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REVIEW ARTICLE

NANOMATERIALS IN FOOD CONTACT MATERIALS; CONSIDERATIONS FOR RISK ASSESSMENT

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ABSTRACT

Nanotechnology applications in the food industry, including food contact materials, offer many potential benefits for consumers and manufacturers alike. The article discusses the migration of nanoparticles from food contact materials and the possible health risks associated with in the context of insufficient knowledge of the potential exposure to nanomaterial. The importance of gaps in the general knowledge on the behaviour and biological interactions of nanomaterials in biological systems becomes crucial for risk assessment. The article also discussed numerous doubts concerning the measurements of biological reactions in animal tests and the need for new approaches in the interpretation of data from nanoparticles studies *in vivo*. The article underlines the need to develop predictive and validated toxicological tests that can be used to screen for potential hazards, and also to develop new methodology for measuring nanoparticles in biological matrices to assess human exposure. Further studies should focus on understanding the mechanisms of action. Nanoparticles exhibit chemical and physical properties that significantly differ from those substances at a large size. Different properties of nanoparticles may lead to different toxicological properties. From that reason nanoparticles, in each case, are individually assessed by the European Food Standard Agency (EFSA) in terms of health risk before the European Commission authorizes them to be used in food contact materials.

Key words: *nanomaterials, nanotechnology, food contact materials, FCM, risk assessment, toxicity testing, consumer safety*

STRESZCZENIE

Zastosowanie nanotechnologii w przemyśle spożywczym, w tym w materiałach do kontaktu z żywnością, przynosi wiele potencjalnych korzyści konsumentom i producentom. W artykule omówiono zagadnienie migracji nanocząsteczek z materiałów kontaktujących się z żywnością oraz możliwe ryzyko dla zdrowia konsumenta, w kontekście niewystarczającej wiedzy na temat potencjalnego narażenia na nanomateriały. Brak wiedzy na temat zachowania i biologicznych interakcji nanomateriałów w systemach biologicznych stanowi podstawowy problem przy ocenie ryzyka. W artykule omówiono również liczne wątpliwości dotyczące pomiarów odpowiedzi biologicznej w testach na zwierzętach oraz potrzebę nowych podejść w interpretacji danych otrzymanych z badań nanocząsteczek w warunkach *in vivo*. Podkreślono potrzebę opracowania predyktywnych i zatwierdzonych testów toksykologicznych, które można wykorzystać przy badaniu potencjalnych zagrożeń, a także opracowania nowej metodologii oznaczania nanocząsteczek w matrycach biologicznych w celu oceny narażenia ludzi. Dalsze badania powinny koncentrować się na poznaniu mechanizmów ich działania. Właściwości chemiczne i fizyczne nanocząsteczek znacznie różnią się od takich substancji o większych rozmiarach. Różne właściwości nanocząsteczek mogą też powodować różne właściwości toksykologiczne. Z tego powodu substancje w postaci nanocząsteczek, w każdym przypadku, oceniane są indywidualnie przez Europejski Urząd ds. Bezpieczeństwa Żywności (EFSA) pod względem ryzyka dla zdrowia, zanim Komisja Europejska zezwoli na ich stosowanie w materiałach do kontaktu z żywnością.

Słowa kluczowe: *nanomateriały, nanotechnologia, materiały do kontaktu z żywnością, ocena ryzyka, badania toksyczności, bezpieczeństwo konsumenta*

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NANOMATERIALS DEFINITIONS, GENERAL INFORMATION

Nanoparticles can occur naturally (e.g. in ashes, as soil particles or biomolecules), or they can be produced unintentionally (e.g. in diesel exhaust) or intentionally engineered [59]. It is generally acknowledged that the term nanomaterials refers to objects with at least one size measurement not exceeding a length of 100 nm [22, 34, 42]. The nano-dimensional objects are generally characterized by their shape: nanospheres, nanoparticles, nanowhiskers, nanorods, nanotubes, nanosheets and nanoplatelets [6].

In the area of food contact materials (FCMs) definition for the term 'nanomaterial' (NM) was adopted in 2011 by the Commission Recommendation no 2011/696/EU [22], which states that "nanomaterial means a natural, incidental or manufactured material containing particle, 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 from 1 to 100 nm. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50% may be replaced by a threshold between 1 and 50%". This definition is as a reference for determining whether a material should be considered as a 'nanomaterial' for legislative and policy purposes in the European Union.

Nanocomposites is another term used in this paper, meaning a fusion of traditional food packaging material with nanoparticles. Nanocomposites are usually made up of a polymer matrix as a continuous phase and the nanomaterial(s) as a discontinuous phase.

The past several years have witnessed a remarkable and dynamic development of nanotechnology concerning the design and research of nanomaterials in practically all areas of use, including those closely related to health i.e. medicine [28], cosmetics [42, 56] and food production, including food packaging [3, 16, 61].

Like any other food technology, nanotechnology raises many questions about consumer safety arising from a lack of understanding on the possible health-related effects of exposure to nanoparticles [43]. A feature article by *Duncan* [16] stresses that there are numerous data gaps that need to be filled in order to demonstrate product safety. Some of these data gaps are on nanomaterial migration through polymer films, the interaction of nanomaterial biomolecules and cellular components, the interrelationships between nanoparticle characteristics (size, shape, surface charge, etc.) with toxicity or pharmacokinetic properties, appropriate methods to identify, characterize and quantify nanomaterials in complex food matrices and the chronic toxicity of nanomaterials upon oral exposure. Some of these data gaps are addressed in this article. These issues together with legislation are currently considered as barriers to acceptance of introduction and use of nanotechnology in food packaging applications.

APPLICATIONS OF NMs IN FCM

One of the promising and rapidly developing nanotechnologies currently applied to the food chain concerns the manufacturing phase that includes food packaging. Such an application to food is considered as being the most important nanotechnology for the near future. Nevertheless, contamination of food can be expected when NMs become incorporated into food packaging materials or storage containers to extend the shelf-life and keep the food products fresh.

Since a solely used polymer is unable to offer all the desired properties expected for efficient food packaging, one major application of nanotechnology to food contact materials (FCMs) is adding NMs to polymeric matrices. Polymers used for manufacturing FCM have been traditionally filled with synthetic or natural additives to enhance their properties. Such additions have permitted many uses in industry, transportation and consumer products, which includes food packaging. Various NMs have found numerous applications like: direct incorporation into food products, into food packaging material and during food processing. Nanomaterials in packaging has created a new generation of packaging technology, becoming one of the most developed areas in nanotechnology and represents an alternative to conventional food packaging [33].

Mixtures of polymers with inorganic or organic fillers with particular geometric shapes (fibres, flakes, spheres and particulates) have recently been introduced as novel packaging materials [49]. The ratio of the largest to smallest filler in packaging filling material plays a critical role in determining the physical properties of packaging. Filling materials having a higher large to small ratio possess higher reinforcing properties 15, 50]. Various nanomaterials are being extensively used as fillers such as graphene [38], silica [5], clay and organo-clay [29, 53], chitosan [7], polysaccharide nanocrystals [40], carbon nanotubes [58], cellulose-based [52] and also metal nanoparticles, such as ZnO_2 [21], colloidal Cu [8] or Ti [39].

Nanomaterials like nanoparticles, nanoclays and nanoemulsions have found numerous applications in the food sector as nanocomposites and constitute innovative food packaging.

Clay and silicate nanoplatelets are extensively used fillers of which montmorillonite [$Mx(Al_4$-$xMgx)$ $Si_8O_{20}(OH_4)$] demonstrates the desired properties due to the form of its octahedral sheets of $Al(OH)_3$ between silica tetrahedral bi-layers [60]. Nanoclay (1%) containing polypropylene was successfully tested as a proposed FCM to provide for an efficient air barrier in a potential

packaging material so that the shelf-life of food products is prolonged when stored in a CO_2 atmosphere [45].

Carbon nanotubes have been widely used as a non-food application of nanotechnology. Certain globular proteins from milk (such as hydrolyzed a-lactalbumin) can be made to self-assemble into similarly structured nanotubes under appropriate environmental conditions. This technique can be applied to other proteins as well [60].

Cellulose-based nanofibres have also been investigated as support structures for many nanomaterials. Using cellulose increases the nanoparticle surface area and thus enhances their activity. Such additive features make cellulose nanofibres an attractive class of nanomaterials. Reinforcements based on cellulose nanofibres improve the strength and thermal properties of polymers [48]. The assimilation of cellulose nanowhiskers and starch improves their thermo-mechanical properties, together with a reduced water sensitivity and intact biodegradability. Cellulose nano reinforcements may also form a moisture barrier property to polymer films [54].

These advantages are responsible for making nanotechnology-derived food packaging materials the most common nanotechnology application for the food sector, with the most prominent examples. This includes FCMs incorporating nanomaterials to improve packaging properties (flexibility, gas barrier properties and temperature/moisture stability), 'active' FCMs that give nanoparticles antimicrobial or oxygen scavenging properties, 'intelligent' FCMs incorporating nanosensors for monitoring and reporting food conditions and biodegradable polymer–nanomaterial composites [9, 10, 61].

Behind incorporating nanomaterials into food packaging, there are numerous data gaps to be filled in order to demonstrate product safety to consumers. Among these are: migration of NMs into food and food simulants, toxicological and legal aspects, toxic potential of NMs and safety assessment.

MIGRATION OF NMs FROM FCM

When NMs are used in food packaging materials, direct contact with food becomes possible following their migration. The risk of consumers being exposed to nanoparticles from food packaging is likely to occur via potential and unintended migration of nanoparticles from the packaging material into food and beverages. Because of general concerns to consumers over the possible adverse health effect of migrating food packaging components, this is considered a critical factor in the risk assessment of FCMs. The question if nanoparticles can at all migrate from plastic polymers remains unanswered and many studies devoted to this problem show greatly inconsistent findings [2, 32, 57, 61]. NMs migration has been discussed as a possibility by *Jokar* et al. [35], who following extensive literature searches did not exclude this and suggested three factors involved: the nature of the nanoparticle, the nature of the matrix (polymer) and the analytical methods for detecting nanoparticles migrating into food or food simulants.

The aim of nanoparticles migration studies was to assess whether they can migrate from plastic FCM into foodstuffs. Several studies have been published on nanoparticles migration from polymers. Most of them concerned nanosilver, which is used as an antimicrobial agent [10, 11, 12, 18, 32, 44]. *Huang* et al. [33] studied the migration of nanosilver from commercially available polyethylene plastic bags into food simulants. The lowest migration was observed into 95% ethanol, although there were no significant differences in migration recorded for all food simulants which were used. Nanosilver migration studied by *Echegoyen* and *Nerin* [18] was below the maximum limit for silver allowed by legislation. *Bott* et al. [2] demonstrated in the study that carbon black nanoparticles incorporated in polyethylene or polystyrene did not migrate regardless the time, temperature and food simulants were used.

A number of studies on migration from FCMs has assessed the safety of stored food products, as is particularly the case for plastic packaging, because it can be a source of harmful substances including carcinogenic primary aromatic amines [14], or endocrine disrupters [13]. Firstly, such studies need to determine the migration of the substances from FCM into food or food simulants, thereby estimating exposure which then gives the opportunity to risk assessment.

According to EFSA's scientific opinion [19], the potential risk arising from nanoscience and nanotechnology in food and FCMs has to be clarified and approval for a substance as nanoparticle to FCMs is required. According to EU Regulation No. 10/2011 [23], only those nanoparticles which were assessed by EFSA and authorized by the European Commission may be used in plastic FCMs.

The role played by the physical appearance of the nanoparticle was appreciated by *Jokar* et al. [35] who suggested that whenever there is no migration found, then the limit of determination (LOD) of the analytical method should be included, not only for particle mass or number concentration but also for particle size.

Using migration experiments and mathematical models this issue was investigated from another perspective by *Abreu* et al. [1] who found that the migration of chemicals from food packaging may be lower in nanocomposite polyamide film than in conventional polyamide film. It was concluded that this kind of packaging may reduce the potentially adverse health effects that may result from exposure to toxic compounds present in FCMs. Such findings thus add a new dimension to the risk-benefit discussion on nanomaterials in FCMs.

TOXICOLOGICAL AND LEGAL ASPECTS

The consumer safety implications from applications of nanotechnology in food and FCMs concern the physicochemical nature of the nanoparticles and the likelihood of exposure to NMs. When particle size becomes less than 100 nm, its physicochemical properties are significantly different to macroscale materials composed of the same substance. It also appears that the toxic effects of otherwise inert materials are very different in the nanoscale; as the surface area of particles increases, a greater proportion of their atoms may interact with potential receptors in the organism [30]. These effects pose numerous toxicological concerns because 'classic' toxicometric testing seems to be inadequate for any safety evaluation based on toxicological properties, even if they are well known for the same material but on the macro scale. The current understanding on possible consequences of exposure to NMs remains an open question because of the many uncertainties. The main concerns stem from the insufficient knowledge over the potential effects and possible impacts of such materials on human health. To address these concerns, four key risk assessment components should be identified: hazard identification and hazard characterisation followed by exposure assessment and risk characterisation [20].

The principle underlying the European Regulation (EC) 1935/2004 [17] is that any material or article intended to come into contact with food must be sufficiently inert to preclude substances from being transferred to the food in quantities large enough to endanger human health or to bring about an unacceptable change in the composition of the food or a deterioration in its organoleptic properties.

The European Union Regulation on plastic food contact materials and articles [23] emphasises that because of the new technologies used to engineer substances of a particle size that possess chemical and physical properties significantly different from those at a larger scale may lead to different toxicological properties and therefore these substances should be health-risk assessed on a case-by-case basis by EFSA. It should therefore be made clear that any authorisations based on a risk assessment of conventional particle sizes of a given substance do not cover nanoparticles. This statement reflects an uncertainty because toxicological data is lacking upon exposure to NMs.

It should also be stressed that existing toxicity testing methods, (e.g. OECD guidelines for testing chemicals), may need methodological modifications including application modes.

Under Article 9(2) of the EU No 10/2011 Regulation [23], substances in nanoform are only to be used if explicitly are authorised and mentioned in the Annex I; see Table 1.

At present, the number of nanoparticles allowed for use in FCMs is small. Any application for authorization of a nanoparticle substance is subject to a risk assessment by EFSA. This is also when the nanomaterial is used behind the functional barrier [23].

Table 1. Nanoparticles authorized for use in plastic food contact materials (FCMs), according to EU legislation

FCM substance No	Substance name	Restrictions and specifications	Reference
87	Silicon dioxide, silanated (produced using primary particles in nanoform in the final material)	For synthetic amorphous silicon dioxide, silanated: primary particles of 1–100 nm which are aggregated to a size of 0,1–1 μm and may form agglomerates within the size distribution of 0,3 μm to the mm size.'	Regulation EU No 2016/1416 [25]
807	Titanium nitride (TiN), nanoparticles	No migration of titanium nitride nanoparticles. Only to be used in PET bottles up to 20 mg/kg. In the PET, the agglomerates have a diameter of 100 – 500 nm consisting of primary titanium nitride nanoparticles; primary particles have a diameter of approximately 20 nm.	Regulation EU No 1183/2012 [24]
1050	Zinc oxide, nanoparticles, uncoated	Only to be used in unplasticised polymers.	Regulation EU No 2016/1416 [25]
1046	Zinc oxide, nanoparticles, coated with [3-(methacryloxy)propyl] trimethoxysilane (FCM No 788)	Only to be used in unplasticised polymers. The restrictions and specifications specified for FCM substance No 788 shall be respected (0.05 mg/kg).	Regulation EU No 2016/1416 [25]

It should however be noted that whenever nanoparticles are deliberately released, (eg. in inteligent antimicrobial packaging), then they should be treated as food additives rather than a packaging component and are thus covered by different regulations. As early as 2006, the USA's Food and Drug Aministartion (FDA) formed the Nanotechnology Task Force, responsible in developing regulatory approaches for nano-based products to ensure safety and efficacy, while also facilitating beneficial technological innovation. The task force then issued a report recommending an evaluation of agency-guidance, pointing out to the manufacturer what data needs reporting to the FDA on nano-products [55]. The FDA also recommended continuing an individual case by case approach for any nanotechnology products appearing in food.

Another uncertainty arises from the absence of appropriate analytical methods for quantifying NMs in such a complex matrix as food. *Huang* et al. [33] reviewed various analytical techniques that may be applicable, showing the weaknesses of each for performing quantitative NM analysis. This explains why there are no current labelling requirements. Monitoring nano-based products and assessing their safety is thus difficult for the authorities responsible; e.g. EFSA, FDA or other competent authorities at the national level.

TOXIC POTENTIAL OF NMs

The toxicity of chemicals depends on a number of factors including their bioavailability expressed as the amount of substance absorbed by the body from the site of first exposure, e.g. the gastrointestinal (GI) tract. The most important entry route of NMs that may be present in food from FCMs migration is oral, and discussion on the toxicokinetics of nanoparticles is thereby largely confined to oral intake in this review. To date, studies on exposure, absorption, bioavailability, distribution, metabolism and excretion have focused on the toxicokinetic processes following inhalation and dermal routes of exposure to NMs. Little is known however about the fate of nanoparticles following oral exposure, particularly in relation to ingestion of NMs contained in food. Nanoparticles can enter the GI tract in many ways such as ingestion directly from food, water and from nano-drugs. Inhaled nanoparticles can also be swallowed and enter the GI tract following clearance from the respiratory tract [31]. As a potential entry point for further distribution pathways, the oral route includes cells as possible target sites for NM action. After penetration, the particles are mainly distributed in the body according to their surface characteristics. The toxic potential of nanoparticles can be greatly enhanced by their free movement throughout cells, thereby promoting interactions with intracellular proteins and organelles, including nuclear DNA. There may be no means to prevent, affect or to direct nanoparticle uptake once they enter the cell. *Geisler* et al.[26] who following exposure of rats to titanium dioxide (TiO_2) via aerosol inhalation found ultrafine TiO_2 particles in connective tissue from heart sections and concluded that a transport mechanism with adhesive interactions, particle diffusion and uptake might play a role in particle transport across membranes. They also stated that following deposition of nanometre-size particles, their further fate may be largely independent of particle surface chemistry and charge. Consequently, ultrafine TiO_2 particles may be transferred from the lungs to other organs. This is consistent with a study by *Nel* et al. [46] who described mechanisms involved in cell death following exposure to nanoparticles. The mechanisms of NMs' absorption through the GI tract walls are complex and little is known about the behaviour and fate of NMs after they reach the GI tract; however it may not be excluded that GI tract absorption is affected by the different NM coatings [19] which could alter their surface properties. EFSA [20] recommends that the transformation and stability of NMs in the GI tract merits studying e.g. by *in vitro* digestion testing.

There is however another aspect of presence of nanoparticles in a food matrix. It is well recognised that the toxicity of many chemicals in the diet is markedly influenced by the food matrix, and that toxicity outcomes based on *in vitro* or *in vivo* testing will not be necessarily identical as when the same chemical is administered in a dietary matrix. The same phenomenon may obviously occur if nanoparticles are likewise introduced via the diet. The presence of NMs in food may cause increased bioavailability of other substances normally found in the diet, because actively charged surfaces can absorb biomolecules during their passage through the GI tract [27]. These so-called 'Trojan horses' [41] may transport any toxins appearing in food into the intestinal mucosa which may have associations with *Crohn's* disease. Another possible scenario is that active NM surfaces may adsorb beneficial food components thereby weakening their bioavailability. It is therefore advisable to consider the nutritional implications when NMs are present or expected in food [3]. Unfortunately there is no currently available information on toxic effects after low dose chronic or acute oral exposure; being a serious concern for assessing future health consequences arising from prolonged exposure to NMs. Their effects on the immune and cardiovascular systems and other organs e.g. lungs, liver, heart and brain remain unknown. Not enough is also known on genotoxicity, carcinogenicity, teratogenicity, neurotoxicity, reproduction toxicology nor the endocrine disrupting potential. Such huge areas of uncertainty give rise to inter- and intra-species

differences in toxicokinetics and toxicodynamics. Thus if the available scientific data does not indicate otherwise, conventional default uncertainty factors should be applied, (10 for inter- and 10 for intra-species differences), as currently there are no science-based indications necessary to modify these factors [20]. Appropriate *in vitro* and *in vivo* studies on NM are obviously required as a priority for identifying and characterising the hazards and for obtaining dose-response data. Further studies should be focused on understanding the mechanisms of action.

SAFETY ASSESSMENT

Although the beneficial effects of nanotechnologies are generally recognised, the potentially toxicological effects and impacts of NMs have so far received little attention. The rapid and massive introduction of consumer goods containing nanoparticles requires a better understanding of their potentially adverse effects on health. The general approach to assessing and controlling risk firstly involves hazard identification, understood as the potential of a given substance to cause harm, followed by a structured approach to determine the likelihood of exposure to the hazard and the associated consequences thereof [51].

The oral route is the most likely way of exposure to NMs from FCM, because when contained in food they have direct access to the GI tract. The biotransformation of nanoparticles in biological matrixes, (which mimic human oral exposure), is a complex process influenced by many factors (ie. Nanomaterial size, aggregation state, solubility, etc.), but also by matrix conditions, ie. pH, temperature and ionic strength. *Bove* et al. [4] described the silver nanoparticle dissolution process by means of an *in vitro* assay using human digestive simulating matrixes. They found that >90% Ag-NM dissolution took place during Ag-NM passage through the stomach. However, the resulting ions were found not to be all bioavailable, as most bind to the digestive matrices. These findings have been supported by *in vivo* studies showing that most of the nanoparticles were present in the faeces.

The gastrointestinal tract constitutes a complex barrier of around 2000 m^2 that fulfils different functions. Nutrients are taken up via the intestinal epithelium and are distributed throughout the body by the bloodstream. Since blood vessels are only one or several cell layers below the intestinal epithelium, it seems to be efficient barrier preventing macromolecules or nanoparticles from migrating into the bloodstream [37]. There is no consensus about the fate of nanomaterials once they get into the GI tract. While some animal experiments found that 50 to 100 nm-sized polystyrene particles were absorbed through the intestinal wall to get into the lymphoid system, other studies maintain that there is no such uptake at all [36]. While 98% of the nanoparticles administered orally to the test animals were excreted, approximately 80% of the intravenously administered material accumulated in the liver. The uptake of nanoparticles by the GI tract could accordingly, therefore be rather of minor significance.

Another doubt arises because of the complex nature of food and it is important that the NM is characterized within the context of the food matrix administered to test systems which should reflect the consumer's exposure to the matrix-NM complex, but is not necessarily reflected by animal experiments. *Oberdorster* et al. [47] pointed out that it is essential that NMs are accurately characterised, so that the potential toxicity of NMs in biological systems can become understood. He further proposed that specific NM parameters be additionally measured within such a matrix; thus being an important criterion for safety assessment. However, this poses considerable difficulties for routinely using analytical techniques to characterise NMs because a completely different approach of *in vitro* 'effect screening' is used to determine the presence of NMs. This implies that measuring the effect of exposure as a biological response should be sufficiently specific to serve as a screening tool. By such means the presence of NMs can be confirmed using assay systems that focus on biomarkers of exposure or its effects thereof. The *in vitro* assays could be used as a first tier for detecting nanoparticles in food [3]. It is therefore vital that methods for evaluating the toxicity of engineered nanomaterials are developed. Both *in vitro* and *in vivo* tests will be needed to achieve a reliable risk assessment outcome, the more so because computational methods (eg. QSAR) appear to be inadequate for nanoscale particles when together with the growth of the surface area of particle greater proportion of the atoms may interact with receptors in the organism as compared to the classical situation of exposure to chemical compounds [30]. The generally accepted risk assessment paradigm for non-nanomaterials is nevertheless considered also applicable for NMs. However, account should be taken of specific NMs features such as their unique biological properties arising from surface/body-receptors interactions, chemical composition, other physico-chemical properties and interaction with tissues. Hazard identification and characterisation resulting from NM exposure should include specific properties, with data on the non-nanoform of the same chemical being compared [19, 20].

The risk to human health can be characterised by comparing exposure levels; with a no-effect level expressed as an appropriate reference dose obtained from toxicity data. The most important issues for risk assessment are the sensitivity and validity of currently existing test systems. This requires developing models to predict the potential impact of engineered nanomaterials on human health. Such models should

include the physical and chemical characteristics of nanomaterials and an estimate of their health effects on humans. The NM toxicity literature is constantly growing, nevertheless results are often obtained for only one type of nanomaterial, where other types may give different results. Another difficulty is that test animals may be generally exposed to much higher concentrations than in reality. These obstacles limit the usefulness of animal experiment data. Moreover, there is insufficient science-based data to permit official extrapolation from one type of NM to another. According to present day knowledge, it is still impossible to determine which type of effects are to be expected for which type of NM. It is also unlikely whether the extent of surface properties, chemical composition and their proportions can be judged to affect an organism's biological response following exposure to NM.

As one of the key steps in risk assessment, hazard characterisation concerns the setting of health-based guidance values such as the acceptable daily intake which are based on toxicological endpoints eg. no-observed-adverse-effect-level (NOAEL), no-observed-effect-level (NOEL) or benchmark-dose-level (BMDL) defined by animal toxicity studies. It seems unacceptable that no relevant toxicity studies have been performed for obtaining these guidance values for NMs. Without any detailed toxicological data, but in view of the potential harm from nanoparticles it is therefore appropriate to consider using the precautionary principle (PP) for nanotechnology applications to food contact materials. Despite the preliminary evidence suggesting that certain engineered nanoparticles are potentially harmful to human health, it is unclear at present whether there is a sufficient scientific basis to use the PP for all applications of nanotechnology to food contact materials. More research is needed to better understand the levels of risk, but it would be prudent to consider using the PP to certain high-risk applications, particularly whenever there is evidence of engineered nanoparticles migrating from FCM into food [9, 10].

CONCLUSIONS

Polymer nanocomposites consist of a new class of materials that have the potential to introduce novel properties and features to the food-packaging industry. There are however numerous knowledge gaps on the behaviour and biological interactions of NMs which thus burden any risk assessment with serious uncertainty. These may include degradation of NMs during digestion and the availability of hazard information. The potential effects of nanosized food additives on the function of the GI tract and its natural microflora, gut epithelial cells and behaviour of NM free particles are far from being understood.

Another data omission is a deficiency of suitable analytical methods for detecting sufficiently low NM amounts and small NM sizes. At present, determining NM in the food or feed matrix is not possible, leading to increased uncertainty in exposure assessment. Despite the huge number of studies, this lack does not allow for a conclusive answer on whether NMs can or cannot migrate from FCM into food or if there are any circumstances that are likely to affect this process.

Numerous experiments suggest that adverse health outcomes associated with the uncontrolled presence of nanoscale particles in tissues require further attention.

Although study data indicates a rather limited likelihood of NMs migrating from composite packaging material to foodstuffs, the health risk issues still remain unanswered. Much more has to be therefore done, not only regarding the amounts of nanoparticles but also in showing how their surface area affects their biological potential. Future research needs to develop predictive and validated toxicological tests (*in vitro* and *in vivo*) that can be used to screen for potential hazards and also to develop new methodologies for measuring nanoparticles in biological matrices, necessary for assessing human exposure. This should include studies for better understanding the relationships between NMs' physicochemical surface characteristics with the body's receptors.

REFERENCES

1. *Abreu de A. P., Cruz J. M., Angulo I. and Losada P. P.*: Mass transport studies of different additives in polyamide and exfoliated nanocomposite polyamide films for food industry. Packag Technol Sci. 2010;23:59-68. doi: 10.1002/pts.879.
2. *Bott J. Stoermer A., Franz R.*: Migration of nanoparticles from plastic packaging materials containing carbon black into foodstuffs. Food Add Contam: Part A 2014;31(10):1769-1782.
3. *Bouwmeester H., Dekkers S., Noordam M. Y., Hagens W. I., Bulder A. S., De Heer C., Ten Voorde S. C. G., Susan W. P. Wijnhoven S. W. P., Marvin H. J. P., Sips A. J.A.M.*: Review of health safety aspects of nanotechnologies in food production. Regul Toxicol Pharmacol 2009;53: 52–62.
4. *Bove P., Malvindi M. A., Kote S. S., Bertorelli R., Summa M., Sabella S.*: Dissolution test for risk assessment of nanoparticles: a pilot study. Nanoscale 2017;9:6315-6326. doi: 10.1039/c6nr08131b.
5. *Bracho D., Dougnac V. N., Palza H., and Quijada R.:* Fictionalization of silica nanoparticles for polypropylene nanocomposite applications. J. Nanomater. 2012:263915. doi: 10.1155/2012/263915.
6. *Bratovcic A., Odobašić A., Ćatić S., Šestan* I.: Application of polymer nanocomposite materials in food packaging. Croat. J. Food Sci. Technol. 2015;7:86-94. doi: 10.17508/CJFST.2015.7.2.06.

7. *Chang P. R., Jian R., Yu, J., Ma X.:* Starch-based composites reinforced with novel chitin nanoparticles. Carbohydr. Polym. 2010;80:420-425. doi: 10.1016/j.carbpol.2009.11.041.
8. *Cardenas G., Díaz J., Meléndrez M., Cruzat C., Cancino A.G.*: Colloidal Cu nanoparticles/chitosan composite film obtained by microwave heating for food package applications. Polym. Bull. 2009;62:511-524. doi: 10.1007/s00289-008-0031-x.
9. *Chaudhry Q., Scotter M., Blackburn J., Ross B., Boxall A., Castle L., Aitken R, Watkins R.*: Applications and implications of nanotechnologies for the food sector. Food Addit Contam 2008;25(3):241-258.
10. *Chaundhry Q., Castle L., Bradley E., Blackburn J., Aitken R., Boxall A.*: Assessment of current and projected applications of nanontechnology for food contact materials in relation to consumer safety and regulatory implications. Project A03063, UK Food Standards Agency & Central Science Laboratory, London 2008
11. *Cushen M., Kerry J., Morris M., Cruz-Romero M., Cummins E.*: Evaluation and simulation of silver and copper nanoparticle migration from polyethylene nanocomposites to food an associated exposure assessment. J. Agric Food Chem. 2014;62(6):1403-1411.
12. *Cushen M., Kerry J., Morris M., Cruz-Romero M., Cummins E.*: Migration and exposure assessment of silver from a PVC nanocomposite. Food Chem. 2013;139(1-4):389-397.
13. *Cwiek-Ludwicka K., Ludwicki J. K.*: Endocrine disruptors in food contact materials; is there a health threat? Rocz Panstw Zakl Hig. 2014;65:169-177.
14. *Cwiek-Ludwicka K., Pawlicka M., Starski A., Półtorak H., Karłowski K.*: Badanie migracji pierwszorzędowych amin aromatycznych (PAAs) z wielowarstwowych opakowań żywności metodą HPLC. [Studies on primary aromatic amines (PAAs) migration from multilayer plastic food packaging by HPLC method]. Rocz Panstw Zakl Hig. 2011;62:371-375.
15. *Dalmas F., Cavaille J. Y., Gauthier C., Chazeau L., Dendievel R.*: Visco elastic behaviour and electrical properties of flexible nanofiber filled polymer nanocomposites. Influence of processing conditions. Composites Sci. Technol. 2007;67:829–839. doi: 10.1016/j.compscitech.2006.01.030.
16. *Duncan T. V.*: Applications of nanotechnology in food packaging and food safety: Barrier materials, antimicrobials and sensors. J Colloid Interface Sci 2011;363:1-24.
17. EC. Regulation (EC) No. 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. Off J Eur Union L 338/4
18. *Echegoyen Y., Nerin C.*: Nanoparticle release from nano-silver antimicrobial food containers. Food Chem Toxicol 2013;62:16-22.
19. EFSA. Scientific opinion of the scientific committee on a request from the European Commission on the potential risks arising from nanoscience and nanotechnologies on food and feed safety. EFSA J. 2009;958:1-39.
20. EFSA. Guidance of the risk assessment of the application of nanosciences and nanotechnologies in the food and feed chain. EFSA J. 2011:95(5):21-40.
21. *Esthappan S. K., Sinha M. K. Katiyar P., Srivastav A., Joseph R.*: Polypropylene/zinc oxide nanocomposite fibers: morphology and thermal analysis. J. Polym. Mater. 2013;30:79-89.
22. EU 2011. Commission Recommendation No 2011/696 of 18 October 2011 on the definition of nanomaterial. Off J Eur Union L 275, 38-40
23. EU 2011. Regulation (EU) No. 10/2011 of the European Parliament and of the Council of 14 January 2011 on plastic materials and articles intended to come into contact with food. Off J Eur Union L 12/1.
24. EU 2012. Commission Regulation (EU) No 1183/2012 of 30 November 2012 amending and correcting Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food. Off J Eur Union L 338/11.
25. EU 2016. Commission Regulation (EU) No 2016/1416 of 24 August 2016 amending and correcting Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food. Off J Eur Union L 230, 22-42
26. *Geiser M., Rothen-Rutishauser B., Kapp N., Schürch S., Kreyling W., Schulz H. Semmler M., Hof V. I. Heyder J., and Gehr P.*: Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. Environ Health Perspect. 2005;113:1555–1560. doi:10.1289/ehp.8006.
27. *Govers M., Termont D., Vanaken G.A., Vandermeer R.*: Characterization of the adsorption of conjugated and unconjugated bile-acids to insoluble amorphous calcium-phosphate. Journal of Lipid Research 1994;35(5):741-748.
28. *Grudziński I.P.*: Bezpieczeństwo nanoproduktów leczniczych: nowe obszary badań toksykologicznych. [Safety of medicinal nanoproducts: new areas of toxicological research]. Rocz Panstw Zakl Hig 2011;62:239-246.
29. *Ham M., Kim J.C., Chang J.H.*: Thermal property, morphology, optical transparency, and gas permeability of PVA/SPT nanocomposite films and equi-biaxial stretching films. Polym. Korea 2013;37:579-586. doi: 10.7317/pk.2013.37.5.579.
30. *Hatzigrigoriu N.B., Papaspirydes C.D.*: Nanotechnology in plastic food-contact materials. J. Appl. Polymer Sci. 2011;122:3719-3738.
31. *Hoet P., Bruske-Hohlfeld I., Salata O.*: Nanoparticles – known and unknown health risks. J Nanobiotechnol. 2004;2:12. doi:10.1186/1477-3155-2-12.
32. *Huang Y., Chen S., Bing X.:* Nanosilver migrated into food-simulating solutions from commercialy available food fresh containers. Packag Technol Sci 2011;24(5):291:297.
33. *Huang J. I., Li X., Zhou W.*: Safety assessment of nanocomposite for food packaging application. Trends Food Sci Technol 2015;45:187-199.
34. ISO. International Organization for Standardization, ISO/TS 27687: Nanotechnologies - Terminology and definitions for nano-objects - Nanoparticle, nanofibre and nanoplate. 2008:1-14.
35. *Jokar M., Pedersen G.A., Loeschner K.*: Six open questions about the migration of engineered nano-objects from polymer-based food-contact materials: a review. Food Addit Contam. Part A. 2017;34(3):434-450, doi: 10.1080/19440049.2016.1271462.

36. *Kanapilly G.M., Diel J.H.:* Ultrafine 239PuO_2 Aerosol Generation, Characterization and Short-term Inhalation Study in the Rat. Health Phys. 1980;39:505-519.
37. *Krug H. F, Wick P.*: Nanotoxicology: an interdisciplinary challenge. Angew Chem Int Ed Engl. 2011;50:1260-1278.
38. *Lee Y., Kim, D., Seo, J., Han, H., Khan, S. B.*: Preparation and characterization of poly(propylene carbonate)/exfoliated graphite nanocomposite films with improved thermal stability, mechanical properties and barrier properties. Polym. Int. 2013;62:1386-1394. doi: 10.1002/pi.4434.
39. *Li R., Liu C. H., Ma J., Yang Y. J. and Wu H. X.*: Effect of orgtitanium phosphonate on the properties of chitosan films. Polym. Bull. 2011;67: 77-89. doi: 10.1007/s00289-010-0404-9.
40. *Lin N., Huang J., Dufresne A.*: Preparation properties and applications of polysaccharide nanocrystals in advanced functional nanomaterials: a review. Nanoscale 2012;4(11):3274-3294; doi: 10.1039/c2nr30260h.
41. *Lomer M. C., Thompson R. P., Powell J. J.*: Fine and ultrafine particles of the diet: influence on the mucosal immune response and association with Crohn's disease. Proc. Nutr. Soc. 2002;61(1): 123-130.
42. *Lövenstam G., Rauscher H., Roebben G., Sokull Klütten B., Gibson N., Putaud J.P. and Stamm H.*: Considerations on a definition of nanomaterial for regulatory purposes. JRC (Joint Research Centre) 2010 Reference Report, EUR 24403 EN, doi 10.2788/98686.
43. *Maynard, A.D., Aitken, R.J., Butz, T., Colvin, V., Donaldson, K., Oberdorster, G., Philbert, M.A., Ryan, J., Seaton, A., Stone, V.*, *Tinkle S.S., Tran L., Walker N.J., Warheitet D.B.*: Safe handling of nanotechnology. Nature 2006;444:267-269.
44. *Metak A.M., Nabhani F., Connoly S.N.*: Migration of engineered nanoparticles from packaging into food products. LWT – Food Sci Technol. 2015;64(2):781-787.
45. *Nalçabasmaz S., Zehra Ayhan Z.,2 Sossio Cimmino S., Silvestre C. and Duraccio D.*: Effects of PP-based nanopackaging on the overall quality and shelf life of ready-to-eat salami. Packag. Technol. Sci. 2017. DOI: 10.1002/pts.2309.
46. *Nel A., Xia T., Mädler L., Li N.*: Toxic potential of materials at the nanolevel. Science 2006;311:622-627.
47. *Oberdorster G., Maynard A., Donaldson K., Castranova V., Fitzpatrick J., Ausman K., Carter J., Karn B., Kreyling W., Lai D.*: Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Part Fibre Toxicol 2005;2:8.
48. *Podsiadlo P., Choi S. Y., Shim B., Lee J., Cuddihy M., and Kotov N. A.*: Molecularly engineered nanocomposites: layer-by-layer assembly of cellulose nanocrystals. Biomacromolecules. 2005;6:2914-2918. doi: 10.1021/bm050333u
49. *Prateek T. V. K, Gupta R. K.*: Recent progress on ferroelectric polymer-based nanocomposites for high energy density capacitors: synthesis, dielectric properties, and future aspects. Chem. Rev. 2016;116, 4260–4317. doi: 10.1021/acs.chemrev.5b00495.
50. *Rafieian F., Simonsen J.*: Fabrication and characterization of carboxylated cellulose nanocrystals reinforced glutenin nanocomposite. Cellulose 2014;21:4167-4180. doi: 10.1007/s10570-014-0305-4.
51. Royal Society. Nanoscience and Nanotechnologies: Opportunities and Uncertainties. The Royal Society and the Royal Academy of Engineering, London, 2004.
52. *Sandquist D.*: New horizons for microfibrillated cellulose. Appita J. 2013;66,:156-162.
53. *Schuetz M. R., Kalo H., Linkebein T., Groschel A. H., Muller A. H. E., Wilkie C. A.*: Shear stiff, surface modified, mica-like nanoplatelets: a novel filler for polymer nanocomposites. J. Mater. Chem. 2011;21:12110-12116. doi: 10.1039/C1JM11443C.
54. *Sharma Ch., Dhiman R., Rokana N., Panwar H.*: Nanotechnology: An untapped resource for food packaging. Frontiers in Microbiology 2017;8:1735. doi: 10.3389/fmicb.2017.01735.
55. *Silvestre C., Duraccio D., Cimmino S.*: Food packaging based on polymer nanomaterials. Progress in Polymer Science, 2011;36(12), 1766-1782.
56. *Snopczyński T., Góralczyk K., Czaja K., Struciński P., Hernik A., Korcz W., Ludwicki J. K.*: Nanotechnologia – możliwości i zagrożenia. [Nanotechnology – possibilities and hazards]. Rocz Panstw Zakl Hig. 2009;60(2):101-111.
57. *Störmer A., Bott J., Kemmer D., Franz R.*: Critical review of the migration potential of nanoparticles in food contact plastics. Trends Food Sci Technol 2017;63:39-50.
58. *Swain S. K., Pradhan, A. K., and Sahu, H. S.*: Synthesis of gas barrier starch by dispersion of functionalized multiwalled carbon nanotubes. Carbohydr. Polym. 2013;94:663-668. doi: 10.1016/j.carbpol.2013.01.056.
59. *Tiede K., Boxall, A. B. A., Tear S. P., Lewis J., David H., Hassellöv M.*: Detection and characterization of engineered nanoparticles in food and the environment. Food Addit Contam. Part A, 2008;25(7):795-821.
60. *Weiss J., Takhistov, P., Mc Clements D. J.*: Functional materials in food nanotechnology. J. Food Sci. 2006;71:R107–R116. doi: 10.1111/j.1750-3841.2006.00195.x.
61. *Wyser Y., Adams M., Avella M., Carlander D., Garcia L., Pieper G., Rennen M., Schuermans J., Weiss* J.: Outlook and challenges of nanotechnologies for food packaging. Packag Technol Sci. 2016;29:615-648.

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