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Outlook and Challenges of Nanotechnologies for Food Packaging

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Nanotechnology has been considered to have high potential for food packaging applications very early on. The ability to provide additional consumer benefits through the improvement of key properties of packaging materials and the creation of new functionalities means that the increased use of nanomaterials and nanotechnologies is highly likely. It has however up to now failed to reach the widespread use that was initially expected, mainly because of remaining uncertainties on the safety of these materials during the various stages of their life-cycle, which limit legal and consumer acceptance.

This paper aims at presenting the latest developments in the field of nanotechnologies for food packaging applications, describing the legal framework linked to their usage and attempts to clarify the current knowledge of the safety of these materials both for the consumer and the environment.

It is shown that particulate migration into foodstuff is absent in many applications, which drastically reduces the potential risk during the use phase of packaging materials, i.e. the exposure of the consumer to nanoparticles. Other release routes are also evaluated, showing that, although safe in normal use conditions, prudence should still be used, especially with regard to release after disposal of the materials. Copyright © 2016 The Authors Packaging Technology and Science Published by John Wiley & Sons Ltd

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INTRODUCTION

The concept of nanotechnology refers to the manipulation of materials at a nanometric scale to benefit from the specific physico-chemical properties occurring in this size range. The concept was first mentioned in a speech by Richard Feynman given in December 1959 at the annual meeting of the American Physical Society.[1] Theoretical knowledge and analytical tools were developed over the next two decades leading to the discovery of fullerenes in 1985 (resulting in a Nobel prize in 1996)[2] and carbon nanotubes a few years later.[3]

From the early days nanotechnology was identified by the packaging industry as a potential enabler of increased functionality in packaging materials. This was initially in the domain of barrier and

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mechanical property improvement[4] and later in the broader context of active and intelligent packaging.[5] Nanotechnology-enabled packaging materials have since grown to become a major area for innovation within the food sector.[6,7]

Innovations in packaging are being driven by the notable changes in consumer demand and behaviour, which are expected to influence the way we use, and what we expect from packaging in the future. For instance, (a) Observable shifts towards smaller households, (b) increased an out-of-home food consumption, (c) a greater awareness of, and increasing expectations from the nutrition, health and wellness aspects of food, (d) an increased desire for freshness and naturalness and (e) more environmentally aware consumers. These trends are placing clear new demands on both food and the packaging, which accompanies it, some of which are listed hereafter:

1. Changes in household size will inevitably lead to the adaptation of pack sizes, which will affect packaging surface to volume ratios. Consequently, the protection requirements will need to be adapted.
2. Increased consumption of products outside of the home may require additional functionalities to facilitate convenient access and provide means to reclose.
3. Expectations from consumers regarding the health, wellness and nutritional aspects need to be met but also communicated. There are consequently greater demands from packaging to provide the consumer with more and increasingly detailed information.
4. Fresher and more natural products are more sensitive to degradation (e.g. reduction of preservatives and use of unsaturated fats) consequently there will be higher performance expectations from the packaging to protect the contained food product.

These shifting requirements have led to the development of new packaging technologies. Materials have been developed with improved barrier and mechanical properties, and there has been a steady increase in work to develop materials from renewable materials to address protection and sustainability requirements. Active and intelligent packaging technologies have also continued to emerge to address food waste concerns in the form of oxygen scavengers, antimicrobial packaging and freshness indicators, while interactive packaging offers the potential to enable greater consumer engagement and communication through augmented reality, digital packaging and QR codes.

The market pull on packaging development is therefore very strong, and many of the newer requirements can be addressed through the use of technologies available or in development today that is either based on or use nanoscience or nanotechnology in one way or another.

Despite the interest and also the potential benefits of using nanotechnologies, in recent years attitudes towards them have changed considerably. Initially, this technology was seen as a 'must have'; however, concerns were later raised over potential health risks in certain applications. This led to consumer acceptance issues, especially in application areas related to food.[7–15]

Although the understanding of nanotechnology is highly advanced in some domains, there are still safety, toxicological and eco-toxicological questions, which have to be fully addressed. These open questions, together with a legislative framework, which is still in formation, are currently considered as barriers towards a more broadly accepted introduction and use of nanotechnology in packaging applications.

A principle that is often applied when a new technology is used in a pioneering application is that of balanced risk and benefit. This principle is valid when the risks and the benefits can be clearly identified and related, such as in medical applications. When considering food applications, however, the same approach would mean that any risk associated with food would have to be balanced against potential consumer benefits, which would most likely be quality related. However, jeopardizing safety to achieve quality related benefits is an approach that is unacceptable. It is therefore necessary to prove the safety of a specific technology prior to any application aimed at improving product quality. This should be done either by proving the innocuousness of a potential exposure or by preventing the possibility of exposure to a potentially harmful substance during the entire life-cycle of the packaging material (i.e. from production to final disposal).

If the use of nanotechnology in packaging applications is to become relevant in terms of volume and scope, the safety and inertness of these new materials with respect to health (human and animal) and ecosystems must be proven, and public acceptance improved by demonstrating consumer benefits,

which are linked to actual needs.[10,11,16] Furthermore, given the numerous technologies and potential applications, nanotechnology cannot be assessed as a single technology. Instead each of the main categories will have to be treated independently with respect to their specific benefits and safety considerations.

A key message from these earlier experiences is that driver for using nanotechnology should not be because it is seen as the latest 'must have' technology. Nanotechnology must rather be seen as an enabler, which can be used to achieve specific and desired material properties that deliver measurable benefits. These material properties and associated benefits must be achieved without jeopardizing product safety or quality.

This paper provides a state-of-the-art review of work relevant to the application of nanotechnologies in food packaging applications. The purpose is to provide a consolidated review of current state-of-the-art research on the topic in order to inform a current broader public debate on the use of nanomaterials in food packaging.

Definitions are first given and new nanoscalar materials and processes for use in food packaging applications are reviewed along with the current applicable regulatory framework. The important aspect of safety is then addressed by first examining the exposure aspect of risk assessments and then reviewing recent work on the migration of nanomaterials from packaging materials to foods and the technologies allowing characterization of nanostructures and migration testing. Considerations are also given towards potential environmental implications, which could arise from the use of such materials over the complete life cycle of the products they are containing. Finally, there is a discussion of the findings and their implications on risk assessment.

DEFINITION

Definitions relating to nanotechnologies can be found in a series of technical specifications edited by the International Organization for Standardization (ISO), namely, the ISO TS 80004 series, comprising 8 parts edited between 2010 and 2015.[17–24]

Nanotechnology is defined in part 1[17] as being:

> The application of scientific knowledge to manipulate and control matter in the nanoscale in order to make use of size- and structure-dependent properties and phenomena, as distinct from those associated with individual atoms or molecules or with bulk materials. The nanoscale being the size range from approximately 1 nm to 100 nm.

Nanomaterials are defined as materials with any external dimension in the nanoscale (nano-objects) or having internal structure or surface structure in the nanoscale (nanostructured materials).

In part 2,[24] nano-objects are further clustered into three classes:

1. Nanoparticles: nano-objects with all external dimensions in the nanoscale where the lengths of the longest and the shortest axes of the nano-object do not differ significantly.
2. Nanofibers: nano-objects with two external dimensions in the nanoscale and the third dimension significantly larger.
3. Nanoplates: nano-objects with one external dimension in the nanoscale and the other two external dimensions significantly larger.

Other nano-objects are nanorods, nanotubes and nanowires, which are specific examples of nanofibers that are solid, hollow and (semi-)conducting, respectively. Nanoribbons are a special case of nanoplates where one planar dimension is significantly larger than the other. Figure 1 shows selected nano-objects.

Terms related to nanostructured materials are defined in part 4.[19] A nanophase is a physically or chemically distinct region or collective term for physically distinct regions of the same kind in a material with the discrete regions having one, two or three dimensions in the nanoscale. A nanocomposite is defined as a solid comprising a mixture of two or more phase-separated materials, one or more being a nanophase.

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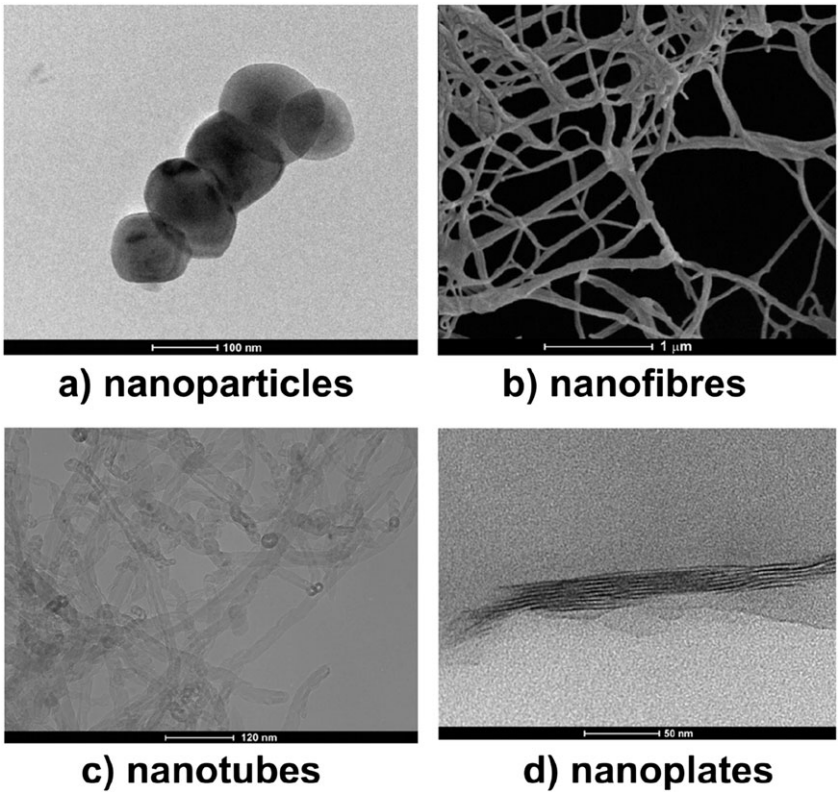

Figure 1. Examples of shapes of nano-objects

Under the classification format mentioned earlier packaging materials produced by applying nanotechnology for improved performance can be considered as nanomaterials, which can be termed as nanostructured.

The conclusion will not be the same when looking at some of the regulatory definitions[25,26] that have been implemented, which will be discussed later in the paper. All terminology used in the following section are based on the ISO standards, except when otherwise mentioned.

REGULATORY ISSUES AND IMPLICATIONS

Legal background

A number of studies have been carried out to identify regulatory gaps in the field of nanotechnology. These studies have covered different jurisdictions, such as the European Union, the United States, and Australia, and have concluded that existing frameworks are broad enough to 'capture' nanotechnology applications for food and food packaging.[27–30] This section describes and discusses the relevant regulatory frameworks in place in Europe and the USA and the current regulatory position regarding the applications of nanotechnologies for food packaging.

Regulatory frameworks in the European Union

Food contact materials (FCM) are regulated within the field of food legislation. They are governed by regulation (EC) No. 1935/2004 on materials and articles intended to come into contact with food.[31] This regulation sets the general rules for FCM with regard to their safety, labelling and traceability. It also establishes the authorization procedure for substances used in FCM and empowers the Commission to adopt specific measures for specific materials. Up to date, specific measures have been adopted for plastics,[32] ceramics,[33] regenerated cellulose film,[34] recycled plastics[35] and active and intelligent

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materials and articles.[36] Materials not covered by the European Union (EU) harmonized specific measures are subject to national legislations.

Certain EU regulations include requirements for nanomaterials, however no definition is given. For this reason, it is usually accepted that the 696/2011 Recommendation for a definition of nanomaterial adopted by the European Commission in 2011 applies,[25] which states

'Nanomaterial' 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. 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%.

The definition also considers fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm as nanomaterials.

It differs from the ISO definitions presented earlier by excluding nanostructured materials, except aggregates and agglomerated of primary nanoparticles, and defines a threshold of 50% on the number-based size distribution.

The Recommendation also contained a deadline for its revision by December 2014, which was not achieved. Nevertheless, the Joint Research Center of the European commission has recently completed a series of three reports that evaluate the current definition and presents options in view of its revision.[37–39] It can therefore be reasonably expected that the definition contained in Recommendation 696/2011 might evolve in the coming months.

MATERIALS COVERED BY THE EUROPEAN UNION HARMONIZED SPECIFIC MEASURES

Plastics

Plastic materials and articles are covered by Regulation 10/2011[32] and its amendments. Naturally occurring macromolecules not chemically modified are not within the scope of the Plastics Regulation, e.g. starch based polymers are not in scope while polymers based on chemically modified starch are covered. The use of additives to modify the macromolecule as such is not considered a chemical modification. For example, plasticized starch-based polymers would not be covered by the Plastic Regulations.

Only nanoparticles authorized and specifically mentioned in the specification of Annex I of the Regulation can be used in plastic packaging. This applies also to nanoparticles intended to be used behind a functional barrier.[1] Nanoparticles, which were initially listed in the specifications and thus authorized, are silicon dioxide and carbon black,[2] Titanium nitride (in nanoform) is also authorized in Annex 1 but under specific use conditions. The Annex has since then been amended based on several opinions published by the European Food Safety Authority (EFSA) on nanomaterial usage in FCM, and other materials have been added, however, with specific conditions of use. These are mentioned later.[40–44]

Given the few materials currently authorized for use, most of the nanoparticles with potential for improving functionality in plastic packaging materials mentioned in the next chapters are not authorized at EU level, even if their bulk form is authorized. Before these substances can be authorized and used in plastic FCMs, an application for their authorization will need to be submitted containing specific information regarding toxicology and possible exposure.

Nanoparticles used as antimicrobials to keep the surface of FCMs free from microbial contamination (surface biocides) and which do not exhibit an antimicrobial function on the food are not currently included in the list of authorized substance in the EU. However, 10 silver containing antimicrobials have been evaluated to which the EFSA has responded favourably.

[1]Functional barrier is a layer that reduces the migration of a substance from behind the layer into food to a non-detectable level with a detection limit of 10 ppb.

[2]This does not exclude the use of these additives in the bulk form.

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Active and intelligent materials and articles

Active and intelligent materials are covered by Regulation 450/2009.[36]

Active materials are materials and articles that are intended to extend shelf-life or to maintain or improve the condition of packaged food; they are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food. Intelligent materials are materials and articles, which monitor the condition of packaged food or the environment surrounding the food.

Substances forming the passive part of the active or intelligent material are regulated by their corresponding specific measure, e.g. plastics by the Plastics Regulation and rubber by the framework regulation and national legislation.

Substances released into food to become a component of the food must comply with food legislation. For example, a released antioxidant must be authorized in food as antioxidant in the food additives legislation.[45] Substances released or grafted that are intended to exhibit an antimicrobial function on the food need to be authorized as preservatives in the food additives legislation. Food additives are authorized for particular food types only. Currently no nanoparticles have been authorized as food preservatives or antioxidants in the EU. If such a nanoparticle were to be used in active materials then an application for authorization would need to be submitted under the authorization scheme for food additives. For substances forming part of the active or intelligent component, and which are not intentionally released, the Regulation (EU) No. 450/2009[36] provides the authorization scheme.

Currently, a list of authorized substances for use in active and intelligent packaging applications for the EU is under development. Once complete, only those substances listed will be authorized for use in active and intelligent packaging components. This will also apply to nanomaterials such as those in intelligent indicators, e.g. nanopigments or nano-based colour systems even if used behind a functional barrier. Until the EU list is established, the general rules of the Framework Regulation and national legislation apply.

Regenerated cellulose film and ceramics

In the following sections, no applications of nanomaterials are described for the use in regenerated cellulose film (RCF) or ceramics. If uses in RCF were envisaged in the future, they would be covered by the authorization scheme foreseen for RCF.[34] The current list on substances that can be used in RCF does not cover nanomaterials. Regulation (EC) No. 1935/2004[31] requires any user of an authorized substance to inform the Commission on any new scientific information, which might affect the safety assessment of the authorized substance. Therefore, any authorized substances that were envisaged to be used as nanoparticle would need a new application for authorization.

Legislation on ceramics[33] is only harmonized for the migration of lead and cadmium. Any use of nanoparticles would be covered by national legislation.

MATERIALS NOT COVERED BY EU HARMONIZED SPECIFIC MEASURES

All other materials have to comply with the general safety requirements of the Framework Regulation and specific national legislation. The Framework Regulation requires, in particular, that materials and articles should not release its constituents in concentrations that could

- endanger human health,
- bring about an unacceptable change in the composition of the food, or
- bring about a deterioration in the organoleptic characteristics thereof.

SPECIFIC CASES

Nanocoatings of, e.g. silicon dioxide that are applied on a plastic layer are covered by the rules of the Framework Regulation. Inorganic coatings such as silicon dioxide coatings are not covered by Regulation (EU) No. 10/2011.[32] EFSA has given a positive opinion on one silicon dioxide nanocoating.[46]

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Pigments used in printing inks or in plastics are not covered by an EU specific measure. They have to comply with the general safety requirements of the Framework Regulation and specific national legislation. If they would be used in intelligent components of intelligent materials they would fall under Regulation (EC) No. 450/2009.[36]

Surface biocides used in other FCMs than plastic FCMs are not covered by an EU specific measures. However, they are covered by the biocidal products regulation.[47]

REGULATORY FRAMEWORKS IN THE UNITED STATES

The responsibility of the US food and drug administration

The US Food and Drug Administration (FDA), an Agency within the US Department of Health and Human Services, has the responsibility for safety and efficacy of drugs and devices for humans and animals, products that emit radiation, biological products for humans, foods (including direct and indirect food additives, food contact substances and dietary supplements), colour additives and cosmetics. The FDA is also responsible for advancing the public health by helping to speed innovations that make foods and medicines more effective, safer and more affordable and helping the public get the accurate, science-based information they need to use medicine and foods to improve their health.

Nanotechnology and food contact substances

A new food contact substance (FCS) must be the subject of an effective food contact notification (FCN) to be lawfully used in the United States. For new authorization of an FCS, FDA focuses on particle size when it is important for the identity of the food contact substance, when it impacts the functionality of the food substance or when it impacts the intended technical effect. The FDA does not have a bias either for or against nanotechnology as it applies to food additives or food packaging. If the nanomaterial in question can be shown to be safe under the intended conditions of use, it can be used in contact with food.

Guidance for approval of new food contact substances

The FDA offers guidance to the regulated industry on the submission of FCNs, for new FCMs. While the current guidance documents for FCNs[48] do not presently make specific recommendations regarding nanomaterials, the chemistry guidance does address the issue of properties that are specific to particle size. A document entitled *Guidance for Industry*: *Assessing the Effects of Significant Manufacturing Process Changes*, *Including Emerging Technologies*, *on the Safety and Regulatory Status of Food Ingredients and Food Contact Substances*, *Including Food Ingredients that are Color Additives* was issued in June, 2014, and provides some of the Agency's current thinking on FCS manufacturing relating to nanotechnology.[49] More specific requirements will be addressed in future revisions of the guidance documents. Until that time, the FDA offers informal advice on the issues of interest when evaluating these materials. Its general recommendations are presented hereafter.

Chemistry considerations

Identity. If the particle size is important for the FCS to achieve its intended technical effect, such that the additive is produced or processed using techniques or tools that manipulate the particle size and may contain altered particles that are formed as manufacturing by-products, data on the size (average and distribution), shape, surface area (average and distribution), surface charge (zeta potential) and morphology of the particles, as well as any other size-dependent properties (e.g. agglomeration, aggregation and dispersion) should be included as appropriate.

Specifications for identity and purity. Parameters related to the particle size, shape and surface properties of the FCS, as appropriate, if particle size is important for the identity and functionality of the

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FCS would need to be described. Replacing an existing FCS with a nanoscalar version might have significant safety implications.

Intended technical effect and use. A clear statement of the intended technical effect(s) of the FCS in food is a necessary component of an FCN. If the technical effect of the FCS is related to particle size, the statement should explain how size-dependent properties of the FCS affect functionality (e.g. solubility, viscosity, stability, antibacterial properties and antioxidant properties).

Impact on safety of the food contact substance

The replacement of an existing FCS with a nanoscalar version, or the introduction of a new nanoscalar additive might require safety considerations in addition to those in use for traditional (non-nano) additives. For example, are the uptake, absorption and bioavailability of the modified product different than the conventional product? Are new impurities detected at concentrations that are of concern? Are there new toxicology issues that were not previously addressed?

Considering the basic feature of nanomaterials, the very small particle size, it is understandable that introduction of a new nanomaterial as an FCS might introduce new issues that warrant additional or different evaluation during a safety assessment of a food substance or might raise new safety issues that have not been seen in their traditionally sized counterparts.

There is significant debate in the toxicology community regarding the correct and appropriate testing to judge the safety of nanomaterials. Extrapolation from data on traditionally manufactured food substances can generally be conducted only on a case-by-case basis. Safety assessments should be based on data relevant to the nanoscalar version of the FCS.

Presently, all nanoscale FCSs may not fit the general guidance; hence, the tiered toxicity testing recommendations as outlined in the guidance to industry may not apply to all nanoparticles. Moreover, little is known currently concerning the *in vivo* toxicity of nanoparticles *via* the oral route of administration, and none of the *in vitro* assays used to evaluate genotoxicity have been validated for use with nanoparticles. As nanoparticles may present unique challenges in the assessment of their genotoxicity *in vitro* and their toxicity *in vivo*, the FDA suggests that any submission concerning these nanoparticles consider the validity of the test protocol and the applicability of that protocol to the test substance to assure safety with regard to its relationship to the substance notified for food contact uses.

In addition to addressing the safety of the nanoparticles based on the dietary exposure as outlined in FDA's toxicology guidance, the following should be considered:

- The form of the FCS migrating to food is important. As an example, if only the disassociated ionic form of a nanoparticle metal FCS migrates to food, it may be appropriately conservative to use available toxicity data for the appropriate metal salt to support the safety of the FCS. Should the nanoparticle itself migrate to food, it would be necessary to address the safety of the nanoparticle form of the FCS.
- As stated earlier, the applicability and suitability of the use of a given *in vitro* genotoxicity assay in the safety assessment of a given nanoparticle should be carefully considered, with particular attention paid to the issues of excess cytotoxicity or precipitation. Assessment of the agglomeration/aggregation characteristics and other relevant physico-chemical characteristics of the nanoparticles in the media used in the *in vitro* test system should be performed.
- For *in vivo* studies *via* the oral route of administration, the test substance would be given either in the drinking water or in the diet. Given the fact that agglomeration of nanomaterials becomes more of an issue at high concentrations, gavage with a concentrated solution of a nanomaterial may induce high levels of agglomeration *in vivo*, decreasing the bioavailability of the nanomaterial. Assessment of agglomeration/aggregation characteristics in the drinking water or feed matrix would be important, but *in vitro* pH studies of particle agglomeration at pH 1 and 9 in the absence of feed matrix will not be very relevant to the agglomeration state of the particle in the gut.
- Should the *in vivo* micronucleus assay be the only genotoxicity test able to be used with the test substance, the toxicity of the test substance to the bone marrow should be evaluated to ensure that the test substance reached the target site. Routes of administration other than oral (inhalation and

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intraperitoneal) may be used for this assay, should oral administration not deliver the test substance to the target site.
- Should the notifier wish to use alternative methods for the assessment of the genotoxicity of the nanoscalar FCS that are not currently recommended in the FDA Redbook 2000, consultation with the FDA *via* a pre-notification consultation (PNC) is strongly recommended to discuss the feasibility of this approach.
- In conducting *in vivo* toxicity studies, careful attention should be paid to the issue of dosimetrics. Consideration of surface area and particle number, as well as mass concentration, in the study design is appropriate.

IMPACT OF NANOTECHNOLOGY ON REGULATORY STATUS

The regulatory status of nanoscale versions of FCSs is frequently questioned. In the absence of formal guidance, FDA has been addressing these questions on a case-by-case basis.

For currently approved *direct and indirect food additives*, and food contact substances, the use of the additive is no longer in compliance with an existing regulation if the change in manufacturing practice alters the chemical such that the chemical identity and composition are no longer the same as the approved compound, the use or intended use is no longer in conformity with the regulation in Title 21 of the US Code of Federal Regulations, or the quantity of the additive in food renders it injurious to health.

For FCSs, as defined under Section 409 (h)(2)(C) of the Federal Food, Drug and Cosmetic Act, a FCS approval does not apply to a similar or identical substance manufactured or prepared by a person other than the manufacturer identified in the notification. FDA's long-standing guidance to industry on administrative aspects of a food contact notification advises that a new notification should be submitted if substantive changes are made in the specifications for the FCS or if significant changes are made in the manufacturing method that result in substantive changes in the identity of the product or its impurities, and/or levels of impurities.

For substances *Generally Recognized As Safe* (i.e. having GRAS status) under the provisions of Title 21 of the US Code of Federal Regulations, it is the obligation of the manufacturer to demonstrate whether the ingredient has been affirmed as, or is otherwise, GRAS.[49] Relevant to such a determination are the identity of the food substance and its conditions of use as described in the administrative record for a substance affirmed or identified as GRAS.

Responsibility of the manufacturer or user

Ultimately, it is the manufacturer's responsibility to ensure that the foods and food components that they bring to market are safe and lawful. Thus, the manufacturer or user has an obligation to take all appropriate steps to ensure that the substance as manufactured is safe and lawful under the conditions of its intended use. FDA encourages food manufacturers to conduct a thorough safety assessment of all manufacturing changes.

Guidance for currently authorized food products

As mentioned earlier, a guidance document has been published in June 2014 to address the impact of manufacturing changes, including nanotechnology, on the regulatory status of FCSs.[49] The FDA encourages manufacturers to consult this guidance document, as well as other relevant guidance documents (available at www.fda.gov/food/IngredientsPackagingLabeling/default.htm) and to consult with the FDA before undertaking any experimental activities in support of submission of a future FCN so that pertinent issues can be discussed and mutual understanding on issues be achieved.

ENHANCEMENT OF FOOD PACKAGING PROPERTIES THROUGH NANOTECHNOLOGY

The primary purpose of food packaging is to keep the packaged product, fresh, safe and secure and to prevent damage during transportation and storage. Packaging can also extend the shelf-life of food

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products by controlling the transfer of moisture, gases, flavours and taints, and thus plays a significant role in reducing food waste worldwide. The food packaging in use today is the result of decades of evolution, and during this period, a wide range of materials have been utilized from wooden crates, cardboard boxes, paper bags and glass bottles, to modern polymers that offer stronger, lightweight, recyclable, and in some cases functional materials.

However, all packaging materials have some drawbacks and limitations.

Glass and tinplate provide a perfect barrier to gases and vapour and are recyclable, provided the right infrastructure is in place. However, they have a high specific weight and their production is considered energy intensive in comparison with other materials.

Paper-based materials, on the other hand, are often considered as materials with low environmental impact, as they are produced from renewable resources and are in most cases recyclable. However, they lack mechanical properties (e.g. tear resistance) and product protection properties because they have very low barrier performance to gases and vapour.

Plastic materials provide barrier to either oxygen (or other gases) or water vapour but rarely both. In addition, they are in many cases transparent, which can be seen as a benefit for some applications but can generate photo-induced degradation in some products.

In the case of flexible packaging applications, these limitations are often overcome through combining different layers of materials with different properties and functions. Multi-layering approaches are also used for rigid packages but are technically more difficult to achieve.

Blending of polymers with different properties is another common way to improve the properties of plastic packaging materials.

The ability to combine different materials allows packaging to be developed with all the desired properties for a specific application. However, it significantly increases complexity and cost and can limit the recovery and valorization potential of the materials.

Nanotechnology has the potential to improve the properties of different packaging materials and thus is seen as technology, which can provide enhanced protection without some of the previously mentioned drawbacks and complexity of currently used approaches thus potentially providing benefits in terms of reduced material use, cost and environmental performance.

The application of nanotechnology in packaging polymers has so far taken two major routes – (a) continuous nanocoatings applied on the polymer surface[3] or (b) dispersion of nano-objects or nanophases within a polymer matrix. These approaches are aimed at enhancing barrier, mechanical and/or other functional properties of the packaging materials.

Nanomaterial-polymer composites for improved barrier properties

The incorporation of nanoparticles into polymer materials is reported to significantly enhance barrier properties.[4,12,50,51] Nanoclay-polymer composite, for instance, have exhibited excellent gas barrier properties. The nanoclay mineral most often used to achieve these improved gas barrier properties has been montmorillonite (also known as bentonite), which has a natural nanoscalar layered structure that can restrict the permeation of gases when incorporated into a polymer. It has the added advantage that it is relatively inexpensive and also widely available.

Nanoclays have been added to a range of polymers, with a variety of suggested food packaging applications, such as for processed meats, cheese, confectionery, cereals and boil-in-the-bag foods. Their application in extrusion-coated packaging materials for fruit juices, dairy products and co-injection processes for the manufacture of beer and soft drink bottles has also been suggested. Polymer materials modified with nanoclay include multi-layered film packaging, polyethylene terephthalate (PET) bottles for carbonated drinks and thermoformed containers. A few of these materials are available commercially, and their use has been reported in some countries for bottling beer and other beverages.[7,8,52] Moisture and gas barrier properties of bio-based polymer packaging materials such as starch and polylactide have also been improved with the use of nanomaterials.[51,53–56]

[3]The term nanocoating is not defined in the ISO norms; it will be used here to describe continuous coating with a thickness in the nanorange, excluding coatings of larger thicknesses containing nano-objects.

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A nanoplate material rapidly gaining attention is graphene. Graphene is a single layer of carbon atoms with each atom bound to three neighbours in a honeycomb structure.[18] Several authors have reported barrier improvements by using graphene sheets or graphene oxide in polymer composites.[57–60]

The mechanisms underlying the barrier improvement of nanoplate-based composites is the well-known 'tortuous path' principle, schematized in Figure 2.

High improvement factors can only be reached when the plates are dispersed in the matrix and oriented perpendicularly to the migration direction.[61] A perfect orientation of the particles is difficult to achieve in bulk materials or even in films. New technologies have been developed to address this such as polymer based coatings highly loaded with nanoplates or more recently with layer-by-layer deposition of nanocomposites alternating organic and inorganic layers.

Layer-by-layer is a method by which a multilayer coating/film of nanometre-thick layers is produced by sequential adsorption of oppositely charged polyelectrolyte on a solid support.

Several authors reported the fabrication of multilayered films by depositing intercalated layers of anionic sodium montmorillonite clay and cationic polymer on a polymer substrate yielding materials with extremely good barrier properties.[53,59,62–65]

Another means of improving the barrier properties of a polymer film is the deposition of a thin, metal or metal-oxide or organic-based coating. Nanocoatings have a thickness in the nanometre range and comparatively infinite size in the other dimensions that are deposited on the surface of a substrate (e.g. metallization or SiOx coating). Nanocoatings have been available for decades, and some are extensively used today (e.g. metallization). The principle is to coat a substrate with a thin layer of organic or inorganic material that can act as a barrier to permeation and migration, as chemical protection for the substrate, or as surface property modifiers.[4]

Nanomaterial-polymer composites for improved mechanical properties

The incorporation of nanomaterials has been shown to improve certain mechanical properties of polymers – such as flexibility, durability, temperature/moisture stability, etc. Nanoclays have been shown to improve the mechanical properties of a range of thermoplastic, thermoset and elastomer polymers, such as polyamides, polyolefin, polystyrene, ethylene-vinylacetate copolymer, epoxy resins, polyurethane, polyimides and polyethylene terephthalate.[50,54,55,57,66–72] Some nanomaterials have been used as UV absorbers (e.g. titanium dioxide) to prevent photo-degradation of plastics such as polystyrene, PE and PVC. Nanozinc sulfide, in combination with organic stabilizers has been reported to improve the durability of plastic polymers.[50]

Special case: bio-based materials

Biopolymers have attracted considerable attention as possible replacements for conventional oil based plastic packaging materials. The term bioplastic encompasses a whole family of materials, which differ from conventional plastics insofar as that they are bio-based or biodegradable or both.[73] Bio-based

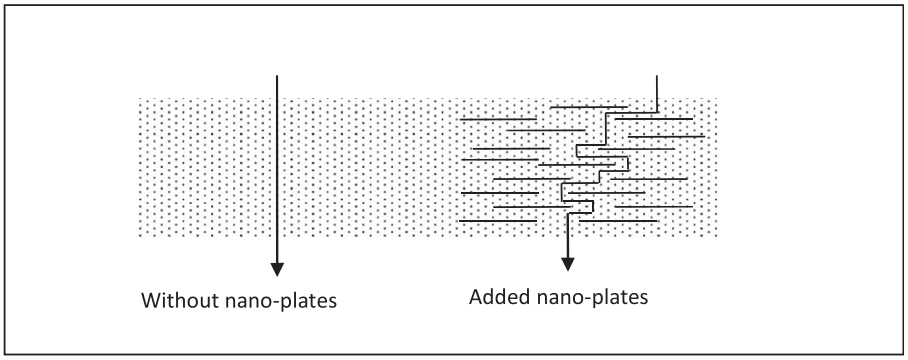

Figure 2. Addition of nanoplates increases tortuosity and decreases permeability

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polymers may be derived from plant materials (starch, cellulose, other polysaccharides and proteins), animal products (proteins and polysaccharides), microbial products (polyhydroxybutyrate and PHB) or polymers synthesized chemically from naturally derived monomers (e.g. polylactide and bio-polyethylene).

For food packaging applications, biopolymers typically have mechanical and barrier properties, lower than conventional plastics, which limit their industrial use. Especially challenging is the development of moisture barrier due to the hydrophilic nature of most biopolymers.

There are also bio-based nanomaterials, which are receiving increasing attention. These are nano or microfibrillated cellulose as well as cellulose nanocrystals. These materials, directly extracted from natural fibres, can improve strength and gas barrier however are still very sensitive to moisture. Their application in stand-alone films, coatings and fillers in nanocomposite systems has been explored. Although still in their infancy, the amount of research performed on these materials is significant, as can be seen for the review articles by Siqueira *et al.*[74] and more recently by Li *et al.*[75]

Research is ongoing to overcome these inherent shortcomings of biopolymer-based packaging materials, with nanotechnology being one avenue of exploration.[51,53–56,59,65,66,68] Nanomaterial-biopolymer materials have the potential to match many of the properties required for packaging applications; hence, their use could increase the potential for biopolymer use.

NOVEL FUNCTIONALITIES OF FOOD PACKAGING PROPERTIES THROUGH NANOTECHNOLOGY

Active packaging

Recent developments on blends have allowed the production of monolayer bottles with oxygen scavenging properties. These consist of blends of PET and oxygen absorbing polymer (typically polyamides or co-polyesters), where the latter is separated through processing and compatibilization of the two polymers into nanoscalar inclusions in the matrix. In most cases, the reaction is catalysed by the presence of cobalt dissolved in the polymer matrix. A strong reduction of the size of the included polymer domains is desirable, both for improving the properties of the blend and retaining a good transparency of the resulting material.[4] To achieve the latter, it is clear that the domain size must be below the wavelength of visible light, hence clearly in the nanometre range (Figure 3).

Antimicrobial packaging has received extensive attention in research, especially through the application of nanotechnology.

As mentioned earlier for oxygen scavenging applications, blending can be used as a means of providing antimicrobial properties to a material, e.g. through controlled release of natural or synthetic antimicrobial agents.[76]

Certain metal and metal oxide nanomaterials are known to have strong antimicrobial properties. Their incorporation into polymer materials has led to the development of FCMs with antimicrobial surface properties. These nanomaterial-polymers are claimed to preserve packaged foodstuffs for longer

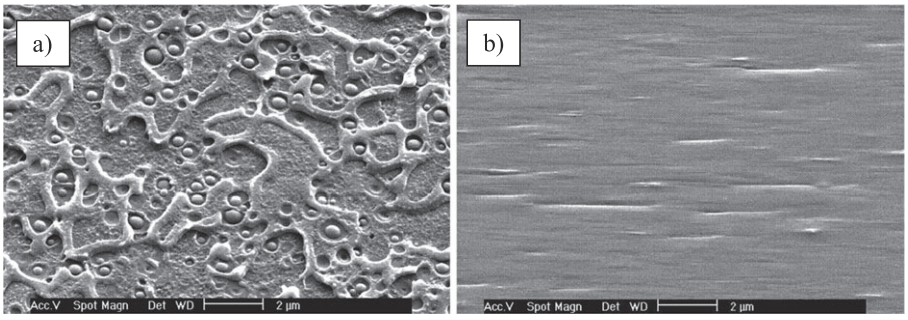

Figure 3. Micrographs of nanostructured oxygen scavenging polymer blends, (a) after injection and (b) after blow moulding

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by inhibiting the growth of micro-organisms at the food contact surfaces. Examples include nanosilver that is claimed to add antimicrobial and anti-odorant properties to plastic food storage containers and bags.[12,67,77–80] The discovery of antimicrobial properties of nanozinc oxide and nanomagnesium oxide may provide further opportunities for the use of less expensive nanomaterials for antimicrobial food packaging materials.[81] A plastic wrap containing nano-zinc oxide is available in some countries, which is claimed to keep the packaging surfaces hygienic under indoor lighting conditions.[27]

Another way to include active functionality through nanotechnology is by grafting active components (anti-microbial, scavengers and anti-oxidant) on clay nanoplates. This approach allows to efficiently disperse the active compound in the matrix, while improving other properties linked to the presence of clay, as detailed earlier.[82–84]

'Intelligent' (or 'Smart') packaging concepts

Nanomaterials can also be used to monitor the condition of packed foods and provide a visual indicator of condition. This functionality is enabling the development of novel intelligent packaging concepts, which can be in the form of sensors (bio or temperature), or nanomaterial-based 'intelligent' inks, which can be printed on labels or incorporated in coatings for food packaging.[85–87] Such labels could, for example, show the consumer that the product is safe to consume or that the package integrity has not been compromised in the supply chain. Other labels can determine the level of microbial activity in a package by indicating when the food starts deteriorating or can provide an indication of time–temperature exposure to indicate if the cold chain has been breached.[88–90]

Nanomaterials are also increasingly found in authentication technologies for food products for instance in nanobarcodes or taggants, incorporated into printing inks or coatings. Another area of foreseen application for these materials is in the development of printed electronics for packaging applications, either as pigment nanoparticles for the inks, or other deposition technologies. Such technologies are expected to appear in anti-counterfeiting, traceability and/or provide other intelligent features. An extreme example of this is given by Vicente *et al*. presenting printable solar cells on liquid carton packaging.[91]

Nanostructured surfaces

The surface of packaging is the part that will interact most with either the consumer or the product. In this sense, modifying its properties can add new functionalities to packaging materials.

Nanostructures at the surface of a material can affect its compatibility with other materials and products as well as induce light diffraction in a controlled way to generate special optical effects.

Structured surfaces can be obtained through material addition (e.g. deposition, coating and printing), material removal (e.g. etching) or by actual structuring of the surface itself (e.g. through nanoimprinting).

Nanostructuring of a surface can drastically change its adhesive properties in one way or another, as can be observed in natural instances, such as the lotus or gecko effects. Such structures have, for example, shown a drastic reduction of biofilm formation by reducing the adhesion of bacterial cells to solid surfaces.[92,93]

Although not yet broadly applied, the authors believe that nanostructured surfaces will play an important role in providing new functionalities to packaging in the future.

MIGRATION, OTHER EXPOSURE ROUTES AND ENVIRONMENTAL CONSIDERATIONS

Despite the anticipated benefits of using nanomaterials in food packaging, their larger scale adoption would require that open questions regarding effects on consumer health and the environment are addressed. These questions, and consequently, challenges to broader use have been highlighted in recent publications,[7,8,52] and discussed at several meetings, workshops and conferences.[94–96] The main issues relate to uncertainties and knowledge gaps regarding the possible health effects of nanomaterials when used in food packaging and the lack of analytical tools and methods to assess the migration of nanomaterials in particulate form to food products, which are necessary to assess potential exposure.

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To address the open questions, it is imperative that the effects and impacts of nanomaterial containing packaging on food quality, consumer health, and the environment are investigated and understood to facilitate further developments in this area.

This chapter provides an overview of the current state-of-knowledge in respect to these issues.

Migration testing

Nanomaterials incorporated in plastic polymers are present in embedded, fixed or bound forms. Like other chemical constituents in FCMs, nanomaterials could have the potential for transfer to foods with which they are in contact. Thus, a fundamental question in relation to compliance and safety is whether or not any nanoparticles can migrate from such materials to packaged foodstuffs in significant quantities.

Nanomaterial migration can occur through mechanisms such as, dissolution of the compound in a simulant or food, actual diffusion of particles or transfer through abrasive action on the surface of the FCMs.

Today, the migration of solubilized nanoparticles can be evaluated as well-established methods of detection are already in existence.

On the other hand, assessing the migration of nano-objects in particulate form is more complex but remains of prime importance as their possible occurrence in food represents the main source of uncertainty regarding safety.

Currently, the level of nanoparticle migration expected is very low (as shown in subsequent sections). Consequently, the methods used need to allow separation, concentration and determination of chemical identity as well as the particulate nature of nanomaterials. The possible creation of new nano-objects for instance, during the concentration phase (e.g. through precipitation of solubilized compounds) must also be avoided.

The behaviour of nanomaterials is governed not only by the chemical composition and the size of the particles but also by other physico-chemical properties such as surface composition, surface morphology, surface charge and distribution of charges and also thickness of interfacial membranes.[97] Multiple techniques are therefore needed for characterization. Generally, methods can be classified as those yielding information on (a) composition, (a) morphological structure and (c) physico-chemical properties.

The description of these methods is out of the scope of this paper but can be found in the following references:[37,97–99]

This paper will instead focus on the methods used for the assessment of migration from FCMs containing nanomaterials.

Migration tests

Regulation require that packaging materials be tested for their suitability for use in food contact applications. These tests are generally carried out using food simulants that mimic the migration effects in different types of foods. In general, it is accepted that this same approach is also appropriate for assessing the migration of nanoparticles.[40–43,100,101] In Europe, migration testing for nanomaterials will need to be carried out in accordance with the existing legislation,[32] which defines the conditions and the simulants for plastic FCMs. Migration testing is carried out under conditions that are equivalent to the worst foreseeable conditions of use. The exposed simulant samples and controls (blanks and overspikes) are subjected to analysis for the detection and quantification of migrating substances using a range of analytical methods.

Chemical characterization of migrants

Any compounds migrating from the packaging to the food or simulant can be assessed by different means, depending on the chemical nature of the expected migrants.

In some cases, the migrants can be directly analysed while other application require extensive samples preparation, especially when migration is directly measured into food rather than food simulants.

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The most commonly used sample preparation method is matrix degradation. Here, either enzymatic or chemical (alkaline or acidic) digestion or thermal degradation of the matrix is carried out, followed by dissolution of the obtained ashes.

Once sample preparation is completed, the resulting solution or suspension can be analysed to detect compounds or elements contained in the nanomaterial of interest.

In the case of inorganic nanoparticles, the most commonly used analytical method is inductively coupled plasma (ICP) coupled with either mass spectroscopy (ICP-MS) or atomic emission spectrometry (ICP-AES), also referred to as optical emission spectroscopy (ICP-OES).

In these methods, the sample is directly vaporized in plasma where the elements composing the substance studied are liberated as atoms or ions.

In the case of ICP-MS, the formed ions are analysed by mass spectroscopy, whereas for ICP-AES, the radiation generated by the transition between excited and relaxed state of the atoms or ions is detected. The energy of these transitions is element dependent and can therefore be used for identification.[102]

Another commonly used analytical technique is atomic absorption spectroscopy, which determines the concentration of specific element in an atomized gas using light absorption characteristic of specific elements. Different atomization technologies can be used; the most common being flame and graphite tube atomizers.

Being the most sensitive, ICP-MS is the most used technique when migration studies are being performed.

Physical characterization of migrants

The chemical identification methods presented earlier allow migrants to be identified along with their concentrations. They do not, however, identify the nature of the migration, i.e. whether it happens through solubilization of components or through actual particle transfer. In order to assess the risk of migration from FCMs, it is essential to understand the mechanism behind the migration as the toxicological properties of a substance in particulate form could differ from that in solution.

In order for particles to be identified, sample preparation must be performed in such a way not to alter potentially present particles or to create new particles.

As in the case of chemical analysis, matrix digestion can be applied. Care must be taken to ensure that target nanostructures are not broken down or digested. For example, if the nanostructure is composed of organic compounds, it may be subject to hydrolysis or oxidation reactions that may alter or destroy it. Where nanostructures are initially present in a packaging material, this scenario is relatively easy to test, as the nanomaterial is known and could be obtained and tested separately.

In some cases, extracts obtained using food simulants can be further processed without digestion.

Once the matrix has been digested, nanostructures present can be concentrated or extracted by filtration, centrifugation or solvent extraction. It should be noted that separating nanostructures using these traditional separation techniques is not trivial, and other separation techniques have been developed recently, some of which are described in the following paragraphs.

A very powerful separation technique that promises to be of particular importance for the determination of the particulate migration is field flow fractionation (FFF). FFF allows the separation of mixtures that contain compounds that vary substantially in their molar mass.[103] Therefore fractionation of macromolecules from nanoparticles or microparticles is made possible.[104] In principle, the technique is a liquid chromatography-type elution method where the separation is accomplished by applying a fractionation flow field perpendicular to the flow field that is established to transport the sample through the separation chamber. During the application of the fractionation flow field, the sample is displaced to the outer walls of the separation chamber. After a brief relaxation period, small molecules or structures rapidly diffuse back into the centre of the separation chamber. The subsequently applied transport flow field that typically has a parabolic flow profile, rapidly transports the small structures in the centre of the chamber, where flow speeds are highest, to the outlet while larger particles exit the chamber later.

A recent evolution of FFF, i.e. Asymmetric flow FFF (AF4), has recently gained much attention as separation technique, especially in the field of determination of nanomaterials in food, but also in testing the migration from FCMs.[105–109] Contrarily to FFF, the cross flow is generated by the presence of a single porous membrane at the bottom of the system, enabling a more efficient size separation.

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Other separation techniques that are under investigation to detect nanostructures in food include hydrodynamic chromatography, where a size dependent exclusion from the wall of a microchannel allows a separation of larger particles from smaller ones. The laminar flow profile that is established in the small microchannel prevents larger structures from entering the slow flowing regimes near the wall. As such, larger structures are ejected faster from the microchannel while smaller ones remain in the separation chamber longer.

These separation techniques can then be coupled with various detection techniques such as Multi-Angle Laser light scattering (MALLS), ICP-MS, UV–Vis spectroscopy and Dynamic Light Scattering, among others, or combinations thereof.[37,98]

Another particle detection method that gains interest is single particle ICP-MS. In this method, a highly diluted suspension of nanoparticles is injected in the ICP-MS. The dilution allows that particles potentially present in suspension will enter the plasma one by one, creating high intensity peaks in the signal; thanks to the sudden high concentration of the analysed element. The presence and intensity of these peaks allow both the identification of particles and the determination of their size. A constant background signal indicates the presence and concentration of the substance in a dissolved state.[109,110]

Transmission and scanning electron microscopy or other imaging techniques are also regularly used to identify the presence of particles, sometimes coupled with elemental analysis. These techniques however imply the extraction of the particles from the fluid they are suspended in, e.g. through evaporation. This sample preparation step could, if the substance is solubilized, generate particles through precipitation, hence potentially leading to erroneous conclusions.

Potential migration of nanomaterials

Despite the need for understanding the migration behaviour of nanoparticles from nanomaterial-polymer based food packaging, the number of studies available in this area remains fairly low and mainly focused on nanosilver and nanoclay materials. It emerges from these few studies that nanoparticles are considered unlikely to migrate to a large extent under normal conditions of use for packaging materials.

Avella *et al.*[51] determined the migration of Fe, Mg and Si from a biodegradable starch/nanoclay composite film. The study used vegetable samples (lettuce and spinach) placed in bags made of either potato starch, potato starch–polyester blend or their respective composites with nanoclay. After storage for 10 days at 40°C, the vegetables were acid-digested, and the migration of minerals was determined by atomic absorption spectrometry. The results showed no significant increase in Fe and Mg in the vegetables compared with controls, whereas an increase in Si content was recorded. The concentrations of Si detected in the vegetables were 16–19 mg/kg in the case of nanoclay composites of potato starch and potato starch–polyester blend, compared with 13 mg/kg for the same polymers without nanoclay and around 3 mg/kg in unpackaged vegetables.

Xia *et al.*[111] studied the migration of nanoclay from polypropylene (PP) and PA nanocomposites, showing low levels of migration in the range of 80 to 150 µg/L, depending on the matrix material and the simulant. The characterization of actual particle release was carried out by evaporating the extract on a copper grid and observed by TEM. A known drawback of this method is that the substance may precipitate during the evaporation. The results showing actual particle migration were, therefore, not conclusive. The authors also studied the evolution of the concentration over time, showing a fairly rapid increase during the first hours or days of the experiment followed by a plateau where the concentration remained constant. This suggests that only particles at the surface of the polymer film were extracted or dissolved.

Farhoodi *et al.*[112] studied the migration of clay nanoparticles from PET bottles into acidic food simulant. In this case, a steady increase in the concentration of both aluminium and silicon was observed over a period of 90 days by ICP-OES. The study concluded that nanoclay particles could migrate from PET nanocomposite, although no data was provided to support the hypothesis. On the contrary, the concentration ratio between aluminium and silicon in the extract was approximately 10 times lower than what is theoretically present in clays. Actual particle migration would be expected to display a ratio much closer to the natural ratio.

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Chaudhry *et al.*[113] studied migration from multilayered PET bottles with nanoclay composites embedded between PET layers. The migration testing was performed using different foods and simulants. ICP-MS analysis showed no detectable migration of clay minerals from PET bottles.

The migration of clays from low density polyethylene bags was studied by Echegoyen *et al.*[114] Here some aluminium was detected, indicating that migration did occur. This paper also showed that, based on single particle ICP-MS results, a certain fraction of the clay migrated as particles.

Huang *et al.*[115] studied the migration of nanosilver from commercially available polyethylene plastic bags containing 100 µg silver per gram of the plastic material into food-simulants. The silver nanoparticles ranged from 100 to 300 nm in diameter. Migration testing was carried out over a range of temperatures between 25°C to 50°C and time intervals between 3 to 15 days. The food simulants used in the study included water, acetic acid (4%), ethanol (95%) and hexane. The study reported migration of nanosilver from the polyethylene bags to food-simulants, which seemed to increase with storage time and temperature. The lowest migration was observed in 95% ethanol, although there was no significant difference in the amounts recorded in all simulants – i.e. they ranged between 0.5 and 1.0 (at 25°C) µg/dm^2 of the polyethylene material, and between 3.0 and 4.0 µg/dm^2 (at 50°C) after 15 days. Scanning electron microscopy and energy-dispersive X-ray analysis (EDX) were used to confirm the presence and morphology of the migrating silver, and quantification was carried out by atomic absorption spectroscopy. The sample preparation method used for microscopy experiments involved solvent evaporation; hence, there was potential for creating particles through precipitation. The likelihood of this occurring is considered high because the particles observed were much larger than what could have been expected (300 nm or larger).

Metak *et al.*[116] studied the migration of silver nanoparticles from polyethylene containers and films into various foods. A combination of ICP-MS and electron microscopy was used to quantify the amount of silver migrating from the packaging material and characterizing the form under which the migration occurred. In the case of milk powder, electron microscopy coupled with energy-dispersive X-ray analysis was used directly on the product stored in the packaging during the migration test and showed that some silver nanoparticles were released to the product. Liquid products first undergo drying, ashing and finally digestion prior to scanning electron microscopy analysis. It can therefore not be concluded whether the observed particles migrated or whether they have been generated during the evaporation process. The conclusions of the paper are, therefore, considered only partly valid.

Chaudhry *et al.*[113] studied migration from polypropylene food containers, which were claimed to have dispersed nanoparticles of silver within the polymer matrix. Extensive migration testing was performed using different foods and simulant systems. ICP-MS analysis showed the migration of silver from polypropylene to be lower than the limit of quantification.

Cushen *et al*. also reported the migration of silver nanoparticles from various matrix materials by ICP-MS in a series of publications.[117,118]No information was gained on the nature of the migration, and the assumption of worst case, i.e. particulate migration, was made for the subsequent exposure assessments. A more recent paper,[119] by the same authors, compared TEM images of the silver particles prior to insertion in the matrix and after migration, which showed a significant reduction in size of the particles. These results led them to conclude that the majority of the silver migrated in the ionic form.

Greiner and Hetzer[120] studied the migration of silver from polyethylene films. The conclusion of this work was that the total amount of silver migrating out of the material, as determined by ICP-MS was low. The authors also used single particle ICP-MS to determine the proportion of particulate migration and found this to be less than 1% of the total silver migration.

The same testing methods were applied by Echegoyen and Nerín;[121] the proportion of particulate migration was much larger, i.e. ranging from 1 to 20% of the total amount of silver migration. It is to be noted that in both cases, the level of migration was below the maximum migration limit for silver found in legislation (valid for solubilized silver).

The difference observed in the fraction of particle migration in the two studies mentioned earlier[120,121] can be due to factors such as the initial concentration or the interaction between the particles and the matrix. It could also be explained by the findings published by Bott *et al*. in their study on the migration of nanosilver from LDPE polymer.[122] In this study, the migration of silver was tested in various simulants and under different conditions, using ICP-MS and AF4/MALLS to assess particulate migration yielding similar conclusions to those drawn in Greiner and Hetzer[120] and Echegoyen and

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Nerín.[121] The authors however further measured the stability of the silver nanoparticles in the food simulants by injecting the same samples hourly over a period of 5 h, which showed a rapid dissolution of the particles into silver ions in acidic food simulants. This could further explain part of the discrepancies observed in different studies. As a comparison, the authors added silver nanoparticles to ultrapure water under these conditions; they were more stable with an 80% retention of particles after 24 h, showing the validity of the approach.

Bott *et al.* also[123] demonstrated that carbon black nanoparticles embedded in polyethylene or polystyrene did not migrate whatever the conditions or simulant used. This study also used a combination of AF4 and MALLS for the detection of particulate carbon black in the extracts. This allows an unambiguous identification of carbon black particles, considered difficult with other techniques due to the chemical nature of the compound, i.e. practically pure carbon. The results were further validated using a simulation approach. The combination of AF4 and MALLS for the determination of migration from nanocomposites was first been described by Schmidt *et al.*[124] in a study aiming to determine whether nanoclay particles migrated from a poly(lactic acid) (PLA) matrix.

In addition to the previously mentioned studies, the EFSA Panel on FCMs, enzymes, flavourings and processing aids (CEF) has issued several scientific opinions on the use of nanomaterials in FCMs. These opinions concern titanium nitride in PET bottles and films,[41,101] silicon dioxide, silanated,[100] kaolin[42] and montmorillonite clay.[44] All these opinions concluded that the use of these materials do not represent a health risk because no exposure would occur, as proven by absence of migration. The techniques used for the determination of migration were ICP-MS and AF4-MALLS.

A more recent scientific opinion was given on the use of Zinc oxide in FCM, based on migration results obtained by ICP-MS and ICP-AES.[43] The results indicate that the migration of Zinc occurs to a significant level, while still remaining compliant with the current specific migration limit (SML) for Zinc. Although no proof of the absence of migration in particulate form was given, the panel concluded that the substance does not migrate in nanoform and that the safety evaluation should focus on the migration of soluble ionic zinc. The conclusion is based on the understanding that even if particulate Zinc oxide would migrate, it would immediately dissolve in acidic foods or stomach acid. The panel however recommends that the current SML for zinc should be reduced to take into account zinc exposure from other sources.

There is an additional concern that nanomaterials added to polymer matrices could also influence the migration of other compounds present in the polymer. Chaudhry *et al.*[113] showed that this was not the case in either of the two nanomaterial-polymer materials tested in their study.

Work by de Abreu *et al.*[125] investigated the effect of nanomaterials on the migration of caprolactam, triclosan, and trans, trans-1,4-diphenyl-1,3-butadiene (DPBD) from polyamide and polyamide/nanoclay composites to different food simulants. The presence of the nanoparticles was reported to slow down the rate of migration of (non-nano) substances from the matrix polymer into the food by up to six times. This reduction was not only related to the tortuous path created by the presence of the nanoplates, which is the basic principle of barrier property improvement, but also to a potential interaction between the non-nano substances and the clay particles.

Modelling as assessment tool for migration

In addition to experimental protocols, modelling is a useful approach, which can provide information about the potential risk that may arise from nanoparticles migrating into food from packaging material.[126–131] A report by the Joint Research Centre of the European Commission[132] provides an excellent overview of available mathematical models that can be used to describe the migration of compounds incorporated in a plastic packaging material with respect to environmental conditions, composition and structure of the FCMs, the nature of the migrant. Available mathematical equations are largely based on a diffusion-driven mass transport process, which is governed by the diffusion coefficient of the migrant and the partition coefficient for the migrant between the plastic and the food simulant.[133] Experimental studies have shown that available models are capable of predicting the migration of compounds from a packaging matrix into a food, as long as the process is indeed diffusion driven and not due to other mechanisms such as erosion, swelling or abrasion of the packaging materials. For nanomaterials, the modelling may also be used to better understand potential exposure scenarios.

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Šimon *et al.*[134] used a physico-chemical approach to model the factors that control migration of nanoparticles from nanomaterial polymer composite materials to food. The modelling performed suggests that migration of nanoparticles is only likely to take place when an equilibrium distribution of nanoparticles is established between the packaging and the packaged food. Because the concentration of nanomaterials in both polymer composite and food is likely to be small, nanoparticle interaction is predominantly expected to be with the surrounding matrix. In such a situation, standard chemical potentials should reflect the strength of the interactions between the nanoparticle and the polymer or the packaged food. Because the equilibrium distribution of nanoparticles between the food and the polymer are likely to establish over a period of time, the resulting migration would also be time dependent. The approach considered that a few important variables influence the migration of nanoparticles, such as (the square root of) time, the temperature and the radius of a nanoparticle. Movement of a nanoparticle would also be affected by interactions with the polymer matrix, hence the dynamic viscosity of the polymer are likely to have a major influence on the overall migration. The model developed by Šimon *et al*. predicted that significant migration of nanoparticles from packaging to food can only be expected if the following conditions are met: (a) very small nanoparticles are present (with a radius in the order of a few nanometre); (b) the polymer matrices have a relatively low dynamic viscosity; and (c) the matrices do not interact with the nanoparticles. Example materials that fulfil these conditions include small nanoparticles of silver in polyolefins (LDPE, HDPE, PP). The model also predicted that detectable migration would be unlikely for larger nanoparticles bound in a polymer matrix with a relatively high dynamic viscosity. Examples of such polymers are polystyrene and PET. The model estimated a migration of 0.26 mg of nanosilver from 0.2 m^2 of LDPE containing nanosilver at a concentration of 1 g/dm^{-3} over a one year contact with food at 25°C. Under comparable conditions, a migration of 0.22 µg of nanosilver was estimated from PET packaging to food.

Recently, Cushen *et al*. presented a new model[117] based on the Simon *et al.* model,[134] showing favourable correlation between experimental and predicted migration values for silver, although the model was much less precise for the migration of copper. The authors attributed the lack of precision to the high variability of copper content in the chicken used for the migration tests. The use of food simulant rather than actual food was consequently recommended for further validation of numerical modelling of migration.

Building on previous work, Bott *et al*. presented a model estimating the migration of chemical substances[132,135] in which the molar cross sectional area[136] is replaced by the volume or size of the nanoparticles. This model suggests that particles larger than 3.5 nm in diameter will not migrate from polymer matrices, when diffusion is considered the sole mechanism. The authors concluded that, due to the usual size, shape and aggregation or agglomeration of the particles used in FCMs, they would actually be immobilized in the matrix, and thus prevent possible consumer exposure.

DISCUSSION

The studies cited earlier show that in the last few years, several analytical techniques have been developed and applied to the assessment of the migration from nano-based packaging materials into food or food simulants. This topic has also been reviewed in a recent paper by Duncan and Pillai,[137] reaching similar conclusions. The knowledge generated by these studies will help to better assess the risk of using nanomaterial in contact with food.

The general conclusions that can be drawn are the following:

- Nanomaterials contained in FCMs are shown to migrate in some applications to a quantifiable level.
- The materials that showed the highest migration are those intentionally designed to fulfil a function that requires a certain migration level, i.e. active packaging materials. All studies nevertheless show that the levels of migration remain in the limits set by regulation for dissolved species.
- The migration of actual particles cannot be excluded, but appears to be limited to particles on the surface of the material directly in contact with the food or the simulant, even in the case of matrices of low dynamic viscosity such as polyolefins. This would mean that, in the case where direct

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food contact is not required to fulfil the function, any direct food contact layer not containing nanomaterials would be sufficient to prevent any particulate migration and act as a functional barrier. This is currently not well taken into consideration by regulations on FCM's containing nanomaterials.

These conclusions would suggest that, in most applications, classical risk assessment could be applied to FCM's containing nanomaterials. In the cases where particle migration could be proven, the risk assessment would have to be based on the potential toxicity of substance in nanoparticulate form, which, as will be shown later in the paper, is not straightforward.

Migration, if at all occurring, is not the only route through which human exposure to nanomaterials could occur. Other exposure routes as well as some environmental consideration are exposed in the next sections.

Possible release and exposure scenarios for nanomaterial-polymer packaging materials

Manufacturing. The handling processes involved at the manufacturing stages – e.g. powder handling, blending and disposal of waste materials involving nanomaterials during dispersion in solvents or polymer melts – may generate particulate emissions. Hence, provide the possibility for worker exposure. Factors that may influence the potential for release of nanoparticles include the physical state of the supplied materials (e.g. powder, dispersion and pre-mixed batches), quantities to be handled, dustiness of the blending process, containment and particulate control measures in place (e.g. ventilated enclosures or LEV). Mixing tasks have demonstrated release potential of nanomaterials (e.g. Han *et al*.[138]).

Similar programmes with other nanomaterials (e.g., TiO2, SiO2 and nanoclays) have included monitoring of the workplace air during continuous melt compounding and post processing operations (grinding and cutting). The grinding and cutting tests of such composites have not thus far indicated any detectable regeneration of nanoparticles.[139]

The few studies carried out so far have indicated that nanoparticle emissions during manufacturing stage can be effectively controlled through appropriate engineering measures. The management of these risks is described in details in the ISO/TS 12901 series.[140,141]

Transportation. Post-production transportation of nanomaterials and nanomaterial-polymer resins is likely to be carried out in sealed containers. Hence, a risk of exposure or release would only be expected in the case of an accident for instance where a sealed container is breached or nanomaterials containing polymers are combusted in an uncontrolled environment.

Use. Releases of any significant quantities of nanomaterials are not anticipated under normal use of the nanomaterial-polymer materials. As described in the previous sections, the use of such materials in food packaging may lead to migration of nanomaterials into food; however, the available studies discussed earlier and the modelling estimates indicate that the levels of nanomaterial migrating from plastic polymer-nanomaterial based packaging materials to food are likely to be either nil or very low. However, depending on the nature of nanomaterials and polymers, there may be some exceptions. For example, more studies are needed to establish whether migration patterns for nanoparticles in different polymer types – especially biopolymers – are not any different from those observed and estimated in the few plastic polymer systems tested so far. The likely contribution of surface aberration of packaging in the transfer of nanoparticles to food (especially during re-use of FCMs) is currently not known. This is further emphasized in a recent review by Duncan[142] on the potential release of nanoparticles through matrix degradation, which clearly shows that this aspect has hardly been studied for packaging material. It should nonetheless be noted that packaging materials, during their use phase are rarely exposed to weathering conditions that are reported to have significant effects on particle release. Abrasion of nanocoating by mechanically aggressive food products has however been observed.[143] This abrasion has however degraded the properties of the packaging material to a point where they were not fit for the protection of the product.

End of life. Even with the increasing emphasis on recycling, a significant proportion of nanomaterial-polymer packaging is likely to be disposed of in landfills or littered into the environment. These

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packaging materials will eventually degrade in the environment because of physical and biological factors, resulting in the possible release of nanoparticles into the environment. Like other environmental contaminants, any persistent nanomaterials released from packaging materials are likely to end up in soil and aquatic environments. A few modelling studies (such as Boxall *et al.*[139]) and reviews[142] have estimated the likely concentrations of nanomaterials in the environment from the current use and disposal of nanomaterial-containing consumer products (including packaging). Boxall *et al.*[139] estimated the expected environmental levels from such route to be very low – in the order of lower parts per billion for most nanomaterials. However, these estimates were based on simple modelling parameters and did not take into account the persistence, concentration or accumulation of some nanomaterials in the environment. The accumulation or concentration of nanomaterials in any of the environmental compartments will be dependent on the chemical reactivity (or inertness) and persistence to physical, chemical or biological degradation.

It is currently not clear how industrial waste from nanomaterial-polymer composite manufacturing facilities should be dealt with, i.e. whether it should be recycled, incinerated or land-filled. Some materials, such as CNTs are known to be completely degraded when heated at 740°C under oxidative conditions. Most organic nanomaterials are also likely to be degraded during incineration. It is, nevertheless, imperative that if final disposal of nanomaterial-polymer based packaging is through incineration; it is carried out under appropriately controlled conditions. Some nanomaterials, such as metals and metal oxides, may survive incineration process and may need subsequent chemical treatment. Nanomaterial-polymer composites and resins are also likely to be disposed of to landfills. It is, however, not known whether nanomaterials may also be released from the packaging materials and migrate to soil/ water environments.

Bio-based polymer can, in some cases, be more sensitive to matrix degradation, which could increase the release rate of the nanomaterials in the environment. This would especially hold true for biodegradable or compostable polymers. Such considerations are rarely taken into consideration in studies claiming environmental benefits for the use of bio-based matrix nanocomposites.

Recycling of the used nanomaterial-polymer materials may involve separating, chemically cleaning, grinding, chopping, milling, melting, mixing, pelletizing, and compounding of the disposed packaging materials. It is currently not clear whether and how separate collection/separation streams for nanomaterial-polymer material will be set up and work. There is a strong likelihood of nanomaterial contamination of other recycled polymer materials if the nanomaterial-containing packaging is not separated prior to recycling. Whether this will be detrimental to the quality and safety of recycled materials still has to be evaluated.

There is a potential for exposure of workers to nanomaterials during recycling, but it will depend on the processes involved, the degree of manual handling, and the safety measures in place. Some of these processes may lead to worker exposure if they are not carried out as closed processes or under appropriate engineering controls.[140,141]

The main emphasis from the exposure point of view may need to be on stages in the lifecycle of products, beyond that of manufacture where emission control measures are unlikely to exist or where the same level of process control may be achieved.

Environmental considerations

Life cycle assessment (LCA) is a methodology, which enables one to quantify the environmental impacts associated with a specific service, manufacturing process or product. The methodology enables one to take a holistic approach and consider the complete product life cycle from the extraction of the required raw materials to the point where all residuals are returned to the earth. Typically the life cycle is partitioned into the main life cycle phases: (a) raw materials, (b) manufacturing, (c) use and (d) end of life. As well as considering the complete life cycle, several environmental impact categories are considered to ensure that a holistic view is obtained and that burden shifting is avoided (where impacts of one sort are reduced at a cost of another). Climate change, ozone depletion, tropospheric ozone creation (smog), eutrophication, acidification, toxicological stress on human health and ecosystems, depletion of resources and land use are all impacts categories, which can be considered. LCA can assist with optimizing the environmental performance of a product or for comparative assessments

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between products to determine the most environmentally favourable. LCA is considered the most widely accepted approach for assessing environmental performance and is supported by a set of standards from the International Organization for Standardization namely ISO 14040[144] and 14044,[145] which provide guidelines on how to define and what to include in an LCA.

A workshop organized by Woodrow Wilson Institute for International Scholars and the EU Commission in 2006 concluded that the ISO-framework for LCA is also applicable to LCA of nanomaterials and nanoproducts.[146] A few limitations were identified, such as nanomaterial-containing products may have new functions for which it may be difficult to use a conventional (benchmark) functional alternative for comparison; the inventory may be difficult to develop because of rapidly evolving production technologies; or the impact assessment of risks of nanomaterials may be difficult because of the lack of data on release exposure and effects.

While the approach is already established and successfully used for many products, there are some limitations with relation to its use for nanotechnology derived products. The current scarcity of data on characterization of both hazard and exposure of nanoparticles is one such gap that is confirmed and even emphasized in recent review papers on the topic.[147–150] It is worth noting, for the non-specialist, that LCA is not a tool for exposure assessment, but information on exposure assessment is essential in LCA for the impact assessment of nanomaterial releases. Despite this, and until these data gaps are addressed, LCA still remains a useful tool for the assessment of nanomaterials containing products. For example, LCA can provide important information to support decisions on, and during, the development of new nanomaterials or nanomaterial-products.[150,151] For instance by identifying opportunities for process improvement along the production cycle or by quantifying potential environmental benefits of use associated with improved functionality or material performance.

Despite the current lack of data on the effects of exposure and release to the environment, there are still examples of where LCA has been used for nanomaterial-containing polymer-nanomaterial composite. One such study is by Roes *et al*. where a PP/layered silicate nanocomposite is assessed as packaging film.[152] The purpose of the LCA was to investigate whether the use of a PP nanocomposite has environmental advantages over the use of conventional polyolefins. The study took into account several impact categories, however again highlighting the discussed gaps in knowledge, did not take into account potential toxicity or ecotoxicity associated with the nanoparticles. The findings of the LCA showed benefits in terms of reduction of materials used to achieve the same level of performance and functionality (e.g. barrier properties) of PP. The material reduction amounted to 9% for packaging film and 36.5% for agricultural film. This reduction in material usage will undoubtedly have a positive effect on environmental performance and highlights some of the advantages that nanotechnologies can bring; however, these benefits will of course have to be assessed against potential negative impacts, which cannot currently be taken into account modelled.

Actual occurrence in the environment. A recent report,[153] published by the Danish Ministry of Environment and Food presents the results of an environmental assessment of nanomaterial use in Denmark. This study evaluated the risk of the current presence of nanomaterials in fresh water and effluents from sewage treatment plants by calculating the ratio between the predicted environmental concentration and the predicted no-effect concentration. For ratios smaller than one, the biological effects of the concentration were considered to be acceptable. The study concludes that the 10 nanomaterials evaluated do not present environmental concern at their current usage state. It however suggests that some of these deserve continued observation, due either to their already high occurrence in the environment (e.g. TiO2 and carbon black) or due to their potentially high eco-toxicity (silver, copper oxides and carbon nanotubes). The source of the materials is clearly not uniquely related to packaging materials, but all of the materials evaluated in the study have been evaluated or used in food contact applications.

IMPLICATIONS FOR RISK ASSESSMENT

Introduction

As a general principle, an FCM must not pose a risk to consumers upon use. Therefore, a risk assessment is performed to determine if an FCM is safe for its intended use. Data concerning the migration of

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components from the FCM to the food matrix provide an indication of the potential exposure of consumers to migrants. The 'Food Contact Materials Note for Guidance', published by EFSA,[154] provides guidance on the interpretation with respect to FCM regulation. This also applies to nanomaterials, which are intended for use in FCM. However, this guidance does not cover nanomaterials that have different characteristics and physico-chemical properties to non-nano equivalents. Recently, EFSA has published a guidance note on the risk assessment of the application of nanosciences and nanotechnologies in the food and feed chain.[97] This guidance provides the latest views on how to deal with nano-related aspects for hazard, exposure and risk characterization within the food chain. In the USA, no guidance is available for the safety evaluation of nanomaterials; the US-FDA performs safety evaluations of nanomaterials on a case-by-case basis, as detailed in a previous section of this paper.

Detailed procedures for risk assessment of nanomaterials are still under development, even though some approaches for safety assessment in food have been published.[155,156] The scientific committee on emerging and newly identified health risks[157] reported that it can be expected that these procedures remain under development until there is sufficient scientific information available to characterize the possible harmful effects on humans and the environment. Therefore, within this chapter, implications for hazard and risk assessment of nanomaterials are provided as a general guide.

Exposure assessment

The starting point in the evaluation of the safety of a nanomaterial is to determine the exposure, which depends on the potential of the nanomaterial to migrate from the FCM to the packaged food, irrespective of its function in the FCM. If adequate migration testing shows no measurable migration of nanomaterials from the FCM to the simulant, no human exposure via food consumption will likely occur. Consequently, there will be no overall risk to the consumer and further toxicity testing will not be needed.

In the case where it is demonstrated that nanomaterials are capable of entering the food matrix, the solubility of the nanomaterial in the food matrix and/or upon gastrointestinal passage is of relevance. For nanomaterials that completely dissolve in the food matrix, the hazard and risk upon exposure will be similar to its non-nanoform. Where it is demonstrated that the nanomaterial originating from an FCM is present in the food matrix but is dissolved completely before intestinal absorption can take place, the safety upon exposure should be evaluated related to the relevant guidance for the non-nanoform. Hazard and risk assessment can be performed in this case using toxicity data of the non-nanoform. In case no conventional counterpart is known or no toxicity data are available, the normal testing strategy for FCM applies.

For further details on exposure assessment for FCMs reference is made to the 7th Framework EU funded project flavourings, additives and food contact materials exposure task, or in short FACET (http://www.ucd.ie/facet/links/).

Determining solubility in the food matrix is relatively straightforward, and tests can be integrated during migration testing.

Solubility in gastro-intestinal fluids can, however, be more challenging to determine depending on the nanomaterial in question. *In vitro* gastro-intestinal absorption systems or stability in gastric fluids will likely provide the most accurate information. For nanomaterials, which are assumed to completely dissolve in gastrointestinal fluids, the dissolution process of the nanomaterials is important to consider. In case the dissolution is relatively slow, it cannot be excluded that nanomaterials may become systemically available and/or some local 'site of contact' effects may occur. Therefore, the location and timing of the dissolution process should be evaluated and discussed to ensure that the nanomaterial is dissolved completely before intestinal absorption takes place.

Although *in vivo* studies seem the most appropriate it should be noted that evaluation of absorption *in vivo* may overestimate the dissolving potency of a nanomaterial, because of the time needed to sample and evaluate the intestinal content for the presence of nanomaterials.

Characteristics of nanomaterials from FCM

Where it is demonstrated that a nanomaterial will migrate to food and will not (completely) dissolve in the food matrix and/or during gastrointestinal passage, the hazard and risk upon exposure of the

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nanomaterial must be evaluated. The hazard upon exposure will be dependent on the characteristics of the nanomaterial in question.

A nanomaterial migrating from an FCM may have different characteristics to the one added during processing. Possible modifications include coating of the nanomaterials by components of the matrix material or the inclusion of the nanomaterial in migrating components of the matrix. Consequently the particle size, shape, available surface area, surface chemistry, etc. may be altered, which may result in a modification of its toxicity.[158] Biopersistence and biodurability of particles are also important for hazard and risk assessment as they may influence long-term toxicity.[159] Therefore, they should be taken into account and the need for specific toxicity testing may arise, although preparing representative test material for a conclusive hazard/risk assessment may be challenging today. The assessment may be simplified, however, if it can be demonstrated that the particles migrating from the FCM have lost their characteristics specifically related to the nanoscale. Nevertheless, it can be considered that, from a worst case point of view, the nanomaterial as used in the FCM is in fact the most critical chemical form for use in hazard and risk assessment.

In general, it could be expected that upon clustering or attaching to FCM particles, a reduction of toxicity could occur (e.g. a nanomaterial coated with polyethylene will have a reduced surface charge and/or chemistry and therefore is likely to have a lower toxicity). There may be an exception where nanomaterials of different types are combined or when the material's structure is strongly affected by processing, such as through the exfoliation of clay particles. Although data would be needed to support this, it is proposed to pragmatically start the hazard assessment with the naked material. Reduction of the likely adverse effects of a nanomaterial, e.g. when evidence shows a reduction in the surface charge or chemistry due to an interaction with the FCM, can be discussed in relation to its potential absorption and in the context of overall risk assessment.

Factors to consider for hazard characterization

As for FCMs in general, the combined hazard characterization and potential exposure of the nanomaterial will determine the overall risk to the consumer. Until now, no generic threshold for nanomaterials [like the most critical threshold of toxicological concern class of 0.15 μg/person/day for bulk chemicals with (potential) genotoxic properties] has been defined. Consequently, any exposure to nanomaterials should be considered for risk assessment. In addition to normal considerations in toxicology, characteristics like chemical reactivity and morphology of a nanomaterial should be considered in relation to the potential toxicity. Some of these characteristics are discussed later.

Chemical composition

The chemical composition, specifically the presence of impurities and/or contamination with other nanomaterials, may influence the characteristics and toxicity of nanomaterials. Therefore, the chemical composition and purity criteria for the nanomaterial to be used in FCMs should be described in detail and be covered by toxicity testing. Lower purity may also be related to a higher or less consistent toxicity profile of the nanomaterial under evaluation, e.g. due to specific catalytic activity of the impurities, etc. Therefore, a representative batch, which falls within the specifications for the identity of the nanomaterial to be used in the FCM, has to be considered before hazard characterization. Furthermore, potential batch-to-batch variations including ageing effects should also be taken into account.

Physico-chemical characteristics

Particle size, morphological form, surface area, surface charge, etc. of a nanomaterial might be of relevance to chemical reactivity, migration characteristics and absorption and distribution in the human body. Therefore, hazard characterization has to be related to specific characteristics of the nanomaterial, and this should be taken into consideration in toxicity testing.[160] Premature choices of nanomaterials for toxicity testing may lead to unusable toxicity data in the case where changes in the characteristics of nanomaterials may occur during FCM development or processing.

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Dose metrics

For non-nanomaterials, the presence in food on a mass basis is the current standard for dose metrics. However, there may be other characteristics, which are relevant discriminators to be considered in relation to dose metrics. Currently, particle size, shape and surface area are considered to be the most relevant parameters for dose metrics, as they have also been shown to correlate with certain adverse effects.[161] It should be noted that depending on the type of nanomaterial, different dose metrics may be considered relevant. As a consequence, for such nanomaterials, the limit values should also be set in the relevant metric. This may have a consequence for (procedures of) monitoring.

Furthermore, the toxic effects of a nanomaterial may not only depend on structural features of the particle itself but also on the nano-bio-interface, i.e. their interaction with (sub) cellular structures and biomolecules. Therefore, it should be noted that for toxicity testing and use in FCMs, well-defined nanomaterials should be used, because a change in, e.g. particle size or area, may result in significant differences in hazard characteristics. Furthermore, a valid comparison for read-across with other nanomaterials having the same chemical composition but slightly altered characteristics will be highly dependent on the physico-chemical characteristics of the nanomaterials.

The mass dose in parts per million or milligram per kilogram can be applied as standard dose metrics when exposure of a well-defined nanomaterial can be related (1 : 1) to its hazard (so all toxicity tests are performed with an equivalent well-defined nanomaterial). In cases where exposure estimates are above the safe human intake values by comparison on a mass dose (and taken into account conventional uncertainty factors[97]), potential health risk cannot be quantified. Furthermore, when an unforeseen change in characteristics of a nanomaterial is concerned (e.g. outside batch variations), interpolation to determine the toxicity between two nanomaterials differing only in shape and/or surface area can be achieved by introducing an intermediate dose metrics to determine dependency of the hazard towards a specific parameter, e.g. the surface area. It should be noted, that this is only valid for well-defined nanomaterials. Depending on the nanomaterial and its characteristics, the parameters available will determine the possibilities for a dose metrics interpolation or comparison.

Prior to toxicity testing

Before toxicity testing is considered, there need to be evidence or reasons to assume that human exposure to nanomaterial might occur (see also paragraph on Exposure assessment). When starting toxicity testing with nanomaterials the following aspects should be taken into account.

Route of administration

In *in vivo* testing the nanomaterial may be administered via feed, water or by gavage. Although bolus gavage administration may be preferential due to a high peak exposure, the presence of a (protein) corona when the nanomaterial is administered via feed may result in higher systemic exposure. The administration method is therefore dependent on the characteristics of a nanomaterial and also the way of exposure. Therefore, the coherence between the nanomaterial and the administration vehicle, and possible interactions, should be investigated prior to toxicity testing.

Kinetics

Toxicity testing of nanomaterials usually starts with determining the absorption, distribution, metabolism and excretion (ADME) to identify the kinetics and possible accumulation of nanomaterials in the body using, e.g. in Peyers patches, and/or to identify possible affinity for specific target organs and metabolic transformation. ADME information is a prerequisite to determine the toxicokinetic behaviour of the nanomaterial, which may be heavily influenced by physico-chemical properties (e.g. size, surface charge and functionalizing groups). Therefore, slight changes of these parameters may have a significant influence on the gastrointestinal absorption and uptake of nanomaterials in the body. Furthermore, partial solubilization, e.g. of some nanometals and/or oxides, could alter the results of ADME studies if absorption was determined by chemical analysis without ascertaining if the materials were absorbed in nanoparticulate form.

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Apart from *in vivo* ADME testing, *in vitro* models can be used to assess permeability and/or barrier integrity of the cell walls. It is essential that before ADME testing starts, analytical methods are available for the detection of nanomaterials and/or their non-nano counterparts in the body. In case labelling is used, one should be able to discriminate between the nanomaterials and their possible non-nano counterparts.

In case the ADME data convincingly demonstrates that no nanomaterial is absorbed in the gastrointestinal tract, i.e. the nanomaterial will not become systemically available, a limited set of toxicity testing is required. Therefore, it is of great importance to have consensus on the limit of detection to be used in these studies. This is especially of importance for the interpretation of test results determining sufficient evidence that no absorption of nanomaterial occurs.

Toxicity testing

In case systemic exposure can be excluded, EFSA requires at least *in vitro* genotoxicity and *in vivo* local effects testing be carried out.

It should be noted that when the nanomaterial is not absorbed it will not be able to reach the cell in genotoxicity testing. In that case the *in vitro* genotoxicity test will only provide information on local effects.

Where absorption of nanomaterials upon oral exposure has been demonstrated, hazard identification of the nanomaterial by appropriate *in vitro* and/or *in vivo* studies for mutagenicity, and repeated dose toxicity (90 day) is required.

In vivo genotoxicity testing is required when initial *in vitro* tests show positive results or when the results return inconclusive results. This may be the case with one of the basic tests for non-nanoform substances (Ames test). If nanomaterials are not able to enter the bacterial membrane through endocytosis, the relevance of this test for nanomaterials is limited. For genotoxicity testing *in vivo*, one should take care that the nanomaterial is capable of reaching the target cells, which may result in a selection of a different dosing route. If this is not the case, the results of the test are considered invalid. Furthermore, the choice of further *in vivo* testing depends on the endpoint in which a positive effect was found. In the case of a positive *in vitro* gene mutation test, a comet assay or transgenic rodent gene mutation assay may be considered. A positive *in vitro* micronucleus test is followed by an *in vivo* micronucleus test. In case the nanomaterial cannot be tested *in vitro*, an *in vivo* comet assay included in the ADME study or repeated dose test may be considered as an alternative.

The main mechanism of nanomaterial toxicity is considered to be from oxidative stress, which triggers inflammation via the activation of oxidative stress-responsive transcription factors. Therefore, the mechanism of genotoxicity may be of relevance to determine, i.e. primary effects such as those resulting from DNA binding, or secondary effects such as those resulting from oxidative stress/ROS formation.

In vivo sub-chronic repeated dose testing (a 90 day study) should include endocrine-related endpoints, cardiovascular and inflammatory parameters, as well as effects to the mononuclear phagocyte system (due to clearing by phagocytosis).[162,163] The need for additional testing for reproduction toxicity, neurotoxicity, allergenicity and/or other endpoints will be determined by the toxic effects observed in the initial tests. Also in the case where an increased hazard is identified by comparing the results of the nanomaterial to its non-nanoform and/or there is potential for accumulation in the body. Additional *in vitro* testing can be performed to generate mechanistic data on, e.g. epithelial permeability, release of inflammatory mediators or other parameters. Triggers for developmental effects cannot be derived from initial test with the nanomaterial. For this endpoint information from the non-nanoform may be helpful. If no non-nanoform data are available, potential developmental effects will remain an uncertainty.

Because of the limited knowledge on nanomaterials, *in vitro* genotoxicity testing, ADME and a repeated-dose (90 day), study in rodents will be required independently of the amount of migration. It should be noted that unrealistically high dosing can lead to effects by overload rather than toxicity of a nanomaterial under evaluation. The dose levels to be tested should therefore be chosen with care, taking into account the expected exposure based on the migration testing including safety factors to convert from animal testing to human exposure.[164] If present, also public human data may be taken

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into account to determine the relevance of animal studies for humans, e.g. when performed with an intention for pharmaceutical use.[165]

If not indicated otherwise by consideration of the data, the conventional default uncertainty factors of 10 for inter-species and 10 for intra-species differences should be applied. There are currently no indications for a need to modify these factors.[97]

CONCLUSIONS

Nanomaterials and nanotechnology are considered to have strong potential to bring a variety of new or improved properties and/or functionalities to food packaging. The food packaging industry sees potential in using these materials, because the properties and functionalities, which accompany their use, can be linked to a number of benefits related to higher product quality, shelf-life extension, better environmental performance, improved consumer experience and security.

Despite the interest and perceived advantages of these materials and technologies in the food packaging sector, so far their use has not been widespread. There have been various barriers to a more extensive implementation, in the form of gaps in knowledge related largely to (a) benefits of use, (b) unclear legislative requirements, and (c) safety. All these contribute to limited consumer acceptance.

The aim of this article was to review the work in the field of nanomaterials and nanotechnology with a specific focus on food packaging applications and thus to provide clarification regarding the existing identified barriers, which have hindered their wider use until now. More specifically, this paper has explored current technological developments along with potential benefits in terms of property enhancement. The legislative framework for these materials has also been investigated, along with potential safety risks, related to both human and environmental exposure.

The range of available nanomaterials and nanotechnologies is extremely broad, and there is continuous research in the field to develop these materials and technologies further. The broad nature of this domain means that the use of those materials can be applicable to a plethora of polymer base materials used to enhance properties in packaging. The processes and materials developed until now have been shown to provide novel properties, which can answer current and emerging industry needs and offer new functionalities, which bring additional benefits to the consumer and other stakeholders along the supply chain. Benefits could be gained through enhanced mechanical, functional and barrier properties, which can be translated into direct benefits for the consumer, retailers and food producers.

These enhanced properties could enable improved protection capability of the packaging materials currently in use, which could lead to several benefits. For instance these properties have the potential to protect food products for longer as the higher barrier properties provide increased protection from various degradation factors such as oxygen and moisture. Food could therefore be kept fresh for an extended amount of time.

The enhanced barrier and mechanical properties could enable lower gauge packaging films to be used, which could potentially bring environmental benefits and cost reduction through light-weighting. This benefit would, of course, have to be weighed against the impact on manufacture. Property enhancement of bio-based materials is a clear potential application for nanotechnology materials because there currently is significant interest in this domain and any improvement of properties would only help to broaden their scope for the use. Finally, although less defined, there is also potential for the development of 'Intelligent' and 'Smart' packaging concepts to ensure safety, security and authenticity of packaged food products.

The legislative framework currently enforced in Europe and the United States was described. In Europe, specific legislation on plastic-based food-contact materials limits the use of nanomaterials to those explicitly mentioned in the positive list. Currently, the number of authorized nanomaterials is limited to a handful of compounds. The fact that all applications have to undergo a specific risk assessment, even those where the nanomaterial is used behind a functional barrier, might partly explain the slow authorization rate for new nanomaterials. A similar approach is defined in the harmonized measure on active and intelligent packaging, while those for ceramics and regenerated cellulose do not mention nanomaterials. FCMs not covered by these harmonized measures have to comply with the general safety requirements of the Framework Regulation and with specific national legislations. In

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the United States, there is no specific legislation for the use of nanomaterials in food contact applications. However, the nature of any components likely to be added is evaluated. Food contact notification is therefore based on a risk assessment, which takes nano-aspects into consideration.

Perceived safety concerns over the use of nanomaterials and technologies have meant that consumer acceptance has not yet reached a level where high volume industrial-scale applications could commence. Any possible safety risk would be dependent on the migration of nanoparticles into packaged foodstuffs. Consequently, specific consideration was given to the likelihood of migration of nanoparticles from packaging materials.

Methods have now been developed, which enable the migration of nanoparticles from packaging materials into foodstuffs to be determined. Such methods are now available for both particulate and non-particulate migration. This is a key message because these methods now are able to address one of the main questions regarding the use of nanomaterials in packaging and enable consumer exposure to be estimated. The studies that are available on this topic so far indicate that any significant migration of nanoparticles from polymer packaging materials into packaged foodstuffs is unlikely. However, more studies are required in this area to ascertain whether the same trend can be observed in other packaging types (biopolymers for instance) that are more prone to matrix degradation. Another area where further work is required is where nanotechnology is used to produce active packaging. Here, a certain level of (non-particulate) migration is desired, such as in the case of anti-microbial packaging materials. Ultimately the available information on the topic of migration does provide some assurance that the use of nanomaterial-polymer composites for food packaging application is unlikely to create a consumer exposure risk during the use phase.

The implications of potential migratory behaviour of nanoparticles have also been explored. Should any migration of nanoparticles be evident, a potential exposure would exist and, consequently, a safety risk assessment would be required. In such cases, non-particulate migration could be addressed with knowledge available from the non-nanoform of the substance in question. However, migration of actual nanoparticles poses a greater challenge in terms of risk assessment. This would require an initial toxicological evaluation using *in vitro* and *in vivo* tests. A positive indication of toxic effects from these initial tests would trigger further more detailed toxicological investigations. In view of this, it is highly recommended that new packaging materials based on nanotechnologies be developed in a manner that reduces risks by minimizing the chances of consumer exposure to nanomaterials during their use and potential exposure in the environment upon disposal. This can be achieved by ensuring that packaging materials are purposely designed to minimize nanoparticle migration to packaged foodstuffs and that the packaging materials are appropriately handled and treated at their end of life.

Ultimately, the findings from this work show that that momentum surrounding nanomaterials and nanotechnologies in terms of interest and research is growing with respect to their use in packaging applications. There are clear benefits linked to their use in terms of performance enhancements and functionalities that can improve material properties in existing and emerging materials. There is an existing legislative framework applicable to these materials, which continues to evolve to meet the requirements of this rapidly evolving field. The various questions regarding the risk of these materials are also in the process of, to being addressed with the emergence of relevant methods and approaches.

It can therefore be expected that the packaging applications, which use nanomaterials and nanotechnology will continue to grow in coming years as the previous barriers are increasingly removed. The benefits of using such materials and their potential to improve environmental performance reduce food waste and provide improved product quality and security, along with reassurances regarding safety could also improve consumer acceptance.

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