A Brief Review of the Occurrence, Use, and Safety of Food-Related Nanomaterials

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Abstract: Nanotechnology and nanomaterials have tremendous potential to enhance the food supply through novel applications, including nutrient and bioactive absorption and delivery systems; ingredient functionality; improved colors and flavors; microbial, allergen, and contaminant detection and control; and food packaging properties and performance. To determine the current state of knowledge regarding the safety of these potential uses of nanomaterials, an appraisal of the published literature on the safety of food-related nanomaterials was undertaken. A method of assessment of reliability of toxicology studies was developed to conduct this appraisal. The review of the toxicology literature on oral exposure to food-related nanomaterials found that the number of studies is limited. Exposure to nanomaterials in the human food chain may occur not only through intentional uses in food manufacturing, but also via uses in agricultural production and carry over from use in other industries. Although a number of analytical methods are useful in physicochemical characterization of manufactured nanomaterials, new methods may be needed to more fully detect and characterize nanomaterials incorporated into foods and in other media. There is a need for additional toxicology studies of sufficient quality and duration on different types of nanomaterials to further our understanding of the characteristics of nanomaterials that affect safety of oral exposure resulting from use in various food applications.

Keywords: food, nanomaterial, nanotechnology, nutrition, safety

Overview of Current and Potential Nanomaterials That May Be in Foods

Nanoscale materials in foods can be naturally occurring, as will be described below, or may be intentionally added. Intentionally added nanomaterials are being developed using naturally occurring food components, or maybe engineered using materials that are not typically present in food substances. An additional source of nanomaterials in foods may be the result of unintentional contamination, such as through the migration from food contact substances or through agricultural uses of nanoengineered pesticides. The Woodrow Wilson Project on Emerging Nanotechnologies compiles an international inventory of commercially available consumer products to assess the trends of development of consumer products using nanotechnology[1]. At the time of publishing of this article, there were 97 food and food-related products listed in this inventory as consumer products purported to be produced with or to contain nanotechnology (out of a total of 1015 products in the database). However, this may be rather misleading as the inventory includes products that are not normally considered food (for example, vitamin and dietary supplements, cookware, and food storage containers). Inclusion in the inventory is also based solely on manufacturers' claims. Whether the products were produced using nanotechnology or contain any engineered nanomaterials at all has not been conclusively determined, as some products may include the term "nano" for marketing purposes.

Natural Presence of Nanomaterials in Foods

All foods that have been consumed for centuries are composed of substances representing naturally occurring nanomaterials. Plant and animal products are composed of components and structures that, based on their dimensions, are nanomaterials. For example, a DNA molecule, with a width of 2.5 nm, is a nanomaterial. The major constituents of milk, such as casein micelles, whey proteins, and lactose would also be nanomaterials as their dimensions range from 0.5 to 300 nm (Tuinier and de Kruif 2002). The structure of muscle in meats and fish represents a complex and highly organized nanostructure. As food scientists begin to utilize more advanced imaging technologies to examine the nanostructure of materials, more specific details on nanomaterials in foods will emerge. For example, Zhang and others (2008) explored the nanostructure of pectin in fruits and demonstrated that the nanostructural characteristics of pectins were closely related to fruit firmness. Other factors, such as cooking and processing of foods (including grinding, homogenization), may modify their nanostructure and consequently their function (Dang and others 2006). As such, the term "nanofood" can aptly be applied to all natural foods.

Improvements in the knowledge of the structure and function of naturally occurring nanomaterials in foods can lead to novel applications of these naturally occurring substances to improve existing foods as well as develop novel ones. For example, use of the self-assembling properties of casein and other milk proteins has lead to their potential use as encapsulating agents, viscosifiers, and coatings (Graveland-Bikker and others 2006; Semo and others 2007). Understanding the nanostructure of proteins in meats and fish provides the promise of future alternative means of producing highly desirable foods from nonanimal protein sources (Yang and others 2007).

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[1] http://www.nanotechproject.org/inventories/consumer/ [Accessed 21/04/2010]

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Engineered Nanoingredients

Nanomaterials are being developed for a variety of food-related applications, including improvements in nutrient and bioactive delivery systems, improved texture and flavor encapsulation, improved microbiological control, improved food processing and packaging, and specific, highly sensitive biosensors that can be used to detect pathogens, allergens, contaminants, and degradants that can affect food quality and safety.

In the area of food processing, nanotechnology is enabling design of nanostructured processing aids, such as filters, membranes, and reactors. A short discussion on applications of engineered nanomaterials will be presented below. Nanotechnology can also be used to design new food ingredients, such as solid-liquid particles, multilayered particles, fibers, assembled aggregates and novel structures using lipids, proteins, and other components that are natural food components, and approved food ingredients. Readers are also referred to more specific reviews for additional information (Chen and others 2006; Weiss and others 2006; Chaudhry and others 2008; Bouwmeester and others 2009; Sozer and Kokini 2009; Weiss and others 2009; FAO/WHO 2010).

Nanomaterials engineered as ingredients for addition to food and food-related products

Use of nanomaterials offers the opportunity for improved health-promoting properties of nutrients and delivery of bioactive compounds in foods by, for example, controlled and sustained release of ingredients (e.g., biopolymer-based hydrogels and encapsulation technologies); reduced interaction between ingredients within a food system; improved dispersion and suspension of water insoluble ingredients using liposomes, nanodispersions, and nanoemulsions; improved bioavailability; and improved stability (IFT 2006). As an example, Canham (2007) investigated nanoscale silicon for use in functional foods to improve stability of specific nutrients during processing and storage, and delivery to the gut, and having the additional benefit of releasing a biodegradation substance—orthosilic acid—which is of interest in bone health. Aquanova[2], a company in Germany, uses nanotechnology to produce nanomicelles to improve solubility of bioactives and change water/fat solubility of nutrients, such as Vitamins A, C, D, E, and K, Coenzyme 10, β-carotene, isoflavones, α-lipoic acid, and omega fatty acids.

Use of novel structures for specific uses

Novel structures that are being developed using nanotechnology include nanoemulsions, solid lipid nanoparticles, double-layered nanocapsules, nanofibres and aggregate structures (Weiss and others 2009). Emulsion technology is widely used in the food industry, and many foods are forms of emulsions. In contrast to traditional emulsions with droplets in the micron-size range (that is, diameters (d) of 0.1 to 100 μm), ultra-high pressure homogenizers (such as microfluidizers) are facilitating the production of droplets in the nano-size range (that is, d <100 nm). These "nanoemulsions" differ appreciably from conventional emulsions in their functionality due to the decreased size and increased relative surface area.

The ability to alter the solubility of functional lipids is an attractive application, as the poor water solubility of lipids makes them problematic in food formulations. However, how the application of nanotechnology to nutrients and food compounds will alter their chemical and biological properties is not well known at this time. Research is indicating that in some cases, these nanomodifications may alter various properties of the materials being investigated. Tan and Nakajima (2005) described the preparation of ß-carotene nanodispersions for improved solubility and bioavailability. However, the ß-carotene in the nanodispersions was chemically unstable, and the authors showed that the degradation was dependent upon the mean particle diameter. Thus, a change in size altered the chemical stability, and further research is needed to develop optimal formulations.

McClements (2010) reviewed the design and potential for use of emulsion-based nano-laminated biopolymer coatings produced using electrostatic deposition to produce novel encapsulation and delivery systems to control the bioavailability of bioactive lipids. They also described the use of *in vitro* and *in vivo* experiments necessary to study the efficacy of such coating systems. Findings included the utility of a chitosan coating to physically or chemically protect an encapsulated bioactive component within a food product and achieve release and bioaccessibiilty in the digestive tract after ingestion.

Huang and others (2010) reviewed development of nanoemulsions and polymer micelles-based delivery systems to achieve enhanced water solubility/dispersibility, oral bioavailability, and biological benefits for phytochemicals. The examples they cited included development of several nanoemulsion delivery systems for increasing the bioavailability of curcumin, a polyphenol extracted from the rhizomes of turmeric (*Curcuma longa*), and polymer micelle encapsulation systems for improving the water dispersibility of β-carotene as well as curcumin. The studies led to their speculation that emulsion size and lipid component are important for nanoemulsion-based delivery systems to increase bioavailability of encapsulated bioactives; they also hypothesized that natural components (for example, hydrophobically modified starch and casein micelles) may facilitate cellular uptake of micelles.

Solid lipid nanoparticles are fundamentally composed of solidified nanoemulsions. The oxidative stability of ß-carotene was improved by encapsulation within solid lipid nanoparticles, with stability being dependent upon the type of surfactant and the physical state of the carrier lipid (Helgason and others 2009). As described by Weiss and others (2009), double-layered nanoparticles can be created by coating the particles with polymers to form monomolecular layers using the so-called layer-by-layer (LbL) electrostatic deposition method, which can greatly improve the stability and functional performance of conventional nanoparticles. Recently, the formation of double-layered particles has been expanded to a variety of other particles, including liposomes. Liposomes are spherical particles that are formed from polar lipids (for example, phosphatidylcholine or phosphatidyl-ethanolamine) or mixtures of polar lipids with cholesterol or ergosterol, components that are available in abundance in nature. For example, a variety of polymers, such as chitosan, β-lactoglobulin, fish gelatin, and casein were successfully adsorbed on the surface of liposomes with dramatic improvements in the long-term stability of the particles.

Other nanoscale structures that are currently being developed for future food applications are nanofibres and aggregate structures. Nanofibres are fibers with average diameters below 100 nm that may be used as food packaging materials, ingredients, sensors, and processing aids. A wide variety of aggregate structures is possible by combining the various nanostructures described above. For further details, readers are referred to the review by Weiss and others (2009).

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[2] Aquanova AG, 2008 (http://www.aquanova.de/. [Accessed 21/04/2010].

Nanotechnology and improved antimicrobials

Two approaches to using nanotechnology to develop novel and improved antimicrobials are: (1) enhancing the activity of a compound by nanosizing, and (2) increasing effectiveness of currently used antimicrobials by improved targeting within a food system.

The antimicrobial activity of various nanomaterials is presented in Table 1. Several important foodborne pathogens are effectively killed by nanomaterials, with nanosilver being the material that has received the greatest attention from the food industry for use in food packaging and food storage containers (Magnuson 2009a).

Use of nanoencapsulation for food antimicrobials may increase the effective concentration of the antimicrobial in areas of the food system where the target microorganisms are preferentially located, such as in water-rich phases or at solid-liquid interfaces (Weiss and others 2009).

Food processing, packaging, and storage

Potential applications of engineered nanomaterials in food processing, food packaging, and storage include monitoring of food quality, safety, and biosecurity (for example, via nanosensors); improved food packaging and enhancement of package biodegradability; and improved food processing (Baeumner 2004; Chen and others 2006; Sozer and Kokini 2009). Examples include nanosensors for detection of foodborne pathogens and contaminants; adhesion-specific nanoparticles for selective binding and removal of pathogens and contaminants (nonantibiotic approach to disease prevention); active antimicrobials such as metal oxides; and tracers that could help determine sources of contamination (Scott and Chen 2003). These nanosensors have several advantageous properties, such as high sensitivity and selectivity, near real-time detection, and low cost and portability. Currently, scientists are using nanotechnology to develop rapid and accurate diagnostics and detection methods for pathogens, such as *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes, Campylobacter jejuni*, and *Salmonella*. Latour and others (2004) have been investigating the potential for synthesized adhesion-specific nanoparticles to irreversibly bind to targeted bacteria (such as *Campylobacter jejuni* in poultry) thus inhibiting them from binding to and infecting their host and reducing the infective capability of foodborne enteropathogens in poultry products. The opportunities for advancement and benefits in this area are great, but still require a significant amount of research.

Potential food packaging benefits include: high-performance packaging with enhanced mechanical and barrier properties; antimicrobial packaging infused with antimicrobial nanoparticles (for example, silver nanoparticles); intelligent packaging technologies that could prevent or respond to spoilage (for example, polymer opal films that change color to indicate spoilage) or DNA biochip nanosensors that detect toxins, contaminants, and pathogens; and water and dirt repellent packages. In a study by de Moura and others (2008), the tensile, water vapor, and oxygen permeability properties of edible films were significantly improved through the application of nanoscience. Sorrentino and others (2007) and Arora and Padua (2010) reviewed the benefits and challenges in the use of nanocomposite technology and materials (for example, polymer/clay) to improve physical properties (for example, mechanical strength, thermal stability, gas barrier, physico-chemical, and recyclability) for increased utilization of food packaging biopolymers. Arora and Padua (2010) reported that montmorillonite and kaolinite clays showed good potential and that the novel carbon-based graphene nanoplates are highly promising. These scientists concluded that further work is needed in this area in the development of more compatible filler-polymer systems, better processing technologies, and a systems approach to the design of polymer-plasticizer-filler. Azeredo and others (2010) described use of cellulose nanofibers and glycerol as a plasticizer to improve the mechanical and water vapor barrier properties of edible chitosan films. They reported that a nanocomposite film having 15% cellulose nanofibers and plasticized with 18% glycerol was comparable in strength and stiffness to some synthetic polymers, although having poorer elongation and water vapor barrier properties. In addition, such films have the important advantage of having environmentally friendly characteristics. Each of these technologies demonstrates the many opportunities for nanotechnology to enhance the safety and quality of the food supply.

Table 1–Representative nanomaterial antimicrobials.

Nanomaterial	Target organism	Reference
Silver	*Listeria monocytogenes; Salmonella typhimurium; Escherichia coli* O157:H7	Chen and Schluesener 2008
Chitosan/Silver	*Staphylococcus aureus; L. monocytogenes*	Rhim and others 2006
Nanoclays	*S. aureus; L. monocytogenes; S. typhimurium, E. coli* O157:H7	Hong and Rhim 2008
Fullerenols	Skin pathogens, cosmetics	Aoshima and others 2009
Nanoglass	Enterococci dental pathogens	Waltimo and others 2007

Nanomaterial Contaminants in Foods

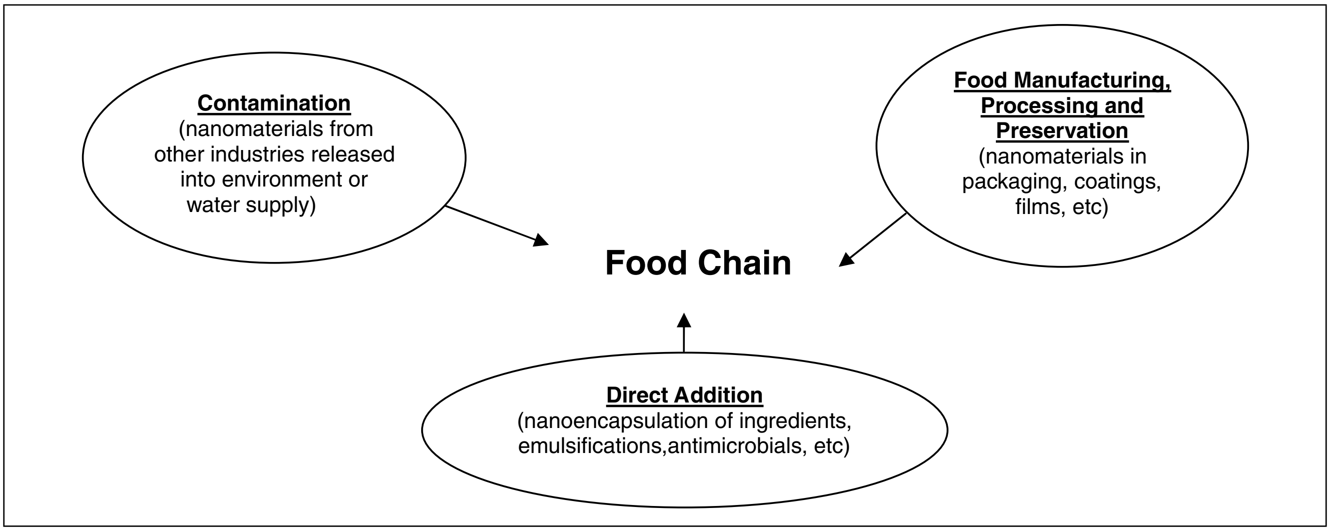
Potential sources of unintentional nanomaterial contaminants in foods include the environment, nanomaterials used during plant and animal production, unintentional release from nanomaterial-containing food packaging materials, and residues in foods from nanomaterials used as food processing aids or surface coatings on food equipment (Magnuson 2009b). For example, nanoemulsion and nanoencapsulation technologies are being explored to improve uptake and efficacy of fertilizers, herbicides, and insecticides, which will alter the levels of these substances in foods (that is, could increase or decrease their presence) (Perez-de-Luque and Rubiales 2009).

Environmental contamination of nanomaterials resulting from use in a number of other industries is also a potential source of nanomaterials in foods, if these materials are present in the environment where food is being produced or in the water being used in food processing. The ability of environmental contamination created by these other industries to potentially affect the food supply has already been illustrated by the presence of many existing industrial contaminants found in foods. A study conducted by Lin and others (2009) demonstrated the uptake, translocation, and transmission of carbon nanoparticles from their growth environment. Rice seeds were grown to seedlings in germination buffer containing natural organic matter-modified fullerenes (C70) or multiwalled carbon nanotubes for 2 wk before seedlings were transplanted to soil (without added nanomaterials) to grow to maturity. Rice seeds were harvested from these 1st-generation plants and grown in germination buffer without addition of nanomaterials to become 2nd-generation plants. Aggregates of C70 were identified frequently in the seeds and roots and occasionally in the stems and leaves of the 1st-generation plants, and surprisingly, also in the leaves of the 2nd-generation plants. Uptake of multiwalled carbon nanotubes was insignificant. The implications of this research are still to be elucidated through additional investigations.

At this time, the types and amounts of nanomaterials in the environment, the potential uptake by plants or animals, or the likelihood of contamination of the food supply due to use of nanomaterials by other industries is poorly understood but an increasingly important area of research as the uses of nanomaterials expand. As the use of nanomaterials is predicted to continue to grow in many industries, many regulatory agencies are working to evaluate the environmental impact of nanomaterials and to establish requirements for environmental assessments of novel nanomaterials prior to approval of commercialization of products. These investigations will be further enhanced through improved detection and monitoring tools, some of which will be further discussed below.

Exposure to Nanomaterials

As research into the field of nanomaterials in the food sector expands, the number of potential applications for nanomaterials in foods and food production will inevitably also increase, and consequently so will the potential human exposure to these substances. In some cases, the exposure is intended, such as through nanoemulsions/encapsulates of nutritional active ingredients or edible coatings. In other cases, exposure may be through unintended routes via pesticides or leaching from specialized packaging. The primary points at which nanomaterials may enter the human food chain can be illustrated as follows:



The potential for human exposure to food-related nanomaterials will depend greatly upon their specific use in the food and food-related industries. For example, incorporation of nanomaterials into multicomposite packaging materials in which the nanomaterial layer is coated with other materials is likely to result in minimal to no transfer to the food and thus present the food consumer with exceedingly low exposure possibilities. In contrast, the use of nanomaterials as carriers of nutrients or bioactive compounds that will be added directly to food products may result in higher levels of exposure that will depend on the concentration in the food and the amount of that food that is consumed. In the latter case, the compositions of the nanomaterials are likely to be modifications of compounds already found in food, such as proteins and lipids, and thus have low hazard potential. Examples of these include nanolaminates that are used in creating coatings or films that are made of polysaccharides, proteins, and lipids, and encapsulating nanoparticles that are predominately made from polylactic acid and polyethylene glycol. These are generally nontoxic and break down in the body to their simple constituent parts (Weiss and others 2006). Similarly, the breakdown and digestion of all food components occurs at the nanoscale (Chaudhry and others 2008); however, whether or not "constituent" nanoparticles may behave differently than bulk materials upon entering the body is a question that still needs to be addressed. (Back and others 2006; Chaudhry and others 2008; Vargas and others 2008; Li and others 2009).

To what degree consumers will be exposed to nanomaterials (outside of direct addition to foods) and through which applicable routes of entry is still being evaluated. There are many data gaps in understanding whether nano-sized pesticides, for example, will accumulate in plant matter and/or progress up the food chain, and in determining how much, if at all, nanoparticles will migrate from food packaging (Chaudhry and others 2008; Bouwmeester and others 2009). In certain cases, it appears that nanoparticles are able to migrate through living plant matter, as was observed when iron-carbon nanoparticles were found to move away from the application site in pumpkins following injection of the particles but not following spray application (Corredor and others 2009). In another study, gold nanoparticles were observed to move from the water column and throughout a laboratory-constructed estuarine mesocosm containing sea water, sediment, sea grass, microbes, biofilms, snails, clams, shrimp, and fish over the course of 12 d (Ferry and others 2009). Though no direct adverse effects were observed in either of these preliminary studies, it should be noted that they were of short duration and very specifically constructed, such that no general conclusions can be drawn to other nanoparticles. With regard to pesticides, the application of nanoscience to improve efficacy may result in a reduced amount of pesticides being required on plants and crops, such that the potential for human exposure is greatly reduced. This possibility remains to be more fully evaluated as research and development of nanoscale pesticides are ongoing efforts and their commercial application is still being evaluated.

Abbott and Maynard (2010) addressed the challenges that the unique characteristics of nanomaterials present for measurement, modeling, and exposure assessment. These authors pointed out that concentration- and mass-based exposure assessments may not adequately measure exposure to functional or structural properties. They also indicated that characterization in foods before and during digestion may require new detection and quantification methods.

Detection and Characterization of Nanomaterials in Foods

The biological properties, including toxicological effects, of nanomaterials are largely related to their physicochemical parameters (Oberdörster and others 2005; Borm and others 2006; Stern and McNeil 2008). Size, shape, and structure (physical and chemical) are key elements of nanomaterials that contribute to their biological effects and their site(s) of deposition and distribution within the body as well as their clearance. Other parameters, such as surface charge and reactivity, may be deliberately modified to provide a desired functionality to a given nanomaterial, which also may influence deposition, distribution, and clearance, as well as inflammatory or other responses of the body to the nanomaterial. Whether a nanomaterial agglomerates in the experimental medium of choice is another important characteristic to consider, as this may alter the size of the material that is "seen" by the test system and also lead to an altered relative surface area for the tested material. As the dose metric (for example, particle number, mass, or relative surface area) may vary among different nanomaterials, it is important to characterize the physicochemical parameters of nanomaterials to the greatest extent possible in order to determine accurate dose-response characteristics of the materials.

The use of nanoscience in foods involves, in part, the nanosizing of regular constituents of food (fats, proteins, and so on) with the result being that it is often difficult to differentiate the nano-sized form from the bulk form of a given material. In addition, the presence of naturally occurring nanomaterials in foods creates a challenge in distinguishing between naturally occurring and engineered nanomaterials (Tiede and others 2008).

Key techniques for detecting nanomaterials in food matrices are:

(1) High-Performance Liquid Chromatography (HPLC),
(2) Ultra-Performance Liquid Chromatography (UPLC),
(3) Field Flow Fractionation (FFF)
(4) Capillary Electrophoresis.

Methods, such as HPLC, specifically size exclusion and ion exchange chromatography (as size and surface charge are key characteristics of nanoparticles), have been used to measure nano-sized materials in various food matrices (Luykx and others 2008). However, a more advanced method is UPLC, which is proving to be more refined and powerful in its resolution and efficiency. The characteristics of these techniques are that they are easily reproducible, highly efficient, and have sensitive selective markers for detection of a variety of compounds. A method similar to HPLC is the FFF method; rather than using a stationary phase as in HPLC, the FFF method uses thermal gradients, or hydraulic, sedimentation and electrical forces to separate various analytes (Luykx and others 2008). The capillary electrophoresis method, while low cost, simple, and rapid, is less reproducible and less sensitive than the chromatographic methods. Additional challenges for detection of engineered nanomaterials in food and food-related products include development of optimal extraction methodologies, artifacts due to sample preparation, interference of measurements by nanomaterials, and lack of reference materials (Tiede and others 2008).

Appraisal of the Literature on Safety of Oral Exposure to Food-Related Nanomaterials

To determine the current state of knowledge regarding the safety of these potential uses of nanomaterials, an appraisal of the published literature on the safety of food-related nanomaterials was undertaken. This work lead to the development of a method to assess the reliability of toxicology studies of nanomaterials, which has been published previously (Card and Magnuson 2010). This method was then applied to the appraisal of published literature on oral exposure to nanomaterials that have potential applications in food or food-related products, such as packaging. The results of that appraisal were recently published by Card and others (2010). A summary of this work is provided below.

Card and others (2010) conducted an extensive search of the scientific literature pertaining to the safety of oral exposure to food-related nanomaterials to assess the state of the toxicity information available in this growing field. Specifics of the literature search strategy are discussed in detail in Card and others (2010). Briefly, 53 nano-related root terms were searched in relation to 58 terms and root words related to food, food packaging, food safety, oral exposure, and *in vitro* studies through 8 databases (search conducted on February 18, 2009, with no limit on publication date). The identified studies were evaluated and ranked using a 2-part method, outlined by Card and Magnuson (2010). The resultant relevant studies were scored using the categorization system for toxicological and ecotoxicological data that was proposed by Klimisch and others (1997) and Schneider and others (2009). Studies that are considered reliable without restriction are assigned a score of "K1," those that are considered reliable with restrictions are assigned a score of "K2," and those that are considered unreliable are assigned a score of "K3". The 2nd step involved the determination of the completeness and reporting of the physicochemical characterization of the nanomaterial(s) that were assessed within the study; this provides a "Nano Study Score," which can range from N0 (worst) to N10 (best) based on the extent of nanomaterial characterization that is conducted and reported. This 2-part scoring method for assessing overall quality of a nanomaterial toxicity study is shown in Figure 1.

The detailed results of the quality evaluation of the identified studies are addressed in Card and others (2010). The number of toxicology studies that have been published using either oral administration or a relevant *in vitro* assay of nanomaterials is small as compared to the number of studies published on toxicity of nanomaterials following dermal, inhalation, or other methods of exposure. When these were limited to nanomaterials that have the potential to be used in food-related applications, a total of 30 studies (21 *in vivo*, 9 *in vitro*) that evaluated a toxicological endpoint were identified.

As shown in Table 2, only 6 of the 21 *in vivo* studies were scored as K1 (reliable without restrictions), whereas 15 were scored as K2 (reliable with restrictions) as the majority of identified publications lacked certain key elements of standard experimental design. Characterization data (for example, information pertaining to agglomeration/aggregation, purity, and surface properties) were notably absent from many studies. The majority of the *in vivo* studies used gavage as the dosing method. Four studies incorporated the nanomaterial into the animal diet (Xu and others 2004; Jia and others 2005; Rohner and others 2007, Chan and others 2009). Twelve of the *in vivo* studies were acute dose studies. Among the repeated dose studies, only 4 studies were longer than 28 d, with the longest exposure period being 90 d. No long-term chronic study was identified. Other *in vivo* studies included assessment of the potential beneficial effect of orally administered nanomaterials on biological endpoints, such as the effect of selenium on levels of detoxifying enzymes, alleviation of inflammatory bowel disease by nanosilver, and prevention of arsenite- and amyloid peptide-induced toxicity by nanoformulations of quercetin and vitamin E, respectively.

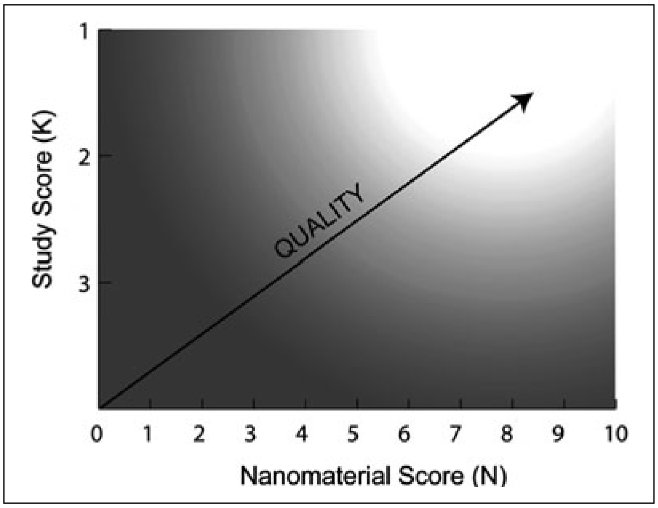


Figure 1–A schematic depiction of the assessment of the overall quality of a nanomaterial toxicity study based on its derived "Nano Study Score." The clear area represents the range of nano study scores for which a study can be considered of high overall quality; conversely, the shaded area represents the region of low overall quality. Used with permission of Sage Publications Inc, from: A method to assess the quality of studies that examine the toxicity of engineered nanomaterials, JW Card and BA Magnuson, *International Journal of Toxicology* 27:408, 2010; permission conveyed through Copyright Clearance Center, Inc.

Table 2–*In vivo* studies discussed in Card and others (2010) designed to assess toxicity of oral exposure to nanomaterials listed according to Nano Study Score.

Nanomaterial(s) studied *in vivo*	Species studied	Oral dosing route	Dosing duration	Nano Study Score
Fullerenes and single-walled carbon nanotubes	Rat	Gavage	Acute	K1-N7
Ferric phosphate	Rat	Dietary	15 d	K1-N6
Red mold rice	Rat	Gavage	28 and 90 d	K1-N4
Selenium	Rat	Dietary	13 wk	K1-N3
Silver	Rat	Gavage	28 d	K1-N3
Fullerenes	Rat	Gavage	Acute	K1-N3
Titanium dioxide	Rat	Gavage	Acute	K2-N8
Polymeric nanoparticles (N-isopropylacrylamide, methylmethacrylate) and acrylic acid (NMA622)	Mouse	Gavage	28 d	K2-N7
Copper	Mouse	Gavage	Acute	K2-N7
Chitosan nanospheres	Rat	Gavage	Acute	K2-N7
Copper	Mouse	Gavage	Acute	K2-N6
Calcium carbonate and calcium citrate	Mouse	Gavage	Acute and 28 d	K2-N5
Zinc	Mouse	Gavage	Acute	K2-N5
Titanium dioxide	Mouse	Gavage	Acute	K2-N5
Selenium	Mouse	Gavage	Acute	K2-N4
Aluminum oxide	Rat	Gavage	Acute	K2-N3
Iron oxide (encapsulated with biodegradable substances)	Mouse	Gavage	Acute	K2-N3
Black soybeans	Mouse	Dietary	12 wk	K2-N2
Fullerenes (polyalkylsulfonated C_{60})	Rat	Gavage	Acute	K2-N2
Selenium	Mouse	Gavage	12 or 15 d	K2-N2
Montmorillonite nanocomposite	Pig	Dietary	83 d	K2-N1

As shown in Table 3, 7 of the 9 *in vitro* studies focused on cytotoxicity, and 2 evaluated genotoxicity. Eight were scored as K1, and 1 was K3 (unreliable based on unacceptable methodology). The high number of K1 studies is somewhat misleading, however, as several limitations that are specific for nanomaterials are not part of the Klimisch scoring system. For example, the potential interference of the nanomaterial itself in the *in vitro* assays was not discussed in these studies. The potential of the nanomaterial to affect cell culture nutrient levels by adsorbing proteins and other nutrients also was not addressed as is discussed by Card and others (2010).

An important finding that must be addressed in future studies is the lack of adequate characterization of the nanomaterials in most studies reviewed. Few of the 30 studies (only 7 of 21 *in vivo* and 5 of 9 *in vitro*) reported more than 5 physicochemical parameters for the nanomaterial(s) being evaluated. Unless nanomaterials are adequately characterized, the results of the toxicology studies cannot be utilized to predict toxicity of other nanomaterials as changes in any of the characteristics may result in changes in biological activity.

It is also noteworthy that adverse biological endpoints and/or toxicity of the nanoformulation of a certain ingredient or material were not necessarily consistently increased as compared to non-nanoformulation, in some cases being the same or actually reduced or even showing beneficial effects.

Due to the limited number of studies and the lack of complete characterization of the nanomaterials studied, it was not possible to derive any overall conclusions regarding the toxicity of nanomaterials for food use and food-related products, or to identify possible structure-function relationships. The same nanomaterial of a different size or surface charge may react very differently than

Table 3–*In vitro* studies discussed in Card and others (2010) designed to assess toxicity of oral exposure to nanomaterials listed according to Nano Study Score.

Nanomaterial(s) studied	Endpoint(s) evaluated	Nano Study Score
Titanium dioxide	Genotoxicity	K1-N8
Gold nanorods with various surface coatings	Cytotoxicity	K1-N7
Quantum dots (cadmium-selenium core)	Cytotoxicity	K1-N6
Polyamidoamine (PAMAM) dendrimers (G2, G2.5, G3, G3.5, and G4) with ethylenediamine cores	Cytotoxicity, integrity, paracellular permeability	K1-N6
Neutral (G2-G4) and anionic (G0.5-G4.5) poly (amidoamine) (PAMAM) dendrimers	Cytotoxicity	K1-N6
Quantum dots (cadmium telluride core with sodium thioglycolate coating)	Cytotoxicity, cell junctions	K1-N5
Native polyamidoamine (PAMAM) dendrimers and arginine (ARG)- and ornithine (ORN)-conjugated PAMAM dendrimers	Cytotoxicity	K1-N5
Poly(amidoamine) dendrimers (G1.5, G2, G3.5, G4)	Cytotoxicity, integrity, paracellular permeability	K1-N5
Nanosilver	Genotoxicity	K3-X

another in a given medium and as such, there is currently insufficient information from which to draw such conclusions. Thus, there is a great need for additional toxicology studies of sufficient quality and duration on different types of nanomaterials with sufficient physicochemical characterization of the nanomaterials.

Conclusions

The use of nanotechnology and naturally occurring or engineered nanomaterials in the food system is a continuously growing area of research with a tremendous potential for valuable advances in the food system, with considerable benefits to human health and the environment. The outcome of the literature assessment conducted and described by Card and others (2010) points to the need for further studies, adequately designed and focused on food-related applications, and human health impacts. Such research will continue to advance as nanomaterial-specific detection and measurement tools improve and well-designed safety studies of nanomaterials with adequate characterization provide additional information. The required resources for this research as a whole are considerable. Global cooperation and strategic planning of research priorities will reduce the time and effort for advances in our understanding to occur.

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