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Review

Nanoparticle Food Applications and Their Toxicity: Current Trends and Needs in Risk Assessment Strategies

JEFFERSON DE OLIVEIRA MALLIA,[1,2] RUSSELL GALEA,[2] RAJAT NAG,[3] ENDA CUMMINS,[3] RUBEN GATT,[2,4] AND VASILIS VALDRAMIDIS *https://orcid.org/0000-0001-6486-3890*[1,4]*

[1]*Department of Food Sciences and Nutrition, Faculty of Health Sciences,* [2]*Metamaterials Unit, Faculty of Science, and* [4]*Centre for Molecular Medicine and Biobanking, University of Malta, Msida MSD2080, Malta; and* [3]*UCD School of Biosystems and Food Engineering, University College Dublin, Belfield, Dublin 4, Ireland*

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ABSTRACT

Nanotechnology has developed into one of the most groundbreaking scientific fields in the last few decades because it exploits the enhanced reactivity of materials at the atomic scale. The current classification of nanoparticles (NPs) used in foods is outlined in relation to the production and physicochemical characteristics. This review aims to concisely present the most popular and widely used inorganic and organic NPs in food industries. Considering that the toxicity of NPs is often associated with chemical reactivity, a series of in vitro toxicity studies are also summarized, integrating information on the type of NP studies and reported specifications, type of cells used, exposure conditions, and assessed end points. The important role of the digestive system in the absorption and distribution of nanoformulated foods within the body and how this affects the resultant cytotoxicity. Examples of how NPs and their accumulation within different organs are presented in relation to the consumption of specific foods. Finally, the role of developing human health risk assessments to characterize both the potential impact of the hazard and the likelihood or level of human exposure is outlined. Uncertainties exist around risk and exposure assessments of NPs due to limited information on several aspects, including toxicity, behavior, and bioaccumulation. Overall, this review presents current trends and needs for future assessments in toxicity evaluation to ensure the safe application of NPs in the food industry.

HIGHLIGHTS

- The use and inclusion of NPs in food production is growing.
- TiO_2 NPs are widely used by the food industry.
- Thorough NP hazard characterization by using more advanced in vitro models is required.
- Individual and multimixture NP interactions require further hazard investigations.
- Unified risk assessment approaches are needed to determine NP health risks.

Key words: Food industry; Hazard characterization; Nanoparticles; Risk assessment

The advent of nanotechnology, which involves the manufacture and use of materials of enhanced reactivity at the atomic scale, has brought great opportunities for the development of new materials. One of the characteristics of these materials is the antimicrobial properties, supporting applications in several fields, such as medicine, agriculture, and food production. Several applications of metal nanoparticles (NPs) are currently available, but their further exploitation in the food sector requires thorough food safety and toxicity assessments.

The use of NPs in food-related applications has put more pressure on regulatory bodies to assess and certify them for safe use. Current public awareness and concern regarding NP use and consumption also increased the necessity for safe applications *(6, 77)*. A "nanomaterial," as defined by the European Parliament and the Council of the European Union (*Off. J. Eur. Union* L 275:40, 2011), "means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm–100 nm." Materials can exhibit new or altered physicochemical properties at nanoscale dimensions, enabling the development of novel products *(113)*. These materials are, therefore, categorized separately from other materials, thus requiring distinct characterization and appropriate safety evaluation.

The application of nanomaterials by the food industry has gathered much interest due to the potential in improving food production efficiency, product shelf life, and sensory properties *(63)*. The most prevalent and consumed nano-

* Author for correspondence. Tel: +356-99648817; E-mail: vasilis.valdramidis@um.edu.mt.

materials used within food products are titanium dioxide (TiO_2) and iron (III) oxide (Fe_2O_3), acting as food colorants. These colorants contain varying percentages of nano-sized fractions that form part of these additives *(54, 119)*. The inclusion of other nanomaterials within food products is hindered, as more in-depth risk assessments are required to support safe application and regulatory or policy reforms. The risk assessment process requires that nanomaterials be evaluated and monitored throughout their life cycle, primarily in development, application, and disposal *(108),* requiring robust frameworks and platforms to thoroughly assess any associated risks. They also have to consider, evaluate, and expand current regulatory guidelines and policies. Studies conducted in the initial nanomaterial development stage can highlight possible issues for safe applications. Thus, the requirement for studies to have a high degree of assessment and resemblance to the real-life environments these nanomaterials would experience is essential *(35)*. The lack of resemblance would impede a precise evaluation; therefore, potential adverse effects might not be determined. Studies must be developed, evaluated, and adapted accordingly to accurately assess potential risks with the highest degree of real-life resemblance. Assessment strategies would also require the reevaluation of currently used approaches and the development of novel and/or inclusion of other methodologies.

This review presents an overview of the kinds of NPs being used in food products, how these are classified, and how current regulations affect categorization and evaluation. The potential effects that NPs might have within the digestive system and studies that have assessed the accumulations of NPs within humans are also discussed. The need for thorough human health risk assessment strategies for NPs within foods is highlighted. Hazard identification and characterization, as well as exposure assessments, and how these are used in risk characterizations are considered. The studies and presented strategies will assist in future assessments for the safe application of NPs in food products.

NPs USED IN FOODS AND THEIR CLASSIFICATION

Foods containing NPs can be classified as either natural or engineered *(66)*. Engineered NPs can be further categorized into three distinctive classes: organic, inorganic, and composite or hybrid NPs *(97)*. The organic (lipid, protein, and carbohydrate) NPs were observed to be more quickly metabolized in the human body compared with the inorganic ones (silver [Ag], Fe_2O_3, TiO_2, silicon dioxide [SiO_2], and zinc oxide [ZnO]) *(78)*. This allows for organic NPs to be used extensively in nanoformulations to deliver drugs and nutraceuticals in humans *(55, 67, 102)*.

Nanoformulations consist of suspensions of organic NPs that are partially encased by an encapsulant *(56)* and are divided into three broad categories on the basis of the encapsulant material: lipid and surfactant-based nanocarriers, polysaccharide-based nanocarriers, and protein-based nanocarriers *(84, 94),* with each type of encapsulant imparting different properties, such as aqueous solubility, bioavailability, and absorption *(102)*. In addition to this, they provide protection from degradation and oxidation for sensitive compounds *(10, 68)* and, in some cases, allow release at target organs *(55, 67, 102)*.

NPs can be further classified into biological, physical, and chemical NPs, depending on the synthesis mechanism *(100)*. Furthermore, according to their degradation nature, NPs can be grouped into biodegradable and nonbiodegradable NPs *(8)*. The most popular and widely used inorganic NPs in food manufacturing are documented in Table 1.

Clay (SiO_4^{4-}), cellulose-based, carbon nanotubes, SiO_2, starch nanocrystals, and chitin or chitosan NPs act as reinforcements to the biodegradable NPs *(106)*. The most popular and widely used organic NPs are documented in Table 2. Polymers most widely used in nanocomposites include gelatin, polylactic acid (PLA), isotactic polypropylene, and low-density polyethylene. Polylactic acid needs an associative compound such as polyethylene glycol to be successful in delivering active components.

EUROPEAN REGULATIONS FOR NP FOOD APPLICATIONS

Several reports and regulations provide detailed information on nanotechnology and its use in food production (Table 3). The term "nanotechnology" first appeared in legislation Regulation (EC) No 1333/2008 of the European Commission (EC) on p. 17 and 23 *(40)*. According to this legislation (p. 17), "a food additive already approved under this regulation but which is prepared by production methods, or using starting materials significantly different (including nanotechnology) from those included in the risk assessment of the authority, or different from those covered by the specifications laid down, should be submitted for evaluation by the authority." Therefore, the uniqueness of the nanoscale state was acknowledged, and according to EC Regulation No 450/2009 (*41*, p. 4), "new technologies that engineer substances in particle size that exhibit chemical and physical properties that significantly differ from those of a larger scale, such as NPs, should be assessed on a case-by-case basis for risk until more information is known about this new technology."

The term "engineered nanomaterial" was further defined in Regulation EU No 1169/2011 of the EU (*43*, p. 26), "as any intentionally produced material that has one or more dimensions of the order of 100 nm or less, or that is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic of the nanoscale. Properties that are characteristic of the nanoscale include (i) those related to the large specific surface area of the materials considered; and/or (ii) specific physicochemical properties that are different from those of the nonnano form of the same material" *(43)*. In addition, according to the U.S. Food and Drug Administration (FDA) (*112*, p. 5), the food industry was asked "whether a material or end product is engineered to have at least one external dimension, or an internal or surface

TABLE 1. *Most popular and widely used inorganic NPs in food industries, use, and classification*[a]

NP(s)	Purpose	Solubility	State	Synthesis	Degradability
TiO_2	Color, lightness, and brightness additives (light-scattering properties), binders for composites	Insoluble	Stable	Chemically	Nonbiodegradable
SiO_2	Color additives, flavors, packaging, anticaking agents in powdered foods	Insoluble	Stable	Chemically	Nonbiodegradable
SiO_2–gallic acid	Antioxidants: scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl radicals	Insoluble	Stable	Chemically	Nonbiodegradable
Silicate (SiO_4^{4-})	Nanosensors	Insoluble	Stable	Chemically	Nonbiodegradable
Clay (SiO_4^{4-})	Bottle industry: lighter and stronger than glass and also less likely to shatter, increases shelf life, prevents spoilage, prevents O_2 absorption	Insoluble	Stable	Natural, yet usually not biological	Nonbiodegradable
ZnO	UV light absorbers, active packaging, an additive in supplements, antimicrobial, antifungal	Insoluble	Stable, can release ionic Zn	Chemically	Nonbiodegradable
Magnesium oxide (MgO)	Active packaging, antifungal	Insoluble	Stable	Chemically	Nonbiodegradable
Copper (II) oxide (CuO)	Active packaging	Insoluble	Stable	Chemically	Nonbiodegradable
Cu	Active packaging	Insoluble	Both stable and ionic	Chemically	Nonbiodegradable
Fe_2O_3		Insoluble	Stable	Chemically	Nonbiodegradable
Zn	Antioxidants increase shelf life, packaging, food supplement, colorant	Insoluble	Stable and ionic	Chemically	Nonbiodegradable
Ag	Disinfectant, antibacterial, antifungal, packaging, chopping boards, storage containers, refrigerators, and health supplements	Insoluble	Both stable and ionic	Chemically	Nonbiodegradable
Gold (Au), platinum (Pt), palladium (Pd), iridium (Ir)	Packaging, metal-based nanosensors, food supplement	Insoluble	Stable	Chemically	Nonbiodegradable
Graphene or graphite oxide (compound of carbon, oxygen, and hydrogen in variable ratios)	Packaging	Insoluble	Stable	Chemically	Biodegradable/ nonbiodegradable
C nanotubes	Nanosensors, active packaging, antibacterial or antifungal, absorb undesirable flavors, used in low-resistance conductors and catalytic reaction vessels, gelation, and viscosifying agent	Insoluble (fullerene)	Stable but can be used to form multiwalled ionic nanocomposites	Physically (vacuum or with process gases)	Nonbiodegradable (stable)
Ag-TiO_2–SiO_2, Ag-N–TiO2, or Au-TiO_2	Packaging	Insoluble	Stable	Chemically	Nonbiodegradable
Nanofilters (many)[b]	Filters microorganisms (even viruses)	Insoluble	Stable	Chemically	Biodegradable/ nonbiodegradable
Nanoceramic particles	Used for clustering of dirt molecules from a liquid media	Insoluble	Stable	Chemically	Nonbiodegradable

[a] Sources: references *6, 49, 52, 59, 95*.
[b] Can be organic.

TABLE 2. *Most popular and widely used organic NPs in food industries, use, and classification*[a]

NP(s)	Purpose	Solubility	State	Synthesis	Degradability
Carbohydrate (digestible or indigestible polysaccharides, such as starch, cellulose, xanthan, carrageenan, alginate, and pectin), proteins, and lipids (combination)	Nanolaminates (extremely thin food-grade film): flavors, colors, antimicrobials, antibrowning agents, antioxidants, enzymes	Lipid soluble	Stable	Biologically	Biodegradable
Lipid and liposomes	Oral delivery systems, liposomes are capable of carrying both water-soluble and oil- or fat-soluble compounds within a single particle	Lipid soluble	Stable	Biologically	Biodegradable
Protein	Catalysis, materials synthesis, drug and gene delivery, and bioimaging	Soluble-insoluble	Stable	Biologically	Biodegradable
Nanofibers (globular proteins)	A platform for bacterial cultures; structural matrix for artificial foods and packaging; thermal stability, increased shelf life; formation of transparent gel network for use as a thickening agent	Usually water soluble	Stable	Physically or chemically: electrospinning	Biodegradable or nonbiodegradable
Nanoemulsions (oil in water calcium) or dispersions, emulsions (calcium carbonate [$CaCO_3$])	Stabilization of biologically active ingredients, delivery of active compounds, extended shelf life, flavor release, low-fat products; increased solubility of calcium carbonate, can be used at higher addition levels	Lipid soluble	Stable	Chemically	Biodegradable (organic $CaCO_3$) or nonbiodegradable (inorganic $CaCO_3$)

[a] Sources: references *6, 49, 52, 59, 95*.

structure, in the nanoscale range (approximately 1 nm to 100 nm) and whether a material or end product is engineered to exhibit properties or phenomena, including physical or chemical properties or biological effects, that are attributable to its dimension(s), even if these dimensions fall outside the nanoscale range, up to one micrometre (1,000 nm)." The agency applies these considerations broadly to all FDA-regulated products, including food substances *(112)*. The FDA also continues postmarket monitoring; however, the industry remains responsible for ensuring that its products meet all applicable legal requirements, including safety standards *(113)*.

The provisions for engineered NPs stated in Regulation EU No 10/2011 (*42*, p. 4) stated that these materials can have "chemical and physical properties that are significantly different from those at the macroscopic scale. These

TABLE 3. *Current European regulations around NPs in food products*

Regulation	About	Reference
(EC) No 258/97	Concerning novel foods and novel food ingredients	*36*
(EC) No 178/2002	Laying down the general principles and requirements of food law	*37*
(EC) No 1935/2004	On materials and articles intended to come into contact with food	*38*
(EC) No 1333/2008	Harmonizes the use of food additives in food products	*40*
(EC) No 282/2008	On recycled plastic materials and articles intended to come into contact with foods	*39*
(EC) No 450/2009	On active and intelligent materials and articles intended to come into contact with food	*41*
(EU) No 10/2011	Plastic materials and articles intended to come into contact with food	*42*
(EU) No 1169/2011	On the provision of food information to consumers	*43*
(EU) 2015/2283	On novel foods	*44*

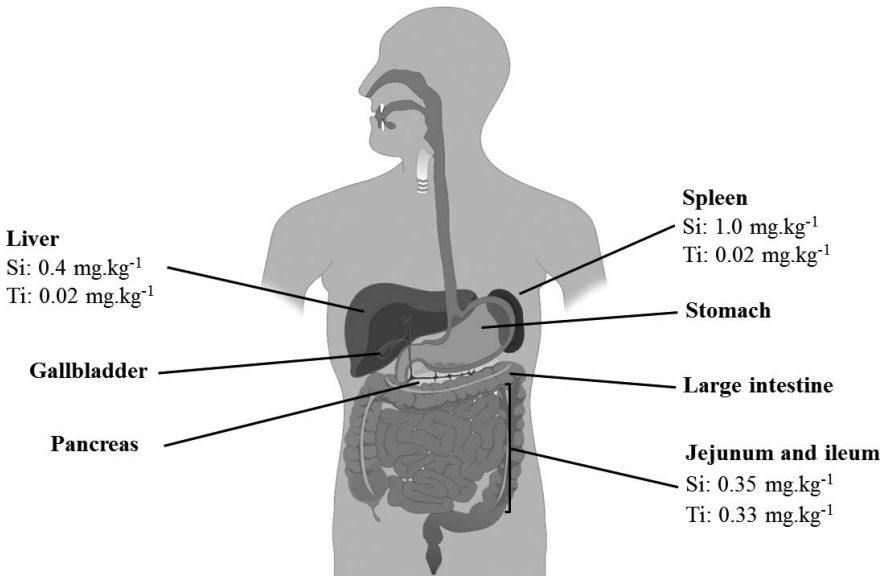

FIGURE 1. *A diagram illustrating organs relevant to the digestive system and the determined quantities of Si and Ti particulate matter found in the human liver, spleen, jejunum, and ileum organs are indicated, as reported by Peters et al. (89).*

different properties may lead to different toxicological properties. These substances, therefore, should be assessed on a case-by-case basis by the authority regarding their risk until more information is known about this new technology." It is made clear that authorizations on the basis of the risk assessment of the conventional particle size of a substance does not cover that of engineered NPs. Also, NPs should not be covered by the functional barrier concept applied for food contact materials to prevent the migration of substances into foods *(42)*.

The most recent legislation Regulation EU 2015/2283 (*44*, p. 2) states that "substances that give rise to significant changes in the composition or structure of a food, affecting its nutritional value, metabolism, or presence of undesirable substances" should also be considered as novel foods. It further states that "limited information is available regarding nanotoxicokinetics, the toxicology of engineered nanomaterials, and existing toxicity testing methods may need methodological modifications" (*44*, p. 5). In this regard, the Organisation for Economic Co-operation and Development concluded that approaches for the testing and assessment of traditional chemicals are, in general, appropriate for assessing the safety of nanomaterials but may have to be adapted to the specificities of nanomaterials *(44)*.

Other regulations that are relevant for the use of NPs in foods are Regulations EC No 258/97 *(36),* EC No 178/2002 *(37),* EC No 1935/2004 *(38),* and EC No 282/2008 *(39),* which look at novel foods and ingredients and the role of the European Food Safety Authority in implementing food safety laws and materials that come in contact with food, including recycled materials. The European Food Safety Authority has issued scientific opinions and reevaluations regarding food additives that might contain NPs of equivalent chemical composition. Since 2015, the scientific opinions and reevaluations regarding TiO_2 (E171) *(4, 124, 126),* iron oxides and hydroxides (E172) *(1),* silver (E174) *(3),* gold (E175) *(2),* and silicon dioxide (E551) *(125)* food additives suggested the need of further toxicological studies due to the absence of toxicity data and characterization of NPs present in these additives and to provide definitive safety evaluations for these materials. Guidance documents have also been issued to streamline and unify the various aspects to be considered in evaluating these NPs *(62),* further emphasizing the need for more in-depth studies investigating the toxicity effects of ingested NPs.

NPs AND THE DIGESTIVE SYSTEM

The digestive system is responsible for the physiological processes of digestion, absorption, motility, and secretion, including that of NPs ingested through foods. Daily, about 8 L of fluid passes through the gastrointestinal tract in addition to 2 L of solids and liquids ingested by the average adult *(103)*. Almost 90% of this fluid is processed and absorbed before entering the large intestine and is mostly absorbed before the colon. The remaining undigested solids and substances excreted are between 5 and 10% of the daily ingested quantities. Optimal metabolic functionality of the digestive system also depends on it being supplied with sufficient oxygenated and nutrient-rich blood *(103),* which is supplied through the splanchnic circulation through the celiac artery, the superior mesenteric artery, and the inferior mesenteric artery. The various digestive system organs transfer this to the portal vein, which passes through the liver. The portal vein supplies about 80% of blood to the liver, while the remainder is from the celiac artery via the hepatic artery. The hepatic veins send back this blood to the heart via the vena cava, recirculated throughout the body. Therefore, the digestive system plays an important role in the absorption and distribution of nanoformulated foods within the body. Some organs of note that are relevant to the digestive system are shown in Figure 1.

Several studies have focused on the toxicity of organic NPs due to their extensive use in food products and health applications. The studies have confirmed that organic NPs, in general, are nontoxic, and do not accumulate in the body, as they are biodegradable. In a study by Frenzel et al. *(51),* quercetin-loaded, whey protein isolate–coated liposomes were observed to successfully deliver water insoluble core materials, showing good stability and nontoxicity as a food additive. Proteins were also investigated in a study by Yi et

al. *(122)* in which a protein-based β-carotene nanocarrier was developed, showing very low cytotoxicity while yielding an increased cellular uptake of β-carotene.

However, in certain cases, the methods used to prepare NPs affect the resultant cytotoxicity. This was shown by Shin et al. *(101)* when comparing the "ethanol injection" method and "dry thin film" method. The study showed that toxic organic solvents used for the dry thin film method restricted the use in food-grade liposomes. Similarly, in nanoemulsions, the use of large quantities of surfactants may result in increased cytotoxicity *(46, 104)*. However, this has been shown to depend on the surfactant used and the amount, as shown by Sessa et al. *(99)*. They produced a Tween 20 and glycerol monooleate–based nanoemulsion and evaluated them on Caco-2 cells with no cytotoxicity observed. Some studies have been undertaken to investigate the presence and impact of NPs that could have been derived through oral administration in humans. A study by Jones et al. *(70)* showed no significant absorption of Ti after the oral administration of TiO_2 NPs by nine human volunteers. The study reported that agglomeration of these NPs of various particles sizes in simulated gastric fluids might have inhibited absorption during in vivo ingestion. Postdose Ti levels in urine and blood were not significantly different from predose levels. Another study by Rompelberg et al. *(96)* investigated the potential intake of TiO_2 NPs through several dietary and oral sources across the age groups of 2 to 6, 7 to 69, and 70+ years in the Dutch population. Chewing gum, coffee creamer, milk, and sauces were the main dietary contributors for the intake of TiO_2 NPs, while for the age group of 2 to 6 years, old toothpaste was the main contributor. The source of these particles was through indirect environmental sources or feed to milk transfer.

A recent study by Peters et al. *(89)* reported the amounts of SiO_2 and TiO_2 NPs in several postmortem human organs. These particles were confirmed by using scanning electron microscopy with energy dispersive x-ray detection and inductively coupled plasma mass spectrometry. Organs assessed were the liver, spleen, kidney, jejunum, and ileum. Particulate quantities determined in the liver, spleen, jejunum, and ileum are shown in Figure 1. It was reported that SiO_2 particulates accounted for 10% of total Si content. These ranged between 0.2 to 25 mg of Si (in particulate form) per kg of tissue, with particle sizes between 250 to 400 nm. Particles of TiO_2 accounted for about 80% of the total Ti content within these tissues. These ranged between 0.01 to 1.8 mg of Ti (in particulate form) per kg of tissue, with particle sizes between 50 and 500 nm. These results reported for the liver and spleen were comparable with those reported by Heringa et al. *(65)*. The authors of both publications indicate that the main source of these particles and the accumulation within the liver and spleen was most likely through food, toothpaste, or medicines.

GUT CYTOTOXICITY OF NPs

The cytotoxicity of NPs within the gut system encompasses both toxicities of the cells of the target animal and microbiota response populating these surfaces. This is of particular importance when considering the accumulation of nonabsorbed foodborne NPs, as they can directly interact with the microorganisms present apart from the animal cells.

In a study by Taylor et al. *(109),* cerium (IV) oxide (CeO_2), TiO_2, and ZnO NPs were compared, and the effect on the gut microbial community was investigated. This revealed significant differences in several phenotypic traits compared with an untreated community, including hydrophobicity and electrophoretic mobility. It was observed that the community's stability when treated with TiO_2 NPs caused extended changes in hydrophobicity. Similar studies *(16, 47)* showed that the changes were deemed nonlethal yet significant enough to affect the properties of the organisms microbial community.

The inorganic Ag NPs stand out as known antimicrobial agents and have been the focus of several studies. In a study by Fröhlich and Fröhlich *(53),* the cytotoxicity of Ag NPs and ZnO NPs on enterocytes and bacteria was investigated and revealed Ag NPs to be antimicrobial agents that selectively damage *Escherichia coli,* while ZnO NPs specifically damaged enterocytes more at lower concentrations than *E. coli*. Cueva et al. *(22)* managed to create a model system simulating the gastrointestinal digestion of food in which Ag NPs were observed to undergo several modifications as they passed through simulated gastric fluids. It was observed that the composition and activity of the intestinal microbiota were not noticeably affected. A similar study *(13)* focused on Ag NPs and their interaction with intestinal microbiota by using in vitro batch fermentation models inoculated with human fecal matter. The core bacterial community was shown to be unaffected; however, a nonlethal concentration of Ag NPs was shown to negatively affect the bacteria: *Faecalibacterium prausnitzii* and *Clostridium coccoides–Eubacterium rectales* taxa in the fermentation cultures.

NPs AND HUMAN HEALTH RISK ASSESSMENT

Risk assessment typically consists of hazard identification and characterization, exposure assessment, and risk characterization *(49)*. A pollutant or substance may be extraordinarily hazardous but have a small human exposure potential; therefore, the resultant risk may be small. However, a hazard may have limited toxicity, but the human exposure is high, and over long periods, the pollutant may pose a much greater risk. Hence, it is essential to characterize both the potential impact of the hazard and the likelihood or amount of human exposure for a risk assessment study *(49)*. Uncertainties exist around the risk assessment and exposure assessment of NPs due to limited information on several aspects, including toxicity, behavior, and bioaccumulation *(23)*. It is, therefore, important when assessing and considering the potential toxicity of these NPs; dosage, bioaccumulation, and metabolic rates have to be synergistically regarded.

The toxicity of NPs is often associated with chemical reactivity; for example, some inorganic NPs dissolve and release ions that promote undesirable chemical or biochemical reactions (e.g., Ag NPs), whereas others are relatively inert (e.g., TiO_2 NPs) *(78)*. It has been reported that

positively charged NPs were more toxic than negative or neutral NPs *(6)*. Insoluble NPs are more readily taken up across the human body's intestinal barrier and can be more immediately bioavailable. The uptake of NPs is determined mainly by particle solubility, charge, and size. Smaller diameter NPs are more likely to be absorbed *(49)*. Note that on the one hand, inorganic NPs have different tendencies to dissolve under specific solution conditions (pH and ionic strength) and chemical reactivities, significantly impacting gastrointestinal fate and toxicity *(6)*.

Hazard identification. The physical and chemical behavior of NPs within the human body is not fully understood and requires further hazard investigation *(75)*. However, the primary pathways of human exposure were identified as inhalation (including intratracheal), ingestion (gavage or food), and dermal contact *(75)*. The potential health consequences of ingestion of NPs may cause Crohn's disease and colon cancer *(6)*. Hemorheology suggests that once the NPs enter the bloodstream, blood circulation cells and other components, such as serum proteins and coagulation factors, are exposed to NPs and may result in cardiovascular disease *(30)*. Once within the bloodstream, it was determined that for medium- and high-dose groups (300 and 1,000 mg kg^{-1}), Ag NPs accumulated in the following descending order, stomach > liver > kidneys > lungs > testis > brain > blood. When administrated with a low dose (30 mg kg^{-1}), the following descending order was determined, stomach > kidneys > testis > liver > brain > lungs > blood *(75)*.

Systematic tools such as NanoRiskCat, a nanomaterial database developed by the Danish Ecological Council and Danish Consumer Council *(27),* and Hansen et al. *(60, 61)* can assist in the determination of NP hazard identification in consumer products. A data filter strategy (NanoRiskCat > search database > filter; categories: food and beverage; potential exposure pathways > oral; CPDAT > food contact) was used to narrow down the list of products in which NPs are used. (The Chemical and Product Database [CPDAT] is a filter search option available on the NanoRiskCat Web site *(27)*.) On the basis of the observation of 34 products, TiO_2 and Ag NPs both appeared on 10 occasions. According to Regulation EC 1333/2008 *(40),* TiO_2 is authorized as a food additive (E171) in the EU in quantum satis in 51 food categories. TiO_2 is mostly used in chocolate products, and Ag NPs are used in packaging and surface protection due to antimicrobial properties, as reported in the literature. Overall observation suggests that humans can be exposed to NPs through food directly, and indirect exposure includes cooking utensils, mostly the coating on the frying pan to make it nonstick and packaging products. With a few exceptions, TiO_2 was categorized as high risk in both components of risk assessment, such as exposure (professional, consumers, environment) and effects (human, environment). In contrast, the exposure to Ag NPs was classified as medium, whereas the effect was labeled as high.

TiO_2 is a white powder, mainly used in products including chewing gum, ice cream, and confectionery products, such as candies, chocolate products, cakes, pastries, biscuits, sauces, dressings, spreads, cheese, or even fish products, to give a white background color *(7)*. Possible health effects are related to the use of E171 as a food additive, which highlighted the importance of examining immune toxicological effects in addition to potential reprotoxicological effects *(33)*. The FDA has limited the total amount of E171 to 1% TiO_2 per weight of food *(114)*. In addition, the French Agency for Food, Environmental and Occupational Health and Safety suspended the use of E171 (food-grade TiO_2) in France *(50)*. More recently the European Food Safety Authority *(33)* has updated its safety assessment of the food additive TiO_2 (E171), following a request by the EC in March 2020.

Exposure assessment. The sustainable development of nanotechnology requires a thorough knowledge of the life cycle of synthesized NPs, including environmental release, deposition, exposure, and potential health risks *(123)*. The life cycle of NPs can be assessed as fabrication stage > stabilization stage > effect of microenvironment during application > degradation > metabolism > excretion *(32)*. Human exposure to NPs through the digestive system is the main focus of this section. Representative studies are presented in Table 4. Very few studies looked at the entire probabilistic approach of the quantitative human exposure model. Also, the experiment-based probabilistic models are mostly conducted on Ag, Cu, and TiO_2 NPs only. Ag and Cu NPs assessments are based on migration studies, whereas TiO_2 assessments are based on the inherent concentration of engineered NPs in food and cosmetics products. Thus, a more unified risk assessment model is required in this field.

In addition, a sensitivity analysis *(24)* can help a probabilistic model investigate the influential parameters of migration studies such as percent fill, storage duration, and temperature. Similar research can be conducted for other NPs. Peters et al. *(90)* raised the need to extend the exposure assessment nodes, as they indicated that the fate of TiO_2 particles in the human digestive tract is unknown. Therefore, the net and cumulative retention data after the metabolism and excretion process can improve the understanding of the fate of NPs inside the human body.

Hazard characterization. NPs may cause significant reactive oxygen stress to the living cells, resulting in cytotoxicity *(127)*. Cytotoxicity is a vast domain, and it can be categorized into specific hazard end points, such as hepatotoxicity, nephrotoxicity, pulmonary toxicity, gastrointestinal toxicity, cardiotoxicity neurotoxicity, reprotoxicity, and embryotoxicity *(75)*. Therefore, there is a need for a standard parameter to compare or rank different toxicities. Human equivalent dose can be such a parameter; it can be derived from animal studies, and it is a function of animal dose, animal correction factor, and human correction factor *(75)*. On the basis of the duration of exposure, toxicity can be categorized as acute (single dose), subacute (14 to 28 days), subchronic (90 days), and chronic (180 days, rodent; 270 days, nonrodent) *(29)*. Li and Cummins *(75)* collated acute, subacute, and subchronic toxicity data on Ag NPs on

TABLE 4. *Representative exposure assessment studies of NPs through the food pathway*

No.	NP(s)	Methodology	Scope	Migration	Level on food	Remarks	Reference
3	Ag	Multifactorial design, probabilistic mathematical human exposure assessment model	Migration of silver from plasticized polyvinyl chloride nanocomposites to chicken meat, following varying storage time and temperature conditions	Yes; percentage fill, time, temperature dependent	Migration was found to occur within a range of 0.03–8.4 mg kg^{-1}	Ag migration from the nanocomposite to the food surface was influenced most by the percentage fill, followed by storage time and storage temperature (negative correlation)	*24*
4	Ag, Cu	Experimental design and parameterization, nanocomposite preparation, migration test, probabilistic human exposure model	Migration from polyethylene nanocomposites to food and an associated exposure assessment	Yes	Migration of Ag 0.003−0.005 mg dm^{-2}, Cu 0.024−0.049 m dm^{-2}; human exposure Ag 5.89×10^{-5} -8.9×10^{-5} mg kg bw^{-1} day^{-1}, Cu $2.26 \times 10^{-5}-1.17 \times 10^{-4}$ mg kg bw^{-1} day^{-1} (95th percentile range)	Still considerable uncertainty regarding the toxicity of materials at the nanoscale; hence, exposure estimates should be reevaluated when more toxicity data become available	*25*
5	Ag	Ag quantification in food simulants after migration tests with laboratory-manufactured composites	Evaluate silver migration from nanosilver and Agion (commercially available filler) polyethylene composites to food simulants by using analytical and imaging techniques	Yes	Mean Ag migration from Agion composites ($n = 12$) <0.001−1.50×10^{-2}, mg L^{-1}; mean Ag migration from Ag NP composites (laboratory) $4.65 \times 10^{-2}-0.38$ mg L^{-1}, and 8.92×10^{-2} and 5.15×10^{-2} mg L^{-1} for Hach-Lange spectrophotometry and inductively coupled plasma atomic emission spectroscopy, respectively	Through careful polymer engineering, composites that conform to European nonauthorized substance migration limits can be developed, while being cognizant of food pH and percentage fill rates	*26*
7	Cu (also ionic form), Ag (also ionic form)	Probabilistic human exposure model looking at NP migration from food packaging (EU 10/2011)	Migration of NPs from packaging to human exposure through food consumption	Time-dependent migration: Ag (0.6%): 0.46, SD 0.19; Cu (0.6%): 0.82, SD 0.08 (mg kg $food^{-1}$)	Ag: 1.41, SD 0.29; Cu: 1.06, SD 0.22 (mg kg $food^{-1}$); partition coefficient Ag: 2.18; Cu: 0.30	Migration levels were found to exceed the EC regulatory limit for unauthorized substances of 0.01 mg kg^{-1}	*59*
9	TiO_2	Electron microscopy, mass spectrometry	7 TiO_2 materials, 24 food products, and 3 personal care products	No	0.02–9.0 mg TiO_2 g^{-1} product, 5–10% of the particles in these products had sizes below 100 nm	The fate of TiO_2 particles in the human digestive tract is unknown	*90*
13	Ag	Experiment-based NP migration study (food and water)	Migration of NPs from packaging to food	Time-dependent migration: 11.9 (SD 2.4), 9.7 (SD 1.6), 23 (SD 5.1), <0.1, <0.1, 37.1 (SD 1.2) μg g^{-1} of polypropylene	Worst case: acute exposure to 4.2 μg of Ag can result from storage of 100 mL of food in a new Ag-doped box of normal size (calculated for 30 ng cm^{-2} migration from a 140-cm^2 surface)	Negligible NP exposure in comparison with the background exposure to Ag for the general population	*117*
15	TiO_2	Mechanistic human health exposure assessment of NPs through selected seafood products (jellyfish, squid, and cuttlefish)	Human exposure to NPs from selected seafood products		6–12 mg g^{-1} (dry weight) in some white-colored seafood products (squid and cuttlefish); relatively low concn were observed in jellyfish 1–3 mg g^{-1}	Consumption of selected food products may be an essential route for TiO_2 NP uptake, especially for younger people aged 20–30 yr	*123*

the basis of animal studies available in the literature. It was also the first study to provide a full picture of the dose-response relationships of potential hazards to humans resulting from Ag NP exposure. Oral exposure was found to be the most critical exposure pathway for most of the toxic end points. Other than acute toxicity on major organs, including the liver, kidney, and lungs, the potential for acute neurotoxicity is also an issue of concern, arising from small long-term doses *(75)*.

For in vitro assessments that were carried out on NPs that can be consumed through the ingestion of foods, these studies are illustrated in Table 5 and mostly focus on TiO_2 NPs. However, interest in Ag and SiO_2 NPs was noted. Most studies report essential physicochemical characteristics, such as size, shape, crystal structure, elemental composition, purity, hydrodynamic diameter, and zeta potential of the assessed NPs. Nevertheless, critical data, such as specific surface area, surface functionalization, and metal ion leaching capacity, are not always reported. Differences between reactive surfaces of NPs with similar chemical composition might induce different responses and have been indicated as an essential parameter in comparing and understanding them *(9, 72)*. Exposure conditions for the studies illustrated in Table 5 mostly investigated concentration ranges between 1 to 300 μg mL^{-1}, with exposures lasting between 2 to 72 h. Note that a considerable number of studies predigested the NPs in liquids to mimic gastric fluids and even assessed them in the presence of other food compounds *(12, 28, 76, 92, 105)*. Studies mostly reported NP uptake, distribution, and cell viability as general end points for the toxicity effects of NPs. Other end points, such as reactive oxygen species, DNA damage, cell cycle, oxidative stress, and gene expression, were reported to understand the observed toxicity interactions further.

Cell lines reported in the various studies illustrated in Table 5 mostly used the human colorectal adenocarcinoma Caco-2 cell line. An increasing interest was noted toward using Caco-2 cells in conjunction with human lymphocyte Raji B cells *(73)* and human mucus-secreting epithelial HT29-MTX cells *(12, 28, 31, 118)* to obtain more complex coculture model systems that resemble more closely in vivo digestive cellular environments. Recently, studies *(64, 91)* have been conducted by using the EpiIntestinal model to assess NP responses with a three-dimensional in vitro model that closely mimics the human in vivo intestine. This three-dimensional cellular model possesses a columnar epithelium with villi structures, brush borders, and tight junctions, which closely imitate in vivo intestinal function. The cellular morphology present within this model allows for spatial interactions and phenotypic crosstalk that is not present in two-dimensional cellular monolayers. Both studies reported that this closer in vivo resemblance makes the reported end points more relevant to the risk assessment of the NPs assessed. The study by Henson et al. *(64)* also assessed NP responses by using rat small intestine epithelial IEC-6 cells and concluded that the different responses observed from using the EpiIntestinal model could have been due to animal to human species differences. These variations have been the subject of debate and concern regarding the use of animal in vivo assessments of novel compounds *(11, 93)*. The minimization for the use of animal models is becoming more relevant and in favor of the implementation of alternative approaches in line with the replacement, reduction, and refinement principle *(69, 71)*.

Several other nonhuman in vivo studies reported the fates of NPs consumed through oral administration. For the last 5 years, research efforts predominantly focused on assessing TiO_2 NPs characterization and toxicity *(5, 14, 15, 17–20, 45, 57, 70, 79, 80, 107)*. There is interest in focusing on TiO_2 because it is a common food additive E171 that has a fraction of about 36% consisting of NPs *(120, 121)*. Recent studies have reported that this could be higher than 60% *(54, 115)*. Several studies that evaluated TiO_2 in vivo are presented in Table 6. Although most studies provide the required basic characterization specifications, such as size, shape, crystal structure, hydrodynamic diameter, and zeta potential, specifications, such as specific surface area and polydispersity index, are not always reported. Assessment of the metal ion leaching capacity of the NPs should be further investigated and reported due to the acidic environments of the digestive system. The release of metal ions within these acidic environments might lead to a different mode of interaction. The further use of aggressive dispersion methods, such as ultrasound sonication, might also cause the release of metal ions. Note that within this selection of studies, only one report was found that investigates the interaction of TiO_2 administered through solid feed *(80)*.

Throughout the studies in Table 6, it was observed that a considerable elevated presence of Ti was found within the liver, stomach, intestines, and colon. Other organs that have accumulated titanium were the spleen, pancreas, and kidneys. The most frequently assessed and reported toxicity end points were biochemical assessments of blood or tissue lysates to assess specific organ functionality within these various studies. It is not always the case that changes in the Ti content of blood or organs postexposure are reported, limiting the comparisons between studies. The inclusion of DNA damage quantification assessments, such as the in vivo mammalian erythrocyte micronucleus *(85)* and alkaline comet assay *(86),* would further add value to the toxicity assessments of these NPs. These should be complemented by oxidative stress and inflammatory cytokine assessments.

Risk characterization. Risk characterization is the final step of the risk assessment paradigm (Fig. 2A). It combines exposure assessment and hazard characterization stages and evaluates the final risk, conducting a series of model analyses such as scenario and sensitivity analysis to capture variability and uncertainty at each node of the model *(48)*. A proposed risk assessment framework is given (Fig. 2B).

Assessment of the daily intake of NPs through food products can also be assessed through the daily dietary index (DDI) on the basis of equation 1 *(58, 111)*.

TABLE 5. *In vitro studies focused on the potential effects of NPs might induce during ingestion*[a]

NP studied	Cells used	Exposure conditions	Assessed end points	Reference
Type: TiO_2 Reported specifications: Size and shape Elemental composition and purity Polydispersity index	Caco-2	Concn used: 50 and 100 μg of TiO_2 per mL Medium: cell culture medium dosed with sonicated particulate suspension Duration: 3 h	NP distribution	*70*
Type: Ag Reported specifications: Size and shape Metal ion leaching potential	Caco-2	Concn used: 20–100 μg of Ag per mL Medium: cell culture medium dosed with undigested and digested particulate suspensions and digested with food components and particulate suspensions Duration: 24 h	Metal content in culture media and cells NP uptake and distribution in cells Cell viability	*76*
Type: TiO_2 Reported specifications: Elemental composition and purity Size and shape Hydrodynamic diameter Zeta potential Metal ion leaching potential	Caco-2	Concn used: 50 and 200 μg of TiO_2 per mL Medium: cell culture medium dosed with digested food components and particulate suspensions Duration: 2, 4, and 24 h	Cell viability Cell proliferation Reactive oxygen species generation NP uptake and distribution in cells	*105*
Type: SiO_2 Reported specifications: Crystal structure Size and shape Hydrodynamic diameter Zeta potential Surface functionalization	Caco-2 Raji B	Concn used: 250 μg of SiO_2 per mL Medium: cell culture medium dosed with particulate suspensions Duration: 6 h	NP distribution Cell viability	*73*
Type: Fe_2O_3 Reported specifications: Crystal structure Size and shape Specific surface area Hydrodynamic diameter Zeta potential	Caco-2 Raji B HT29-MTX	Concn used: 0.05 and 0.1% (w/w) Fe_2O_3 Medium: cell culture medium dosed with digested food components and sonicated particulate suspensions Duration: 2 and 4 h	Metal content in culture media and cells NP uptake and distribution in cells Cell viability Reactive oxygen species	*28*
Type: Ag, Au, CuO, TiO_2, and ZnO Reported specifications: Elemental composition and purity Hydrodynamic diameter Zeta potential Metal ion leaching potential	HT29	Concn used: 2–10 μg of NP per mL Medium: cell culture medium dosed with sonicated particulate suspension Duration: 24 h	Metal content in culture media and cells NP uptake and distribution in cells Cell viability DNA damage Cell cycle	*98*
Type: Iron phosphate ($FePO_4$), SiO_2 Reported specifications: Specific surface area Crystal structure Size and shape Hydrodynamic diameter Zeta potential	HCEC[b] HT29 HT29-MTX	Concn used: 37.5 and 75 μg of NP per mL or 0.01–2.5 mM Fe Medium: cell culture medium dosed with sonicated particulate suspension Duration: 48 h	Cell viability Cellular oxidative stress NP uptake and distribution in cells	*118*
Type: TiO_2 Reported specifications: Crystal structure	MKN-45	Concn used: 10–50 μg of TiO_2 per mL Medium: cell culture medium dosed with sonicated particulate suspension Duration: 24, 48, and 72 h	Cell viability Cell cycle Cell migration	*83*
Type: Ag Reported specifications: Size and shape Hydrodynamic diameter Zeta potential Crystal structure	Caco-2	Concn used: 1–100 μg of Ag NP per mL Medium: cell culture medium dosed with sonicated particulate suspension Duration: 24 h	Cell viability NP uptake and distribution in cells DNA damage Gene expression	*116*

TABLE 5. *Continued*

NP studied	Cells used	Exposure conditions	Assessed end points	Reference
Type: TiO_2 Reported specifications: Size and shape Specific surface area Hydrodynamic diameter Zeta potential Polydispersity index	Caco-2-GFP HT29-MTX	Concn used: 10–250 μg of TiO_2 per mL Medium: cell culture medium dosed with particulate suspension Duration: 6, 48, and 72 h	Cell viability Reactive oxygen species DNA damage Gene expression	*31*
Type: TiO_2 Reported specifications: Size and shape Specific surface area	Caco-2	Concn used: 42 and 84 μg of TiO_2 per mL Medium: cell culture medium dosed with particulate suspension Duration: 4, 24, and 48 h	NP uptake and distribution in cells Metal content in culture media and cells Cell viability Gene expression	*88*
Type: TiO_2 Specifications reported in another article *(74)*: Size and shape Crystal structure Specific surface area Elemental composition and purity Surface functionalization	Caco-2 Raji B HT29-MTX	Concn used: 150 and 300 μg of TiO_2 per mL Medium: cell culture medium dosed with digested food components and sonicated particulate suspensions Duration: 6 or 24 h	Cell viability Reactive oxygen species Proteomic analysis	*12*
Type: TiO_2 Reported specifications: Elemental composition Size and shape Hydrodynamic diameter Zeta potential	Caco-2 HepG2 NL20 A-431	Concn used: 100 μg of TiO_2 per mL Medium: cell culture medium dosed with digested food components and sonicated particulate suspensions Duration: 24 and 72 h	NP distribution Cell viability DNA damage Gene expression	*92*

[a] The table outlines the type of NP studies and reported specifications, type of cells used, exposure conditions, and assessed end points to elucidate the potential in vitro toxicity effects.
[b] HCEC, human corneal epithelial cells.

$$\mathrm{DDI} = A \times B \times \frac{C}{\mathrm{BW}} \quad (1)$$

where A is the NP content in food products (mg $kg_{dry\ weight}^{-1}$), B is the daily intake of food products ($kg_{wet\ weight}$ day^{-1}), C is the conversion factor (0.085 is to convert fresh vegetable weight to dry weight), and BW is the average human body mass (kg). The DDI can be compared with the reference dose (RfD), which is the oral reference dose, specific to the study (mg kg^{-1} day^{-1}). Further, the health risk index (HRI) can be calculated by equation 2 *(58)*. If the value of HRI is <1, the exposed population is said to be safe.

$$\mathrm{HRI} = \frac{\mathrm{DDI}}{\mathrm{RfD}} \quad (2)$$

If the model's inputs are variable instead of fixed value, a probabilistic model may be used *(82)*. A predictive model's most sensitive parameter can be identified in the risk characterization stage by analyzing Spearman's rank order correlation coefficient in the sensitivity analysis applications *(21)*. This approach can be useful to understand the effects of natural variability and uncertainty of the model input parameters on the model outputs.

PROPOSED RISK ASSESSMENT STRATEGIES AND CONCEPTUAL MODELS

1. Future toxicity studies should integrate the effects of different properties of NPs in determining cytotoxicity. Such properties include aggregation or agglomeration state, elemental composition, mass concentration, particle number concentration, shape (aspect ratio), size and size distribution, (water) solubility and dispersibility, speciation, structure, surface area (and porosity), crystallite phase crystallite size, surface charge, surface chemistry *(32, 110)*.
2. For all in vivo applications, biodegradable and biocompatible nanomaterials should be used with defined metabolism and excretion.
3. Nondegradable and nonbiocompatible NPs can be used for in vitro applications, such as diagnostics and industrial use. However, the potential for environmental contamination and hazards should be clearly defined. The use of such NPs in food manufacturing has limitations.
4. Nanomaterials for agricultural use must be assessed carefully due to the possibility of entering the food chain and affecting the environmental ecosystem.

TABLE 6. *In vivo studies conducted for TiO_2 NPs outlining specifications, in vivo animal model used, mode of oral administration, reported organs of concern, and assessed end points to elucidate the potential NP in vivo toxicity effects*

Reported TiO_2 NP parameters	In vivo animal model	Mode of oral administration	Reported organs of concern	Assessed end points	Reference
Size and shape Crystal structure Elemental composition and purity Size and shape Hydrodynamic diameter Specific surface area Surface functionalization Zeta potential	3-wk-old Sprague Dawley rats	Mode: Oral gavage Medium: sonicated ultrapure with occasional glucose supplementation	Liver, kidney, heart, spleen, ovaries, and testis	Whole blood cell count Biochemical organ function assessments Histopathological analysis	*18*
Size and shape Hydrodynamic diameter Crystal structure Zeta potential Metal ion leaching potential	6-wk-old CD-1 mice	Mode: oral injection via syringe Medium: stirred unspecified liquid suspension	Liver, kidney and spleen, heart, stomach, and pancreas	The titanium content in blood and organ tissues Plasma glucose and insulin quantification Inflammatory cytokine quantification Oxidative stress quantification Insulin resistance assessments Histopathological analysis	*57*
Size and shape Crystal structure Hydrodynamic diameter Zeta potential	10- to 12-wk-old Swiss Webster mice	Mode: Oral gavage Medium: sonicated deionized distilled water	Stomach	The titanium content in organ tissues DNA damage quantification Oxidative stress quantification Histopathological analysis	*79*
Size and shape Crystal structure Hydrodynamic diameter Zeta potential	7-wk-old CD-1 mice	Mode: oral gavage Medium: unspecified liquid suspension	Liver, spleen, lung, kidney, stomach, duodenum, ileum, jejunum, and colon	The titanium content in blood and organ tissues Whole blood cell count Disease activity index and colon length Intestinal permeability Gut microbiota Histopathological analysis	*15*
Size Shape, chemical purity, and form[a]	Swiss albino mice	Mode: oral administration Medium: dissolved in physiological saline	Liver	Biochemical organ function assessments Oxidative stress quantification DNA damage quantification Histopathological analysis	*5*
Size and shape	7- to 8-wk-old Swiss albino mice	Mode: oral administration Medium: water suspension	Lungs, heart, liver, and kidneys	Biochemical organ function assessments Whole blood cell count DNA damage quantification Histopathological analysis	*14*
Size and shape Elemental composition and purity Crystal structure Specific surface area Hydrodynamic diameter Zeta potential	3-wk-old Sprague Dawley rats	Mode: oral gavage Medium: sonicated ultrapure water suspension	Liver	Biochemical organ function assessments Inflammatory cytokine quantification Oxidative stress quantification Gut microbiota Metabonomic analysis Histopathological analysis	*19*

TABLE 6. *Continued*

Reported TiO_2 NP parameters	In vivo animal model	Mode of oral administration	Reported organs of concern	Assessed end points	Reference
Size and shape Elemental composition and purity Crystal structure Specific surface area Hydrodynamic diameter Zeta potential	3-wk-old Sprague Dawley rats	Mode: oral gavage Medium: sonicated ultrapure water suspension	Liver	The titanium content in organ tissues Biochemical organ function assessments Whole blood cell count Inflammatory cytokine quantification Oxidative stress quantification Histopathological analysis	*20*
Size and shape Crystal structure Specific surface area Hydrodynamic diameter Zeta potential Polydispersity index	6- to 8-wk-old Swiss mice	Mode: intraperitoneally Medium: sonicated distilled water suspension	Liver and kidney	DNA damage quantification Oxidative stress quantification	*45*
Size and shape Elemental composition and purity Crystal structure Polydispersity index	C57BL/6J mice	Mode: ad libitum Medium: added in the solid feed mix	Mesenteric lymph nodes and colon	Immune cell counts Colon length Gut microbiota	*80*
Size and shape Elemental composition and purity Crystal structure Hydrodynamic diameter Zeta potential Polydispersity index	8-wk-old NFR mice	Mode: oral administration Medium: water suspension	Lung, liver, stomach, spleen, kidney, brain, testes, and whole intestine	The titanium content in blood and organ tissues Oxidative stress quantification Inflammatory cytokine quantification Histopathological analysis	*107*
Size and shape Elemental composition and purity Crystal structure Specific surface area Hydrodynamic diameter Zeta potential	3-wk-old Sprague Dawley rats	Mode: oral gavage Medium: sonicated distilled water suspension	No organs harvested	Biochemical organ function assessments Metabolomics analysis Oxidative stress quantification	*17*
Size and shape Hydrodynamic diameter Zeta potential Polydispersity index	C57BL/6JRj mice	Mode: oral gavage and oropharyngeal aspiration Medium: liquid dispersion	Lung, liver, spleen, and kidney	The titanium content in blood and organ tissues Whole blood cell count Biochemical organ function assessments Oxidative stress quantification DNA damage quantification	*81*

[a] Mentioned but not specified.

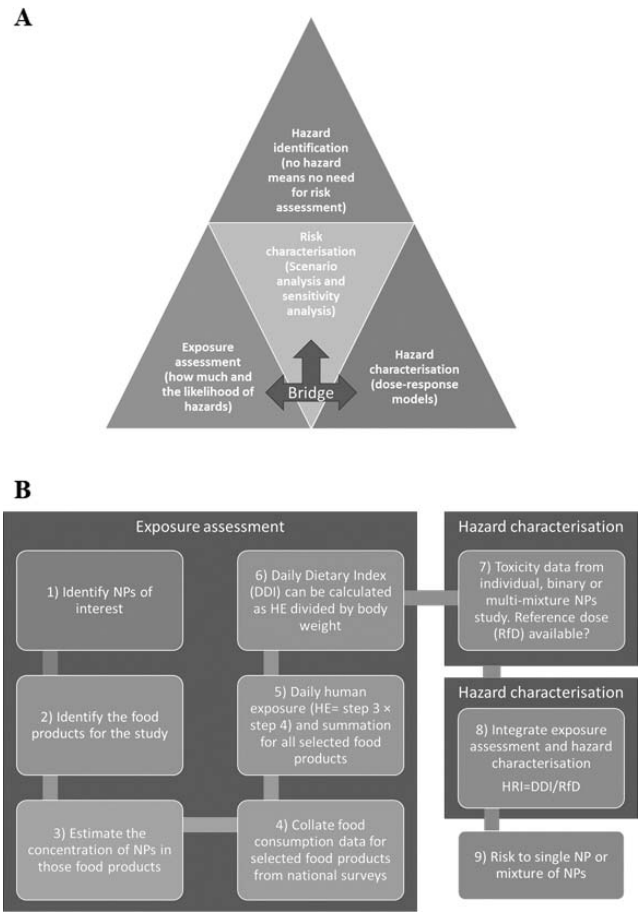

FIGURE 2. *A framework for risk assessment of NPs through the food pathway, showing the four-tier risk assessment approach (A) and the proposed risk assessment framework for NPs (B).*

5. Coating NPs (e.g., with hydrophilic polymer polyethylene glycol) can diminish NP toxic effects *(6),* and this concept can be used as a remedial strategy for reducing risk.
6. Inclusion of more advanced in vitro and in silico models are required to improve current risk assessment approaches at the hazard identification and characterization stages *(34)*.

CONCLUSIONS AND FUTURE CHALLENGES

In the current situation, overregulation may drive away further developments in nanoscience as vast areas of knowledge have not yet been investigated, while underregulation may potentially expose humans to NPs with unintended adverse health effects. The ionic properties (cations, anions) of NPs positively influence the nature of the toxicity pattern of NPs toward bacteria; however, the confirmation in an actual natural setting, such as in rivers and lakes, needs to be established. Ag and ZnO are the most studied NPs in the laboratory, although TiO_2, single or multiwalled C nanotubes, and fullerenes have the broadest range of applications. There is also uncertainty around the transport, partitioning, degradation, transformation, and mutual interaction (environmental fate) of a multimixture of NPs and the interaction with living organisms. Following the settling of NPs on natural organic matter or sediments, or vice versa, the resultant pH, NP concentration, ionic strength, toxicity nature, dissolution or zeta potential of the suspension changes *(87)*. Not enough data are available to bridge the relationship between exposure assessment and hazard characterization. Therefore, limited studies are available on exposure assessments only. In contrast, hazard characterization, or the toxicity studies, especially for Ag NPs have been extensively performed. Risk characterization is the final step toward completing a risk assessment in which scenario and sensitivity analysis of several NPs will need to be performed before being included in food applications. Developing a more unified risk assessment methodology with further enhanced food-based NP modeling techniques and data sets would help advance knowledge in this area.

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