Review of health safety aspects of nanotechnologies in food production

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ABSTRACT

Due to new, previously unknown, properties attributed to engineered nanoparticles many new products are introduced in the agro-food area. Nanotechnologies cover many aspects, such as disease treatment, food security, new materials for pathogen detection, packaging materials and delivery systems. As with most new and evolving technologies, potential benefits are emphasized, while little is known on safety of the application of nanotechnologies in the agro-food sector. This review gives an overview of scientific issues that need to be addressed with priority in order to improve the risk assessment for nanoparticles in food. The following research topics are considered to contribute pivotally to risk assessment of nanotechnologies and nanoparticles in food products.

- Set a definition for NPs to facilitate regulatory discussions, prioritization of research and exchange of study results.
- Develop analytical tools for the characterization of nanoparticles in complex biological matrices like food.
- Establish relevant dose metrics for nanoparticles used for both interpretation of scientific studies as well as regulatory frameworks.
- Search for deviant behavior (kinetics) and novel effects (toxicity) of nanoparticles and assess the validity of currently used test systems following oral exposure.
- Estimate the consumer exposure to nanoparticles.

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1. Introduction

Due to new, previously unknown, properties attributed to engineered nanoparticles (NP) many new consumer products containing these NPs have been launched to the market recently. Application of NPs in electronics, medicine, textiles, defence, food, agriculture, cosmetics, and other areas are already a reality and applications are beginning to impact the food associated industries (Chen et al., 2006a). The potential benefits for consumers and producers of these new products are widely emphasized. In food and agricultural systems nanotechnologies cover many aspects, such as food security, packaging materials, disease treatment, delivery systems, bioavailability, new tools for molecular and cellular biology and new materials for pathogen detection (Maynard et al., 2006; Chen et al., 2006a; Weiss et al., 2006).

Nanotechnology is generally seen as new and fast emerging field that involves the manufacture, processing and application of structures, devices and systems by controlling shape and size at the nanometer scale. Nanoparticles are defined as; “a discrete entity that has three dimensions of the order of 100 nm or less” (SCENIHR, 2007b). It is this small size in combination with the chemical composition and surface structure that gives NPs its unique features and its huge potential for applications.

Although potential beneficial effects of nanotechnologies (NT) are generally well described, the potential (eco)toxicological effects and impacts of NPs have so far received little attention. The high speed of introduction of NP-based consumer products observed nowadays urges the need to generate a better understanding about the potential negative impacts that NPs may have to biological systems. Some recent studies have shown that indeed there are reasons to suspect that NPs may display toxicological effects on biological systems (Nel et al., 2006; Oberdorster et al., 2007b; Donaldson and Seaton, 2007). As nanofood related products are already on the market and uncertainty about potential risks is...
large (Morgan, 2005), the need for science-based adaptation of the regulatory frameworks is high.

This review is focussed on application of engineered NPs and nanotechnology in the agro-food production chain. The current knowledge on possible consequences of oral exposure to NPs is discussed. Emphasis is put on those issues in toxicokinetic and toxicodynamic issues of NPs with importance for risk assessment. We will suggest the most important knowledge gaps in human risk assessment, that if resolved will bring the risk assessment of NPs following oral exposure a step forward. It is acknowledged that over the entire life-cycle of the products NPs will be released into the environment, possibly resulting in human exposure via that route, however, environmental contamination is not discussed in this paper as we have focussed on food related consumer exposure to NPs.

2. Nanotechnologies currently applied in the food chain

To improve the risk analyses of the application of nanotechnology (NT) within food more insight is needed in the availability of different types of food products containing engineered nanomaterials. Therefore, a survey on application of NT in the agro-food production chain was performed for which various sources including published literature and internet web pages were extensively searched. Most products have been obtained from Google™, the database of consumer products of the Nanotechnology project (www.nanotechproject.org) of the Woodrow Wilson International Center for Scholars, the Global New Products Database of Mintel (www.gnpd.com), the Nanotechnology Product Directory (www.nanoshop.com) and the report of nanoforum (Nanotechnology in Agriculture and food; www.nanoforum.org).

The survey clearly demonstrates that NT in the agro-food production chain are claimed to be applied throughout all phases of food production (Table 1).

Inclusion of products into Table 1 is based on labeling information on the product as provided by the manufacturer. The claim that these products contain nanotechnology cannot be verified from the information presented. This also applies to the information on the presence and/or type of NPs in these products. It can be expected that the claim ‘nanotechnology’ is wrongfully present or absent on the label of some products. The latter situation might even be more critical. Nevertheless, the table as shown gives direction on the type of products that are likely, on the market, including areas of use. Both scientists and regulators should be aware of this and use this information in the prioritization of research. In general, NPs can be divided into several classes of applications, including nanosensors, pesticides, packaging materials, supplements, etc. For each application an indication of the likelihood of consumer exposure to free nanoparticles (NPs) is given. Free, insoluble NPs including agglomerates are thought to be of the highest concern to the consumer health (SCENIH, 2007a).

2.1. Food production phase: agricultural production

During primary production nano-formulated agro-chemicals are employed to increase the efficacy of the agro-chemicals compared to conventional formulations. Only some pesticides containing nano-sized or nano-formulated agro-chemicals were identified as available on the market. Residues of these products might be present in products as consumed. Furthermore, this survey indicated applications of NPs for water and soil cleaning purposes. Carry-over of the used NPs to crops cannot be excluded, resulting in potential consumer exposure. Examples of used NPs are aluminium oxide, lanthanum particles and nanoscale iron powder in the process of water purification and/or soil cleaning (see Table 2).

2.2. Food production phase: processing

Nanotechnologies are applied in food production machinery. Examples of this type of nanotechnology are coatings of machines or the use of nano-sieves (e.g., to filter out bacteria). While direct food contact is evident this application of nanotechnology is expected to have negligible additional safety concerns in comparison with conventional techniques, as carry-over to food is expected to be negligible. The type of material (and wear-off as result of the use) of filters or coatings might requires some attention, but this is not exclusively related to safety of nanotechnologies.

2.3. Food production phase: preservation and packaging

Indirect contamination of food can be expected when NPs or nanotechnological devices are incorporated in packaging materials or storage containers in order to lengthen the storage time while keeping the products fresh. This type of application is seen as the most important of nanotechnologies in the food area for the near future (Chaudhry et al., 2008). Recently the Woodrow Wilson International Center for Scholars published a review dealing with both the applications and regulatory (and toxicological) issues related to NPs incorporated in food packaging materials (WWWIS, 2008). NPs are for instance incorporated to increase the barrier properties of packaging materials (e.g., silicate NPs, nanocomposites, and nano-silver, magnesium- and zinc-oxide). When the NPs are applied into the food packaging materials, direct contact with food is only possible following migration of the NPs. The migration of metals from biodegradable starch/clay nanocomposite films used in packaging materials for its gas barrier properties to vegetable samples, was shown to be minimal (Avella et al., 2005), but more studies are needed to reach a conclusive statement.

NPs can also be applied as reactive particles in packaging materials. These so-called nanosensors are designed to respond to environmental changes (e.g., temperature or moisture in storage rooms), degradation products of the food commodities, or contamination by micro-organisms. No data is available on possible migration of NPs into food using these applications. However, it is conceivable to assume that the use of active packaging releasing NPs with antimicrobial functions into the food (e.g., nanosilver and in rarer cases zinc-oxide NP), will lead to direct consumer exposure to (free) NPs. Hence, this urges the need for information on the effects of these NPs to human health following chronic exposure. Moreover, attention should be paid to life-cycle analysis (LCA) and effects on the environment.

2.4. Functionalized foods: engineered NPs as a food additive or supplement

The current survey revealed nano-formulated food additives or supplements to be available on the market, e.g., regulatory peptides from plants, nano-droplets/-clusters and nano-water (see Table 2). The validity of the claimed effects and formulations are low. The applicants claim increased bioavailability but valid studies on relative bioavailability comparing these products with similar non-nano-formulated products are lacking.

An important class of NPs for application within food are nanodelivery systems (Letchford and Burt, 2007; Taylor et al., 2005). Examples (see Table 2) range from novel pesticide formulations (e.g., increased crop adherence) but also oral delivery systems for bioactive compounds. The latter is created for targeted delivery of bioactive compounds and to increase the bioavailability of these

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compounds. When used as a food additive or supplement the delivery systems are commonly build from peptide, carbohydrate or lipid monomers (Chen et al., 2006b; Graveland-Bikker and de Kruif, 2006; Mozafari et al., 2006). It is obvious that this application of NPs might result in unusual high consumer exposure to the enclosed compounds.

Summary of type of nanoparticles applied in the food production chain.

2.5. Conclusions

It is difficult to get a clear overview of products either produced by means of nanotechnology or products actually containing NPs. Information on the type NPs applied and their claimed added value for the product is essential for gaining insight in their potential hazard and associated risks for humans might vary significantly. It is therefore important both for manufacturers as well as for regulatory bodies and consumers that valid information on relevant characteristics of NPs as present in the product is at least known to risk assessors. Consequently, products falsely claiming nanotechnology will be skipped from inventories of agro-food products. On the other hand, special efforts are still required to expand these inventories with products containing NPs but not claiming it.

From the information that is available we expect that consumer exposure to NPs via food is likely to occur. Clearly, instruments need to be developed for the verification of labeling information and validation of databases of nano-products.

3. Applied types of nanoparticles in the agro-food production chain

Current nanotechnology applications in the agro-food production chain are focused on the development of nano-sized food ingredients and additives, delivery systems for bioactive compounds and innovative food packaging (Chaudhry et al., 2008). As a consequence of their small size NPs show different physico-chemical properties compared to their respective conventional-sized materials, likely resulting in different biological interactions. The physico-chemical properties are thus relevant for the assessment of the possible associated hazards. The main characteristics of the various types of NPs are introduced in this section.

3.1. Nanoparticles and nano-emulsions

NPs in food may appear in suspension (mostly solid in liquids) or an emulsion (two liquid phases). Within the agro-food chain, metal or metal-oxide NPs are applied (e.g., nano-Ag, nano-ZnO, nano-Cu, nano-TiO$_2$). These particles are known to have different structures and shapes, which can be (homogeneous or heterogeneous) spherical, tubular, irregularly (non-spherical) shaped, or can exist in fused aggregated or agglomerated forms (Fig. 1). These characteristics are important with respect to potential risks for health or environment and determine their fate and behavior in the environment, humans and other organisms.

3.2. Nano-delivery systems

Nano-encapsulation involves the incorporation, absorption or dispersion of bioactive compounds in/at or on small vesicles with nano (or submicron) diameters. This type of application is especially important in food and is discussed here more in-depth. The incorporated bioactive compounds may be protected against degradation, have improved stability and solubility (e.g., solubilizing a hydrophilic compound in hydrophobic matrices and vice versa) and therefore might increase bioavailability and delivery to cells and tissues (Letchford and Burt, 2007; Taylor et al., 2005). Reducing the size of the encapsulates into the nanoscale offers opportunities related to prolonged gastrointestinal retention time caused by bio-adhesive improvements in the mucus covering the intestinal epithelium (Chen et al., 2006b; Medina et al., 2007). Modulations of surface properties (e.g., coatings or bio-molecular flags) can enable targeted delivery of compounds. The latter field of application is however, more developed in relation to biomedical applications.

Nano-encapsulates may consist of a core composed of one to several types of compounds surrounded by a wall or barrier (Dins-
Table 2
Summary of type of nanoparticles applied in the food production chain.

<table>
<thead>
<tr>
<th>Type of NP</th>
<th>Application</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metal nanoparticles (Silver, ZnO)</td>
<td>Food additive/supplement</td>
<td>Claimed enhanced gastrointestinal uptake of metal</td>
</tr>
<tr>
<td>Packaging materials/Storage</td>
<td>Increase barrier properties</td>
<td></td>
</tr>
<tr>
<td>Food preparation devices</td>
<td>Clean surface</td>
<td></td>
</tr>
<tr>
<td>Refrigerators, storage containers</td>
<td>Anti-bacterial coating</td>
<td></td>
</tr>
<tr>
<td>Water purification/Soil cleaning</td>
<td>Removal/catalysis</td>
<td></td>
</tr>
<tr>
<td>Sprays</td>
<td>Oxidation of contaminants</td>
<td></td>
</tr>
<tr>
<td>Complex nanoscale structures</td>
<td>Detection of food deterioration</td>
<td></td>
</tr>
<tr>
<td>Nanosensors in packaging</td>
<td>Monitoring storage conditions detection of contaminants etc.</td>
<td></td>
</tr>
<tr>
<td>Incorporate active nanoparticles</td>
<td>Migration out of packing materials</td>
<td>Oxygen scavenging, prevention of growth of pathogens</td>
</tr>
<tr>
<td>Filters with nano-pores</td>
<td>Water purification</td>
<td>Removal pathogens, contaminants</td>
</tr>
<tr>
<td>Equal sized emulsions</td>
<td>Product design (e.g., taste, texture)</td>
<td></td>
</tr>
<tr>
<td>Nano-sized nutrients (foods)</td>
<td>Food additive/supplement</td>
<td>Claimed enhanced uptake</td>
</tr>
<tr>
<td>Delivery systems (nano-encapsulates)</td>
<td>Food additive/supplement</td>
<td>Protecting and (targeted) delivery of content</td>
</tr>
<tr>
<td>Pesticide</td>
<td>Increased efficacy, water solubility and crop adherence, triggered (local) release</td>
<td></td>
</tr>
</tbody>
</table>

more, 2002; Letchford and Burt, 2007) (see Fig. 1 for types of delivery systems relevant for food applications). These delivery systems have its roots in the pharmaceutical industry, where often synthetic polymeric nano-encapsulates are employed (Langer, 2003). For application of nano-encapsulates into food, lipid- or natural-polymer-based capsules are most often studied or applied (Chen et al., 2006b). These are introduced in more detail in the sections below.

3.2.1. Lipid-based nano-delivery systems

The main lipid-based nano-encapsulation systems that can potentially be used in food and food supplements are nanoliposomes, archaeosomes and nanocochleates (see Fig. 2) (Mozafari et al., 2006). Nanoliposomes are defined as bilayer lipid vesicles (<30 or 30–100 nm vesicles) possessing and maintaining nanometric size ranges during storage and application. Because of their unique properties, e.g., hydrophilic and hydrophobic regions, they can entrap, deliver and release both water-soluble and lipid-soluble material (Mozafari et al., 2006). These may release their contents into cells upon e.g., encountering specific cellular enzymes, due to pH or thermo-sensitivity or after antigen-binding when antibody-tagged (Taylor et al., 2005).

Archaeosomes, which are liposomes made from Archaeobacteria, are even more thermostable and stress resistant as compared to normal liposomes. These are therefore considered ideal candidates to protect i.e., antioxidants during food processing (Patel, 2000).

Nanocochleates have a multilayered structure consisting of a continuous, solid, lipid layer sheet rolled up in a spiral fashion with little or no internal aqueous space (Mozafari et al., 2006). Nanocochleates have been used to deliver proteins, peptides and DNA for vaccine and gene therapy applications. They are resistant to degradation in the gastrointestinal tract, which makes them ideal candidates for oral delivery (Zarif, 2003). At this moment, it has become possible to use liposomes to deliver functional components such as nutraceuticals, antimicrobials, and flavors to foods (Hentschel et al., 2008; Hsieh et al., 2002; Laridi et al., 2003; Were et al., 2003).

The actual use of these delivery systems in food and food supplements is determined by their preparation procedure, which may involve non-food grade solvents and detergents that might leave residues in the delivery systems (Mozafari et al., 2006). Lipid-based nano-delivery systems were found most frequently in the current survey.

3.2.2. Polymer-based nano-delivery systems

Nano-encapsulates based on polymers are obtained by the polymerization of more than one type of monomer, typically one hydrophobic and one hydrophilic, so that the resulting molecule is composed of regions that have opposite affinities for an aqueous solvent. Natural polymers that are used are albumin (protein), gelatin (protein) / Zwiorek et al., 2004), alginate (saccharide), collagen (protein), chitosan (saccharide) (des Rieux et al., 2006) and the milk protein α-lactalbumin (Graveland-Bikker and de Kruif, 2006). Protein-based nano-encapsulates are particularly interesting because they are relatively easy to prepare and can form complexes with polysaccharides, lipids or other biopolymers. A wide variety of nutrients can be incorporated (Chen et al., 2006b). In addition, to date numerous copolymers have been synthesized leading to the formation of micelles, nanospheres, polymersomes and nanocapsules (see Fig. 2; Kabanov, 2006; Letchford and Burt, 2007).

Micelles are characterized by a core-shell architecture in which the inner core is composed of the hydrophobic regions of the amphiphilic molecules creating a cargo space for the lipophilic bioactive compound (Chen et al., 2006b). Nanospheres can be defined as a solid colloidal particles in which bioactive compounds are dissolved, entrapped, encapsulated, chemically bound or adsorbed to the polymer matrix. However, the central core can become more or less solid-like depending on the copolymer composition, making it difficult to have a clear distinction between micelles and nanospheres (Chen et al., 2006b). Nanocapsules and polymersomes are colloidal-sized, vesicular systems in which the bioactive compound is confined within a cavity surrounded by a polymer membrane or coating. If the core is an oily liquid and the surrounding polymer a single layer the vesicle is referred to as a nanocapsule; these system have found utility in delivery of hydrophobic compound. If the core of the vesicle is an aqueous phase and the surrounding coating is a polymer bilayer, the particle is referred to as a polymersome. These vesicles are analogous to liposomes and find utility in delivery of encapsulation of water-soluble compound, but they differ from liposomes in that the external bilayer is composed of amphiphilic copolymers. Variation in composition, molecular geometry and relative monomer lengths results in various physico-chemical properties and morphologies of the resulting nano-encapsulates (Letchford and Burt, 2007).

Currently, greater fundamental understanding of polymer-polymer and polymer-nutraceutical interactions at the molecular level and their impact on functional properties of the delivery systems is required to ensure design of ideal nutraceutical carriers for use in the food industry.

4. Knowledge gaps for risk assessment of nanotechnologies in food

The remaining part of this paper will address the scientific knowledge gaps that severely hinder the current risk assessment of application of NPs in general but specifically in food and related products. Discussions on safety issues of nanoparticles (NPs) and nanotechnological products can almost entirely be brought back
Discussions on data requirements and expected performance of current assays have demonstrated that it is important to focus the question on what information is additionally required compared to dossier requirements for conventional chemicals. Some research agendas or roadmaps try to circumvent uncertainties which are accepted in risk assessment of conventional chemicals. For example, questions like “are in vitro tests applicable for NPs” should rather be formulated as “are in vitro tests equally applicable for NPs as for conventional chemicals”, as the role of in vitro test results for chemical in risk assessment is also still subject to many uncertain-

<table>
<thead>
<tr>
<th>Particle type and shape</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>'solid' nanoparticles</td>
<td>Spherical or compact particles compositionally homogeneous</td>
</tr>
<tr>
<td></td>
<td>Tubular particles compositionally homogeneous</td>
</tr>
<tr>
<td></td>
<td>Complex non-spherical particles compositionally homogeneous</td>
</tr>
<tr>
<td></td>
<td>Compositionally heterogeneous particles compositional variation core - surface</td>
</tr>
<tr>
<td></td>
<td>Compositionally heterogeneous particles, distributed compositional variation</td>
</tr>
<tr>
<td></td>
<td>Homogeneous aggregates/agglomerates consisting of a single particle class</td>
</tr>
<tr>
<td></td>
<td>Heterogeneous aggregates/agglomerates consisting of diverse particle types</td>
</tr>
</tbody>
</table>

**Nano delivery systems: lipid based**

<table>
<thead>
<tr>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanoliposomes/archaeosomes bilayer lipid vesicles</td>
</tr>
<tr>
<td>Micelle</td>
</tr>
<tr>
<td>single layer lipid vesicles</td>
</tr>
<tr>
<td>Nanocochleates</td>
</tr>
<tr>
<td>lipid layer sheet rolled up in spiral fashion</td>
</tr>
</tbody>
</table>

**Nano delivery systems: polymer based**

<table>
<thead>
<tr>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micelle aggregated copolymers</td>
</tr>
<tr>
<td>Nanosphere aggregated copolymers generating a solid central core</td>
</tr>
<tr>
<td>Nanocapsule / Polymersome polymer membrane surrounding a central cavity</td>
</tr>
<tr>
<td>Nanocapsule: oily liquid cavity, single layer membrane</td>
</tr>
<tr>
<td>Polymersome: aqueous cavity, bilayer membrane (similar to nanoliposome)</td>
</tr>
</tbody>
</table>

Adapted from Maynard and Atken (2007) and Letchford and Burt (2007)

**Fig. 1.** Different forms and shapes of nano-structured particles.
ties. Also from a regulatory point of view the question has been raised what information is additionally required and if the current regulatory system within the EU is suited to deal with nanotechnology. The EU’s approach to nanotechnology is ‘safe, integrated and responsible’ (Communication from the Commission—Towards a European strategy for nanotechnology, 2004). To that end the EU has commissioned its scientific committees and commission services (amongst them the European Food Safety Authority in 2008) to perform a scientific and legislative review on the suitability of the existing regulation for nanotechnologies. From a number of regulatory reports it becomes clear that there is currently no nano-specific regulation in the EU (Chaudhry et al., 2007), or other countries (Hodge, 2007). The US Food and Drug Administration was among the first government agencies around the world having defined nanotechnologies, but the FDA has no nanotechnology specific regulations, as it regulates “products, not technologies”. The same holds true for other important nanotechnology innovative worldwide regions like Japan and China (Chau et al., 2007). However, despite the lack of a nano-specific regulation, any new developments in nanotechnology will at the moment not be taking place ever, despite the lack of a nano-specific regulation, as it regulates “products, not technologies”. The Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR, 2007a) in line with this the Health Council of the Netherlands considered that: “the best course of action would be to modify existing laws and rules as and when developments within the fields of nanoscience and nanotechnologies render such measures necessary” (Health Council Netherlands, 2006). SCENIHR and others deemed adjustments of legislation, guidelines and guidance documents concerning the testing of nanoparticles (NPs) of the substance to be necessary (SCENIHR, 2007a). However, such adaptations can currently not been performed due to the lack of knowledge on this topic.

For regulatory purposes there is a need for a strict definition of NPs. As mentioned recently SCENIHR (2007b) postulated a size definition of “the order of 100 nm or less”. The exact size limit of 100 nm in the present definition of NPs is arbitrary due to lack of knowledge on the relationship between particle size and kinetics or toxicological effects. It will be relevant to explore the legal feasibility of avoiding arbitrary size limits and establish a risk assessment driven definition for NPs.

An important way of focusing the discussion is to keep in mind what will really bring risk assessment to a higher level. In other words, in an area where such an enormous amount of research questions can be/are raised, it is essential to define those questions that represent the ‘needs to know’.

4.1. Physico-chemical characterization of NPs in food

As indicated in previous sections engineered NPs encompass many forms. They can be made bottom up, through assembling molecules into NPs, or derived top down by down sizing conventional substances. A complete and accurate characterization of NPs (Oberdorster et al., 2005a; Powers et al., 2006) is an essential part of both understanding the possible benefits as well as the potential toxicity of NPs in biological systems (The Royal Society and the Royal Academy of Engineering, 2004). Whereas the characterization of chemicals is usually relatively straightforward (e.g., composition, purity), characterization of NPs in biological matrices is more complex from an analytical point of view but also regarding lack of knowledge on which characteristics that need to be identified (Powers et al., 2007).

It might however, not always be possible to fully characterize the NPs. In an attempt to give some guidance on prioritization of characterization of NPs Oberdorster and coworkers (Oberdorster et al., 2005a) proposed three criteria:

- “the context within which a material is being evaluated;
- the importance of measuring a specific parameter within that context;
- the feasibility of measuring the parameter within a specific context”.

Because of the complexity of food it is especially important that the characterization is performed in the matrix containing the NPs as administered to test systems (or as consumed by the consumer). It is clear that the functionalities of the NPs (e.g., particle size, size distribution, potential agglomeration and surface charge), can change in different biological matrices (Powers et al., 2006), depending on compounds that are present in the matrix and thermodynamic conditions (Borm and Kreiling, 2004). In addition, NP interactions with the matrix can change as a result of dilution (Oberdorster et al., 2005a). In practice this means that the appearance of a NP can be expected to change following sample processing (e.g., freeze–thaw cycles, heating, dilution).

At present there is a vast array of analytical techniques to characterize NPs (Oberdorster et al., 2005a; Powers et al., 2006; Thomas and Sayre, 2005; Tiede et al., 2008), but methods for in situ characterization of NPs are currently lacking as well as the detection of nano-delivery systems (Luykx et al., 2008). Therefore priority research should focus on methods that are able of in situ detection and characterization of NPs ideally with methods that are relatively easily performed with equipment that is currently present at laboratories suited for detection of chemicals in food.

The development of routine analytical techniques for the characterization of NPs might be very difficult to achieve. In analogy with screening approaches used for complex mixtures of chemicals and products derived from genetically modified organisms in food, a completely different approach could be to determine the presence of NPs by means of effect screening (e.g., in vitro). In this approach the presence of NPs can be “detected” using developed assay systems that focus on biomarkers of exposure or effects. The in vitro assays could be used in a first tier of detection of NPs in food. However, suspected samples would have to be further characterized by means of analytical techniques.

4.2. Dose metrics

Up to now it has not been possible to establish a single dose-describing parameter that best describes the possible toxicity. It is likely that mass alone is not the good metric (SCENIHR, 2007a). It has become clear that the size will not be the only critical factor to consider, the total surface area may also be relevant, as
well as the number of particles per particle size and perhaps other characteristics. As long as it is not known which metrics should be used to describe the dose (e.g., particle size distribution, number of particles, particle charge, total surface) (Hagens et al., 2007; Oberdorster et al., 2007a), toxicity tests will have to be analyzed case by case using different dose-describing parameters. This has led in literature to a general recommendation that NPs used for (toxicological) studies should be characterized as completely as possible (Oberdorster et al., 2005b; Powers et al., 2006; Thomas and Sayre, 2005). For risk assessors it is important to have access to a clear description of the analytical methods that were used to determine the physico-chemical properties of the respective NP, to the (raw) experimental data and a sound description of the statistical procedure used to analyze the data.

Discussions on definitions and dose metrics are important for several reasons. First it is important for adaptation of the regulatory framework that is in place to ensure the safety of food and food ingredients. A proper definition and dose metrics will help researchers to compare study results and will help regulators to formulate health-based limit values. It will also enable risk assessors to compare and combine exposure and hazard information and conclude on the likelihood of health risks. Currently pragmatic definitions are postulated by scientists and regulators. In future, better understanding of the mechanisms of action and dose-response relations of NPs with biological systems will support the formulation of new definitions. This requires priority research.

4.3. Assessment of consumer exposure to engineered NPs

Exposure assessment is defined as the qualitative and/or quantitative evaluation of the likely intake of biological, chemical or physical agents via food as well as exposure from other sources if relevant (FAO/WHO, 1997).

Basically, the principle of exposure assessment of NPs (via food) will be comparable to the exposure assessment of conventional chemicals. Issues like food sampling and variability within composite samples, variation in concentrations between samples and consumption data on specific food products are not different from the exposure assessment of conventional chemicals. However, some aspects do require specific attention.

4.3.1. Detection of engineered NPs in food

The actual determination of the amount and characterization of the NPs present in the food in its consumable form will be difficult because of the earlier mentioned lack of methods for the detection of engineered NPs in food matrices. Special attention is needed for the determination of nanoscale delivery systems loaded with bioactive compounds or bioactive compounds themselves in nanoscale formulations. For nanoscale delivery systems, both the amount of bioactive compounds within the capsules as well as the free form in the food matrix has to be determined, because these factors determine the bioavailability.

The presence of NP in a food matrix might result in increased bioavailability of other substances (both nutrients and contaminants) normally present in food. For example, food containing NPs with actively charged surfaces can absorb biomolecules as they pass through the GI tract (Govers et al., 1994). These so-called ‘Trojan horses’ (Lomer et al., 2002) may transport toxins into the intestinal mucosa, resulting in changed exposure of the cellular lining of the intestine (Borm and Kreyling, 2004). It is advisable to consider such nutritional implications when NP are present in food.

It will not always be feasible to measure chemicals and NPs in the food matrix as consumed. If chemicals are measured at an early stage of the food chain or at the site of production, effects of processing should be considered in exposure assessment (Kroes et al., 2002). However, processing factors as used for determination of exposure assessment of conventional chemicals (e.g., pesticides [Joint FAO/WHO meeting on pesticide residues]), are not (yet) available for NPs. These might be even more important for NPs because the functionalities of the NPs (e.g., particle size, size distribution, potential agglomeration and surface charge) can change in different biological matrices (Powers et al., 2006), depending on compounds that are present in the matrix and thermodynamic conditions (Borm and Kreyling, 2004). An alternative approach next to the search for NPs by actual measuring food products could be to rely on information from producers. This information could be useful for an initial exposure assessment, but also to prioritize those NPs that are most frequently used at the moment in order to start predicting health effects. However, it can be questioned if reliable information on what NPs are being produced and in which products they are applied will become available for risk assessors. Novel governance approaches are needed to address this basic lack of data in order to make this information available to risk assessors.

4.3.2. Consumption data

Various sources of consumption data are currently utilized ranging from standardized food baskets used in pre-marketing authorizations to household or individual dietary surveys used in post-marketing studies (Kroes et al., 2002). There are no additional requirements on consumption data to perform an exposure assessment of NPs. However, the use of NPs as additive or in specific food products (novel foods or food supplements) might require additional data on consumption of these specific food products, because this information is generally lacking in the regular consumption databases. This is of course a general problem for exposure assessment of food products, but more prominent in evaluating NPs because these particles are likely to be incorporated more frequently in food supplements.

4.3.3. Exposure assessment

The last step in performing exposure assessment is the integration of food consumption and amount of chemicals or NPs present in food. Usually one of the following three approaches is applied for integration of data: (1) point estimated; (2) simple distributions; (3) probabilistic analyses (Kroes et al., 2002). In the end consumer exposure is usually compared to a toxicological reference value (e.g., tolerable daily intake, acceptable daily intake or acute reference dose) or to a nutritional reference value (e.g., recommended daily intake or upper safe intake levels). These reference values are currently lacking for NPs and need to be established.

4.4. Toxicokinetics

Available experimental data so far indicate that the characteristics of NPs (e.g., size, surface charge, functionalized groups) are likely to influence the absorption, metabolism, distribution and excretion (ADME) (Ballou et al., 2004; des Rieux et al., 2006; Florence, 2005; Jani et al., 1990; Roszek et al., 2005; Singh et al., 2006) of NPs present in food. However, not much is known on the relationship between these physico-chemical characteristics and the behavior of NPs in the body. The kinetics of NPs following various exposure routes have recently been reviewed by Hagens and colleagues (Hagens et al., 2007). In this section, current knowledge and focus on ADME characteristics following oral exposure only is discussed, with emphasis on peculiarities of special agro-food related NPs, like nano-encapsulat. Subsequently knowledge gaps are identified that currently hinder the risk assessment of NPs in food.

4.4.1. Gastrointestinal absorption

Uptake of NPs (or particles in general) in the gastrointestinal tract depends on diffusion and accessibility through mucus, initial
contact with the gut epithelium and various uptake and translocation processes. There seems to be a tendency that smaller particles are able to diffuse faster through the mucus layer than larger particles. The diffusion rate also depends on the charge of the particle; anionic particles have been shown to reach the epithelial surface, whereas cationic particles were trapped in the mucus (Szentkuti, 1997) The mucus layer can thus be considered the first barrier NPs have to pass before entering the body.

The gastrointestinal epithelium represents the second barrier. A first possible route of passage is between the cells (e.g., paracellular route). There is a body of evidence that indicates that the intestinal epithelium is permeable to large proteins and polypeptides. Cells of the gastrointestinal epithelium are tightly connected to each other by means of tight junctions. The permeability of the tight junctions can be modulated for instance by specific polymers. These polymers can act as expanders for the tight junctions thereby introducing a port of entry for many particles including toxins, bacteria and immunogens (Salama-Miller and Johnston, 2005). Therefore, passage of nano-encapsulates (and NPs in general) via tight junctions cannot be excluded before hand, they should be considered by risk assessors.

Another uptake route is the transcellular route. This route describes the process by which particles are taken up at the apical side of the intestinal epithelium (by endocytosis), are transported through the M-cells in the Peyers Patches and/or the enterocytes and subsequently released at the basolateral side of the intestinal epithelium (Aprahamian et al., 1987; Jani et al., 1990; Hillery et al., 1994; Carr et al., 1996; Hoet et al., 2004; Florence, 2005; des Rieux et al., 2006). Specific NP characteristics, such as particle size, the surface charge of particles, attachment of ligands or coating with surfactants, may influence the transcellular uptake of particles in the gastrointestinal tract (Hoet et al., 2004; Russell-Jones et al., 1999). If the encapsulates or NPs are protected/prevented from local degradation or metabolism due to the modification, they will enter both the blood and lymphoid circulation intact (Gabor et al., 2004) and can be further distributed in the body.

4.4.2. Distribution, metabolism and excretion

Once nano-encapsulates or NPs pass the gastrointestinal epithelium and end up in the blood circulation, they can interact with various blood-components (i.e., plasma-proteins, coagulation factors, platelets and red and white blood cells) depending on the surface chemistry of the particle (Nemmar et al., 2002). This interaction may have a substantial effect on the distribution and excretion of the NP (Dobrovolskaia, 2007). For instance, the hydrophobic surfaces of nanospheres are highly susceptible to opsonization and clearance by the reticulo-endothelial system, resulting in sequestration of the particles within organs such as the liver and spleen (Letchford and Burt, 2007).

A widespread distribution of NPs inside the (animal) body has been identified. In addition, the smallest NPs revealed a more diverse distribution to e.g., brain, bone marrow, spleen and liver compared to the larger counterparts (Jani et al., 1990; Hillery et al., 1994; Hillyer and Albrecht, 2001; Hoet et al., 2004; De Jong et al., 2008). Crucial for the risk assessor is the potential passage of natural barriers like the cellular barriers, blood–brain barrier, placental barrier and the blood–milk barrier.

There are indications that cellular barriers like membranes may not constitute an obstacle for some NPs. Several different NPs (gold and titanium-oxide) have been identified inside human red blood cells (Rothen-Rutishauser et al., 2006). Interestingly, this cellular uptake of NPs did not involve endocytosis or phagocytosis (Geiser et al., 2005) since erythrocytes do not have phagocytic receptors (Rothen-Rutishauser et al., 2006). This suggests that some NPs are able to cross the cell membrane by processes other than phagocytosis and endocytosis. The permeability of the blood–brain barrier is highly restricted to molecules which are either lipophilic, actively transported or are small soluble molecules (<500 Da). This barrier may therefore represent a strict defense mechanism from blood borne particle exposure that limits the distribution of NPs to the brain. However, evidence exists that distribution to the brain might occur for some NPs (Hillyer and Albrecht, 2001; De Jong et al., 2008). Literature on the effectiveness of the placental barrier in relation to NPs is scarce as is information on passage of the blood–milk barrier.

One of the primary aims of applying nano-encapsulates next to increased bioavailability, is to protect the loaded compounds from liver metabolism and excretion by bile. At this moment, little is known on the prospect and capacity of the liver to metabolize and excrete NPs. However, a size-dependent excretion of NPs has been suggested via bile. In rats, intravenously administrated polystyrene NPs were taken up by the liver and subsequently excreted in the bile (Ogawara et al., 1999). The smaller particles (50 nm) were phagocytosed by Kupffer cells partly and partly taken up by the hepatocytes, whereas polystyrene microparticles (500 nm) were taken up predominantly by the non-parenchymal cells (Kupffer cells and endothelial cells) (Ogawara et al., 1999). Although it is unlikely that the inert NP itself, such as gold and silver particles, fullerenes and carbon nanotubes, can be metabolized effectively by enzymes in the body, there are some indications that functional groups added to the inert nanoparticle are sensitive for metabolism. For instance, the protein cap of a functionalized quantum dot could be cleaved by proteases (Hardman, 2006). Also the metallic core of quantum dots (and other metal-oxides) could be bound by metallothionein and excreted via liver and kidney (Coyle et al., 2002).

4.4.3. Importance of kinetics for the risk assessment of NPs

Although there are some data on kinetics of NPs following oral exposure, there is also a clear need for fundamental research on the absorption, distribution, metabolism and excretion (ADME) of NPs to elucidate the driving forces and mechanisms behind these processes. This knowledge would greatly facilitate the extrapolation and modeling approaches. Moreover, especially important for the risk assessment, this kinetic knowledge will guide the search for target tissues where the NPs could exert a toxic effect. Due to the potential impact of toxicological effects special attention needs to be paid to the possibility that certain NPs can cross the barriers (e.g., gastrointestinal barrier, cellular barrier, blood–brain barrier, placenta barrier, blood–milk barrier).

4.5. Toxicity of NPs

As the application of NPs in consumer products is a phenomenon of recent years, knowledge on the potential toxicity of NPs is limited, but rapidly growing. Several studies suggest that NPs may have a deviating toxicity profile when compared to their conventional chemical analogs (Donaldson et al., 2001; Nel et al., 2006; Oberdoster et al., 2005a). The most important question for risk assessment is the sensitivity and validity of currently existing test systems. It is generally thought that the standard battery will suffice, but special attention is needed for specific endpoints (Babus et al., 2007). These will be discussed in the following paragraphs, with a focus on toxic effects following oral exposure and if not available also from other exposure routes.

While the literature on toxicity of NPs is rapidly growing it should be kept in mind that results are often obtained for only one type and size of NPs. Furthermore test animals are generally exposed to high concentrations under artificial conditions. This limits the usefulness of obtained data for risk assessment. Extrap-
olation from one type of NPs to another or from one size to another is on the basis of present knowledge still impossible. At this moment there is too few data to determine which type of effects are to be expected for which type of NP.

4.5.1. In vitro toxicity

While results of in vivo studies might provide relevant information for the hazard identification of the studied NP, caution has to be exercised when extrapolating results or mechanisms for the hazard characterization and subsequent human risk assessment (Ober dorster et al., 2007b). The search for mechanistic explanations is only now starting. For this in vitro models can be very important. Numerous in vitro studies using various NPs are being published. Many NPs can trigger the release of reactive oxygen species and cause oxidative stress and subsequent inflammation by means of interaction with the reticulo-endothelial system (Nel et al., 2006; Donaldson et al., 2007; Donaldson and Seaton, 2007; Ober dorster et al., 2007b).

Cells are generally exposed under artificial conditions, and a selected number of endpoints are studies. Special attention is required for cellular interactions in order to better understand and predict cellular toxicity and the validity of currently used in vitro models (e.g., for the gastrointestinal absorption). While in vitro studies might be useful in a tiered screening approach it is recommended to develop validated assays and assess sub-lethal changes for example by means of profiling studies (Balbus et al., 2007; Lewinski et al., 2008).

4.5.2. Acute toxicity

Acute, subacute and subchronic toxicity following oral exposure have been investigated in rodents for several different NPs (e.g., copper, selenium, zinc and zinc-oxide and titanium dioxide NPs). The results of the available oral toxicity studies indicate that, depending on the particle size, coating and chemical composition of the NPs, acute toxicity at high doses may occur (Jia et al., 2005; Zhang et al., 2005; Chen et al., 2006c; Wang et al., 2006; Wang et al., 2007; Wang et al., 2008).

4.5.3. Long term toxicity

No information on the toxicity after chronic or acute low dose oral exposure is currently available. Information from toxicity studies with other routes of exposure indicate that several systemic effects on different organ systems may occur after long term exposure to NPs, including the immune, inflammatory and cardiovascular system. Effects on the immune and inflammatory systems may include oxidative stress and/or activation of pro-inflammatory cytokines in the lungs, liver, heart and brain. Effects on the cardiovascular system may include pro-thrombotic effects and adverse effects on the cardiac function (acute myocardial infarction and adverse effects on the heart rate). Furthermore, genotoxicity, and possible carcinogenesis and teratogenicity may occur, but no data on these latter endpoints are available yet.

For the following endpoints research is needed.

4.5.3.1. Neurotoxicity. There is evidence from ADME studies that NP may pass the blood–brain barrier following systemic availability of NPs (Hillyer and Albrecht, 2001; Borm et al., 2006; Silva, 2007). It is not clear if this is a generic effect of all NPs or only a subgroup. This emphasizes the need of kinetic studies, and increased attention of toxicologists to neurotoxicity in their search for potential effects in target tissues. Toxic effects due to the presence (or even accumulation) of NPs in the brain have not been studied so far, but risk assessors should be aware of possible neurological effects when assessing toxicology experiments. Possibly, current guideline tests will need to be adapted to render these tests more sensitive for neurotoxic effects of NPs.

4.5.3.2. Reprotoxicity. Transfer of NPs across the placenta cannot be excluded (including excretion via breast milk (the blood milk barrier), which could lead to embryotoxicity as a result of exposure to NPs (Fujimoto et al., 2005). Data addressing the distribution of NPs to the reproductive cells is currently unavailable. In addition, no clear data showing the distribution of NPs in the fetus are available (Tran et al., 2005). This leads to the recommendation that reproductive toxicity needs to be considered carefully when there is evidence for NP passage of the placenta.

4.5.3.3. Mutagenicity. Intracellular NPs do not appear to be membrane bound and might have direct access to the intracellular proteins, organelles and DNA of the cell, which might imply enhanced toxic potential (Geiser et al., 2005; Kabanov, 2006). However, possible interactions of NPs with cell components are poorly understood and validated assays with meaningful endpoints for genotoxicity are needed (SCENIHR, 2007a).

4.5.3.4. Allergenicity (or sensitization). Even for conventional chemicals little is known on the induction of food allergy and the type of exposure required to induce such responses. In the case of NPs this becomes extra prominent for two reasons. First of all it is the possible adjuvant activity of NPs that introduces additional uncertainty (Dobrovolskaia, 2007). And secondly, because of the actively charged surfaces of NPs it can absorb biomolecules as they pass through the GI tract (Govers et al., 1994). This might result in changed exposure of the cellular lining of the intestine (Borm and Kreylng, 2004). In addition the surface properties (e.g., coatings) are important determinants for the active uptake of encapsulates, but might also be a reason for concern. For example lectins used for coatings are highly immunogenic, can be cytotoxic or induce inflammatory responses and gastrointestinal irritation (Gabor et al., 2004; des Rieux et al., 2006; Hong et al., 2006; Kabanov, 2006).

4.6. Setting health-based guidance values

The last step in the hazard characterization may involve the setting of health-based guidance values such as acceptable or tolerable daily intakes. These are generally based on animal toxicity studies. Reference points (e.g., the no-observed-adverse-effect-level or benchmark-dose-level) for the critical effect of a substance form the starting point of the risk assessment. This is a general approach for all substances either being in a conventional form or at a nano-sized scale. For NPs, using only mass as the dose metric for health-based guidance values will not be sufficient. This is exemplified by the ongoing discussions on dose metrics as mentioned before.

Furthermore, guidance values are based on toxicological studies performed with a substance (or NP) with a given bioavailability. Of special concern is the increased bioavailability of bioactive compounds loaded in nano-delivery systems (or nano-sized bioactive compounds).

Extrapolation of a health-based guidance value between formulations with different bioavailability might therefore not be possible. Ultimately, this might require setting of separate health-based guidance values depending on the formulation (e.g., nano-formulated or not).

5. Consequences for risk assessment of NPs

As indicated, engineered nanoparticles (NPs) have novel or distinct properties that are attributed to a combination of their small size, physiochemical properties, chemical composition and surface structure (Nel et al., 2006). It is the added functionality of NPs that makes the engineered NPs different from natural small sized parti-
frequent use of novel technologies (e.g., profiling approaches) and the more
as a request for additional newly developed studies. It can also im-
in these assumptions for example extrapolations from one com-
to be made because of knowledge gaps. However, uncertainties
studied and used in parallel with conventional techniques.
Assess the validity if currently used toxicological assays for
detecting the effects caused by NPs.
Identify products containing nanoparticles that are on the market
or (being developed), including the type of NPs are (or will
be) used and the estimated consumption of these products.
Elaborate regulatory/policy approaches for the disclosure of
information on NPs being produced including the products they are incorporated in.

This request for extra information is not to be considered solely
as a request for additional newly developed studies. It can also im-
ply that conventional study approaches need to be redesigned. The
use of novel technologies (e.g., profiling approaches) and the more
frequent use of in vitro approaches for risk assessment need to be
studied and used in parallel with conventional techniques.

Logically, present safety and risk assessment requirements are
based on knowledge gathered for conventional chemicals. Also in
the risk assessment of conventional chemicals assumptions have
been made because of knowledge gaps. However, uncertainties
in these assumptions for example extrapolations from one com-
ponent to another are approached on a sound basis of general
knowledge. For NPs such a basis is lacking, moreover uncertainties
in the safety assessment are expected to be larger (Morgan, 2005).
To reduce the uncertainties it is important that currently devel-
oped quality-controlled databases are also suitable to obtain infor-
mation on the occurrence of NPs in food. Such a database can be
used to identify certain patterns of behavior and/or toxicity of
NPs, such as for example toxicological size-thresholds.

At this stage of (lack of) knowledge of nanotoxicology it is
unavoidable that risk assessors need as much information as pos-
sible about NPs and their appearance and behavior in biological
matrices and organisms. Over time it will be possible to evaluate
the data and look for the set of pivotal information. This clearly
needs close collaborations between NP (and products) developers,
risk assessors, regulators and researchers.

Conflict of Interest statements

The authors declare that there are no conflicts of interest.

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