Part Four
Nanotechnology and Society

10
Toxicology of Nanomaterials in Food

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10.1
Introduction

Research on the potential applications of nanotechnology and engineered nanomaterials in the areas of food-borne pathogen detection, antimicrobial activity, food packaging, food processing, food ingredient development, and nutritional studies have demonstrated the potential for nanotechnology to provide significant benefits for the consumer. These benefits may include improved food safety through enhanced detection and control of the pathogens responsible for food poisoning, enhanced shelf-life and quality of food products, and superior health-promoting or nutritional properties of foods. However, as is the case with the development of any new food processing technology, food ingredient or food packaging material, there must also be adequate studies to demonstrate that these potential benefits of nanotechnology and engineered nanomaterials designed for use in foods are not accompanied by any undesirable adverse health effects. Thus evaluation of the potential hazards related to exposure to nanomaterials and nanotechnology-based products has emerged as an important area in toxicology and risk assessment.

10.2
What Makes Nanomaterials Special?

An 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 [1, 2]. Compared to bulk-scale materials, nanomaterials have a very large relative surface area (i.e., surface area per unit mass) and high particle number per unit mass, and the ratio of surface area to total number of atoms or molecules increases exponentially with decreasing particle size. The physicochemical properties of ENMs make them different from micro- and macroscale material and from dissolved chemical of the same material. So, besides

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offering a wide range of novel applications, this may also give rise to altered kinetics and toxicity profiles. This will be discussed in subsequent paragraphs.

The decreased size of nanomaterials results in increased specific surface area, until the properties of the surface molecules dominate. This very high surface area has several consequences and renders them, for example, more reactive, thus generating more effective catalysts in a variety of applications [3, 4]. However, when considering potential health implications, reactive groups on the surface of nanomaterials are likely to influence their biological and/or toxicological effects. As the surface of a nanoparticle provides the initial interaction between the particle and a biological system, and therefore is a crucial determinant of particle response, these unique properties need to be investigated and understood from a physicochemical and toxicological standpoint.

10.3 Characterization of Engineered Nanomaterials

An understanding of the physicochemical properties of ENMs that impact upon their interaction with biological systems can only be gained with sufficient measurement and reporting of these properties in studies that subsequently assess biological activity. Lack of adequate nanomaterial characterization limits the value and significance of a given study and renders it impossible to compare studies and to recognize parameters that might influence biological activity (i.e., desirable effects) or toxicity (i.e., undesirable effects) [5, 6]. Thus, there has been considerable effort by the scientific community to define a minimal set of characteristics of ENMs recommended for research studies. As the outcome of the European FP7 project NanoimpactNet, a set of minimal characteristics and metrics is recommended for every field of research investigating the health impact of nanomaterials [7]:

- size distribution (of primary particles);
- chemical composition;
- nanomaterial surface (i.e., surface area, surface charge, and surface chemistry);
- structure (agglomeration state);
- shape;
- persistence.

This list is very similar to the lists of parameters that have been recommended by other authors and scientific organizations [3, 8–13]. In addition, it is generally recommended to fully characterize the nanomaterials in order to understand the potential toxicity of the nanomaterials and relate toxicity to the physicochemical properties [3, 8, 14, 15].

It is also imperative to document these parameters in the experimental exposure media (cell culture media, oral dosing solution, etc.) to the greatest extent possible,

as many physicochemical parameters differ depending on whether determined in experimental media or in the bulk dry (i.e., "as-received") state.

Analytical methods are available to determine most if not all characteristics of nanomaterials [16–18]. The issue, however, is that these methods are normally only able to determine one single characteristic, making the process of full characterization very labor intensive. In addition, these methods cannot be employed to determine the characteristics directly in the food matrix. This leads to the conclusion that currently not all ENM characteristics can be readily determined. Furthermore, different techniques that are available to measure the same nanomaterial characteristic can produce contrasting results (e.g., reported sizes of ENMs) – the variations typically emerge as a result of intrinsic biases and modeling assumptions of the techniques. Agreement on standard testing methods is lacking and the comparability between various methods to assess a specific metric is still being evaluated. The challenge is initially to prioritize some metrics based on biological dose–response relations and then to develop less labor-intensive analytical methods for characterizing ENMs in biological matrices. Additionally, harmonized sample preparation procedures need to be developed.

10.3.1 Unique Issues for Characterization of Engineered Nanomaterials for Food Applications

Current and foreseen nanotechnology applications in the agri-food production chain are focused on the development of nano-sized food ingredients and additives, delivery systems for bioactive compounds, and innovative food packaging [19]. Nanomaterials in food may appear in suspension (mostly solid in liquids) or emulsion (two liquid phases). Within the agri-food chain, metal or metal oxide nanomaterials (e.g., nano-Ag, nano-ZnO, nano-Cu, nano-TiO_2) are applied in, for example, food packaging materials. Each of these different types of nanomaterial requires a different characterization approach.

The focus for safety evaluation will be on persistent nanomaterials, that is, non-soluble or non-biodegradable particles, since potential risks are predominantly associated with these types of particle. But another category of nanotechnology application in the food sector is represented by nano-encapsulates. It is particularly challenging to detect these types of nanomaterial within the food matrix and to differentiate between naturally occurring micelles and liposomes (e.g., in milk) and the deliberately created nano-encapsulates. Some analytical methods are available for this [17].

Engineered nanomaterials in food may encompass many forms. It is likely that nanomaterials are used in foods in an agglomerated form, but it cannot be excluded that these agglomerates may break down, and that the consumer may ultimately be exposed to free nanomaterials. Owing to their specific physicochemical properties, it is to be expected that nanomaterials could interact with proteins, lipids, carbohydrates, nucleic acids, ions, minerals, and water in food, feed, and biological

tissues. For nanomaterials present in food, their interactions with proteins are important [20, 21]. Therefore, it is important that the nanomaterials are characterized in the relevant food matrix [22, 23].

10.4 Safety Assessment of Oral-Exposure Engineered Nanomaterials for Food Application

There are several approaches to assessing the safety of ENMs. These include: (i) investigating the toxicokinetics of nanomaterials to determine if they are absorbed into the body, and how they are handled within the body after absorption; (ii) investigating the toxicodynamics of nanomaterials to determine how they interact with tissues, cells, and cellular components; and (iii) conducting classic oral toxicity studies. A brief review of studies conducted in each of these areas will be presented below. However, to put these studies into context, it is important for the reader first to be presented with several important considerations for toxicology studies on nanomaterials.

10.4.1 Experimental Design Considerations for Toxicology Studies

The basic tenet of the study of toxicology is from Paracelsus, who wrote: "All substances are poisons; there is none that is not a poison. The right dose differentiates a poison from a remedy. The dose makes the poison." In other words, at some level of exposure, all compounds will illicit an adverse effect. Thus, to demonstrate clearly the reported toxicological properties, evidence of a dose–response is required. This means that multiple doses of the materials must be given, to see that, with increasing dose, the magnitude or incidence of an adverse effect also increases. Ultimately this leads to the derivation of a concentration at which no significant effect is observed. Traditionally, this point is called the "no observed adverse effect level" (i.e., the dose at which no adverse effects are observed), though nowadays the more efficient "benchmark dose" is often derived [24]. These reference points are used further in the risk assessment and the establishment of toxicological safety values.

Unfortunately, many of the reported toxicology studies of nanomaterials either do not provide sufficient information on the doses used, do not use more than one dose, or conclude that, because adverse effects are observed at one dose, the nanomaterial is "toxic". This is unfortunate and provides limited useful data for risk assessment purposes. In order to put the results of toxicology experiments into perspective for human health implications, more doses need to be investigated, and a rationale for the doses chosen, or a comparison with likely human exposures, should be provided when possible.

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 a

good metric [25]. As discussed earlier, the characterization of the exposure is crucial. 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) [26, 27], the used doses should be expressed using different dose-describing parameters. 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 to conclude on the likelihood of health risks.

The importance of good experimental design for toxicological studies of nanomaterials cannot be over-emphasized. The following factors must be considered to assess the quality of the experimental design and data resulting from experiments.

- As discussed above, adequate nanomaterial characterization in general and specifically within the surrounding matrix is clearly needed to establish metrics other than mass alone that are relevant to the toxicity of nanomaterials.
- Inclusion of positive and negative controls, as in every scientific experiment, is obviously required. Importantly, in nanotoxicology the administration of nanomaterials to the testing system needs to be accompanied by larger-sized materials and conventional forms of the materials (i.e., ions). Without these experimental groups, the studies have very much less added value to the scientific literature and are not useful for risk assessment purposes.
- Nanomaterials are known to interfere with optical and other detection measurements and to adsorb essential growth factors and nutrients from the growth medium, leading to non-specific indirect growth inhibition and apparent cytotoxicity. Therefore, adequate controls need to be used to eliminate potential interference with colorimetric and fluorometric dyes as used in cell cytotoxicity assays, interference with assays for measurement of reactive oxygen species, and alteration of the nutritional properties of the growth medium. Several authors have discussed the limitations and high likelihood of false positives of these assays [28–32], indicating that improvements in sensitivity, reliability, and sophistication, and a clear correlation with *in vivo* activity is needed in order for *in vitro* assays to yield informative data.
- One of the most important questions for the safety assessment is the sensitivity and validity of currently used test assays [25]. The question of appropriate test methods for evaluating nanomaterials has been addressed by the Organisation for Economic Co-operation and Development (OECD) in a recently published document [33]. This provides a starting point from which researchers across the globe can design testing strategies that would standardize the testing of nanomaterials. Currently, there are 118 published OECD testing guidelines covering physicochemical characterization, effects on biotic systems, degradation and/or accumulation, health effects, and other endpoints. In general, the OECD guidelines were judged to be applicable for investigating the health effects of nanomaterials [33]. An important caveat was that additional consid-

eration needs to be given to the physicochemical characteristics of the material tested (17 physicochemical properties have been suggested as the necessary prerequisite for toxicological testing), including such characteristics in the actual dosing solution. Additional pathology following certain tests was also suggested.

- Assessment for endotoxin contamination, which is exceedingly common due to the ubiquitous nature of endotoxins, is a critical step in this cascade. As endotoxin contamination generates a cellular inflammatory response, it is necessary to establish whether any inflammatory response observed in biological systems exposed to nanomaterials is due to endotoxin contamination or the nanomaterial or both [28, 29, 34].
- High variability and the cost of manufacturing a sufficient amount of nanomaterials for animal studies with uniform characteristics represents a significant hurdle for toxicity testing of some nanomaterials. The stability of nanomaterials during storage and dosing formulation must also be considered.

10.4.2 Toxicokinetics

The ability of micro- and nanomaterials to cross over the intact healthy gastrointestinal tract in humans has been recognized for over 100 years, as citations of absorption date back to the early 1900s (see review by Florence [35]). Absorption of nanomaterials through the gastrointestinal tract has also been reported in the mouse, rat, sheep, pig, and cow (see review by Florence [36]). Thus the study of the pharmacokinetics and toxicokinetics of orally administered particles is not new, and, owing to improved methods for developing nanomaterials with very specific characteristics, there is greater need for understanding the specific parameters of nanomaterials that affect their pharmacokinetics and toxicokinetics.

The absorption, distribution, metabolism, and excretion (ADME) of orally administered nanomaterials is influenced by their characteristics, such as shape, size, hydrophobicity, surface charge, and functionalized groups [26, 36–39]. However, it is unclear to what extent the different physicochemical characteristics of nanomaterials contribute to their kinetics. In this section, current knowledge on the ADME characteristics of nanomaterials that may be relevant to oral exposures is discussed.

10.4.2.1 Absorption

The gastrointestinal (GI) tract represents a port of entry for nanomaterials, not only through the ingestion of food, dietary supplements, drugs, and water that may contain nanomaterials, but also by way of ingestion of the inhaled nanomaterials that are cleared by the respiratory tract [40].

Nanomaterials may gain entry into the body by crossing the intestinal wall through the M-cells, through normal enterocytes, and/or through paracellular spaces. Uptake in M-cells, which are specialized phagocytic enterocytes found in

Peyer's patches, occurs through adsorptive endocytosis involving clathrin-coated pits and vesicles, endocytosis, and phagocytosis [36]. Uptake of particles can also occur through normal enterocytes at the apical side of the intestinal epithelial cells (by endocytosis), transport through cells, and subsequent release at the basolateral side of the epithelial cells into the lymphatic system [36, 38, 40].

Another possible uptake route for nanomaterials is via the paracellular pathway or passage between the cells [41–43]. In this pathway, also known as persorption, nanomaterials rely on the gaps and the tight junctions between the endothelial cells to pass through the epithelial cell layer. Studies have shown that the permeability of the tight junctions between the endothelium to nanomaterials can be modulated by synthetic peptides such as E-cadherin-derived peptides, which can act on the aqueous-filled pores of the paracellular pathways and expand the tight junctions [44, 45].

Uptake of nanomaterials in the GI tract depends on a variety of factors, including the diffusion of particles through mucous, initial contact with the GI epithelium, cellular trafficking, and various uptake and translocation processes, which are governed at least in part by the characteristics of the nanomaterials. Specific characteristics of nanomaterials – including particle size, surface charge, attachment of ligands or coating with surfactants, shape and elasticity, and physical and chemical stability – have been shown to influence the transcellular uptake of particles in the GI tract [43, 46–50]. Protein adsorption to engineered nanomaterials may enhance membrane crossing and cellular penetration [51–53].

Studies have demonstrated that diffusion of nanomaterials across the mucus layer depends on the size of the particles: the smaller the particle diameter, the faster they may diffuse through GI secretion to reach the colonic enterocytes [37, 54–56]. For example, when polystyrene microspheres ranging from 50 nm to 3 μm were fed by gavage to female rats at a dose of 1.25 mg per kilogram body weight, the absorption rates were highest for 50 nm particles, lower for 100 nm particles, and particles larger than 300 nm were not detectable in the blood, indicating no absorption [37]. However, increased absorption with decreased size is not always observed. For example, no significant differences in absorption or accumulation was observed in guinea pigs administered customary (10 000–90 000 nm) or nanosized (200–300 nm) sitosterol in the diet for two weeks [57]. Concentrations were measured in plasma, blood cells, bile, liver, kidney, jejunal mucosa/serosa, cecum, colon, and feces.

Diffusion across the mucous layer also depends on the surface charge of the nanomaterial. Anionic or repulsive nanomaterials have been shown to reach the epithelial surface [46], while cationic particles became entrapped in the negatively charged mucus [55]. For example, polystyrene latex nanomaterials (14 nm) rapidly adhered to the mucosal layer of the rat intestine but did not enter the epithelial cells, and were observed to move further away from epithelial cell surfaces over time [55]. A comparative study of the uptake of nanomaterials in a human intestinal cell culture model (Caco-2 cells), in a mucus-secreting cell line (NTX-E12), and *in vivo* using intra-duodenal delivery in the rat, clearly illustrated that the mucous layer of the intestine has a profound effect on uptake of certain

nanomaterials [58]. Thus, the GI tract can also act as a significant barrier to systemic exposure for many nanomaterials [5].

10.4.2.2 Distribution

Following absorption from the GI tract, nanomaterials can reach the systemic circulation, distribute to various tissues and organs, or potentially interact with various blood components, such as plasma proteins, red or white blood cells, coagulation factors, and platelets [34, 59–62]. Variables that can affect distribution and tissue localization of nanomaterials include flow in the lymph vessels, entrapment in lymph nodes, rate of transport between lymph and blood, blood flow, adhesion to capillary walls, extravasation and movement into tissues, and cellular components within tissues [36, 39].

A number of studies report a size-dependent distribution of nanomaterials to various tissues and organs, following their uptake from the GI tract [43, 63, 64]. For example, for gold nanomaterials, clear differences in biodistribution have been observed for 10 nm compared to 50 and 250 nm nanoparticles. While the 10 nm nanoparticles were present in the liver, spleen, kidney, testis, thymus, heart, lung, and brain following an intravenous administration to rats, the 50 and 250 nm nanoparticles were present only in the liver and spleen [63] . Others have found similar trends, where smaller gold nanomaterials (15 nm) showed greater biodistribution compared to larger gold nanomaterials (50, 100, and 200 nm) [64]. Hillyer and Albrecht [43] demonstrated that, following oral administration of metallic colloidal gold nanomaterials of different sizes (58, 28, 10, and 4 nm) to mice, the smallest particles (4 nm) were identified in the kidney, liver, spleen, lungs, and brain, while the largest particles (58 nm) were detected almost solely inside the GI tract.

Although direct comparisons between the above-mentioned studies may not be possible, as there were several differences in the experimental conditions, ports of entry, or study designs, it appears that there is a trend for greater biodistribution for smaller-sized nanomaterials as compared to larger-sized nanomaterials. The extent to which nanomaterials can cross the blood–brain barrier (BBB) is not well known. Although the permeability of the BBB is highly restricted to lipophilic molecules and actively transported or small soluble molecules, evidence exists that this distribution might be relevant for some nanomaterials, as low concentrations of gold were found in the brain after oral administration of gold nanomaterials [43]. In addition, widespread distribution was observed in females administered 60 nm silver nanomaterials in a 28-day subchronic studies [65].

Binding of proteins to the surface of nanomaterials has been shown to have a significant effect on the distribution and excretion of nanomaterials, and, therefore, to influence their potential toxic effects [66]. For example, binding of nanomaterials to serum proteins resulted in a reduction in cytotoxicity of silica [59] and quantum dots [60].

10.4.2.3 Metabolism

There is little known on the metabolism of nanomaterials. It is unlikely that inert nanomaterials, such as gold and silver particles, fullerenes, and carbon nanotubes,

can be metabolized effectively upon absorption. However, there are some indications that functional groups added to inert nanomaterials may be susceptible to metabolism. For instance, the protein cap of a functionalized quantum dot could be cleaved by proteases [67].

10.4.2.4 **Excretion**

Similarly, there is limited information on the excretion of orally administered nanomaterials. Clearly, nanomaterials that are not absorbed are eliminated from the body in the feces. Renal clearance was reported for fullerenes and single-walled carbon nanotubes [68, 69]. Ogawara *et al.* [70] reported that, following intravenous administration in rats, 4% of the dose of polystyrene nanomaterials (50 nm) was excreted into bile; however, larger polystyrene microparticles (500 nm) were not transported to the bile. Similarly, liposomal-based nanomaterials have been reported to be primarily eliminated through the hepatobiliary system [71, 72].

In conclusion, assessing the toxicokinetic properties of nanomaterials as compared to larger macro- or bulk-state materials can be useful in predicting the likelihood that the nano-form of the materials will have altered biological effects. For example, an increase in absorption or a change in the distribution pattern may result in increased dose of the nanomaterial at the target site for toxicity and/or may change the target site. Currently, there is an insufficient number of well-conducted studies on oral exposure to various nanomaterials to develop accurate predictive models. Studies have demonstrated that the qualitatively different physicochemical characteristics of nanoparticles, such as their relatively large and active surface area, can result in altered absorption and body distribution compared with that of bulk materials, although this depends also on surface chemistry, charge, and the specific nanomaterial under investigation.

10.4.3
Toxicodynamics

Knowledge on the potential toxicity of nanomaterials is limited but rapidly growing. There is a body of review papers available [3, 73–75] that suggest that nanomaterials may have different toxicity profiles from their bulk equivalents. 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 [76]. Stern and McNeil [5] point out that current data support the need for a material-specific risk approach, as a generalized risk paradigm for nanomaterials is not emerging from studies evaluating biological properties in which careful and adequate characterization of materials has been reported.

Most of the work that has been done so far addresses primarily the occupational hazards associated with the manufacture and handling of nanostructured materials. Some nanomaterials may initiate catalytic reactions and increase their fire and explosion potential and could potentially present a higher risk than similar

quantities of a coarser material with the same chemical composition [77, 78]. Experimental studies in rodents and cell cultures have shown that the toxicity of nanomaterials may be greater than that of the same mass of larger particles of similar chemical composition, although it is often not clear if this is truly due to the interaction of the nanomaterials with the cell or due to the interference of the nanomaterial with the assay or measurement. In addition to particle surface area, other particle characteristics may influence the toxicity, including solubility, shape, and surface chemistry [3, 78, 79].

10.4.3.1 *In Vivo* Toxicity

There are only a limited number of published *oral* toxicity studies using ENMs, mostly using insoluble metals and metal oxides. Acute, subacute, and subchronic toxicity following oral exposure have been investigated in rodents for several different nanoparticles (e.g., silver, copper, selenium, zinc and zinc oxide, and titanium dioxide nanoparticles). There is a great demand for studies using chronic oral exposure to nanomaterials combined with a broad screen for potential effects [80]. The results of the available oral toxicity studies indicate that, depending on the particle size, coating, and chemical composition of the nanoparticles, acute toxicity at high doses may occur [81–86]. In a subchronic 28-day study of 60 nm silver nanomaterial given by oral gavage at dose levels of 0, 30, 300, or 1000 mg per kilogram body weight per day, modest effects on body and organ weights, and on some blood parameters were observed in the mid- and high-dose groups. Histological studies revealed dose-dependent hyperplasia of the ventral vein in the liver [65]. The *in vivo* micronucleus test revealed no effects upon exposure. In a follow-up study, the same group [87] reported data on gender-specific silver accumulation in the kidneys of rats; however, interpretation of the findings of the study is difficult, as the treatment groups received different doses of carboxymethylcellulose and the dose of nano-silver was not reported.

It is not only the ENM itself that might trigger biological effects. Since ENMs can absorb or bind different compounds on their surfaces [88], including proteins [21], it has been speculated that a so-called "Trojan horse" effect is possible, where ENMs can act as carriers of potentially harmful chemicals and foreign substances into the organism [1]. The use of nano-encapsulates to increase the bioavailability of bioactive compounds raises similar concerns. These carrier systems might introduce unintended macromolecules, for example, undigested or unmetabolized compounds across the GI tract, leading to unknown distribution and accumulation and ultimate toxicological effects. However, clear demonstration of such an effect has yet to be reported for nanomaterials designed for food-related applications.

10.4.3.2 *In Vitro* Toxicity

Numerous *in vitro* studies using various nanomaterials are available in the scientific literature. It is beyond the scope of the current chapter to discuss all these studies. Recent reviews suggest that nanomaterials *in vitro* can trigger the release of reactive oxygen species and cause oxidative stress and subsequent inflammation by means of interaction with the reticulo-endothelial system [74, 75, 89–91]. While

these results are useful for hazard identification of nanomaterials, caution has to be exercised when extrapolating results or mechanisms for the hazard characterization and subsequent human risk assessment [74]. Especially for the *in vitro* studies, a solid description and understanding of the interactions of nanomaterials with the cell culture medium is required. In addition, colorimetric techniques are frequently used as read-out systems. This might be problematic because of the interaction of the nanomaterials with the dyes used in these assays [92, 93]. Thus, while *in vitro* studies might be useful in a tiered screening approach, development of validated assays and assessment of sublethal changes, for example by means of profiling studies, are recommended [76, 94].

10.4.3.3 **Study Reliability**

Only a very limited number of repeated-dose oral-exposure studies are available. The quality of many studies, however, is disputable, severely limiting the use of this information for risk assessment purposes [1, 95]. For example, in most studies, only a single-sized, poorly characterized nanoparticle is used, or nanomaterials are administered at unrealistically high doses, or a narrow range of effects are generally studied [74]. Evaluation of the quality of a study and thus the reliability of the data reported is critical for risk assessment of a nanomaterial. A two-step method to objectively evaluate the reliability of safety studies of nanomaterials has recently been developed [95]. The first step utilizes a publicly available tool to rank the reliability of the study based on adequacy of design and documentation of methods, materials, and results, providing a "study score". The second step determines the completeness of physicochemical characterization of the nanomaterial(s) assessed within the study, providing a "nanomaterial score". This approach is encouraged to promote the notion that, for studies conducted with nanomaterials, the combination of a reliable study and sufficient nanomaterial characterization is of significantly greater value than either of these alone.

In addition, when evaluating the plethora of *in vitro* studies with nanomaterials, caution has to be exercised when extrapolating their results or mechanisms for hazard characterization to subsequent human risk assessment [74]. The *in vitro* studies might be suitable in searching for mechanistic explanations of toxic effects, or as screening methods in combination with profiling studies in a tiered hazard assessment approach [76, 94].

10.5 Conclusions

It is the added functionality of nanomaterials – due to a combination of their small size, physiochemical properties, chemical composition, and surface structure – that makes these materials different not only from natural small-sized particles, but also from their conventional counterparts [8, 75, 89, 96]. Because of this, unexpected toxicological effects might occur. The introduction of nanomaterial-based consumer products into the marketplace in various industrial sectors increases the

urgency for a better understanding of the potential negative impacts that nanomaterials may have on biological systems. 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 [3, 80]. In addition to scientific risk assessment-related concerns, consumer concerns regarding a new technology such as nanotechnology application in food products are mainly related to safety issues [97].

On the other hand, potential beneficial effects of nanotechnologies are generally well described. Nanotechnologies used to improve certain properties of food products can range from the use of so-called soft nanomaterials like micelles and vesicles to encapsulate nutrients and deliver them to specific locations in the gastrointestinal tract, to the use of nano-formulated substances to improve the flow behavior of powdered foodstuffs. It is generally agreed among toxicologists that the supramolecular structures that are designed to break down within the gastrointestinal tract constitute relatively low risks, assuming that the molecules used to make these structures are safe. Also, nanomaterials that easily dissolve in water or are biodegradable will most likely not be very hazardous.

Most of the concerns of applications of nanotechnologies in food are focused on insoluble, free, and persistent nanomaterials that potentially can pass certain barriers and enter the body, and subsequently enter certain tissues or even individual cells. Because of their persistent nature, they can stay there for prolonged periods and induce harmful effects. A special, food-related case of concern is represented by nano-formulations designed to increase the bioavailability of the bulk equivalent. This might impact on the toxic profile of these compounds, and needs to be assessed. Importantly, future studies on the safety of nanomaterials must address the considerations discussed earlier in this chapter, including adequate characterization of the nanomaterial, dose metrics, method validation, and study design, to facilitate interpretation of the data and comparison of results from study to study. Only when sufficient studies of high quality are available will we achieve a greater understanding of the biological effects of nanomaterials.

Techniques in biotechnology, X-omics, and next-generation sequencing might offer valuable instruments to generate an understanding of the mechanism of biological action of nanomaterials, offering a battery of responses from biological systems (e.g., a fingerprint of the ENM in a biological matrix). In addition, combining physiochemical properties integrated with dose–response information from biokinetic and biodynamic studies should be combined in cross reading approaches, like quantitative structure–activity relationships (QSARs), to allow the prediction of the toxicity of a substance using a computer model. These *in silico* approaches are still under development for conventional chemicals and are driven by the European REACH (Registration, Evaluation, Authorization, and Restriction of Chemicals) initiative.

Globally, the scientific and industrial communities need to come together to resolve the key issues of safety of the use of nanomaterials in food. At this stage of lack of knowledge of nanotoxicology, it is unavoidable that risk assessors need as much information as possible about nanoparticles and their appearance and

behavior in biological matrices and organisms. This is a prerequisite to fully exploit the benefits of nanomaterials without exposing the public to harm.

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