterns were not consistent: in some aspects, the nanoparticles were more toxic (anemia, kidneys, heart) than the MP, 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 ENM (20 and 120 nm) were compared to conventional macroscale material (Wang *et al.*, 2008). The sizes of the ENM were checked in the gavage, and were found to average 44.8 and 187.5 nm, respectively. Again, the toxicity pattern was complex: the 120 nm ENM were most toxic in stomach, liver, heart, spleen, kidneys and blood, while the 20 nm ENM were similar to the toxicity of the macroscale material (except in pancreas, where they were the most toxic). However, no dose-dependency was observed.

Titanium dioxide (TiO_2) nanoparticles (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). The 80 nm particles accumulated predominantly in the liver, the 25 and 155 nm ones accumulated primarily in spleen. Kidney, liver and heart damage was observed with all sizes, with 80 and 155 nm particles producing the most pronounced effects, while blood effects (e.g. increased serum lactate dehydrogenase and alpha-hydroxybutyrate dehydrogenase levels) were most pronounced for the 25 nm particles. Administration of TiO_2 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 silver nanoparticles (60 nm in doses 30, 300 and 1000 mg/kg/day) showed minimal dose-dependent biochemical liver toxicity.

4.4.1.2. Other ENM

Only a few studies have been reported on non-metal ENM. 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 nanoparticles (~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 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 ENM and *in vitro* studies

Some information on routes other than oral may be useful to assess oral toxicity. Data on toxicity is 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.*, 2005; Oberdorster *et al.*, 2005; Borm *et al.*, 2006; Oberdorster *et al.*, 2007). Effects of inhaled ENM 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 ENM in human or animal cells (including on the cell nucleus) and a wide range of ENM (e.g, Ti, Ag, Zn, Mn, Se and Si), concentrations and exposure times have been studied. 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 ENM studied, seems to be the generation of reactive oxygen species (Donaldson and Borm, 2004; Oberdorster *et al.*, 2005; 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. Generally these effects are observed only after exposure to high concentrations of ENM and it is difficult to know whether the effects are physiologically relevant (Lewinski *et al.*, 2008).

In vitro studies have also indicated genotoxicity and clastogenicity (Barnes *et al.*, 2008; De Jong and Borm, 2008). Cobalt nanoparticles have been shown to induce more DNA damage than micronsized particles using human fibroblasts in tissue culture in the alkaline comet assay (Papageorgiou *et al.*, 2007); (Colognato *et al.*, 2008).

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 is not a good metric, as it does not incorporate the specific characteristics of ENM (SCENIHR, 2006; SCENIHR, 2007a). Number concentration and surface area may be more appropriate. Morphology may also be important since a recent intraperitoneal study indicate that fibrous shape of some ENM might be important in determining toxicity (Poland et al., 2008). It is clearly desirable to characterize ENM as completely as possible (Oberdorster *et al.*, 2005; Thomas and Sayre, 2005; Powers *et al.*, 2006; OECD, 2008).

4.4.4. Additional considerations

Some other aspects increase the uncertainty in assessment of ENM. The presence of ENM in food might affect normal food components or contaminants. Hence, food containing ENM with actively charged surfaces can absorb proteins, lipids, nucleic acids and carbohydrates. It has been speculated that absorption of ENM is accompanied by transport of food components/molecules that are not normally absorbed and thus may create an (unwanted) port of entry ("Trojan horse" effect), and that this might change their toxicity (Lomer et al., 2002; Borm and Kreyling, 2004). If particles that pass through the epithelial cells via transcytosis by M-cells this may lead to accumulation within the Peyers Patches and subsequently a possible immune reaction. The surface properties (e.g. coatings) that increase the active uptake of encapsulates might also be a reason for concern. Thus, lectins used for coatings of nano-encapsulates can be cytotoxic or induce inflammatory responses (Govers et al., 1994; Des Rieux et al., 2006).

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

There are microscale products (e.g. Mn and SiO_2) used in the food and feed area, which, due to natural size range variation may contain a nanoscale fraction. No oral toxicity studies of such materials with a fully characterised size range have been identified.

4.4.5. Conclusion on Toxicity of ENM

- The understanding of the potential toxicity after oral intake of ENM is in its infancy. Only a very limited number of ENM have been studied after oral administration, mainly

metals and metal oxides. The ENM used in the toxicity studies were often characterized only to a very limited extent.

- Only a narrow range of effects have been studied in the toxicity tests.
- Only a few studies have compared the toxicity of nanoformulated and conventional (dissolved or 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 ENM were administered in artificial dispersions (i.e. via gavage).
- Most of the reported oral *in vivo* studies are on acute toxicity of ENM. Long-term studies have not been conducted.
- There is no adequate information that allows conclusions on the relationship between physico-chemical properties (size, surface properties, etc.) of ENM and toxicity *in vivo* or *in vitro*.
- It is generally not possible to extrapolate the potential toxicity of ENM from information on dissolved or macroscale chemicals.
- Numerous *in vitro* studies have shown that some ENM induce oxidative stress at high concentrations. There are some data to indicate possible genotoxic and inflammatory responses *in vitro*.

5. Environmental impact of nanotechnologies in food and feed area

During production, use and disposal of ENM in the food and feed area, dispersal of ENM to the environment is likely. Possible environmental impacts are influenced by the characteristics and properties of the ENM and may be more or less pronounced depending on the specific ENM. In some instances, there is the possibility of re-entry of certain ENM as contaminants in the food and feed chain. Such contamination may arise from the traditional processes of food and feed waste disposal, e.g. via sewage, from waste incineration or leakage from landfills.

Recycling processes of food packaging material containing ENM should be considered, as the process may affect the migration of the ENM in the recycled material. There may also be secondary environmental implications during disposal from possible release of antimicrobial ENM from FCM. However, there is presently only limited information available of these processes related to ENM in food and feed.

6. Proposed guidance for risk assessment (RA) of ENM in food and feed area

Properties of materials at nanoscale may be different from chemicals in the macroscale or dissolved forms, and existing toxicological knowledge on chemicals cannot be fully extrapolated to ENM (e.g. SCENIHR, 2007a). A number of national and international advisory committees have recommended strategies for the RA of ENM (e.g. SCENIHR, 2007a; SCCP, 2007). In agreement with these, the Scientific Committee view is that the general paradigm can also be applied to the RA of ENM in the food and feed area.

A difficulty at the present time in giving detailed specific risk assessment guidance is the lack of sufficient data and information, which would allow for a comprehensive understanding of potential hazards of ENM. The conventional toxicological testing methods should be used as a starting point to identify hazards from ENM. However, additional issues, specific for the properties of ENM, e.g. toxicokinetics and the possibility of additional endpoints, 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 ENM in food and feed. Hence, until a sufficient body of data is developed, RA of ENM will have to be carried out on a case-by-case basis. Current guidance documents in the food and feed area do not address ENM.

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 additive area) will in general still be applicable but modifications may be necessary to take account of the special properties of the ENM in these areas.

A first step of the RA of ENM 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 of ENM (OECD 2008a; b). At the present time, at least the following characteristics/parameters should be provided: size (including distribution), mass, surface area, specific surface area, number, shape, chemical composition (including impurities and processing chemicals), surface properties (e.g. coating, charge) and solubility (including hydrophilicity). For this purpose standard methodologies (including additional reference/benchmark materials) are needed.

It should be emphasised that characterization of the ENM, both as manufactured or added, as well as of the ENM as present in the food/feed is desirable as it is likely that ENM will interact with food/feed components. A crucial step is to define (confirm) qualitatively and quantitatively the presence of 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 ENM, a conservative approach in the RA is to assume that the entire amount of ENM 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 nanomaterial, 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 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. If evidence is present that ENM dissolve in the lumen, this may be sufficient to allow the conclusion that, if absorbed, the ENM would behave 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 ENM 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, 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 ENM 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 ENM, 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 ENM. However, experience in using these guidelines/tests with ENM is very limited and the adequacy of the existing toxicological tests to detect all aspects of potential toxicity of ENM has yet to be established.

In vivo toxicology studies on food chemicals are normally conducted using admixture into the diet. For ENM, the way of administration must be considered in the context of the likely interaction of the ENM with food/feed components. This is an argument for inclusion of the testing material into food/feed for toxicology and exposure assessment. On the other hand, administration via gavage is a more well-defined mode, and may, if adequately performed, and as an initial step, represent a worst case, conservative approach. The choice of administration method should always be justified.

Concerning *in vitro* tests, the sensitivity and validity of available assays for assessing risks of ENM 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, but they have not yet been validated for ENM. They are generally suited for screening purposes and studies on mechanisms of toxicity (COT, 2005; 2007).

For the risk characterization step, the strategy for ENM would not, in principle, differ from that followed for soluble chemicals or the macroscale material. However, as was also stressed by SCENIHR (2007a), 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 concentration.

Finally, the limited database on ENM assessments should be considered in the choice of appropriate uncertainty factors in the risk characterization step.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS[12]

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 is 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 possible products that may be present on the EU market. The nanospecific properties and characteristics of ENM are likely to affect their toxicokinetic behaviour and toxicity profile. The guidance section indicates the general data needs and aspects to consider when performing a risk assessment of ENM.

The Scientific Committee specifically concludes that;

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

- 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 ENM in food/feed and biological matrices and the limited information available in relation to aspects of toxicokinetics and toxicology. There is limited knowledge of (likely) exposure from possible applications and products in the food and feed area or of environmental impacts of such applications and products. The current usage levels of ENM in the food and feed area is unknown. The limited database on ENM assessments should be considered in the choice of appropriate uncertainty factors in the risk characterization step.
- Whilst recognising these limitations, **t**he currently used risk-assessment paradigm (hazard identification, hazard characterization, exposure assessment and risk characterization) is considered applicable for ENM.
- Risk assessment of ENM in the food and feed area should consider the specific properties of ENM in addition to those common to the equivalent non-nanoforms.
- The available data on oral exposure to specific ENM and any consequent toxicity is extremely limited; the majority of the available information on toxicity of ENM is from *in vitro* studies or *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 RA of ENMs. However, the adequacy of currently existing toxicological tests to detect all aspects of potential toxicity of ENM has yet to be established. Toxicity-testing methods may need methodological modifications. Specific uncertainties arise due to limited experience of testing ENM in currently applied standard testing protocols. There may also be additional toxic effects caused by ENM that are not readily detectable by current standard protocols. Additional endpoints not routinely addressed and pharmacological endpoints 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 ENM compared to conventional dissolved and macroscale chemical counterparts 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 ENM 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 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.

RECOMMENDATIONS

General recommendations

- This opinion should be updated in the light of developments in the area and/or with new relevant data.

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

Additional recommendations

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

- Monitor current and future commercial applications of ENM in the food and feed sectors and developments of nanotechnologies, especially since more complex ENM 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 ENM on physicochemical properties, compared to those of the dissolved chemical or macroscale materials.
- Investigate the interaction and stability of ENM 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 ENM in FCM, food and feed matrices and in biological tissues.
- Generate information on the effects of processing on the characteristics of ENM.

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

- Generate information on the amount and form (dispersed or aggregated) of ENM 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 ENM from FCM into food and feed.

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

- Generate information on toxicokinetic properties of ENM after oral exposure. Correlate these data with the physicochemical characteristics to see whether different ENM can be grouped. Generate information on appropriate dose metrics in relation to toxicity of ENM.
- Generate information on the bioavailability from food and feed of a range of ENM 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 ENM 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 ENM (including reliability and relevance of the test methods).
- Develop understanding of the toxicity (including chronic exposure and carcinogenicity) following oral intake of a wide range of ENM for which there is likely exposure, including studies on the mechanisms of toxicity.
- Develop understanding on whether ENM interact with biomolecules (e.g. enzymes), nutrients and foreign compounds (“Trojan horse effect”), and the significance of such interactions for human and animal health.

- There are substances approved for use in food and feed (e.g., MnO, SiO_2), which have been claimed to also be available in nanoscale dimensions. Oral toxicity studies of these materials with a fully characterised size range should be performed.
- Generate information on the efficacy of applications which claim antimicrobial activity as this may have important downstream consequences for food safety.

In relation to impacts on the environment

- Investigate the contribution and fate of ENM used in the agro-food sector in the environment, including re-entry of ENM into the food and feed chain.

DOCUMENTATION PROVIDED TO EFSA

EFSA published a call for data on its website between 23 January and 28 March 2008. Information, via e-mail, 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 Nanotechnologei Stand März-2008. Pages 1-4.
Progress report and position paper Nanotechnology March 2008. Pages 1-4

CIAA (Confederation of the Food and Drink Industries of the EU)
Communication of information, e-mail 11/03/2008, 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
Nano Risk Framework, Output worksheet. Pages 1-14

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.

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SCCP 2007 (Scientific Committee on Consumer Products). 19 June 2007, Preliminary Opinion on Safety of Nanomaterials in Cosmetic Products, at http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_099.pdf

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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.
Chyme	The seimifluid mass of partly digested food that is expelled from the stomach into the duodenum
Coalescence	The formation of a new homogeneous entity out of two initial ones, e.g. after the collision of two nanoparticles
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.
Nanocrystalline material	A material that is comprised of many crystals, the majority of which 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 three dimensions of the order of 100 nm or less.
Nanoparticulate matter	A substance comprising of particles, the substantial majority of which have three dimensions of the order of 100 nm or less.
Nanorod (nanofibre, nanowire,nanowhisker)	A discrete entity which has two dimensions that are of the order of 100 nm or less, and one long dimension. Note: Other entities such as nanofibre, nanowire, and nanowhisker comply with this definition, but may differ from each other by other characteristics (e.g. rotational symmetry, flexibility). In general a nanorod or nanofibre can be characterised by the aspect ratio.
Nanoscale	A feature characterised by dimensions of the order of 100 nm or less.
Nanosheet	A discrete entity which has one dimension of the order of 100nm or less and two long dimensions. Note: Other entities such as nanofilm and nanolayer comply with this definition, but may differ from each other by other characteristics (e.g. sheet is usually free and a layer is usually supported; there may be considerable differences in flexibility).

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.
Solubilisation	The process of dissolution.

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1353 **Abbreviations**

Term	Abbreviation
ADME	Science dealing with absorption, distribution, metabolism and excretion of substances in the body
ENM	Engineered Nanomaterial
FCM	Food Contact Materials
Nm	Nanometer, 10^{-9} meter
NP	Nanoparticle
WG	Working Group

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