Title: RECENT ADVANCES AND CHALLENGES ON APPLICATIONS OF NANOTECHNOLOGY IN RE-DESIGNING THE TRADITIONAL FOOD PACKAGING AS AN ACTIVE AND INTELLIGENT ONE. A COMPREHENSIVE REVIEW

Article Type: Review Article

Keywords: Migration/Standard food model for evaluating the toxicity and fate of ingested ENPs;
Measurement methods to evaluate ENPs release from food contact materials;
Cytotoxicity;
Innovation


Corresponding Author: Dr. Daniela ENESCU,

Corresponding Author's Institution: INTERNATIONAL IBERIAN NANOTECHNOLOGY

First Author: Daniela ENESCU

Order of Authors: Daniela ENESCU

Abstract: Nanotechnology applied to food and beverage packaging has created enormous interest in recent years, but in the same time there are many controversial issues surrounding nanotechnology and food. The benefits of engineered nanoparticles (ENPs) in food-contact applications are accompanied by safety concerns due to gaps in understanding the toxicology. In case of incorporation in food contact polymers, the first step to consumer exposure is the transfer of ENPs from the polymer to the food. Hence, to improve understanding of risk and benefit, the key questions are whether nanoparticles can be released from food contact polymers and under which conditions.
This review has two main goals. Firstly, it will be presented the current advancements in the application of ENPs in food and beverage packaging sector to grant active and intelligent properties. A particular focus will be placed on current demands in terms of risk assessment strategies associated with the use ENPs in food contact materials (FCMs), i.e. up-to-date migration / cytotoxicity studies of ENPs which are partly contradictory. Secondly, the review provide an extensive analysis of present market dynamics on ENPs in food/beverage packaging moving beyond concept to current industrial applications.

Expertise on food packaging, standardization food modelling

Mohammadi Hamid
Food Hygiene and Control Department, Veterinary Medicine Faculty,
University of Tehran, Iran
mohammadi.hamid@ut.ac.ir
Expertise on food packaging

Hu Bin
Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry
of Education) Department of Chemistry, Wuhan University, China
binhu@whu.edu.cn
Expertise on cytotoxicity

Ko Seonghyuk
Deparment of Packaging, College of Science and Technology, Yansei
University, Gangwon-do, Republic of Korea
s.ko@yonsei.ac.kr
Expertise food packaging

Opposed Reviewers:

**RECENT ADVANCES AND CHALLENGES ON APPLICATIONS OF NANOTECHNOLOGY IN RE-DESIGNING THE TRADITIONAL FOOD PACKAGING AS AN ACTIVE AND INTELLIGENT ONE. A COMPREHENSIVE REVIEW**

**ABSTRACT**

Nanotechnology applied to food and beverage packaging has created enormous interest in recent years, but in the same time there are many controversial issues surrounding nanotechnology and food. The benefits of engineered nanoparticles (ENPs) in food-contact applications are accompanied by safety concerns due to gaps in understanding the toxicology. In case of incorporation in food contact polymers, the first step to consumer exposure is the transfer of ENPs from the polymer to the food. Hence, to improve understanding of risk and benefit, the key questions are whether nanoparticles can be released from food contact polymers and under which conditions.

This review has two main goals. Firstly, it will be presented the current advancements in the application of ENPs in food and beverage packaging sector to grant active and intelligent properties. A particular focus will be placed on current demands in terms of risk assessment strategies associated with the use ENPs in food contact materials (FCMs), i.e. up-to-date migration / cytotoxicity studies of ENPs which are partly contradictory. Food matrix effects are often ignored, and may have a pronounced impact on the behaviour of ENPs in the gastrointestinal tract (GIT).

A standardized food model (SFM) for evaluating the toxicity and fate of ingested ENPs was recently proposed (2019) and herein discussed with the aims to offer an overview to the reader. It is therefore clear that further systematic research is needed, which must account for interactions and transformations of ENMs in foods (food matrix effect) and in the gastrointestinal tract (GIT) that are likely to determine nano-biointeractions.

Secondly, the review provide an extensive analysis of present market dynamics on ENPs in food/beverage packaging moving beyond concept to current industrial applications.

# RECENT ADVANCES AND CHALLENGES ON APPLICATIONS OF NANOTECHNOLOGY IN RE-DESIGNING THE TRADITIONAL FOOD PACKAGING AS AN ACTIVE AND INTELLIGENT ONE. A COMPREHENSIVE REVIEW

**Daniela Enescu[1]**

International Iberian Nanotechnology Laboratory (INL), Department Life Sciences, Research Unit: Nano for Food/Food Processing, Av. Mestre Jose Veiga s/n, 4715-330 Braga, Portugal.

**ABSTRACT**

Nanotechnology applied to food and beverage packaging has created enormous interest in recent years, but in the same time there are many controversial issues surrounding nanotechnology and food. The benefits of engineered nanoparticles (ENPs) in food-contact applications are accompanied by safety concerns due to gaps in understanding the toxicology. In case of incorporation in food contact polymers, the first step to consumer exposure is the transfer of ENPs from the polymer to the food. Hence, to improve understanding of risk and benefit, the key questions are whether nanoparticles can be released from food contact polymers and under which conditions.

---

**[1] Corresponding author.**

*E-mail address*: ✉ daniela.enescu@inl.int (D. Enescu).

This review has two main goals. Firstly, it will be presented the current advancements in the application of ENPs in food and beverage packaging sector to grant active and intelligent properties. A particular focus will be placed on current demands in terms of risk assessment strategies associated with the use ENPs in food contact materials (FCMs), i.e. up-to-date migration / cytotoxicity studies of ENPs which are partly contradictory. Food matrix effects are often ignored, and may have a pronounced impact on the behaviour of ENPs in the gastrointestinal tract (GIT).

A standardized food model (SFM) for evaluating the toxicity and fate of ingested ENPs was recently proposed (2019) and herein discussed with the aims to offer an overview to the reader. It is therefore clear that further systematic research is needed, which must account for interactions and transformations of ENMs in foods (food matrix effect) and in the gastrointestinal tract (GIT) that are likely to determine nano-biointeractions.

Secondly, the review provide an extensive analysis of present market dynamics on ENPs in food/beverage packaging moving beyond concept to current industrial applications.

**KEYWORDS**

Migration/Standard food model for evaluating the toxicity and fate of ingested ENPs;

Measurement methods to evaluate ENPs release from food contact materials;

Cytotoxicity;

Innovation

## Introduction

### *Statistics regarding increasing interest of nanotechnology*

Nanotechnology seems to be new source of key improvements for the current challenges in food security and food sustainability. The concept of nanotechnology was introduced in 1959 by Richard Feynman (Khademhosseini, and Lager 2006). Nanotechnology is the ability to work on a scale of about 1-100 nm in order to understand, create, characterize and use material structures, devices and systems with new properties derived from their nanostructures (Roco 2003).

According to statistics published on the StatNano website, ~166.000 nanotechnology articles were indexed in the Web of Science (WoS) Database in 2018 (StatNano 2018), which represents 10 % of all articles indexed in the database. In brief, in current years, China, United States, and India were ranked 1st, 2nd, and 3rd for publishing research in the related field of nanotechnology, thus showing increasing interest in the similar topics in the field of nanotechnology by the researchers of these countries. Hence, of these ~ 166.000 articles, 39.47 % were published in China, 14.75 % in the United States, and India 8.45 % (StatNano 2018).

In food/food and beverage packaging most application of nanotechnology include engineered nanoparticles [2] (ENPs, called also manufactured nanomaterials) of transition metals, such as silver and iron; alkaline earth metals, such as calcium and magnesium; and non-metals, such as selenium and silicates. Other ENPs that can potentially be used in food applications include zinc oxide, titanium dioxide. Food packaging is the major area of applications of ENPs, which redefine traditional food packaging in *active* and *intelligent* packaging.

This updated review comes as a tool for the reader to understand where we are and where we are heading in terms of the *active* food and beverage packaging. A particular focus of this

---

[2] metal (oxide)

updated review was focused on a overview on (i) regulations due to a possible migration of active additives from packaging into foodstuff, which might have detrimental effects on consumator´health; (ii) cytotoxicty of the active additives; (iii) appropriate measurement techiques for a reliable detection of active additives migration from food packaging into foodstuff; (iv) current packaging industrial applications, based on ENPs, aiming to extend the shelf life of food and drinks and also to improve the food safety.

### *ENPs in food and beverage packaging. Comparative study of nano- vs micro- sized particles*

In 2011, the European Commission (EC) (EC No. 10/2011) adopted the following Recommendation (2011/696/EU) for a definition of the term nanomaterial (NM) (EC No. 696/2011),"a natural, incidental or manufactured materials containg 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 dimentions is in the size range 1-100 nm. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % maybe replaced by a threshold between 1 and 50 %". The EC NM definition further specifies: 'particle', 'agglomerate' and 'aggregate' are defined as follows: (a) 'particle' means a minute piece of matter with defined physical boundaries; (b) 'agglomerate' means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components; (c) 'aggregate' means a particle comprising of strongly bound or fused particles.

EC NM definition is not intended to cover solid products, as defined in EU regulation (EU Directive 2001/95/EC 2002), even if they contain nanomaterials or have an internal structure at

the nanoscale. The EC NM definition applies only to the nanomaterial in terms of particulate matter itself and not to the resulting product of a combination of nanomaterial(s) with other components. Therefore, a consumer product or an end product is not a nanomaterial itself if it contains nanomaterial(s) as component(s). Accordingly, *a food packaging*, for instance, does not become a nanomaterial itself, if it contains a nanomaterial ingredient. Thus, even if a product contains one or more nanomaterials as components, or if it is designed to release nanomaterials, or releases nanomaterials as wear debris during use or ageing, it is not a nanomaterial itself. This also means that the criterion that a material is a nanomaterial if 50 % or more of the particles have one or more dimensions in the range of 1 nm to 100 nm should be applied only to the material itself, and not to a product or parts of it that contain the material, unless the product is a particulate material itself. The potential for nanotechnology to imporve all aspects of life has generated high interest, but in the same times it generated also concerns[3]. The major links between nanotechnology and the food and beverage industry are (i) enhancing food security, (ii) extending storage life, and (iii) improving flavor and nutrient delivery. Hence, nanotechnology redefines food and beverage packaging as *active* and *intelligent* packaging that goes beyond product protection and brand presentation, imparting functions like moisture control (*e.g. pads*[4]

---

[3] the small dimentions of nanoparticles also means they can reach locations in the hyman body not normally accessible to micro counterparts.

[4] e.g. of pads

*used for example to absorb the drip from meat, poultry and fish in display packaging trays)*; antioxidant activity (e.g. *the small sachets[5] that scavenge or capture residual oxygen or ethylene from inside the packaging (from the environment surrounding the foodstuff or from the foodstuff itself) to reduce exposure to oxygen. Exposure to oxygen may result in microbiological growth on the food, chemical changes to the food, etc. An oxygen scavenger is meant to reduce these effects thereby prolonging the shelf-life of the foodstuffs. Applications of oxygen scavengers could be in packaging pasta, milk powder, biscuits, etc.*); and antimicrobial activity (*e.g. sachet systems slowly releasing an antimicrobial agent, and therefore maintain freshness and extending the product shelf-life within the package*). Intelligent packaging denotes features that deliver informaton about brand protection or information for consumator on safety and quallity of the food products like smart labels[6], etc. (*label which can contain for e.g. time-temperature*

---



[5]e.g. of small sachets that scavenge or capture residual oxygen or ethylene



[6] e.g. of *inteligent packaging with a time-temperature indicators*:

Time-temperature indicators are meant to give information on whether a threshold temperature has been exceeded over time and/or to estimate the minium amount of time a product has spent above the threshold temperature (time temperature history) e.g. from the moment the food it is packed until consumption. Below are presented some inteligent packages that are already on the market. As it can be seen from these images, the temperature indicator can be read by the customer with the naked eye and which indicate that the food is no longer fresh or inappropriate for consumption:

[i]*Tempix time/temperature indicator*® (Sweden). This indicator destroys barcodes on labels if the foodstuff or other temperature sensitive products are exposed to too high temperature. How it works: the indicator is built up from an absorbent (paper label) and a container/label cover including an activator. The activator migrates in the paper when the set temperature is exceeded and erases the bar-code and the optical signal bar after the time period stipulated. Temperature ranges between -30 to +30 °C (-22 to +86 °F) with an accuracy of ±0.5 °C (±0.9 °F). Hence, what is unique about the Tempix solution is that it is coupled to the barcode on the price tag. If the product has been exposed to too much heat, liquid flows over the barcode destroying it, making it illegible for the scanner (*see* details: http://www.voyagerventure.com/new-page).

[ii]*"Fresh Meter" time-temperature indicator* from Bizerba's "OnVu" (USA). How it works: the dynamic indicator is based on "intelligent" temperature-sensitive ink. The specialty ink comprises the bulls-eye on the Fresh Meter label that's surrounded by an outer ring printed in regular ink that serves as the standard. The preprinted labels are provided to the processing and packaging plant and are activated inline after tray sealing. That's done by a specialized, microwave-oven sized activator/labeler near the end of the processor's packaging line that activates the Smart Meter using ultraviolet light. When activated, the Fresh Meter indicator's

*indicator; RF Sensor tag; etc. it's acts as interactive marketing platform to engage with consumers*). *Active* packaging includes, e.g. the usage of nanoparticles (metal (oxides)).

---

dynamic inner circle turns bright blue and immediately begins sensing the temperature of the products over time. Consumers can compare that dynamic center circle to the surrounding static-color ring; the latter is printed blue to gray to know when the seafood is fresh (blue) and when it is not (gray).

*[iii]To-Genkyo® time temeprature indicator* (Japan). How it works: the label is printed with a special ammonia-detecting ink that darkens according to how much of the chemical is present in the meat.

The more ammonia it contains, the less fresh the meat. Moreover, the darkened label also effectively obscures the product's bar code, making it impossible for it to be scanned at the checkout counter when the meat is past its prime).

Nanoparticles because of their size[7] have considerable greater surface area to mass ratio and

consequently more surface atoms than their microscale counterparts, which can alter physical

---

Other exmaples of time tempearature indicators are (On Vu$^{TM}$ from Ciba Speciality Chemical and Presh Point; Timestrip$^®$ from Timestrio Plc; Check Point$^®$ from Vitsab; Fresh-Check$^®$ from LifeLines; Cook-Che$^®$ from Keep-it Technology; Colour-Therm$^®$ from Colour-Terms; eO$^®$ from 3M$^{TM}$, Minnesota; TopCro$^{TM}$ from TRACEO (http://cryolog.com).

e.g. of inteligent packaging with a RF Sensor Tag (advanced data carrier with a data storage up to 1 MB; these data include traceability, inventory management and promotion of quality and safety (Kumar et al., 2009).

and chemical properties such as reactivity and surface charge (e.g. gold at the macro scale conducts electricity, is chemically unreactive and yellow in color. In contrast, gold at the nanoscale is a semiconductor, highly reactive and varies in color from pink to red or orange, depending on how small the particles are). The small dimensions of nanoparticles can favor a better interaction with the polymer matrix, and the performance of the resulting material. Thus, the application of nanotechnology to plastic/bio-based materials may open new possibilities for improving not only their physical properties (gas/water vapor/aroma barriers, mechanical properties, etc.) or adding other functions such as antimicrobial/antioxidant properties; biosensing; reducing the weight of food packaging materials etc., but also the cost-price-efficiency (Kahn 2006; Sorrentino et al., 2007).

*Regulations on active food and beverage packaging. Is consumer exposure likely?*



[7] "nano" denotes nanometer, $10^{-9}$ m

Although, active packaging towards traditional[8] packaging is designed to add valuable properties to foodstuff, nevertheless there are serious challenge faced by its commercialization, such as the possible migration of the active additive (i.e. ENPs), from food packaging into foodstuff, which might have detrimental effects on consumator´health.

Indeed, theoretically, there may be potential consumer exposure to ENPs incorporated into food packaging if they migrate[9] into foodstuffs or drinks from the packaging. Until 2004, in Europe there was a lack of legislation for active and intelligent packaging, decreasing their penetration in the EU market. To address the problem, Regulation 1935/2004/EC (EC Regulation No.

---

[8] Unlike traditional packaging, which must be totally inert, *active* packaging is designed to extend the 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 (EC Regulation No. 1935/2004: Article 2.2a; EC Regulation No. 450/2009: Article 3a).



[9] Migration can theoretically occur if nanopartciles desorb from the surface of the packaging material due to weak bounding at the surface (only really relevant for coating), diffuse into foods as a result of a concentration gradient, or dissolve resulting in ions into food (Noonan et al., 2014).

1935/2004) and, more specifically, Regulation 450/2009/EC (EC Regulation No. 450/2009), set

a new legal basis for their correct use, safety and marketing.

 Active and inteligent packaging**:** the definitions laid down in Regulation (EC) No 1935/2004

(EC Regulation No. 1935/2004) and in Regulation (EC) No 450 /2009 shall apply (EU

Regulation  No. 450/2009).

*"Active materials and articles"* means materials and articles that are intended to extend the 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 (EC Regulation No. 1935/2004: Article 2.2a; EU

Regulation   No. 450/2009: Article 3a). This definition of active materials and articles is

interpreted as including all materials and articles that are designed to deliberately interact with

food and/or the food surrounding environment, and bring about change in their composition or

characteristics.

*"Intelligent materials and articles"* means materials and articles which monitor the condition of

packaged food or the environment surrounding the food (EC Regulation No. 1935/2004: Article

2.2b; EU Regulation  No. 450/2009: Article 3b).

 *"Functional barrier"* means a barrier consisting of one or more layers of food contact materials

which ensures that the finished material or article complies with Article 3 of Regulation (EC) No

1935/2004 and with Regulation (EC) No 450/2009. This barrier is a layer within the food contact

materials or articles preventing the migration of substances from behind that barrier into the

food. The maximum tolerated migration level is **0.01 mg** substance/kg food for a substance. This

migration limit is applicable to a group of substances if they are structurally and toxicologically

related, in particular isomers or substances with the same relevant functional group; it also

includes possible set-off transfer. This limit shall always be expressed as a concentration in foods. If it is demonstrated that the packaging material or a layer acts as a functional barrier to migration then nonauthorised substances can be used in the layer (s) behind the barrier (not on the food contact side) provided they don't fall under one of the following categories:

✓ Substances that are mutagenic, carcinogenic or toxic to reproduction should not be used in food contact materials or articles without previous authorisation and are therefore not covered by the functional barrier concept.

✓ New technologies that engineer substances in particle size that exhibit chemical and physical properties that significantly differ from those at a larger scale, for example, nanoparticles, should be assessed on a case-bycase basis as regards their risk until more information is known about such new technology. Therefore, they are not covered by the functional barrier concept. This should be demonstrated in the declaration of compliance and the supporting documentation (EU Regulation No. 450/2009: Article 13 and Annex II (10)).

*"Releasing active materials and articles"* are those active materials and articles designed to deliberately incorporate components that would release substances into or onto the packaged food or the environment surrounding the food (EU Regulation No. 450/2009: Article 3e).

*"Released active substances"* are those substances intended to be released from releasing active materials and articles into or onto the packaged food or the environment surrounding the food and fulfilling a purpose in the food (EU Regulation No. 450/2009: Article 3f).

The Framework Regulation (EC) 1935/2004 states that food contact materials shall not transfer constituents to food in quantities, which could endanger consumers health, bring about an unacceptable change in the composition and bring about deterioration in organoleptic

characteristics thereof (Article 3). The Regulation provides specific requirements for active and intelligent materials and articles (Article 4) and includes the following provisions:

- Substances deliberately incorporated into active materials and articles to be released into the food or the environment surrounding the food shall be authorised and used in accordance with the relevant EU provisions applicable to food.

- Active materials and articles shall not bring about changes in the composition or organoleptic characteristics of food, for instance by masking the spoilage of food, which could mislead consumers.

- Active and intelligent materials and articles already brought into contact with food shall be adequately labeled to allow identification by the consumer of non-edible parts.

- Active and intelligent materials and articles shall be adequately labeled to indicate that the materials or articles are active and/or intelligent.

- The overall migration from active releasing materials can exceed the overall migration limits described in EU or national legislation as long as the levels transferred to the food comply with restrictions in the existing food law (e.g. as authorised food additives). The transfer of these active substance/substances should not be included in the calculation of the overall migration limit (OML) (EC No. 10/2011; EU Regulation No. 450/2009). For materials such as paper and board for which the specific requirements are not regulated at EU level existing national legislation should be applied (EU Regulation No. 450/2009). Intelligent systems that are on the non-food contact surface of the package can be separated from the foodstuff by a functional barrier, i.e. a barrier to any migration. If it is demonstrated that the packaging material acts as a functional barrier to migration then non-authorised substances can be used

providing they meet specific criteria defined in the Regulation (EU Regulation No. 450/2009).

### *ENPs per se migration from active[10] packaging into foodstuffs*

The current challenges facing active packaging implementation are due to a potential migration[11] of ENPs which, unlike traditional packaging (totally inert), are added to exentend the shelf life or to maintain or improve the condition of packaged food. Hence, safety assessment of ENPs used in food packaging first requires an understanding of potential exposure via migration into food. If there is no exposure, it follows there is no risk of adverse effects in consumers. Migration of ENPs from polymer composites into food or food simulants has been assessed by various authors using standard migration tests. These tests are European and USA (FDA) standardised methods used to evaluate migration from food packaging, and are carried out using different food simulant solutions characterised by varying levels of water solubility and acidity (*see* an overview of standard migration tests for food packaging from two jurisdictions in **Table 1**). Although, these tests were originally developed for plastic materials, they can be applied also to other materials printed or unprinted. In the case of paper and board, the standard liquid

---

[10] Metal (oxide): ENPs.

[11] Migration refers to the release of a substance from one medium to another, in other words is the unintentional transfer of packaging materials into the food. This problem may influence the food's safety and, subsequently, consumers'health (Torres et al., 2012). Nevertheless, in some active packaging, ENPs are intended to be released deliberately. Hence, performing migration tests under controlled conditions is essential for safety problems.

simulants are not suitable, as they penetrate into the material. They are therefore replaced by modified polyphenylene oxide (Tenax) according to a European standard BS EN14338 2003.

*The state of science up to date* revealed that there are various issues that complicate the interpretation of ENPs migration studies from food/food packaging. One of this issue being the uncertainty in the ability of the analytical techniques used to detect nanoparticles *per se* in food/food simulants (*see* detalied information at section "Analytical techniques for quantification of nanoparticles per se as well as migration of these from food contact materials " as well as **Table 2**).

### *The main factors that control migration*

Following Fick's first law of diffusion, the substance will migrate due to a concentration gradient between both mediums (Simon et al., 2008). If there are no ENPs present in the food, any ENPs that are loosely bound in the food packaging will migrate from the packaging to the food. This occurs due to the lower concentration of ENPs in the food which drives migration. Others factors that affect migration apart concentration gradient, include: the ENPs' properties (e.g. particle size, molecular weight, solubility, and diffusivity in the polymer); environmental conditions (temperature, mechanical stress); food conditions (pH value (Metak et al., 2015), composition); packaging characteristics (polymer structure and viscosity); position of the ENPs in the packaging material, interaction between the ENPs and the material; and contact time (Noonan et al., 2014; Huang et al., 2015; Hannon et al., 2015).  For instance, higher temperatures and lower pH values increase the solubility of ENPs in aqueous solution, leading to an increase in their migration (Song et al., 2011). Low molecular weigth polymers that have more free volume

accelerate the migration rate and ENPs diffusivity (Schimidt et al., 2011). There also is an inverse relationship between the migration rate of the system and the size of the ENPs (Cushen et al., 2014). If the nature of the food is compatible with the type of packaging, the food itself may be absorbed into the polymer matrix, enlarging the gaps between the polymer chains, thereby increasing the migration rate (Huang et al., 2015). For example, fats have high affinity for polyethylene and polypropylene, so they  may be absorbed by the packaging and cause an increase in plastic mobility and higher migration rates (Huang et al., 2015). The preservation method of foods also is important and can affect ENPs' migration. It has been reported that microwave heating cause structural modification of the packaging, thereby speeding up the migration of silver ions (Echegoyen et al., 2013). High surface volume ratios in nanomaterials induce an active surface chemistry followed by probably unwanted chemical ractions. This would be troublesome if their presence in the packaging enhances the formation of unforeseen reaction by-products during processing (Bradley et al., 2011). ENPs also can impact the migration of other packaging constituies, either speeding  up or slowing down their migration. The migration potential and diffusion mechanisms for ENPs from food packaging materials is an area of nanotechnology which has not received the same attention as such areas as nano-aerosols (Savolainen et al., 2010) and nano-medicines (Lether et al., 2013).

*Standard migration tests*

This article is not meant to provide an exhaustive review of European and USA standard migration tests; but it is intended to provide the reader an indication of what is involved in food packaging migration testing. Standard migration tests are conducted with food simulants under

certainly time and temperature conditions which are chosen according to the type of food that will be packaged. These standard migration methods are used to measure the 'overall' or 'specific migration', which is compared to the respective limits. The standard methods, although were originally developed for plastic materials, may be modified to cover specialist uses, for e.g. for use in microwave ovens, and more than that it might be modified also for printed or unprinted materials; paper and board.

*The overall migration limit* (OML) means the maximum permitted amount of non-volatile substances released from a material or article into food simulants (EC No. 10/2011). Thus, is used to clarify that no packaging additive or contaminate migrates from the packaging to food.

*The specific migration limit* (SML) is defined as the maximum permitted level of a named substance migrating from the final material or article into food or food simulants (EC No. 10/2011).

In migration testing, only those parts of the sample intended to come into contact with foodstuffs in actual use should ideally be in contact with the simulant. With a focus on presenting a worst case migration scenario[12] as well as a most likely scenario, multiple packaging and environmental factors have been investigated mainly storage time, storage temperature, ENPs percentage fill and ENPs size. Natural light has the potential to degrade polymers via photo-oxidation and increase migration of substances from those polymers in applications involving long term exposure (Kumar et al., 2009). Anyway, due to the short exposure period for food packaging in service it is unlikely that natural light will cause significant deterioration and have any effect on migration.

---

[12] i.e. a total immersion test, in which both inner and outer surfaces are in contact with the food simulant.

**Figure 1** display a standardized migration cell with double function, i.e. (i) specific/overall migration for **mono-materials** testing, and (ii) specific/overall migration for **multi-layer materials** testing. If a food packaging article is intended to come into repeated contact with foodstuffs, migration tests are typically carried out three times on the same test sample, using a fresh sample of food simulant on each occasion. However, if there is conclusive evidence that the level of migration does not increase in the second and third test and if the migration limit is not exceeded on the first test, no further test is necessary JECFA 2007. Addition, according to EN 13130-1:2004 (E) (EN 13130-1:2004) where the surface-to-volume ratio to be used in contact with food **is known** this shall be used in the migration tests. An example of this is where a bottle or other container is intended to contain a specified volume of contents, even if this does not completely fill the article. In this case the article shall be tested with the specified volume of simulant. Where the surface-to-volume ratio to be used in contact with foodstuff **is not known**, conventional exposure conditions shall be used, i.e. 0.6 $dm^2$ of surface area of plastics in contact with 100 g of foodstuff or 100 ml of food simulant, in both tests: total immersion as well as single surface using a cell. But, according to EN 1186-1:2002 (EN 1186-1:2002), the surface to volume ratio in a total immersion test is typically 1 $dm^2$ of food contact area to 100 ml of food simulant, and for single surface test using a cell, the surface to volume ratio is conventionally 2.5 $dm^2$ of food contact area to 125 ml of food simulant (EN 1186-1:2002).

**Figure 1**

**Table 1**

The European Commission has published a Union list of authorised substances for use in manufacturing polymer food contact materials (EC No. 10/2011). The ENPs included in the list are (EC No. 10/2011; Chaudhry et al., 2008):   e.g. nano-clay, titanium nitride, nano-silver,

silanated silicon dioxide, titanium oxide, zinc oxide, iron oxide. **Table 2** display *the state of science up to date* of the ENPs migration from polymer food packaging to foodstuffs/food simulants.

*Nano-silver*: Ag nanoparticles are currently used in more manufacturer identified products than any other nanomaterial (Luoma 2008). Up to date, there are least 435 products that utilize some form of nanosilver for their function (Vance et al., 2015): textiles (socks and linens), cosmetics/hygiene products (toothpastes, make-ups), appliances (washing machines and refrigerators), cleaning agents (detergents, soaps), toys and building materials (paints, caulks, glues), as well as into a range of packaging and food contact materials (nanosilver is often incorporated into coatings on products including food storage containers, mugs, dishes, cutlery, chopping boards, etc where the antimicrobial action occurs at the surface), including polymer nanocomposites for 'active' packaging to improve the shelf-life of food or beverages through its antimicrobial properties (nanosilver has been found to be a potent antimicrobial against numerous species of bacteria including E. Coli, Enterococcus faecalis, Staphylococcus aureus and epidermidis, Vibrio cholera, Pseudomonas aeruginosa and putida and fluorescens and oleovorans, Shigella flexneri, Bacillus anthracis and subtilisi and cereus, Proteus mirabilis, Salmonella enterica Typhmurium, Micrococcus luteus, Listeria monocytogenes, and Klebsiella pneumoniae (Duncan 2011; Hajipour et al., 2012; Gaillet et al., 2015; Pulit-Prociak et al., 2015). Nanosilver is also effective against strains of these organisms that are resistance to potent chemical antimicrobials, as well as being toxic to fungi (Hannon et al., 2015; Duncan 2011; Mohammed Fayaz et al., 2003). The use of Ag nanoparticles as an antimicrobial, antiodor, and a health supplement has already surpassed all other engineered nanomaterials currently in use in different sectors (Luoma 2008). In the European Union,  silver is permitted for use in food

colouring (E174 - only allowed for external coating of confectionary, decoration of chocolates, and in liqueurs) (EU Council Directive 94/36/EC 1994). According to GMP, it is also approved in Australia and New Zealand for use as a food additive in confectionary, spirits and liqueurs (GMP 2015).

Current research indicates the antimicrobial action of nanosilver is caused by the release of silver ions from the surface of particles (Cushen et al., 2013; de Azeredo et al., 2013). Hence, in order to be effective, Ag nanoparticles must migrate from food contact materials. In the light of these mentioned, a **compromise** must be made between the level of migration and antimicrobial activity (Hannon et al., 2015). It has been argued that the perceived increased antimicrobial effectiveness of nanosilver compared to conventional particles is not necessarily a result of increased cell toxicity, but rather a result of increased reactive surface area available for oxidation of silver into silver ions (Hannon et al., 2015). Migration of silver into foods from silver containing materials is regulated in the European Union by a specific migration limit (SML) of **0.05 mg/kg** food or food simulant (EFSA 2004; EFSA 2005). It has been estimated that adults may consume between 20 and 80 μg/day of silver, with only a fraction of this being in the form of nanoparticles (Wu et al., 2014).

*Nano-clay*: Polymer nanocomposites incorporating nano-clay are among the first known applications of nanomaterial for food packaging (FAO/WHO 2009). The nano-clay used most commonly is montmorillonite (i.e. bentonite), a natural clay commonly obtained from volcanic ash/rocks  (Hannon et al., 2015; Enescu et al., 2019). Montmorillonite (**Figure 2**) is a hydrated magnesium aluminum silicate layered clay, which belongs to the general family of 2:1 layered silicates. Its crystal structure consists of edge-shared octahedral sheets of either magnesium or aluminum-hydroxide, between two silica tetrahedral layers. The isomorphous substitution in the

nanoclay layers creates a net negative surface charge distributed within the plane of the clay platelets. The imbalance of the negative charges at the surface is compensated by exchangeable cations (typically alkali or alkaline earth cations), located between the platelets (galleries). The parallel layers are linked together by weak electrostatic forces. When montmorillonite is introduced into a polymer matrix, the non-permeable inorganic crystal structure of the nanofiller is expected to cause a decrease in permeability of gases due to a longer and more tortuous pathway (Enescu et al., 2015; Enescu et al., 2019). Thus this peculiar propriety, i.e. restriction of the permeation of gases, has led to its use in food packaging for a variety of food and drinks, e.g. processed cheese, meats, cereals, fruit juices, dairy products, co-extrusion processes for manufacturing bottles for beer and carbonated drinks (Chaudhry et al., 2008; de Abreu et al., 2010; Mahalik and Nambiar 2010).

**Figure 2**

In the European Union, bentonite clay is authorised as an additive for plastic materials and articles in contact with foods with no specific restrictions (EC No. 10/2011). The substance is also an approved food additive (E558) included in European Directive 95/2/EC (Directive 95/2/EC 1995).

 It can be used as a carrier for colours with a maximum of **5 % w/w in food**, and is generally recognised as safe (GRAS) in the United States (CFR 2014). In Australia and New Zealand, bentonite is approved as a food additive in processed foods according to GMP.  No JECFA toxicological monograph has been prepared for bentonite. However information on the hazards of aluminium may be applicable since bentonite contains high amounts of aluminium. Previously the Scientific Committee for Food (SCF) evaluated the safety of aluminium containing food

additives in 1990 at which time they endorsed the Provisional Tolerable Weekly Intake (PTWI[13]) of 7 mg/kg bw for aluminium for all intake sources. Recently, JECFA reevaluated aluminium from all sources, including food additives, and established a PTWI of 1 mg/kg bw which is 7 times lower than the previous PTWI (JECFA 2007).

*Titanium nitride (TiN)*: TiN is a very hard compound, with hardness comparable to steel; it is insoluble in water and stable against cold acids, has a high melting point (3000 °C) but at temperatures $> 500°$ C it may form titanium oxides in air. Titanium nitride nanoparticles are authorised for use as an additive or polymer production aid, specifically to be used as reheat additive in the production of PET botteles for its thermal stability (up to **20 mg/kg**). The listing states no migration of the nanoparticles into food is allowed, and the agglomerates are to have a diameter of 100-500 nm consisting of primary titanium nitride nanoparticles with a diameter of approximately 20 nm. The safety assessment underpinning this evaluation was based on no migration of Ti having been observed in standard migration experiments. As a result, the European Food Safety Authority concluded the nanoparticulate substance would not give rise to consumer exposure via food, therefore it is not of toxicological concern (EFSA 2012; EFSA 2008).

*Silanated silicon dioxide (SiO$_2$)*: SiO$_2$ (silanisation of silica refers to reacting silicon dioxide with methoxy or ethoxy molecules to form alkoxysilane molecules. When these are applied to surfaces it increases the hydrophobicity and generally reduces the adsorption of other molecules to the surface. Alkoxysilanes are also used to silanise glassware to reduce adherence of cells to

---

[13] PTWI was based on a study in which no treatment-related effects were observed in beagle dogs given diets containing sodium aluminium phosphate (acidic) at a concentration of 3 % for 189 days, equivalent to approximately 110 mg/kg by aluminium (JECFA 2007), established previously by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 1988).

the surface). Silanated silicon dioxide was authorised in 1999 in Europe to be used as an additive for the manufacture of plastic food contact materials and is currently listed in the Union list of EU Regulation 10/2011 (EC No. 10/2011). Although this entry "silanated silicon dioxide" is not for nanoparticles *per se*, EFSA (2014) (EFSA 2014). was recently informed that the substance had always been produced using synthetic amorphous silica in nano-form. Amorphous $SiO_2$ has been used for many years in food applications, such as for clearing beers and wines, as anticaking agent to maintain flow properties in powder food products and to thicken pastes (Dekkers et al., 2011), and as a carrier for fragrances or flavors in food and nanofood products (OECD, Regulation (EC) No. 1333/2008). The conventional form of $SiO_2$ is known as the food additive E551. The majority of particles in food-grade $SiO_2$ ingredients (E551) are usually in the 100 to 1000 nm range, but there may also be a significant population of smaller particles. The EFSA Panel (EFSA 2014) concluded silanated $SiO_2$ does not raise a safety concern for the consumer in the currently authorised conditions of use. Silanised $SiO_2$ nanoparticles in the stomach will be subject to acid hydrolysis. Hence, accoring to EFSA exposures to $SiO_2$ equating to 700 mg Si/day (i.e. 12 mg Si/kg/d) in food supplements and typical dietary intakes of 20-50 mg Si/day (i.e. 0.3-0.8 mg/kg/d) are of no safety concern (EFSA 2009). Dekkers et al., (2011) estimated that the food products including E551 contain nanosilica ranged from <0.1–6.9 mg/g product based on the total silica concentration, where the particles sizes were ranging from 30–200 nm. The authors estimated that intake of $SiO_2$ nanoparticles based on consumption of food products analyzed for their nanosilica or silica concentration is about 124 mg/day.

***Titanium dioxide (TiO₂)***: $TiO_2$ is naturally occurring and poorly soluble. $TiO_2$ is in the top five of nanoparticles used in consumer products (Shukla et al., 2011), accounting for 70 % of the total production volume of pigments (Baan et al., 2006) and consumed annually at about 4 million

tons worldwide (Ortlieb et al., 2010). $TiO_2$ has been used  for decades (Shukla et al., 2011) as a pigment for food colouring (i.e. to provide characteristic optical properties such as increased brightness and/or whitening) or added to granular and powdered foods as anti-caking agents (Lomer et al., 2011). There is no evidence of Ti being an essential element for human beings or animals. The Ti compound concentration in drinking water is generally low. A typical diet may contribute 300–400 µg/day (Shi et al., 2013).

The U.S. Food and Drug Administration (FDA) and FAO/WHO Codex Alimentarius approved $TiO_2$ as a food color additive (E171) in Europe and US with the stipulation that the additive should not to exceed **1% w/w**, and without the need to include it on the ingredient label (EC 1994; FDA 2002; FDA 2015). In Australia, it is approved as pigment for use in processed human foods in accordance with Good Manufacture Practice (GMP) (Standard 1.3.1 2013).

The **nano-form** of $TiO_2$ is **not an approved** additive **for food**, however the grade used in food does not have any particle size specifications. Studies have shown it may contain up to approximately 36 % of particles in the nanoscale (Weir et al., 2012). Therefore the presence of 'nano-$TiO_2$' in food is not new. The estimated dietary exposure of humans to $TiO_2$ nanoparticles has been reported to be up to 1.1 and 2.2 mg/kg body weight/day in the UK and US, respectively (Weir et al., 2012). It is noteworthy that the amount of $TiO_2$ nanoparticles consumed was 2–4 times higher for children than for adults, which may be due to the fact that products heavily consumed by children had some of the highest levels of $TiO_2$ nanoparticles, such as  gums[14],

---

14

candies, desserts, and beverages. A recent study found that candies, sweets and chewing gums contained the highest amount of $TiO_2$ in the scale of $< 100$ nm (Weir et al., 2012). Chewing one piece of chewing gum can result in an intake of 1.5–5.1 mg of $TiO_2$ nanoparticles (Chen et al., 2013).

Smaller $TiO_2$ particles have higher UV-blocking properties, which can be advantageous for food storage (Lin et al., 2011) as well as nanosized $TiO_2$ prevents microbial growth (Ge et al., 2012). $TiO_2$-coated packaging film has been shown to considerably reduce E. coli contamination of food surfaces (Chawengkijwanich et al., 2008) and has also been shown to disinfect water from fecal coliform (Gelover et al., 2006).

*Zinc oxide (ZnO)*: ZnO is another commonly used particle with similar utility to $TiO_2$. ZnO nanoparticles are widely used in various applications including cosmetics, paints, as drug carriers and fillings in medical materials (Dufour et al., 2006). ZnO nanoparticles may be used as a

source of zinc in  nutritional supplements (such as multivitamins (Wiking et al., 2008), and functional foods, since this is an essential trace element, needed to maintain human health and wellbeing, with an average requirment of up to 10.2 mg/day for women and up to 12.7 mg/day for men (Wang et al. 2014; EFSA NDA  Panel 2014). ZnO nanoparticles may also be utilized in food packaging as antimicrobial agents to prevent contamination of foods with harmful bacteria (Sirelkhatim et al., 2015) or as ultraviolet (UV) light absorbers to protect foods that are sensitive to UV light exposure  (EFSA 2016). ZnO exhibits antibacterial activity that increases with decreasing particle size (Yammamoto 2001), which could be stimulated by visible light (Esmaeillou et al., 2013). In addition, ZnO effectively absorbs UV light without re-emitting it as heat and therefore improves the stability of polymer composites.

Zinc oxide in bulk form is authorised in Europe as an additive for plastic materials and articles in contact with food with a SML of **25 mg/kg** food or food simulant (as zinc) (EC No. 10/2011). Recently, the European Commission (EC) published in 2016 the Regulation (EU) No 2016/1416 (EC Regulation 1416/2016) amending and correcting the Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food ('The Plastics Regulation') (EC No. 10/2011). Hence, in 2016, EFSA Regulation (EU) No 2016/1416 required a specific migration limit applicable to ionic zinc already limited into (EU) 10/2011, and set a new specific migration limit (SML) of **5 mg/kg** food or food simulant express as zinc.

*Iron oxide*: $Fe_2O_3$ nanoparticles may be utilized in foods as colorants or as a health suppliment, and is used also in the treatment of contaminated water, where it is claimed to decontaminate water by breaking down organic polluants and killing microbial pathogens (Wu et al., 2014; Raspopov et al., 2011; Hilty et al., 2010). The range of applications of iron oxide as a food colorant in the United States are highly limited, i.e., up to **0.1 wt %** in sausages as part of

casings (WHO 2000). It was estimated that the mean intake of iron oxide from consumers of these products was around 450 μg/day (WHO 2000). However, the levels of iron consumed may be considerably higher for consumers who take mineral-fortified supplements or functional foods (Fulgoni et al., 2011). For example, iron taken in the form of enriched/fortified foods ranges from 10 to 23 mg/day, while that from dietary supplements may range from 10 to 32 mg/day (Fulgoni et al., 2011). $Fe_2O_3$ is authorised in Europe as an additive for plastic materials and articles in contact with food with a SML of **48 mg/kg** food or food simulant (as iron) (EC No. 10/2011).

*Nano sized calcium and magnesium*: Nano-calcium salts are the subject of patent applications (Sustech GMBH and Co. 2003) for intended use in chewing gums. Nano-calcium and nano-magnesium salts are also available as health supplements (Erfanian et al., 2017).

*Nano sized selenium:* Nano-selenium is being marketed as an additive to a green tea product, with a number of (proclaimed) health benefits resulting from enhanced uptake of selenium (Vera et al., 2018). Recently, Bai et al., (2017) proposed nano-selenium encapsualted into polysccharide matrices as a potential candiate for Se supplement with antioxidant properties and be used against Se deficiency in animals and human beings.

**Table 2**

***Mathematic and numerical modelling applied for predicting the migration of ENPs from FCMs***

Basically the right way to assess the exposure to a substance contained in a FCMs is to conduct a migration test in which the concentration of the migrated substances in a food is measured under prescribed conditions. Nevertheless, it is not always practical to perform migration experiments

because they can be challenging, expensive, time consuming and difficult to generalize. In the light of the above mentioned, mathematic/numerical migration modelling might be a valuable tool; a mathematical model provides an analytical formula containing a compact relationship between relevant variables in a system. Numerical models are applied in situations where mathematical models cannot be solved analytically and involve an interactive computational procedure (Barnes et al., 2010). Therefore, mathematical and numerical models can be highly beneficial when used for ENPs migration modeling as they can produce comparable migration results, for a range of different system conditions. There is the potential for mathematical and numerical models to be used as an alternative for costly and time consuming migration studies.

Simon et al., (2008) reported a mathematical model which focusses *specifically* on ENPs migration from polymer food packaging to food. The interphase between the packaging and food was assumed to present no obstacle to the migration of ENPs. So, in their work was predicted migration of nanoparticles[15] *per se* from packaging to food would occur only when very small nanoparticles (i.e. ~1 nm) are embedded in polymer matrices which have low dynamic viscosities (e.g. polyolefins such as LDPE, HDPE, and PP) and do not interact with the nanoparticles. Such conditions could be met by nano-silver with polyolefins composite, but not by nano-silver with polyethylene terephthalate (PET) and polystyrene (PS) composites, nor by any surface-modfied nano-clay embedded in polymer matrices (Simon et al., 2008). After developing a particle volume (size) related migration model, other authors (Bott et al., 2014) (based on an existing model for migration of conventional plastic additives (Simon et al., 2008))

---

[15] The type of ENPs was not accounted for, instead the size of ENPs and the viscous properties of the polymer were used to generate equations for the migratability, diffusion rate and amount of migrating particles (Simon et al., 2008).

came to similar conclusion. The modelling suggested only the smallest spherical ENPs up to 3.5 nm in diameter may result in measurable migration, if present at high concentrations in a polymer. Thus, consumer exposure to ENPs via migration from food contact plastics into foods is not expected according to Bott et al., (2014).

Cuchen et al., (2014) developed a mathematic migration model (Williams-Landel-Ferry equation for time-temperature superposition) of ENPs, i.e. silver and copper; the study revealed that the percentage of ENPs in the FCMs was one of the most curial parameters driving migration, more so than particle size, temperature, or contact time.

Nevertheless, Noonan et al., (2014) have emphasized that till experimental methods are sufficiently developed to generate reliable ENPs migration data for FCMs, such theoretical models as described above cannot be validated and are only of academic relevance.

Numerical models dealing with ENPs migration from packaging to food are lacking. To date, a numerical model presented by von Goetz et al., (2013) is the only model which deals with ENPs migration from food packaging. The model assumes Fickian diffusion and takes account of the ENPs diffusion from within the polymer to the plastic/liquid interphase.

The affect that the penetration of food has on migration of particles from food packaging is a major factor that has been intentionally neglected in all the aforementioned migration models.


*Standardized food model*

Zhang et al., (2019) proposed for the first time a standardized food model (SFM) for evaluating the toxicity and fate of ingested ENPs. So its efficacy was assessed by examining the impact of food matrix on the toxicity of $TiO_2$ nanoparticles. The formulation of the SFM was based on the average nutrient composition of the U.S. diet (proteins, fats, dietary fibers, and carbohydrates at

levels typically found in the human diet): 3.4% protein (sodium caseinate); 4.6% sugar (sucrose); 5.2% digestible carbohydrate (modified corn starch); 0.7% dietary fiber (pectin); 3.4% fat (corn oil); and, 0.5% minerals (sodium chloride). The SFM consisted of an oil-in-water emulsion suitable for use in both wet and dried forms. SFM was passed through a commonly used simulated gastrointestinal model and changes in its structural properties were characterized. The main food components in the SFM were shown to be digested when exposed to the digestive enzymes in the simulated GIT. The authors showed that the potential toxicity of the ENPs, such as $TiO_2$ nanoparticles, was reduced in the presence of the SFM, which highlighted the importance of food matrix effects.

This information should be useful when designing experiments to test food matrix effects on the gastrointestinal fate and toxicity of ENPs. In particular, the availability of a standardized food model will facilitate the comparison of results obtained on food matrix effects from different laboratories.

***Analytical techniques for quantification of ENPs per se as well as thier migration from FCMs***

Quantification of migrated ENPs from FCMs is a very important issue because of the safety concerns of these. The lack of exhaustive and complete toxicological data (*see* detailed information at section "Toxicological data. The current challenges faced by implementation of the active food packaging-associated engineered nanoparticles") is also due to the actual difficulty to characterize, detect and measure ENPs per *se* and in complex matrices like food (Tiede et al., 2008). Hence, discussing the potential risks of ENPs for consumers requires thorough insight about their characteristics (Huang et al., 2015). For the characterization of

nanocomposite structures, aspects that are more likely to be considered are particle dispersion, changes in the mass matrix, and the type of particle-polymer interface (Arora et al., 2010).

In the migration test, the specimen to be tested is brought into (i) single-sided contact (multi-layer materials) or (ii) double-sided contact (mono-materials) with food simulants (*see* **Figure 1**). After exposure, the simulant is analyzed by different methods to detect the quantitative as well as qualitative presence of ENPs; there is no "stand-alone" technique for their detection in practical applications. A recently published overview of measurement methods for evaluation of ENPs *per se* (Mourdikoudis et al., 2018) as well as ENPs *per se* released from food contact materials is given by Peters and Hoekstra (2019); Noonan et al., (2014). Due to the inherent properties of ENPs, the detection of ENPs in complex media like foods and food simulants can be very problematic (Singh et al., 2014). ENPs show poor dispersion stability when they are dispersed in an inappropriate medium. The nature of the surrounding matrix strongly determines whether ENPs remain stable in dispersion or are "lost" either due to dissolution of particles or sedimentation (Blasco et al., 2011). Furthermore, for an unambiguous detection of ENPs in complex matrices, the analytical technique must allow a clear distinction or separation of ENPs from other interfering matrix components. Therefore, detecting the migration of ENPs into food matrices requires sensitive analytical techniques due to the complexity of the ENPs, and their representing only a very small portion of the bulk food. Thus, detection of ENPs in complex matrices, such as food, is a challenging task which requires combined methodologies, since no individual techniques can give all the detailed information. Spectroscopic, microscopic and quantitative analytical techniques are all useful for this purpose. For quantitative determination, only few techniques are available, which may allow sensitive and selective detection and characterisation of ENPs. Scanning electron microscopy (SEM), transmission electron

microscopy (TEM) and atomic force microscopy (AFM) are good examples of microscopic methods, each of which has its own advantages and disadvantages. By these methods, nanoparticles' general features, such as size, shape, structure, dispersion, and aggregation state, are accessible. Nevertheless, these techniques usually are destructive, meaning that verification tests for a better analysis are not feasible on the same sample (Huang et al., 2015). Other technique can be use also for size investigation: Dynamic Light Scattering (DLS), single particle Inductive Couple Plasma Mass Spectroscopy ( sp-ICP-MS), Field Flow Fractionation (FFF), FFF-ICP-MS. Spectroscopic techniques, such as UV-vis spectroscopy and X-ray diffraction (XRD), can be used for characterization and analysis of ENPs as well. The supporting method UV-vis spectroscopy has been used extensively due to its cost effectiveness and ease of operation. This technique confers information about the presence of nanoparticles (Huang et al., 2015). XRD, both wide angle (WAXS) and small angle (SAXS), are the conventional methods that are used to examine nanocomposite structures (Arora et al., 2010). Elemental composition or the crystalline arrangement of nanomaterials, both in natural and engineered form, can be investigated by the XRD techniques (Huang et al., 2015; Sharma et al., 2012). Despite microscopic methods, this technique is not destructive, and there is no need for difficult sample pre-treatment. These attributes contribute to its extensive application in the characterization of materials (Huang et al., 2015; Alexandre et al., 2000; Koo et al., 2006). Combination of TEM with energy-dispersive X-ray spectroscopy (EDX), apart particle shape, size and aggregates structures information, allows analysis of the chemical composition (Smith 2007), but data evaluation is very elaborate and time-consuming. More suitable analytical techniques is asymmetric flow field-flow fractionation (AF4) either coupled with a multi angle light scattering (MALS) or dynamic light scattering (DLS) detector which allows separation of nanomaterials

according to their size and to measure particle size distributions accurately (Schmidt et al., 2009; Baalousha et al., 2006; Fraunhofer et al., 2004; Kammer et al., 2005). To acquire chemical information AF4 can be coupled with element specific technique, like inductively coupled plasma[16] - atomic emission spectroscopy (ICP-AES/ICP-OES), inductive coupled plasma - mass spectroscopy (ICP-MS) (Montoro Bustos et al., 2013; Loeschner et al., 2015), which are three important techniques for quantification and elemental analyses (Huang et al., 2015). The advantages of these methods include their high selectivity, sensitivity, and accuracy. Using these methods, ENPs can be detected in amounts as low as 0.1-10 ppm (Huang et al., 2015). Introducing ENPs into the ICP, a beam of gaseous ions is produced by the atoms of the analyte in the plasma, resulting in an individual pulse measured by the detector, which appears as a peak on the graph (Echegoyen et al., 2013). Prior to injecting the sample, a digestion process is required because the presence of food components could impede good atomization of the sample (Huang et al., 2015). In addition, basic ICP-MS does not differentiate between nanosized element metal and metal ions, which  for e.g. in case of nanosilver migration studies means dissolved silver ions and dispersed silver in its particulate form (Fabricius et al., 2014). Whereas, sp-ICP-MS is a analytical technique able to distinguish quantitatively between dissolved (ionic material) and particulate species (Mackevica et al., 2016; Laborda et al., 2014; Laborda et al., 2011; Mitrano et al., 2012; von Goetz  et al., 2016) in one analyses. However, according to Fabricius et al., (2014) and Olesik et al., (2012) there are some limitations of this technique. In

---

[16] Inductively coupled plasma spectometer is well-suited for trace detection of metals in solution, in which a liquid sample is injected into argon gas plasma contained by a strong magnetic filed. The elements in the sample become excited and the electrons emit energy at a characteristic wavelength as they return to ground state. The emitted light is then measured by optical spectrometry; method known as ICP-OES or ICP-AES.

case of metal species which reversibly can change the oxidation state from metal (0) to metal ions (+1 or +2) depending on the chemical environment (e.g. incompatible matrices, e.g. organic solvents; ENPs with broad particle size distributions), sp-ICP-MS can even be misleading and lead to false conclusions. E.g. in the case of silver nanoparticles, one of the most frequently investigated polymer nanocomposite systems, migrating silver (+1) ions may either be reduced to silver (0) particles during the migration test or sample handling and then be false-positive measured as migrated silver nanoparticles by sp-ICP-MS (Turioniemi 2013; Gover et al., 2011; Ntim et al., 2016).

An alternative approach to ICP is atomic absorption spectroscopy (AAS) for the detection of ENPs. In many cases, AAS is used for the quantitative determination of chemical elements within samples, such as food, by measuring the absorption of optical radiation (light) by free atoms in the gaseous state. However, analysis of more than one element is not possible by AAS (Liu et al., 2012).

In short, there is no "stand-alone" technique for the detection and characterizations of nanomaterials in practical applications. More than that, taking into account that the environment can also affects nanoparticles properties, the approach is even more complicated with complex matrices where also sample preparation becomes a determinative step. Complex matrices usually contain different type of nanoparticles that can interfere with the analytical detection. At the same time, sample clean up and preparation might lead to artefacts (e.g. aggregates among particles, aggregate among particles and food proteins or lipids, particle dissolution) and therefore modify the original state of the sample.Thus, the appropriate sample work preparation are crucial for quantitative measurements of nanomaterials. All these aspects and the lack of

reference materials for a large number of food/particle combinations make the development and validation of analytical methods to detect ENPs in food still a challenging and ongoing issue.

*Toxicological aspects. The current challenges faced by implementation of the active food packaging-associated engineered nanoparticles*

The potential risks of the ENPs embedded into FCMs on the consumer 'health is connected to their possible migration from food/beverage packaging into foodstuff; thus the main concern is that, ENPs are not normally eaten and metabolized. Furthermore, nanoparticles are able to enter the cells, tissues and organs of our bodies much more easily than their microscale counterparts (larger particles).

 Ironically, although the active food packaging-associated engineered nanoparticles offer new opportunities, e.g. the extension of the shelf-life of foodstuff, might also pose new risks to human health and the environment (*see* below an overview of these statements).

*Overview on Food "nano-fabricate" by nature vs ENPs in food industry*

Nanotechnology in food industry covers three classes of nanomaterials: (i) natural and processed nanostructures in foods; (ii) particulate nanomaterials metabolized or excreted on digestion; and (iii) particulate nanomaterials not broken down on digestion, which accumulate in the body.

 Natural nanostructures are not regarded as products of nanotechnology, and they need to be differentiated from deliberately manufactured nanomaterials when considering regulatory requirements and definitions (Cockburn et al., 2012).

 Food itself contains many nanostructured materials, i.e. proteins, complex carbohydrates and fats, with sizes extending down to the nanoscale (e.g., casein, alginic acids, etc.). The best

example of a food nano-fabricated by nature itself is the milk in our fridges. Milk proteins (i.e., casein and the globular proteins β-lactoglobulin and α-lactalbumin) are synthesized in the cells of the cow's udder and transported out into the plasma or aqueous phase (Heid et al., 2005). Casein is secreted as a micelle (300−400 nm in size) assembled from different casein subunits held together by colloidal calcium phosphate. Triacylglycerides are gathered together as small droplets that fuse as the growing lipid droplet (100 nm−10 μm) moves toward the apical plasma membrane. Lipid droplets are released from inside the cell surrounded by the cellular membrane and become the fat globules in milk. In turn, the milk fat globule membrane is a complex lipid bilayer, 4−25 nm thick, containing several types of bioactive molecules within its structure (Heid et al., 2005). The dairy industry utilizes three basic *micro-* and *nano-* sized structures (casein micelles, fat globules, whey proteins) to build all sorts of emulsions (butter), foams (ice cream and whipped cream), complex liquids (milk), plastic solids (cheese), and gel networks (yogurt) (Aguilera et al., 1999). In fact, dairy technology is not just a microtechnology, but also a nanotechnology, and it has existed for a long time. Furthermore, casein micelles may be useful as nanovehicles for entrapment, protection and delivery of sensitive hydrophobic nutraceuticals within other food products (Semo et al., 2007). Other times, nature builds hierarchical structures from the macromolecular to the tissue level. Devine and Dikeman (2014); and Sheeparamatti, et al., (2007) described in details these aspects in "Enclyclopedia of meat sciences" (Devine and Dikeman 2014) and "Nanotechnology: Inspiration from Nature" (Sheeparamatti et al., 2007).

 This is important to note since the distinction between natural nanostructures and deliberately manufacturied nanomaterials produced to a particular specification is not always clear. Manufacturied nanomaterials entering the body through ingestion (for e.g. ENPs used as pigment for food colouring (*such as TiO$_2$ used to provide characteristic optical properties such*

*as increased brightness)* in candies, sweets, chewing gums, etc.; or for e.g. migration of ENPs from active packaing into foodstuff) are subject to digestive processes in the gastrointestinal tract (GIT). The GIT and its mucosal layer should play the role of a selective barrier to systemic exposure of materials, including particulate matter, in which case the ENPs may remain in the gut lumen, perhaps with a potential for interaction with GIT surfaces or with inhabitants of the lumen (e.g., microbiota), but essentially being fully eliminated from the body via the faeces. The behavior of ENPs entering the body through ingestion are described by Martirosyan et al., (2012). Nevertheless, up to date, the ENPs have not been comprehensively assessed in regard to the potential effects on human health. Below it will be discussed the existing information on hazard identification of mostly applied metal-based nanoparticles (ENPs) on the GIT based on both *in vitro* cell-based assays and in *vivo* animal experimentation.

Legislation addressing health and safety aspects of nanomaterials in consumator products and ensuring their use is being continously updated in the European Union and globally. Thus, this leads to a growing need for tools to implement this developing legislation. In 2018 was launched on the market a new standard for evaluation of ENP'cytotoxicity "ISO 19007, Nanotechnologies − in vitro MTS assay for measuring the cytotoxic effect of nanoparticles" (ISO 19007 - 2018).


*ENPs and their toxicity profiles: cytotoxicity and cellular malfunction*

ENPs may produce toxicity in cells through a variety of different mechanisms, depending on their composition and structure (Frohlich et al., 2016). One of the most important factors contributing to the toxicity of ENPs is their ability to generate reactive oxygen species (ROS), such as singlet oxygen, superoxide, hydrogen peroxide and hydroxyl radicals (Wu et al., 2014). These ROS may then cause damage to cell membranes, organelles, and the nucleus by

interacting with lipids, proteins, or nucleic acids (Wu et al., 2014; Gaillet et al., 2015; Sarma et al., 2014). As a result, many biochemical functions required to maintain cell viability, such as ATP production, DNA replication, and gene expression, may be adversely affected (Sarma et al., 2014). Below will be discussed the current studies that have reported the ability of ENPs (i) to increase the generation of ROS in cells and to produce cytotoxicity, including silver nanoparticles, silicon dioxide nanoparticles, titanium dioxide nanoparticles, zinc oxide nanoparticles, and iron oxide nanoparticles, and (ii) to generate ions and to produce toxicity (such as $Ag^+$ from silver nanoparticles or $Zn^{2+}$ from zinc oxide nanoparticles) that interact with the normal functioning cellular components (such as proteins, nucleic acids, or lipids) required to maintain biochemical processes. These mechanisms of action are most likely to be important for ENPs (inorganic nanoparticles) that are absorbed by the intestinal cells, since most organic nanoparticles are digested before being absorbed.

*Silver nanoparticles*: Studies suggest that nanosilver can kill 650 disease-causing pathogens in food, whereas most traditional antimicrobials kill only 5–6 such pathogens (Bumbudsanpharoke et al., 2015). At present there are controversal information about the potential toxicity of silver nanoparticles ingested with foods (Gaillet et al., 2015; Ahamed et al.,2010), some studies reporting no toxicity (Hendrickson et al., 2016; Echegoyen et al., 2013; Kim et al., 2008; Fondevila et al., 2009) and others reporting appreciable toxicity (Liao et al. 2019; Cho et al. 2018; Dubey et al. 2015; Chen et al., 2016; Georgantzopoulou et al., 2016; Perde-Schrepler et al., 2019; Ahamed et al., 2010; Trickler et al., 2010; Sharma et al., 2010; Sharma et al., 2009; Frohlich et al., 2016; Williams et al., 2015; Lichtenstein et al., 2015; Kawata et al., 2009; Martirosyan et al., 2014; Sharma et al., 2014; Kim et al., 2012; Cha et al., 2008; Kim et al., 2009; Shahare et al., 2013; Jeong et al., 2010; Park et al., 2010; Garcia et al., 2010). The GIT

may be particularly susceptible to silver nanoparticle-induced toxicity since it contains the first tissues exposed to dietary nanoparticles after ingestion. Nevertheless, the adverse effects of silver nanoparticles on the GIT remain inconclusive. Therefore, there are very controversial data in this relation and there is a need of more thorough investigation with consideration of environmental conditions, e.g., the presence of complex food matrix, time, temperature, etc. While there is a growing number of *in vitro* studies showing that silver nanoparticles are cytotoxic to a variety of mammalian cell types, in *vivo* studies that have investigated the systemic effects of silver nanoparticles upon exposure by oral route are more ambiguous. Taking into account the small number of *in vivo* toxicological studies for silver nanoparticles, a limited generalized conclusion on the effects of silver nanoparticles exposure via food-relevant routes could be done. For example, it is still unclear:

✓ to what extent nanoparticles pass through the intestinal lining intact or as dissolved $Ag^+$ due to the highly acidic environment of the stomach. Further studies are required to determine if silver nanoparticles dissolve in gastrointestinal fluids, and to assess whether there is a difference in behavior of silver when ingested in a soluble or nanoparticle form (Loeschner et al., 2011). Indeed, a study in which rats were fed either soluble or nanoparticle forms of silver found that the organ distribution of the silver was similar in both cases (Loeschner et al., 2011; Wen et al., 2017).

✓ to what extent silver nanopartciles can pass through natural biological barriers such as the gut epithelium, blood-brain barrier, the placenta or get into the breast milk. There is also a knowledge gap concerning the relationship between nanoparticles characteristics (size, shape, charge, coating, etc.) and toxicity.

In short, animal studies have shown that silver nanoparticles may accumulate in the body and have toxic effects when ingested at sufficiently high levels, but it is not clear whether these levels are close to those actually achievable through food consumption. Hence, will be important in future studies to carry out long-term chronic toxicity studies using nanoparticle levels that are more similar to those actually consumed in the human diet.

**Titanium nanoparticles**: A recent review *in vitro* and *in vivo* animal studies concluded that TiO$_2$ nanoparticles not only, accumulate in the tissues of mammals and other vertebrates, but that they also have a very limited elimination rate ( Kononenko et al., 2019; Aliakbari et al., 2019; Dorier et al., 2015; Kruger et al., 2014; Gerloff et al., 2012; Cho et al., 2013; Shi et al., 2013; Jovanović 2015; Wang et al., 2007; Powell et al., 2000; Wang et al., 2007; Trouiller et al., 2009; Valant et al., 2011; Duan et al., 2010; Nogueira et al., 2012; Bu et al., 2010; Tada-Oikawa et al., 2016; Song et al., 2013; Brun et al., 2014; Chalew et al., 2013; Gerloff et al., 2009; Monopoli et al., 2011; Lesniak et al., 2013; Wang et al., 2013). Nevertheless, the results are conflicting, some of them have reported little accumulation or toxicity (Monopoli et al., 2011) of ingested TiO$_2$ nanoparticles. In addition to this, the doses employed were high. For example, a study where TiO$_2$ nanoparticles (mixture of anatase and rutile at size: 21 nm) were repeatedly administered (260–1041mg/ kg) to rats did not report any significant toxicity or TiO$_2$ accumulation in tissues or urine, but reported high concentrations of titanium dioxide in feces, suggesting that the TiO$_2$ nanoparticles were mostly eliminated (Monopoli et al., 2011). The observed contradictions between different animal studies on the accumulation and toxicity of TiO$_2$ nanoparticles may arise for a number of reasons:

✓ Firstly, there are differences in the oral dose, crystal form, particle size/shape, aggregation state, and surface characteristics of the nanoparticles used.

✓ Second, the impact of the food matrix and GIT passage on the properties of the nanoparticles are often ignored, or taken into account differently.

✓ Third, the type of animal model and analytical methods used to determine accumulation and toxicity may vary. For example, it has been shown that the *age* of the experimental animals used is an important factor. The same doses (up to 200 mg/kg body weight per day for 30 days) of $TiO_2$ nanoparticles (anatase at 75 nm) were used to treat both young (3-week-old) and adult (8-week-old) rats (Lomer et al., 2004). Heart injuries, liver edema, and non-allergic mast cell activation in stomach tissue were observed in young animals, but only slight toxic effects were observed in adult animals (Lomer et al., 2004). This finding is particularly important given that the amount of $TiO_2$ nanoparticles consumed by humans is estimated to be appreciably higher for children than adults (*see* section "European and USA standard migration tests: Titanium oxide"). $TiO_2$ is an approved food additive with the limit set at 1 % by weight of the food; however, neigher the size nor the structure is defined (EC 1994; FDA 2002; FDA 2015). It has been estimated that the average daily exposure to $TiO_2$ from food, medicines and toothpaste is around 5 mg/individual (i.e., about 0.07 mg/kg BW) (Athinarayanan et al., 2014), which is a much lower dose than those that showed adverse effects in experimental animals (Lomer et al., 2004). In short, there is no data if, and what proportion of $TiO_2$ nanoparticles is absorbed at doses relevant to human exposure, and how different food matrices affect behaviour and absorption of $TiO_2$ nanoparticles.

No conclusion about the risk of nano-sized $TiO_2$ by oral exposure is possible, until in vivo toxicokinetic data for nano sized $TiO_2$ are available.

Nevertheless, some important questions which should not be neglected in case there is low exposure at nano-sized $TiO_2$ are:

(i)       may trigger symptoms in subjects with an underlying susceptibility,

(ii)      and throught a constant lifetime oral exposure, reach concentrations that would trigger adverse effects?

***Silicon dioxide nanoparticles***: Cell culture and animal feeding studies suggest that high levels of $SiO_2$ nanoparticles may cause adverse effects, such as cytotoxicity and generation of ROS (So et al., 2008). A recent study suggested that the $SiO_2$ nanoparticles accumulate in liver at levels that could cause a health risk (van Kesteren et al., 2015; Dekkers et al., 2013). Dekkers et al., (2011) concluded that single $SiO_2$ nanoparticles may be more easily absorbed from the human intestine (Dekkers et al., 2011; Yun et al., 2015), but that no conclusion on the oral bioavailability of synthetic amorphous silica or nano-sized silica can be drawn so far. Conversely, a study that employed oral administration of silicon dioxide nanoparticles to rats over a 13-week period reported no accumulation or toxicity (Pati et al., 2015). Therefore, no clear conclusion on the toxicity of silicon dioxide nanoparticles can be drawn based on the available evidence.

***Iron oxide nanoparticles***: Crystalline forms, shapes and diferent sizes may alter their toxicity (Yun et al., 2015). It has been proposed that the ability of iron oxide nanoparticles to generate ROS is the most likely mechanism for their potential toxicity (Wu et al., 2014). Some studies where iron oxide nanoparticles were orally administered at about 3 mg/kg body weight or at much higher dose 250–1000 mg/kg body weight to both male or female rats over a 13-week period reported that they did not accumulate in tissues or produce toxicity (Wang et al., 2014; Hilty et al., 2010).

***Zinc oxide nanoparticles***: The antimicrobial activity of ZnO nanoparticles has been partly attributed to their ability to penetrate into microbial cells and generate ROS that damage key

cellular components thereby leading to cytotoxicity (Chen et al., 2019; Sirelkhatim et al., 2015; Choi et al., 2015; Vandebriel et al., 2012). This mechanism could lead to adverse health effects in humans if this type of nanoparticle were ingested in sufficient quantities and then absorbed by the human body.

Chen et al., (2019) reported that the cytotoxicity of ZnO nanoparticles, depend on both the size and concentration, and was attributed to the release of $Zn^{2+}$, induction of oxidative stress and inflammatory response; the death mode of HepG2 cells incubated with ZnO nanoparticles was necrotic rather than programmed cell death. Several rodent feeding studies have demonstrated particle size - dependent effects on the intestinal uptake of ZnO nanoparticles, with a smaller particle size leading to a higher uptake (Pasupuleti et al., 2012;  Wang et al., 2008; Wang et al., 2013; Kang  et al., 2013; Safar et al., 2019). A recent study demonstrated time- and dose-dependent cytotoxicity of ZnO nanoparticles on Caco-2 cells after 24 h exposure (Scherzad et al., 2017). The authors reported that ZnO nanopartciles exert different size-dependent cytotoxic effects with the highest toxicity to Caco-2 cells at 26 nm. These ZnO nanoparticles at 26 nm could also reduce the G1 phase, increase the S phase and the G2 phase cells to repair damaged genes, while no differences were obtained between 62 nm and 90 nm ZnO nanoparticles (Scherzad et al., 2017). The *in vivo* studies on mice have shown that the Zn concentration in liver, spleen and kidney was higher after administration of zinc in *nano-* form compared to similar amounts of ZnO *micro-* particles. Oral nanoparticles administration resulted in transient histopathology of the liver that was not seen after administration of *micro-* sized ZnO particles (Yammamoto 2001). Oral administration of 100 nm ZnO nanoparticles (2.5 g/kg of body weight) resulted in their accumulation in the liver, spleen, lung, and kidney. In contrast to intraperitoneal administration, ZnO nanopartilces did not accumulate in the heart (Esmaeillou et al., 2013).

More information on the existing literature on mammalian toxicity of ZnO nanoparticles, both *in vitro* and *in vivo*, is summarized by Bacchetta et al., (2014).

 Wang et al., (2014) showed in their work that ZnO nanoparticles were not toxic when used in isolation, but that they become toxic when mixed with ascorbic acid. This suggests that it is important to measure the impact of specific food components on the toxicity of this type of nanoparticle. ZnO nanoparticles may be spherical or non-spherical solid particles that are usually highly aggregated when dispersed in aqueous solutions (Wang  et al., 2014; Vandebriel et al., 2012). These aggregates are typically many times larger than the individual nanoparticles, with their size and structure depending on solution conditions, which is likely to have a major effect on their GIT fate and toxicity. Feeding studies with frogs have shown that zinc oxide nanoparticles exhibit greater toxicity than a dissolved form of zinc, which was attributed to their greater capacity to induce oxidative damage in cells (Orfi et al., 2016). This study highlights the importance of establishing the physical form of zinc when ZnO nanoparticles are ingested.

*General comments*

The data of science in this area emphasize that there is currently a lack of detailed understanding about the gastrointestinal fate and toxicity of different kinds of ENPs, and that there are often inconsistencies between different studies. There are a number of factors that may contribute to this uncertainty. The types and levels of nanoparticles used in different studies vary considerably, and the levels used in cell culture and animal studies are often much higher than those that would ever be consumed by humans. In addition, simple cell culture models (such as Caco 2 cells) cannot mimic the complexity of animal and human GITs. It should also be noted that the levels of ENPs reported to accumulate in tissues are often misleading, since the analytical techniques used only measure the concentration of specific elements present (such as

Ag, Zn, Fe, Ti, or Si) rather than the physical form (e.g., dissolved, nanoparticle, or microparticle).

Finally, food matrix effects are often ignored, and may have a pronounced impact on the behavior of nanoparticles in the GIT (ISO 19007:2018; Frohlich et al., 2016). It is therefore clear that further systematic research using well-defined nanoparticles and test methods are urgently needed.

Recently, Zhang et al., (2019) proposed for the first time a standardized food model (SFM) for evaluating the toxicity and fate of ingested ENPs (Zhang et al., 2019). The authors showed that the potential toxicity of the ENPs, such as $TiO_2$ nanoparticles, was reduced in the presence of the SFM, which highlighted the importance of food matrix effects. This information should be useful when designing experiments to test food matrix effects on the gastrointestinal fate and toxicity of ENPs. In particular, the availability of a standardized food model will facilitate the comparison of results obtained on food matrix effects from different laboratories.

In conclusion, the lack of standardized or validated methods estabilished for nanotoxicity testing has led to the publication of confusing and often inconsistent data, and is hindering the development of nanoparticles risk assessment strategies. All these aspects have emerged in the EFSA opinion " The Potential Risks Arising from Nanoscience and Nanotechnologies and Food and Feed Safety" published in 2009 and are still a current issue. It is therefore clear that further systematic research using well-defined nanoparticles and test methods are urgently needed. In 2011 EFSA has published a Guide specifically related to nanoscience and nanotechnologies in food to evaluate the risk of possible impact of the nanoparticles on human health (EFSA 2011). More than that in April 2018 was launched a new normative for nanocytotoxicity ISO

19007:2018 (E) "Nanotechnologies -- In vitro MTS assay for measuring the cytotoxic effect of nanoparticles" (ISO 19007:2018).

***Moving beyond concept to current industrial applications***

New consumer products containing ENPs have been launched to the market and are beginning to impact on the food associated to industries.

In 2008 the global "*Nano-Enabled Food and Beverage Packaging Market*" was 4.13 billion US dollars (iRAP 2009; Ducan 2011; FAO/WHO 2010), in 2017 was valued at 30.60 billion US dollars, and is estimated to reach USD 89.0 billion by 2026 growing at a CARR of 12.7 % during the forecast period, according to a new study published by Polaris Market Research (Polaris Market Research 2018[17]). Thus, numerous companies are already engaged in production of packaging materials based on nanotechnology that are extending the shelf life of food and drinks, and also improving the food safety. Mishra (2019); FoE (2008); Hannon et al., (2015); Reig et al. (2014); Han et al., (2011); Chaudhry et al., (2008) listed a number of examples of commercially available food packaging products containing ENPs.

They include:

- Debbie Meyer® Green Bags (Debbie Meyer® Innovation, USA) containing nano-clay to preserve the freshness and prolong the shelf-life of fruits and vegetables.

- Nano-silver Baby Milk Bottle (Baby Dream Co. Ltd®, Korea) containing nanosilver.

---

[17] The report *'Nano-Enabled Packaging Market Share, Size, Trends, & Industry Analysis Report, By Type (Active, Intelligent, Others); By End-User (Food and Beverages, Pharmaceutical, Personal Care, Others); By Regions: Segment Forecast, 2018 – 2026'* provides an extensive analysis of present market dynamics and predicted future trends.

- NanoSeal$^{TM}$ - Barrier Coating and NanoSeal$^{TM}$ - Bairicade XT$^{TM}$ Barrier Coating (from NanoPack Inc. $^®$, USA): described as a water based coating comprised of a masterbatch and a liquid dispersion of nano-clay platelets. The coating is applied to traditional packaging films (such as polyester, polypropylene, nylon and polylactic acid) to enhance gas barrier properties, and is stated to be approved for indirect food contact (i.e. used with dry and moderately dry food applications).

- Silver-nano Noble one-touch mug cup (Baby Dream Co. Ltd$^®$, South Korea).

- Debbie Meyer$^®$ Bread Bags™ (Debbie Meyer$^®$ Innovation, USA) containing nano-clay for bread storage.

- PET bottles containing nano-titanium nitride (Colormatrix$^®$, USA) to confer barrier properties.

- Plastic beer bottles containing nano-clay (Miller Brewing Co$^®$, USA and Hite Brewery Co.$^®$, South Korea) to confer barrier properties.

- Zeomic$^®$ silver zeolites (Zeomic Co Ltd$^®$, Japan) packaging film.

- Fresh food containers (Oso Fresh$^®$, USA) containing nanosilver (size particles 40-30 nm).

- Nano-silver food containers (A-DO Global$^®$, South Korea).

- Nano-silver NS-315 water bottle (A-DO Global$^®$, South Korea).

- Nano-silver salad bowl (Changmin Chemicals$^®$, South Korea).

- FresherLonger$^{TM}$ Miracle Food Storage (Sharper Image$^®$,USA) containing nanosilver.

- FresherLonger$^{TM}$ Plastic Storage Bags (Sharper Image$^®$, USA) containing nanosilver.

- Fresh box silver nanoparticles food storage containers (BlueMoonGoods$^{TM®}$, USA).

- Smartwist food storage with nanosilver (Kinetic Go Green$^®$, USA).

- Clear Silver Reclosable Mylar Zip Lock Bags Aluminium Foil Packaging Plastic Valve Zipper Paunches Bulk: Food Storage, Coffee, Candy (Pabck®, USA).

- Aegis® OX (Honeywell®, USA): polymerised nanocomposite film is an oxygen-scavenging barrier resin formulation for use in co-injection PET bottle applications, e.g. beer, fruit juice and soft drinks. The resins are a blend of active and passive nylon using $O_2$ scavengers and passive nano-clay particles to enhance barrier properties, retain $CO_2$ but keep $O_2$ out.

- Nano-silver storage box (Quan Zhou Hu Zeng Nano Technology Co. Ltd®, China).

- Nanobox (Hopack®, Australia), a paper food box/container containing nanosilver.

- Agion® (Agion Technologies®, USA) containing silver zeolites for controlled release of antimicrobial ions. The technology is marketed as being applicable to virtually any material or surface.

- Nano Plastic Wrap (SongSing Nano Technology Co. Ltd®, Taiwan) containing nanozinc oxide as a light catalyst to sterilise in indoor lighting.

- Imperm® (Nanocor Inc®) used in nano-clay containing multi-layer polyethylene terephthalate (PET) bottles and sheets for food and beverage packaging. It works by minimising the loss of $CO_2$ from drinks and penetration of $O_2$ into bottles, thus keeping the beverage fresher and extending the shelf life by up to 6 months.

- Nanolok™ (InMat® Inc, USA) is a water based environmentally friendly nano-clay based-composite coating with high oxygen barrier intended for transparent packaging applications.

- Duretham® LDPU 601 (Bayer AG®, Germany): a transparent plastic film with nylon enriched with silicate particles. Its primary purpose is to prevent packaging contents from drying out and protects them from moisture and $CO_2$.

- Plantic™ biodegradable packaging (Plantic Technologies Ltd®, Australia) is a biodegradable and completely compostable bioplastics packaging, prepared from organic corn starch using nanotechnology (www.plantic.com.au, Neethirajan and Jayas 2011). These biopolymer-based nanocomposites are supplied to 80 % of the Australian chocolate tray market.

**Summary and conclusions**

Redesigning traditional packaging into an active and intelligent one by using ENPs has generated a great deal of interest in recent years. Nevertheless, the risks and benefits of ENPs in food and beverage packaging receive conflicting international attention across expert stakeholder groups as well as in news media coverage and published scientific research. Against the generally predicted growth of nano food contact applications the number of the currently and legally authorized nanocomposites materials is rather small. This is linked to the fact that up to date there is insufficient toxicological data/or conflicting data regarding the dose associated with potential health effect and safety assessments are still ongoing.

*Remaining knowledge gaps*

A few examples below highlight the range of needs that still exist:

✓ Methods to monitor release of ENPs from products should further developed, prioritized and linked with consumer exposure assessments. An evaluation of the current literature on the

migration of ENPs from FCMs showed that despite the increasing number of experimental studies, still many open questions remain:

(i)     whether ENPs can migrate from FCMs at all; e.g. in the EU Regulation No 10/2011, article 11, it is stipulated that the Specific Migration Limits (SML) - express in mg of substance per kg of food (mg/kg) for ZnO and $TiO_2$ are not applicable (Art. 11, Annex I, Table 1, Column 8), but for Zn metal the specific migration limit is **25 mg/kg** to food or food simulant. In 2016, the European Commission (EC) published the Regulation (EU) No 2016/1416 amending and correcting the Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food ('The Plastics Regulation'). It was set a new SML for zinc (**5 mg/kg** food or food simulant), aluminum (**1 mg/kg** food or food simulant), and for silver (**0.05 mg/kg** food or food simulant). Furthermore, it was stipulated that the provisions on the specific migration limits for zinc and the assignments of food simulants fresh and peeled fruits and vegetables will apply "on 14 September 2018".

(ii)    potential release mechanism of ENPs;

(iii)   detection and characterizing ENPs in migration studies and the suitability of the most frequently used analytical techniques are still a challenge. Due to different limitations (e.g. regarding size and concentration range) in every single applied techniques, a combination of analytical techniques should preferable be used to improve the detection of ENPs. A combination of ICP-MS, AAS, sp-ICP-MS (*due to their high selectivity, sensitivity, and accuracy allow the detection of very low particle concentrations in food simulants, e.g. 0.1-10 ppm*), and TEM-EDX (*give information*

*on particle shape and elemental composition, and can detect smaller ENPs than sp-ICP-MS*) for studying the migration of ENPs is suggested.

(iv) consideration regarding the risk for the consumer associated with migration ENPs form FCMs were discussed. This is the most complex question because data are lacking in relation to all aspect of risk assessment including the fate of migrated ENPs in food and gastrointestinal tract exposure to ENPs. Interaction of ENPs with food should be further studied, and ENPs characterized in different food matrices. Possible changes of the food matrix by interaction with (migrated) ENPs should be considered. More detailed studies on the influence of physico-chemical characteristics of ENPs in the gastrointestinal tract are needed. The use of in vitro digestion models for predicting the fate of ENPs in the gastrointestinal tract is recommended.

(v) a standardized food model (SFM) for evaluating the toxicity and fate of ingested ENPs was discussed (Zhang et al., 2019 proposed for the first time a standardized food model for evaluating the toxicity and fate of ingested ENPs). This information should be useful when designing experiments to test food matrix effects on the gastrointestinal fate and toxicity of inorganic nanoparticles. In particular, the availability of a standardized food model will facilitate the comparison of results obtained on food matrix effects from different laboratories.

✓ Data sets for the validation of exposure models and *in silico* approaches for the prediction of toxicity and fate using standardized methods should be generated by close cooperation of experimentalist with modelers.

✓ *In vitro* tests that better predict chronic effects on human health should be developed (e.g. ISO 19007: 2018).

✓ More robust data sets that contain curated information on migration and toxicity (e.g . there are various issues that complicate the interpretation of food packaging migration studies conducted with nanomaterials. These include uncertainty in the ability of the analytical techniques utilized to detect nanoparticles *per se* in food simulants, uncertainty in the influence of sample preparation methods, and the often limited level of description provided of how these methods were carried out) of ENPs in food-contact materials should be provided.

In the light of the mentioned, although nanotechnology may hold some hidden threats, in essence it hold out a great deal of promise for the food security and food sustainability.

**References**

Aguilera, J. M., and Stanley, D. W., 1990. Microstructural Principles of Food Processing and Engineering, 2nd ed. Aspen Publishers Inc., Gaithersburg (Germany).

Ahamed, M. M., S. Alsalhi, and Siddiqui, M. K., 2010. Silver Nanoparticle Applications and Human Health. Clinica Chimica Acta, 411, 1841-1848.

Alexandre, M., and Dubois, P., 2000. Polymer-layered silicate nanocomposites: preparation, properties and uses of a new class of materials. Materials Science and Engineering R: Reports, 28, 1-63.

Aliakbari, F., Hosseinali, H. Sarokhalil, K. et al., 2019. Reactive oxygen species generated by titanium oxide nanoparticles stimulate the hemoglobin denaturation and cytotoxicity against human lymphocyte cell. Journal of Biomolecular Structure and Dynamics, 22, 1-7 DOI:10D.1080/07391102.2019.1568305.

Artiaga, G., Ramos, K., Ramos, L. Cámara, C. and Gómez-Gómez. M., 2015. Migration and characterisation of nanosilver from food containers by AF4-ICP-MS. Food Chemistry, 166, 76-85.

Arora, A., and Padua. G. W., 2010. Review: nanocomposites in food packaging. Journal of Food Science, 75, 43-9.

Athinarayanan, J., Periasamy, V. S., Alsaif, M. A., Al-Warthan, A. A., Alshatwi, A. A., 2014. Presence of nanosilica (E551) in commercial food products: TNF-mediated oxidative stress and altered cell cycle progression in human lung fibroblast cells. Cell Biology and Toxicology, 30, 89-100.

Baan, R. K., Straif, Y., Grosse, B., et al., 2006. Carcinogenicity of carbon black, titanium dioxide, and talc. The Lancet Oncology, 7, 295-296.

Baalousha, M., Kammer, F. V. D., Motelica-Heino, M., et al., 2006. Size fractionation and characterization of natural colloids by flow-field flow fractionation coupled to multi-angle laser light scattering. Journal of Chromatography A, 1104, 272-281.

Bacchetta, R., et al., 2014. Evidence and uptake routes for Zinc oxide nanoparticles through the gastrointestinal barrier in Xenopus laevis. Nanotoxicology, 8, 728-744.

Bai, K., Hong, B., He, J., Hong, Z., Tan, R., 2017. Preparation and antioxidant properties of selenium nanoparticles-loaded chitosan microspheres. International Journal of Nanomedicine, 12, 4527-4539.

Barnes, D., Chu, D., 2010. Introduction to Modeling for Bioscience. Spring-Verlag London Limit. DOI 10.1007/978-1-84996-326-1.

Blasco, C., Pico, Y., 2011. Determining nanomaterials in food. TrAC Trends in Analytical Chemistry, 30, 84-99.

Bott, J., Stormer, A., Franz, R., 2014a. Migration of nanoparticles from plastic packaging materials containing carbon black into foodstuffs. Food Additives Contaminants: Part A, 31(10), 1769-1782.

Bott, J., Stormer, A. Franz, R. 2014b. A model study into the migration potential of nanoparticles from plastics nanocomposites for food contact. Food Packaging and Shelf Life, 2, 73-80.

Bott J., Franz, R., 2018. Investigation into the Potential Migration of Nanoparticles from Laponite-Polymer Nanocomposites. Nanaomaterials, 8, 723.

Bu, Q., et al., 2010. NMR-based metabolic study of the sub-acute toxicity of titanium dioxide nanoparticles in rats after oral administration. Nanotechnology, 2, 125105.

Bumbudsanpharoke, N., Choi, J., Park, J. H., Ko, S., 2019. Zinc migration and its effect on the functionallity of a low density polyethylene-ZnO nanocomposite film. Food Packaging and Shelf Life, 20, 100301.

Bumbudsanpharoke, N., Ko, S., 2015. Nano-food packaging: An overview of market, migration research, and safety regulations. Journal of Food Science, 80:R910-R923.

Bradley, E. L., Castle, C., Chaudhry, Q., 2011. Applications of nanomaterials in food packaging with a consideration of opportuities for developing countries. Trends in Food Science & Technology, 22, 604-610.

Brun, E., et al., 2014. Titanium dioxide nanoparticle impact and translocation through ex vivo, in vivo and in vitro gut epithelia. Particle and Fibre Toxiclogy, 11,13.

BS EN14338, 2003. Paper and board intended to come into contact with foodstuffs. Conditions for determination of migration from paper and board using modified polyphenylene oxide (MPPO) as a simulant.

Carbone, M., Donia, D. T., Sabbatella, G., Antiochia, R., 2016. Silver nanoparticles in polymeric matrices for fresh food packaging. Journal of King Saud University-Science, 28:273-279.

Cha, K., Hong, H. W., Choi, Y. G., et al., 2008. Comparition of acute responses of mice livers to short-term exposure to nano-sized or micro-sized silver particles. Biotechnology Letters, 30, 1893–1899.

Chaudhry, Q., Castle, L., Bradley, E., Blackburn, J., Aitken, R., Boxall, A., 2008. Assessment of current and projected applications of nanotechnology for food contact materials in relation to consumer safety and regulatory implications. Final report Project A03063. Food Standards Agency. http://www.food.gov.uk/sites/default/files/a03063.pdf.

Chalew, T. E. A., Schwab, K. J., 2013. Toxicity of commercially available engineered nanoparticles to Caco-2 and SW48o human intestinal epithelial cells. Cell Biology and Toxicology, 29,101-116.

Chawengkijwanich, C., Hayata, Y., 2008. Development of TiO2 powder-coated food packaging film and its ability to inactive Escherichia coli in vitro and in actual tests. International Journal of Food Microbiology, 123, 288–292.

Chen, N., et al., 2016. Toxicological effects of Caco-2 cells following short-term and long-term exposure to Ag nanoparticles. International Journal of Molecular Science, 17, 974.

Chen, P., Wang, H., Chen, B., et al., 2019. Size-dependent cytotoxicity study of ZnO nanoparticles in HepG2 cells. Ecotoxicology and Environmental Safety, 171, 337-346.

Chen, J., Dong, X., Zhang, Q., Ding, S., 2019. Migration of titanium dioxide from PET/TiO2 composite film for polymer-laminated steel. Food Additives & Contaminants: Part A, 36, 483-491.

Chen, X. X., Cheng, B., Yang, X. X., et al., 2013. Characterization and preliminary toxicity assay of nano-titanium dioxide additive in sugar-ciated chewing gum. Small, 9, 1765–1774.

Cho, W.-S., Duffin, R., Bradley, M., et al., 2013. Predictive value of in vitro assays depends on the mechanism of toxicity of metal oxide nanoparticles. Particle and Fibre Toxicology 10:55.

Cho, Y. M., Mizuta, Y., Akagi, J., Toyoda, T., et al., 2018. Size-dependent acute toxicity of silver nanoparticles in mice. Journal of Toxicology Patholology, 31, 73-80.

Choi, J., et al., 2015. Toxicity of Zinc oxide nanoparticles in rats treated by two different routes: single intravenous injection and single oral administration. Journal of Toxicology and Environmental Health, Part A, 78, 226–243.

Choi, J., Chae, S. J., Kim, J. M., Choi, J. C., et al., 2018. Potential silver nanoparticles migration from commercially available polymeric baby products into food simulants. Food Additives & Contaminants:Part A, 35, 996-1005.

Cockburn, A., Bradford, R., Buck, N., Constable, A., Edwards, G., Haber, B., Hepburn, P., Howlett, J., Kampers, F., Klein, C., et al., 2012. Approaches to the safety assessment of engineered nanomaterials (ENM) in food. Food and Chemical Toxicology, 50, 2224–2242.

Council Directive 85/572/EEC, 1985. Commission of the European Communities, Council Directive of 19 December 1985 laying down the list of simulants to be used for testing migration of constitutients of plastics materials and articles intended to come into contact with foodstuffs (85/572/EEC). Official Journal of the European Communities, 31 December 1985, no. L372, p.14.

Council Directive 82/711/EEC,1982. Commission of the European Communities, Council Directive of 18 October 1982 laying down the basic rules necessary for testing migration of the constituents of plastics materials and articles intended to come into contact with foodstuffs (82/711/EEC). Official Journal of the European Communities, 23 October 1982, no. L. 297, p 26.

Commission Directives 93/8/EEC, 1993. Commission of the European Communities, Commission Directive of 15 March 1993 amending Council Directive 82/711/EEC laying down the basic rules necessary for testing migration of the constitutients of plastics materials and articles intended to come into contact with foodstuffs (93/8/EEC). Official Journal of the European Communities, 14 April 1993, no. L 90, p22.

Commission Directive, 2002. Directive 2002/72/EC of 6 August 2002 on plastic materials and articles intended to come into contact with foodstuffs. Official Journal European Union L 220, 15.8.2002, p.18.

CFR, (2014). Title 21, Volume 3. Direct food substances affirmed as generally recognized as safe. Section 184.1155 Bentonite. Code of Federal Regulations, Food and Drug Administration.

http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=184.1155.

Cushen, M., Kerry, J., Morris, J., Cruz-Romero, M., Cummins, E., 2014. Evaluation and Simulation of Silver and Copper Nanoparticle Migration from Polyethylene Nanocomposites to Food and and Associated Exposure Assessment. Journal of Agricultural and Food Chemistry, 62, 1403-1411.

Cushen, M., Kerry, J., Morris, M., Cruz-Romero, M., Cummins, E., 2012. Nanotechnologyies in the food industry – Recent developments, risks and regulation. Trends in Food Science & Technology, 24, 30-46.

Cushen, M., Kerry, J., Morris, M., Cruz-Romero, M., Cummins, E., 2013. Migration and exposure assessment of silver from a PVC nanocomposite. Food Chemistry, 139, 389-397.

de Abreu, D. A. P., Cruz, J. M., Angulo, I., Losada, P. P., 2010. Mass transport studies of different additives in polyamide and exfoliated nanocomposite polyamide films for food industry. Packaging Technology and Science, 23: 59-68.

de Azeredo, H. M. C., 2013. Antimicrobial nanostructures in food packaging. Trends in Food Science & Technology, 30, 56-69.

Dekkers, S., Krystek, P., Peters, R. J, Lankveld, D. X, Bokkers, B. G., van Hoeven-Arentzen, P. H., Bouwmeester, H., Oomen, A .G., 2011. Presence and risks of nanosilica in food products. Nanotoxicology, 5, 393–405.

Dekkers, S., Bouwmeester, H., Bos, P. M., Peters, R. J., Rietveld, A. G., Oomen, A. G., 2013. Knowledge gaps in risk assessment of nanosilica in food: evaluation of the dissolution and toxicity of different forms of silica. Nanotoxicology, 7, 367-377.

Devine, D., Dikeman, M., 2014. Encyclopedia of Meat Science, 2nd edition. Eds M. Dikeman and C. Devine. Meat Science 80:960−967. ISBN: 978-0-12-384731-7

Dorier, M., et al., 2015. Impact of anatase and rutile titanium dioxide nanoparticles on uptake carriers and efflux pumps in Caco-2 gut epithelial cells. Nanoscale, 7, 7352–7360.

Duncan, T. V., 2011. Applications of nanotechnology in food packaging and food safety: barrier materials, antimicrobials and sensors. Journal of Colloid and Interface Science 363,1-24.

Dubey, P., Matai, I., Kumar, S. U., et al., 2015. Perturbation of cellular mechanistic system by silver nanoparticle toxicity: cytotoxic, genotoxic and epigenetic potentials. Journal of Colloid and Interface Science 221, 4-21.

Dufour, E. K., Kumaravel, T., Nohynek, G. J., Kirkland, D., Toutain. H., 2006. Clastogenicity, photo-clastogenicity or pseudo-photo-clastogenicity: Genotoxic effects of zinc oxide in the dark, in pre-irradiated or simultaneously irradiated Chinese hamster ovary cells. Mutation Research 607, 215-224.

Echegoyen, Y., Nerín, C., 2013. Nanoparticle release from nano-silver antimicrobial food containers. Food and Chemical Toxicology 62, 16-22.

EFSA, 2004. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission related to a 4th list of substances for food contact materials (Question No EFSA-Q-2003-210, EFSA-Q-2003-223, EFSA-Q2003-187, EFSA-Q-2003-202, EFSA-Q-2003-203) adopted on 26 May 2004 by written procedure. European Food Safety Authority. The EFSA Journal 65:1-17. http://www.efsa.europa.eu/en/efsajournal/doc/65a.pdf.

EFSA, 2005. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission related to a 7th list of substances for food contact materials (Question No EFSA-Q-2003-076, EFSA-Q-2004-144, EFSA-Q2004-166, EFSA-Q-2004-082, EFSA-Q-2003-204, EFSA-Q-2003-205, EFSA-Q-2003-206) adopted on 29 March 2005 by written procedure. European Food Safety Authority. The EFSA Journal (2005) 201, 1-28. http://www.efsa.europa.eu/en/efsajournal/doc/201a.pdf- The specific migration limit has been derived to limit the intake of silver from food to <12.5 % of the human NOAEL for argyria of 10 g of silver over a lifetime (i.e. 0.39 mg/person/day, or

0.006 mg/kg/d) (the NOAEL is from the WHO Guidelines for Drinking Water Quality - WHO (2011). Guidelines for drinking-water quality. Fourth edition. World Health Organization. Geneva. http://whqlibdoc.who.int/publications/2011/9789241548151_eng.pdf\

EFSA, 2012. Scientific opinion on the safety evaluation of the substance, titanium nitride, nanoparticles, for use in food contact materials. European Food Safety Authority Panel on food contact materials, enzymes, flavourings and processing aids. EFSA Journal 10(3):2641. http://www.efsa.europa.eu/en/efsajournal/doc/2641.pdf.

EFSA, 2008. 21st list of substances for food contact materials. Scientific Opinion of the Panel on food contact materials, enzymes, flavourings and processing aids. The EFSA Journal 888-890: 1-14. http://www.efsa.europa.eu/en/efsajournal/doc/888.pdf

EFSA, 2014. Statement on the safety assessment of the substance silicon dioxide, silanated, FCM Substance No 87 for use in food contact materials. EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids. The EFSA Journal 12(6), 3712.

EFSA, 2016. Safety assessment of the substance zinc oxide, nanoparticles, for use in food contact materials. EFSA Panel on Food Contact Materials, Enzymes, Flavouring and Processing Aids (CEF). EFSA Journal 14(3), 4408.

Emamifar, A., Kadivar, M., Shahedi, M., Soleimanian- Zad, M., 2011. Effect of nanocomposite packaging containing Ag and ZnO on inactivation of Lactobacillus plantarum in oringe juice. Food Control 22, 408-413.

Emamifar, A., Kadivar, M., Shahedi, M., Zad, S. S., 2010. Evaluation of nanocomposite packaging containing Ag and ZnO on shelf life of fresh orange juice. Innovative Food Science and Emerging Technologies 11, 742-748.

Erfanian, A., Rasti, B., Manap, Y., 2017. Comparing the calcium bioavailavility from two types of nano-sized enriched milk using in-vivo assay. Food Chemsitry 214, 606-613.

Esmaeillou, M., M. Moharamnejad, R. Hsankhani, A. A. Tehrani, and H. Maadi. 2013. Toxicity of ZnO nanoparticles in healthy adult mice. Environmental Toxicology and Pharmacology 35, 67-71**.**

EU, 2011. European Commission, 'Commission recommendation of 18 October 2011 on the definition of nanomaterial' (EC No. 696/2011). Official Journal European Union L275, p38–40, 2011.

EU, 2002. European Parliament and Council, Directive 2001/95/EC of the European Parliament and Council of 3 December 2001 on general product safety. Official Journal of the European Union L11: 4-17, 2002.

EU, 2002. EN 1186-1:2002, Materials and articles in contact with foodstuffs- Plastics- Part 1: Guide to the selection of conditions and test methods for overall migration, pag. 24.

EU, 2004. EN 13130-1:2004 " Materials and articles in contact with foodstuffs- Plastic substances subject to limitation, concerned with the determination of specific migration from plastic materials into food stuffs and food simulants and the determination of specific monomers and additives in plastics.

EC,1994. European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. Official Journal of the European Communities. No L237/13. http://ec.europa.eu/food/fs/sfp/addit_flavor/flav08_en.pdf.

EC,1995. European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. Official Journal of the European Communities. No

L61/1.                                   http://eur-lex.europa.eu/legal-
content/EN/TXT/PDF/?uri=CELEX:31995L0002&from=en.

EC, 1997. Commission directive of 29 July 1997 amending for the second time Council
Directive 82/711/EEC laying down the basic rules necessary for testing migration of the
constituents of plastic materials and articles intended to come into contact with foodstuffs
(97/48/EC). Official Journal of the European Communities. No L222/10. http://eur-
lex.europa.eu/legalcontent/EN/TXT/PDF/?uri=CELEX:31997L0048&from=en.

EU, 2016. COMMISSION REGULATION (EU) 2016/1416 of 24 August 2016 amending and
correcting Regulation (EU) No 10/2011 on plastic materials and articles intended to come into
contact with food. Official Journal of the European Union, 2016, p. L 230/22.

Enescu, D., Gardrat, C., Cramail, H., Le Coz, C., Sebe, G., Coma, V., 2019. Bio-inspired films
based on chitosan, nanoclays and cellulose nanocrystals: structuring and properties
improvement by using water-evaporation-induced self-assembly. Cellulose 26, 2389-2401.

Enescu, D., Frache, A., Geobaldo, F., 2015. Formation and oxygen diffusion barrier properties of
fish gelatin/natural sodium montmorillonite clay self-assamble multilayers onto the
biopolyester surface. Royal Society of Chemistry Advances 5, 61465.

FAO/WHO, 2009. The State of Food and Agriculture. Food and Agriculture Organization of the
United Nations and World Health Organization, Rome (Italy)
http://www.fao.org/3/i0680e/i0680e.pdf

FAO/WHO, 2010. Expert Meeting on the Application of Nanotechnologies in the Food and
Agriculture Sectors: Potential Food Safety Implications. Meeting Report.  Food and
Agriculture Organization of the United Nations and World Health Organization, Rome (Italy).
Available from: www.worldvet.org/node/5688; http://www.fao.org/ag/agn/agns/.

Farhoodi, M., Mousavi, S. M., Sotudeh- Gharebagh, R., Emam-Djomeh, Z., et al., 2014. Migration of Aluminium and Silicon from PET/Clay nanocomposite Bottles into Acidic Food Simulant. Packaging Technology and Science 27, 161-168.

FDA, 2002. Listing of Color Additives Exempt from Certification. In Code of Federal Regulations Title 21—Food and Drugs; U.S. Government Printing Office: Washington, DC, USA, 2002.

FDA, 2007. Guidance for industry: preparation of premarket submissions for food contact substances: chemistry recommendations. US Department of Health and Human Services, Food and Drug Administration. December 2007. http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Ingredients AdditivesGRASPackaging/ucm081818.htm.

FDA, 2015. Summary of color additives for use in the United States in in foods, drugs, cosmetics, and medical devices. United States Food and Drug Administration. http://www.fda.gov/ForIndustry/ColorAdditives/ColorAdditiveInventories/ucm115641.htm#cfr

Fondevila, M., Herrer, R., Casallas, M. C., Abecia, L., Ducha, J. J., 2009. Silver nanoparticles as a potential antimicrobial additive for weaned pigs. Animal Feed Science and Technology 150, 259–269.

FoE, 2008. Out of the laboratory and on to our plates: Nanotechnology in food and agriculture. Friends of the Earth Australia, Europe and USA. https://friendsoftheearth.uk/latest/publications

Fraunhofer, W., Winter, G., Coester, C., 2004. Asymmmetrical Flow Field-Flow Fractionation and Multilangle Light Scattering for Analysis of Gelatin nanoparticles Drug Carrier Systems. Analytical Chemistry 76, 1909-1920.

Frohlich, E. E., Frohlich, E., 2016. Cytotoxicity of Nanoparticles Contained in Food of Intestinal Cells and the Gut Microbiota. International Journal of Molecular Sciences 17, 509.

Fulgoni, V. L., Keast, D. R., Bailey, R. L., Dwyer, J., 2011. Foods, Fortificants, and Supplements: Where Do Americans Get Their Nutrients? The Journal of Nutrition 141,1847–1854.

Gaillet, S., Rouanet, J. M., 2015. Silver nanoparticles: their potential toxic effects after oral exposure and underlying mechanisms-a review. Food and Chemical Toxicology 77, 58–63.

Garcia, T., et al., 2010. Oral subchronic exposure to silver nanoparticles in rats. Food and Chemical Toxicology 7, 20.

Ge, Y., Schimel, J. P., Holden, P. A., 2012. Identification of Soil Bacteria Susceptible to TiO2 and ZnO nanoparticles. Applied and. Environmental Microbiology 78, 6749–6758.

Georgantzopoulou, A., et al., 2016. Effect of silver nanoparticles and ions on a coculture model for the gastrointestinal epithelium. Particle and Fibere Toxicology 13, 9.

Gerloff, K., et al., 2012. Distinctive toxicity of TiO2 rutile/anatase mixed phase nanoparticles on Caco-2 cells. Chemical Research in Toxicology 25, 646–655.

Gerloff, K., Albrecht, C., Boots, A. W., Forster, I., Schins, R. P. F., 2009. Cytotoxicity and oxidative DNA damage by nanoparticles in human intestinal Caco-2 cells. Nanotoxicology 3, 355–364.

Gover, R. D., Mille, J. M., Hutchison, J. E., 2011. Generation of metal nanoparticles from silver and copper objects: nanoparticle dynamics on surfaces and potential sources of nanoparticles in the environment. ACS Nano 5, 8950-8951.

GMP, 2015. Australia New Zealand Food Standards Code-Schedule 15-Substances that may be used as food additives. Commonwealth of Australia Gazette No. FSC 96 on 10 April 2015. https://www.legislation.gov.au/Details/F2015L00439

Hajipour, M. J., et al., 2012. Antibacterial properties of nanoparticles. Trends in Biotechnology 30, 499-511.

Hannon, J. C., Cummins, E., Kerry, J., Cruz-Romero, M., Morris, M., 2015. Advances and challenges for the use of engineered nanoparticles in food contact materials. Trends in Food Science & Technology 43, 43-62.

Heid, H. W., Keenan, T. W., 2005. Intracellulor origin and secretion of milk fat globules. European Journal of Cell Biology 84, 245-258.

Hilty, F. M., et al., 2010. Iron from nanocompounds containing iron and zinc is highly bioavailable in rats without tissue accumulation. Nature Nanotechnology 5, 374-380.

Hendrickson, O. D., et al., 2016. Toxicity of nanosilver in intragastric studies: Biodistribution and metabolic effects. Toxicology Letters 241, 184-192.

Huang, Y., Chen, S., Bing, X., Gao, C., Wang, T., Yuan, B., 2011. Nanosilver Migrated into Food-Simulating Solutions from Commercially Available Food Fresh Contaiers. Packaging Technology and Science 24, 291-297.

Huang, J. Y., Li, X., Zhou W., 2015. Safety assessment of nanocomposite for food packaging application. Trends in Food Science & Techology 45, 187-99.

Huang, H. K., Tang, Z., Luo, H., Zhang, Qin. Y., 2017. Migration of Ti and Zn from Nanoparticle Modified LDPE films into Food Simulants. Food Science and Technology Research 23 (6), 827-834.

http://cryolog.com/blog/topcryo-chez-franprix-la-garantie-fraicheur-et-le-gaspillage-alimentaire-on-en-parle-sur-france-2-chez-telematin/

iRAP, 2009. Nano-enabled packaging for the food and beverage industry - A global technology industry and market analysis. Innovative Research and Products Inc. Report Code: FT-102, pag. 107, 2009 http://www.innoresearch.net/report.

ISO 19007:2018 (E) "Nanotechnologies – In vitro MTS assay for measuring the cytotoxic effect of nanoparticles".

JECFA, 2007. Safety evaluation of certainfood additives and contaminants. Prepared by the Sixty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO food additives series: 58. World Health Organization, Geneva, 2007. IPCS - International Programme on Chemical Safety. ISBN 978 92 4 166058 7 http://www.inchem.org/documents/jecfa/jecmono/v58je01.pdf

JECFA,1988. Summary of evaluations performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA): aluminum powder. http://www.inchem.org/documents/jecfa/jeceval/jec_87.htm

Jeong, G. N., Jo, U. B., Ryu, H. Y., et al., 2010. Histochemical study of intestinal mucis afte administration of silver nanoparticles in Sprague-Dawley rats. Archives of Toxicology 84, 63-69.

Jovanović, B., 2015. Critical review of public health regulations of titaniu dioxide, a human food additive. Integrated Environment Assessment and Management 11, 10-20.

Jokar, M., Abdul Rahman, R., 2014. Study of silver ion migration from melt-blended and layered-deposited silver polyethylene nanocomposite into food simulants and apple juice. Food Additives & Contaminants: Part A 31, 734-742.

Kahn, J., 2006. Welcome to the world of nanotechnology. National Geographical 209, 98-119.

Kammer, Fvd., Baborowski, M., Friese, K., 2005. Field-Flow fractionation coupled to multi-angle laser light scattering detectors: Applicability and analytical benefits for the analysis of environmental colloids. Analytica Chimica Acta 552, 166-174.

Kang, T., Guan, R., Chen, X., Song, Y., Jiang, H., Zhao, J., 2013. In vitro toxicity of different-sized ZnO nanoparticles in Caco-2 cells. Nanoscale Research Letters 8:496 doi: 10.1186/1556-276X-8-496

Kawata, K., Osawa, M., Okabe, S., 2009. In Vitro Toxicity of Silver Nanoparticles at Nanocytotoxic Doses to HepG2 Human Hepatoma Cells. Environmental Science and Technolgoy 43, 6046–6051.

Kruger, K., Cossais, F., Neve, H., Klempt, M., 2014. Titanium dioxide nanoparticles activate IL8-related inflammatory pathways in human colonic epithelial Caco-2 cells. Journal of Nanoparticle Research 16, 2402.

Khademhosseini, A., Lager, R., 2006. Nanobiotechnology from Tissue Engineering and Drug Delivery. Chemical Engineering Progress 102, 38-42.

Kim, T. H., et al., 2012. Size-dependent cellular toxicity of silver nanoparticles. Journal of Biomedical Materials Research Part A 100A,1033–1043.

Kim, Y. S., et al., 2008. Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats. Inhalation Toxicology 20, 575–583.

Kim, W. Y., Kim, J., Park, J. D., Ryu, H. Y., Yu, I. J., 2009. Histological study of gender differences in accumulation of silver nanoparticles in kidneys of Fischer 344 rats. Journal of Toxicology and Environment Health A, 72, 1279–1284.

Koo, B., Joseph, H., 2006. Polymer Nanocomposites-Omar Manasreh. McGraw-Hill Professional Pub 272.

Kononenko, V., Drobne, D., 2019. In Vitro Cytotoxicity Evaluation of the Magnéli Phase Titanium Suboxides ($Ti_xO_{2x-1}$) on A549 Human Lung Cells. International Journal of Molecular Science 20:196 https://doi.org/10.3390/ijms20010196.

Kumar, A. P., Depan, D., Singh Tomer, N., Singh, R. P., 2009. Nanoscale particles for polymer degradation and stabilization −Trends and future perspectives. Progress in Polymer Science 34, 479-515.

Kumar, P., Reinitz, H. W., Simunovic, J., Sandeep, K. P., Franzon, P. D., 2009. Overview of RFID technology and its applications in the food industry. Journal of Food Science 74, R101-R106.

Laborda, F., Bolea, E., Jimenez-Lamana, J., 2014. Single particle inductively coupled plasma mass spectroscopy: powerful tool for nanoanalysis. Analytical Chemistry 84, 2270-2278.

Laborda, F., Jimenez-Lamana, J., Bolea, E., Castillo, J. R., 2011. Selective identification, characterization and determination of dissolved silver (I) and silver nanoparticles based on single particle detection by inductively coupled plasma mass spectrometry. Journal of Analytical Atomic Spectroscopy 26, 1362-1371.

Lesniak, A., et al., 2013. Nanoparticle Adhesion to the Cell Membrane and Its Effect on Nanoparticle Uptake Efficiency. Journal of Americal Chemical Society 135, 1438–1444.

Lether, R., Wang, X., Marsch, S., Hunziker, P., 2013. Intelligent nanomaterials for medicine: carrier platforms and targeting strategies in the context of clinical application. Nanomedicine: Nanotechnology, Biology and Medicine 9, 742-757.

Liao, C., Li, Y., Tjong, S. C., 2019. Bactericidal and Cytotoxic Properties of Silver Nanoparticles. International Journal of Molecular Science 20:449 doi: 10.3390/ijms20020449.

Lin, Q., Li, B. H., Zhong, H. N., et al., 2014. Migration of Ti from nano-$TiO_2$-polyethylene composite packaging into food simulants. Food Additives & Contaminants: Part A 31, 1284-1290.

Liu, J. F., Yu, S. J., Yin, Yg., Chao, Jb., 2012. Methods for separation, identification, characterization and quantification of silver nanoparticles. TrAC Trends in Analytical Chemistry 33, 95-106.

Li, W., Zhang, C., Chi, H., Li, L., Lan, T., Han, P., Chen, H., Qin, Y., 2017. Development of Antimicrobial Packaginging Film Made from Poly (Lactic Acid) Incorporating Titanium Dioxide and Silver Nanoparticles. Molecules 22, 1170.

Lichtenstein, D., Ebmeyer, J., Juling, P., et al., 2015. Impact of food components during in vitro digestion of silver nanoparticles on cellular uptake and cytotoxicity in intestinal cells. Biological Chemistry 396, 1255–1264.

Loeschner, K., et al., 2011. Distribution of silver in rats following 28 days of repeated oral exposure to silver nanoparticles or silver acetate. Particle and Fibere Toxicology 8, 18.

Loeschner, K., Navratilova, J., Grombe, R., Linsinger, T. P., Kobler, C., Molhave, K., Larsen, E. H., 2015. In-house validation of a method for determination of silver nanoparticles in chicken meat based on asymmetric flow field-flow fractionation and inductively coupled plasma mass spectrometric detection. Food Chemistry 181, 78-84.

Lomer, M. C. E., Hutchinson, C., Volkert, S., et al., 2004. Dietary spources of inorganic microparticles and their intake in healthy subjects and patients with Crohn"s disease. British Journal of Nutrition 92, 947-55.

Luoma, S. N., 2018. Silver Nanotechnologies and the Environment: Old Problems or New Challenges? Woodrow Wilson International Center for Scholars: The Project of Emerging Nanotechnologies. 9 September 2008. Available online: http://www.nanotechproject.org/publications/archive/silver/ (accessed on 8novemer 2018, Woodrow Wilson Institute's Project of Emerging Nanotechnologies: Analysis of Consumer Products. Available online: http://www.nanotechproject.org/cpi/browse/nanomaterials/silvernanoparticle/ (accessed on 8 November 2018.

Mackevica, A., Olsson, M. E., Hansen, S. F., 2016. Silver nanoparticle release from commercially available plastic food containers into food simulants. Journal of Nanoparticle Research 18, 5-11.

Mahalik, N. P., Nambiar, A. N., 2010. Trends in food packaging and manufacturing systems and technology. Trends in Food Science & Technology 21, 117-128.

Martirosyan, A., Bazes, A., Schneider, Y. J., 2014. In vitro toxicity assessment of silver nanoparticles in the presence of phenolic compounds preventive agents against the harmful effect? Nanotoxicology 8, 573–582.

Martirosyan, A., Polet, M., Bazes, A., Sergent, T., Schneider, Y. J., 2012. Food Nanoparticles and Intestinal Inflammation: A Real Risk? In Inflammatory Bowel Disease; Szabo, I., Ed.; InTech: Rijeka, Croatia, Chapter 8, 259–282.

Metak, A. M., Nabhani, F., Connolly, S. N., 2015. Migration of engineered nanoparticles from packaging into food products. LWT- Food Sci. Technol. 64, 781-787.

Mitrano, D. M., et al., 2012. Detecting nanoparticles silver using single-particle inductively coupled plasma-mass spectroscopy. Environmental Toxicology and Chemistry 31, 115-121.

Mishra, M., 2019. Encyclopedia of Polymer Applications, CRC Press Taylor & Francis Groups, LLC, NW, Book Nr. 13:978-1-4987-2993-2.

Montoro Bustos, A. R., Ruiz Encinar, J., Sanz-Medel, A., 2013. Mass spectrometry for the characterization of nanoparticles. Analytical and Bioanalytical Chem. 405, 5637-5643.

Monopoli, M. P., et al., 2011. Physical-chemical aspects of protein corona: relevance to in vitro and vivo biological impacts of nanoparticles. Journal of the American Chemical Society 133, 2525–2534.

Mohammed Fayaz, A., Balaji, K., Girilal, M., Kalaichelvan, P. T., Venkatesan, R., 2009. Mycobased synthesis of silver nanoparticles and their incorporation into sodium alginate films for vedetable and fruit preservation. Journal of Agricultural and Food Chemistry 57, 6246-6252.

Mourdikoudis, S., Pallares, R. M., Thanh, N. T. K., 2018. Characterization techniques for nanoparticles: comparison and complementary upon studying nanoparticles properties. Nanoscales 10, 12871.

Neethirajan, S., Jayas, D. S., 2011. Nanotechnology for the Food and Bioprocessing Industries. Food and Bioprocess Technology 4(1), 39-47.

Ntim, S. A., Thomas, T. A., Begley, T. H., et al., 2015. Characterisation and potential migration of silver nanoparticles from commercially available polymeric food contact materials. Food Additives & Contaminants: A 32, 1003-1011.

Ntim, A. S., Thomas, T. A., Noonan, G. O., 2016. Influence of aqueous food simulants on potential nanoparticle detection in migration studies involving nanoenabled food-contact substances. Food Additives and Contaminants: Part A, 33, 905-912.

Ntim, S. A., Norris, S., Goodwin, D. G., et al., 2018. Effects of consumer use practices on nanosilver release from commercially available food contact materials. Food Additives & Contaminants: Part A 35, 2279-2290.

Noonan, G.O., Whelton, A. J., Carlander, D., Ducan,T. V., 2014. Measurement Methods to Evaluate Engineered Nanomaterials Release from Food Contact Materials. Comprehensive Reviews in Food Science and Food Safety, 13, 679-692.

OECD Synthetic Amorphous Silica and Silicates. Available online: http://www.chem. unep.ch/irptc/sids/OECDSIDS/Silicates.pdf (accessed on 17 December 2013). Regulation (EC) No. 1333/2008 of the European Parliament and of the Council of 16 December 2008 on Food Additives. Official Journal of the European Union 2008. Available online: http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32008R1333 (accessed on 19 May 2014)

Olesik, J. W., Gray, P. J., 2012. Consideration for measurement of individual nanoparticles or microparticles by ICP-MS: determination of the number of particles and the analyte mass in each particle. Journal of Analytical Atomic Spectroscopy 27, 1143.

Orfi, E., Szebeni, J., 2016. The immune system of the gut and potential adverse effects of oral nanocarries on its function. Advanced Drug Delivery Review 106, 402–409.

Ortlieb, M., 2010. White Giant or White Dwarf?: Particle Size Distribution Measurments of TiO2. GIT. Laboratory Journal Europe 14, 42–43.

Ozaki, A., Kishi, E., Ooshim, T., Hase, A., et al., 2016. Contents of Ag and other metals in food-contact plastics with nanosilver or Ag ion and their migration into food simulants. Food Additives & Contaminants: Part A 33,1490-1498.

Patil, U. S., et al., 2015. In Vitro/In Vivo Toxicity Evaluation and Quantification of Iron Oxide Nanoparticles. International Journal of Molecular Sciences 16, 24417–24450.

Pasupuleti, S., Alapati, S., Ganapathy, S., Anumolu, G., Pully, N. R., Prakhya, B. M., 2012. Toxicity of zinc oxide nanoparticles through oral route. Toxicology and Industrial Health 28, 675–686.

Perde-Schrepler, M., Florea, A., Brie, I., et al., 2019. Size-Dependent Cytotoxicity and Genotoxicity of Silver Nanoparticles in Cochlear Cells In Vitro. Journal of Nanomaterials ID 6090259, 12 pages. https://doi.org/10.1155/2019/6090259

Peters, R. J. B, Hoekstra, E. J., 2019. Migration of Nanomaterials from Food Contact Materials. Chapter 9. pag. 226. Food Contact Materials Analysis: Mass Spectrometry Techniques, Editied by M. Suman. Published by the Royal Society of Chemsitry. Food Chemistry, Function and Analysis No. 10. ISSN: 2398-0664.

Pulit-Prociak, J., Stoklosa, K., Banach, M., 2015. Nanosilver products and toxicity. Environmental Chemistry Letters 13, 59 -68.

Polaris Market Research, 2018. Nano-Enabled Packaging Market Share, Size, Trends, & Industry Analysis Report, By Type (Active, Intellingent, Others); By End –User (Food and Beverages, Pharmaceutical, Personal Care, others); By Regions: Segment Forecast, 2018-2026. Nano Enabled Packaging Market, Report ID: PM1577, pag 106, 2018. https://www.polarismarketresearch.com/industry-analysis/nano-enabled-packaging-market

(accessed on 29/04/2019), https://www.polarismarketresearch.com/press-releases/global-nano-enabled-packaging-market, accessed on 29/04/2019).

Powell, J. J., Harvey, R. S., Ashwood, P., Wolstencroft, R., Gershwin, M. E., Thompson, R. P. H., 2009. Particles in Normal Subjects and Patients with Inflmmatory Bowel Disease. Journal of Autoimmunity 14, 99–105.

Ramos, K., Gomez-Gomez, M. M., Camara, C., Ramos, L., 2016. Silver speciation and characterization of nanoparticles released from plastic food containers by single particle ICPMS. Talanta 151, 83-90.

Raspopov, R. V., Trushina, E. N., Gmoshinsky, I. V., Khotimchenko, S. A., 2011. Bioavailability of nanoparticles of ferric oxide when used in nutrision. Experimental results in rats. Voprosy Pitaniia 80, 25 –30.

Reig, S., Lopez, A. D., Ramos, M. H., Ballester V. A. C., 2014. Nanomaterials: a Map for Their Selection in Food Packaging Applications. Packaging Technology and Science 27, 839-866.

Roco, A. M. C., 2003. Nanotechnology: convergence with modern biology and medicine. Current Opinion in Biotechnology 14, 337-346.

Safar, R., Doumandji, Z., Saidou, T., et al., 2019. Cytotoxicity and global transcriptional responses induced by zinc oxide nanoparticles NM 110 in PMA-differentiated THP-1 cells. Toxicology Letters 308, 65-73.

Sarma, V. K., Siskova, K. M., Zboril, R., Gardea-Torresdey, J. L., 2014. Organic-coated silver nanoparticles in biological and environmental conditions: fate, stability and toxicity. Advances in Colloid and Interface Science 204, 15-34.

Savolainen, K., Alenius, H., Norppa, H., Pylkkanen, I., Tuomi, T., Kasper, G., 2010. Risk assessment of engineered nanomaterials and nanotechnologies- a review. Toxicology 269, 92-104.

Schmidt, B., Katiyar, V., Plackett, D., Larsen, E. H., Gerds, N., Koch, C. B., Petersen, J. H., 2011. Migration of nanosized layered double hydroxide platelets from polylactide nanocomposite films. Food Additives and Contaminants 28 (7), 956-966.

Schmidt, B., Petersen, J. H., Bender, C., Koch, Plackett, D. et al., 2009. Combining asymmetrical flow field-flow fractionation with light –scattering and inductively coupled plasma mass spectrometric detection for characterization of nanoclay used in biopolymer nanocomposites. Food Additives and Contaminants 26, 1619-1627.

Scherzad, A., Meyer, T., Kleinsasser, N., Hackenberg, S., 2017. Molecular Mechanism of Zinc oxide nanoaprticles-Induced Genotoxicity short running title: Genotoxicity of ZnO NPs. Materials 10:1427; doi: 10.3390/ma1012142

Semo, E., Kesselman, E., Danino, D., Livney, Y. D., 2007. Casein micelle as a natural nao-capsular vehicle for nutraceuticals. Food Hydrocolloids 21, 936–942.

Shahare, B., Yashpal, M., 2013. Toxic effects of repeated oral exposure of silver nanoparticles on small intestine mucosa of mice. Toxicology Mechanisms and Methods 23, 161–167.

Sharma, R., Bisen, D. P., Shukla, U., Sharma, B. G., 2012. X-ray diffraction: a powerful method of characterizing nanomaterials. Recent Research in Science and Technology 4, 77-79.

Sharma, H. S., Hussain, S., Schlager, J., Ali, S. F., Sharma, A., 2010. Influence of nanoparticles on blood-brain barrier permeability and brain edema formation in rats. Acta Neurochirurgica Supplement 106, 359–364.

Sharma H. S., Ali, S. F., Hussain, S. M., Schlager, J. J., Sharma, A., 2009. Infulence of engineered nanoparticles from metals on the blood-brain barrier permeability cerebral blood flow, brain edema and neurotoxicity. An experimental study in the rat and mice using biochemical and morphological approaches. Journal of Nanoscience and Nanotechnology 9, 5055–5072.

Sharma, V. K., Siskova, K. M., Zboril, R., Gardea-Torresdey, J. L., 2014. Organic-coated silver nanoparticles in biological and environmental coditions: fate, stability, and toxicity. Advance Colloid Interface Science 204, 15-34.

Sheeparamatti, G., et al., 2007. Nanotechnology: Inspiration from nature. IETE Techical Review 24, 1.

Shi, H., Magaye, R., Castranova, V., Zhao, J., 2013. Titanium dioxide nanoparticles: a review of current toxicological data. Particle and Fibre Toxicology 10: doi:10.1186/1743-8977-10-15.

Shukla, R. K. V., Sharma, A. K., Pandey, S., Sultana, S., Dhawan, A., 2011. ROS- mediated genotoxicity induced by titanium dioxide nanaoparticles in human epidermal cells. Toxicology in Vitro 25, 231-241.

Simon, P., Chaudhry, Q., Bakos, D., 2008. Migration of engineered nanoparticles from polymer packaging to food-a physicochemical view. Journal of Food and Nutrition Research 47, 105-113.

Singh, S., Stephan, C., Westerhoff, P., et al., 2014. Measurement Methods to Detect, Characterize, and Quantify Engineered Nanomaterials in Foods. Compressive Review in Food Science Food Safety 13, 693-704.

Sirelkhatim, A., et al., 2015. Review of Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism. Nano-Micro Letters 7, 219–242.

Smith, D. J., 2007. Characterisation of nanomaterials using transmission electron microscopy. In J. Hutchison & A. Kirkland (Eds.). Nanocharacterisation (pp.1-27). RSC publishing.

Song, H., Li, B., Lin, Q. B., Wu, H. J., Chen, Y., 2011. Migration of silver from nanosilver-polyethylene composite packaging into food simulants. Food Additives and Contaminants Part A 28, 1758-1762.

So, S. J., Jang, I. S., Han, C. S., 2008. Effect of micro/nano silica particle feeding for mice. Journal of Nanoscience and Nanotechnology 8, 5367–5371.

Sorrentino, A., Gorrasi, G., Vittoria V., 2007. Potential perspectives of bio-pplications. Trends in Food Science Technnology 18, 84-95.

Song, Z. M., et al., 2013. Biological effect of food additive titanium dioxide nanoparticles on intestine: an in vitro study. Journal of Applied Toxicology 35, 1169–1178.

StatNano, Nano Science, Technology and Industry Scoreboard 2018. Nanotechnology Publications of 2018: An Overview; https://statnano.com/news/65056

Standard 1.3.1. GMP. 2013. Miscellaneous additives permitted in acordance with GMP in processed foods specified in Schedule 1". Federal Register of Legislative Instruments F2013C00984, Standard 1.3.1., 5 December 2013, pag. 47.

Tada-Oikawa, S., et al., 2016. Titanium Dioxide Particle Type and Concentration Influence the Inflamatory Response in Caco-2 Cells. International Journal of Molecular Sciences 17:576.

The Commission of the European Communities. Regulation (EC) No. 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. Official Journal of the European Union, L338, (2004).

The Commission of the European Communities. Commission Regulation (EC) No. 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food. Official Journal of the European Union, L135, (2009).

The Commission of the European Communities. Commission Regulation (EC) No. 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. Official Journal of the European Union, L12, 2011.

Tiede, K., Boxall, A. B. A., Tear, S. P., et al., 2008. Detection and characterization of engineered nanoparticles in food and the environment. Food Additives and Contaminants Part A 7, 795-821.

Torres, A., Guarda, A., Moranga, N., et al., 2012. Experimental and theoretical study of thermodynamics and transport properties of multilayer polymeric food packaging. Eurpeanean Food Reserch and Techology 234, 13-722.

Trickler, W. J., Lantz, S.M., Murdock, R. C., et al., 2010. Silver nanoparticle induced blood-brain barrier inflammation and increased permeability in primary rat brain microvessel endothelial cells. Toxicological Science 118, 160–170.

Turioniemi J., 2013. New single particle methods for detection and characterisation of nanoparticles in environmental samples. Univ of Gothenburg, Sweeden.

Vandebriel, R., De Jong, J. W. H., 2012. A review of mammalian toxicity of ZnO nanoparticles. Nanotechnology, Science and Applciations 5, 61-71.

van Kesteren, P. C. E., et al., 2015. Novel insights into the risk assessment of the nanomaterial synthetic amorphous silica, additive E551, in food. Nanotoxicology 9, 442–452.

Vance, M. E., Kuiken, T., Vejerano, E. P., McGinnis, S. P., Hochella Jr., M. F., Rejeski, D., Hull, M. S., 2015. Nanotechnology in the real world: Redeveloping the nanomaterial

consumer products inventory Beilstein J. Nanotechnol. 6:1769–1780. doi:10.3762/bjnano.6.181

Vera, P., Canellas, E., Nerín, C., 2018. New antioxidant multilayer packaging with nanoslenium to enhance the shelf-life of market food products. Nanomaterials 8:837; doi: 10.3390/nano8100837

von Goetz, N., Fabricius, L., Glaus, R., Weitbrecht, V., Günther, D., Hungerbühler, K., 2013. Migration of silver from commercial plastic food containers and implications for consumer exposure assessment Food Additives & Contaminants: Part A 30, 612–620.

Wang, H., Du, L. J., Song, Z. M., Chen, X. X., 2013. Progress in the characterization and safety evaluation of engineered inorganic nanomaterials in food. Nanomedicine 8, 2007–2025.

Wang, J., Zhou, G., Chen, C., Yu, H., Wang, T., Ma, Y., Jia, G., Gao, Y., Li, B., Sun, J., et al., 2007. Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration. Toxicology Letters 168, 176–185.

Wang, B., et al., 2008. Acute toxicological impact of nano- and submicro- scaled zinc oxide powder on healthy adult mice. Journal of Nanoparticle Research 10, 263–276.

Wang, Y. L., et al., 2014. A combined toxicity study of zinc oxide nanoparticles and vitamin C in food additives. Nanoscale 6, 15333–15342.

Wang, Y., et al., 2013. Susceptibility of young and adulty rats to the oral toxicity of titanium dioxide nanoparticles. Small 9, 1742–1752.

WHO in Safety Evaluation of Certain Food Additives and Contaminants (World Health Organization) 44 (2000) 1–2.

Wiking, L., Larsen, T., Sehested, J., Dairy J., 2008. Transfer of dietary Zinc and fat to milk-evaluation of milk fat quality, milk fat precursors, and mastitis indicators. Journal of Diary Science 91, 1544–1551.

Williams, K., et al., 2015. Effect of subchronic exposure of silver nanoparticles on intestinal microbiota and gut associated immune responses in the ileum of Sprague-Dawley rats. Nanotoxicology 9, 279–289.

Wu, H. H., Yin, J. J., Wamer, W. G., Zeng, M. Y., Lo, Y. M., 2014. Reactive oxygen species-related activities of nano-iron metal and nano-iron oxides. Journal of Food and Drug Analysis 22, 86-94.

Weir, A., Westerhoff, P., Fabricius, L., Hristovski, K., von Goetz, N., 2012. Titanium dioxide nanoparticles in food and personal care products. Environmental Science and Technology 46, 2242-2250.

Yun, J. W., et al., 2015. Comparative toxicity of silicon dioxide, silver and ion oxide nanoparticles after repeated oral administration to rats. Journal of Applied Toxicology 35, 681–693.

Yan, J. W., et al., 2019. Release of graphene from graphene-polyethylene composite films into food simulants. Food Packaging and Shelf Life 20, 100310

Yammamoto, O., 2001. Influence of particle size on the antibacterial activity. International Journal of Inorganic Materials 3, 643–646.

Zhang, Z., Zhang, H., Xiao, K., Bhattacharya, D., Demokritou, P., McClements, D. J., 2019. Development of a standardized food model for studying the impact of food matrix effects on the gastrointestinal fate and toxicity of ingested nanomaterials. NanoImpact 13, 13-15.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

**Table 1**

Overview of European and USA (FDA) standard migration tests for food packaging.

| Jurisdiction | Legislation (Reference) | Comments |
|---|---|---|
| **Europe** | European Standard BS EN 1186-1:2002; EN 1935/2004; EN 13130:2004; EN No 10:2011. Food simulants specified in Council Directive 85/572/EEC 1982; Council Directive 82/711/EEC 1982; Commission Directives 93/8/EEC 1993; 97/48/EEC 1997; 2002/72/EC 2002 | *European standardized testing conditions:*<br>- Four types of food simulants (A-D)[a]; choice depends on type of food (aqueous, acidic, alcoholic, or fatty foods). A list of food types and corresponding simulants which should be used is provided.<br>- Times & temperature selected to correspond to worst foreseeable conditions of contact & to any labelling information on maximum temperature for use.<br>- Where no labelling given, depending on food type(s), simulant(s) A and/or B and/or C shall be used for 4 h at 100 °C or for 4 h at reflux temperature and/or simulant D shall be used only for 2 h at 175 °C.<br>- Where materials are labelled for use at room temperature or below, test shall be carried out at 40°C for 10 days. |
| **USA** | FDA 2007 | *USA standardized testing conditions:* |

|  | - Comparable to European test strategy, except recommended testing conditions slightly differ. [b] Recommended migration protocol depends on the thermal treatment and extended storage conditions of the food packaging, as well as the type of polymer used. |
| --- | --- |

[a] Food Simulant A: distilled water or water of equivalent quality (used for only aqueous food, pH > 4.5);

[a] Food Simulant B: 3 % w/v acetic acid (used for only acidic foods, pH ≤ 4.5);

[a] Food Simulant C: 10 % v/v ethanol, shall be adjusted to actual alcoholic strength of food if >10 % (v/v) (used for alcoholic foods);

[a] Food Simulant D: rectified olive oil or other fatty food simulant (used for fatty foods). According to EN13130-1:2004 "When testing with fat simulants, simulant D, at 5 $^o$C the simulant can partially or totally solidified. Hence, to avoid this issue, the test should be carry out at 10 $^o$C. According to this normative, olive oil and sunflower oil at 10 $^o$C does not presence this issue of solidification. Nevertheless, at 10 $^o$C is a" more severe" test, but if the migration does not exceed the specified limit when tested at 10 $^o$C then it would also not have exceed the limit at 5 $^o$C according to EN13130-1:2004 (EN 13130-1: 2004). Alternatives to Food Simulant D include 95 % ethanol in aqueous solution or isooctane.

[b] The 3 % w/v acetic acid simulant is less frequently recommended for use by the FDA. The standard simulant is typically 10 % v/v ethanol (or 50 % v/v ethanol for foods with higher alcoholic content), or food oil (e.g. corn oil).

Not all recommended standardized testing conditions are listed here, but examples include:

| Contact time in days or hours at contact temperature in (°C) | Intended food contact conditions |
|---|---|
| 10 days at 20 °C | Any food contact at frozen and refrigerated conditions. |
| 10 days at 40 °C | Any long term storage at room temperature or below, including heating up to 70 °C for up to 2 hours or heating up to 100 °C for up to 15 minutes. |
| 2 hours at 70 °C | Any contact conditions that include heating up to 70 °C for up to 2 hours or up to 100 °C for up to 15 minutes which are not followed by long term room or refrigerated temperature storage. |
| 1 hour at 100 °C | High temperature application for all food simulants at temperature up to 100 °C. |
| *2 hours at 100 °C or at reflux or alternatively 1 hours at 121 °C | High temperature applications up to 121 °C. (It represents the worst case conditions for all food simulants in contact with polyolefins). |
| 4 hours at 100 °C or at reflux | Any food contact conditions with food |

| | |
|---|---|
| | simulants A, B, or C, at temperature exceeding 40 °C. (It represents worst case conditions for food simulants A, B and C in contact with non-polyolefins). |
| 2 hours at 175 °C | High temperature applications with fatty foods exceeding the conditions of * <br> (It represents the worst case conditions for fatty food simulants in contact with non-polyolefins). |

**Table**

**Table 2**

The updated data on specific migration studies of ENPs from FCMs.

| Reference | ENPs | Polymer | Test material (FCMs) | Migration simulant | Test conditions | Analytical method (s) | Concentration of ENPs in FCM | Migration result (given as total element determinatination if not otherwise stated) |
|---|---|---|---|---|---|---|---|---|
| Bumbudsanpharoke et al., (2019) | Nano-Zinc oxide | LDPE | Films | W<br>4% AA (v/v)<br>50% E (v/v)<br>n-heptane | 30 min at 70 $^o$C | ICP-OES | 1wt% and 5 wt.% | W: 0.009-0.029 mg/L<br>4%AA:0.017-3.416 mg/L<br>50%E: 0.006-0.013 mg/L<br>n-heptane: n.d. |
| Chen et al., (2019) | Nano-Titanium oxide | PET | Fims | 4% AA (w/v) | 10 d at 40 $^o$C<br>8 h at 80 $^o$C | ICP-OES | 583.8 mg/kg | 4%AA: 1.88 mg/kg<br>4% AA: 3.32 mg/kg |
| Yan et al., (2019) | Graphene | LDPE | Films (thickness: 0.043 mm) | 10% E (v/v)<br>50% E (v/v)<br>95% E (v/v) | 8 d at 70 $^o$C | UV-vis | 1.5 wt% | 10% E: 1.02 mg/kg<br>50% E: 1.10 mg/kg<br>95% E: 1.29 mg/kg |

| Choi et al., (2018) | Silver nanoparticle | LDPE | Comercial baby products (Breastmilk storage bags and baby bottles) | 4%AA (w/v) | 10 d at 40 °C | ICP-MS | 24.80-51.17 mg/kg | 4%AA: 1.05-2.25 ng/L |
|---|---|---|---|---|---|---|---|---|
| Bott et al., (2018) | Nano-clay (Laponite) | LDPE-EVA | Films | Surfactant solution as alternative food simulant | 10 d at 60 °C | AF4-MALLS | 2 wt%, 4wt% and 6 wt% | Nano-clay: n.d. |
| Ntim et al., (2018) | Nano-Silver | LDPE | Comercial cuting board (purchased through the US-FDA office in China) | W | Abrasion using Taber Industries Model 5750, US 1000 cycles 5000 cycles | ICP-MS | 7.20 µg/g | 0.24 µg/g |

| | | | | | | | | 0.58 µg/g |
| | | | | | | | | 0.60 µg/g |
| Li et al., (2017) | Nano-Titanium oxide | PLA | Films | 3%AA (w/v) | 40 d at 25 °C | ICP-AS | 1 wt% 5 wt% | 3% AA: 2.19 ug/kg 3% AA:3.12 ug/kg |
| | Nano-Titanium oxide/Nano-Silver | PLA | Films | 3%AA (w/v) | 40 d at 25 °C | ICP-AS | 1wt%/0.5wt% 5%wt/0.5wt% | 3% AA: 2.36 ug /kg 3% AA: 3.5 ug/kg |
| | Nano-Titanium oxide | PLA | Films | 50% E (v/v) | 40 d at 25 °C | ICP-AS | 1 wt% 5 wt% | 50 % E: 0.593 ug/kg 50 % E: 0.80 ug/kg |
| | Nano- | | | | | | | |

| | Titanium oxide/Nano-Siver | PLA | Films | 50% E (v/v) | 40 d at 25 $^o$C | ICP-AS | 1 wt%/0.5 wt% 5 wt%/0.5 wt% | 50 % E: 0.72 ug/kg 50 % E: 0.99 ug/kg |
|---|---|---|---|---|---|---|---|---|
| Huang et al., (2017) | Nano-Titanium oxide | LDPE | Films (thickness: 40 ±2μm) | 3%AA (w/v) | 7 d at 40 $^o$C | ICP-MS | 1 wt% | 3 % AA: 0.61 mg/kg |
| | Nano- Zinc oxide | LDPE | Films (thickness: 46± 2μm) | 3%AA (w/v) | 7 d at 40 $^o$C | ICP-MS | 1 wt% | 14.17 mg/kg |
| EFSA (2016) | Nano-Zinc oxide | LDPE | Plaques (thickness: 2 mm) | 3%AA (w/v) 10% E (v/v) 50% E (v/v) | 10 d at 60 $^o$C | ICP-MS and ICP-OES | 2 wt% | 3% AA: 2 mg/kg 10% E: 0.05 mg/kg 50% E: 0.06 mg/kg |
| Ramos | Nano-silver | PP | Tow plastic | 3%AA (w/v) | 10 d at 20-70 | sp-ICP- | | 3% AA: |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| et al., (2016) | | PC | containers: | | $^o$C | MS | | | |
| | | | - a baby feeding bottle (Nano BeBe$^+$, Baby dream Co.Ltd., Korea) -a food box (T-7003, Tifuco Co., Korea) | | | | | 62 mg/kg 28 mg/kg | 62 ng/dm$^2$ 18887 ng/dm$^2$ |
| Mackevica et al., (2016) | Nano-Silver | PE | 2 brands of commercial food storage boxes and 1 brand of commercial storage bag: | W 10% E (v/v) 3% AA (w/v) | 10 d at 40 $^o$C | ICP-MS | | | W: n.d. (<0.04, <0.05, <0.06). 10% E: n.d. (<0.04, <0.05,< 0.06). 3% AA: 0.20, 0.27, 0.28, 0.31μg/g |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | -Kinetic Go Green™ Premium Food Storage Containers® (Kinetic, USA) | | | | 22.5 µg/g (0.2 µg/cm$^2$) |
| | | | -The Original Always Fresh Containers™ ®(Gourment Trends, USA) | | | | 11.9 µg/g (1.4 µg/cm$^2$) |
| | | | - PE zipper storage bag® | | | | <1.0 |

| | | HDPE | (Fresher Longer TM Miracle Food StorageTM bags (Sharper Image, USA) | | | | (<0.2 µg/cm$^2$) | |
|---|---|---|---|---|---|---|---|---|
| | | | -HDPE bag for breast milk storage (Special Nnaosilver Mother's milk pack (Jaco, Korea) | | | | 31.2µg/g (0.2µg /cm$^2$) | |
| Ozaki et al., (2016) | Nano Zinc oxide | PE HDPE | Commercial containers, bags, | 4%AA (w/v) | 10 d at 5 $^o$C and 40 $^o$C | ICP-MS | 8.4-140 mg/Kg | 4% AA: 0.54-29 µg/L |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | dishes, cups. | | and 30 min at 60 °C and 95 °C | | | 1.4-46 µg/L<br><br>n.d.-26 µg/L<br><br>n.d.-32 µg/L |
| | Nano-Silver | PE<br><br>HDPE | Commercial containers | 4%AA (w/v)<br><br>W<br><br>20%E (v/v) | 10 d at 40 °C | ICP-MS | 21-200 mg/Kg | 4% AA: 1.4µg/L<br><br>W: 0.5 µg/L<br><br>20% E: n.d. |
| | | | Commercial bags | 4%AA (w/v)<br><br>W<br><br>20%E (v/v) | | | | 4% AA: 46 µg/L<br><br>W: 5.7 µg/L<br><br>20%E: 4.7 µg/L |
| Metak et al., (2015) | Nano-Silver | PE | Commercial containers ("Fresh Box® from Blue Moon Goods, USA) | Orange juice<br><br>Orange juice | 7-10 d at 40 °C<br><br><br>7-10 d at 40 °C | ICP-MS<br><br><br>AAS | 1 wt%<br><br><br>1 wt% | Oringe juice: 3.17&5.66 ± 0.02 µg/L<br><br><br>Nano-silver: n.d. |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Nano-Silver | PE with surface coating to 10 µm thickness with nano-silver | Commercial cling films(from Huzheng Nano Technology Co. Ltd., China) | Orange juice<br><br>W<br><br>Orange juice | 7-10 d at 40 ᵒC<br><br>7-10 d at 40 ᵒC<br><br>7-10 d at 40 ᵒC | ICP-MS<br><br>ICP-MS<br><br>AAS | 1 wt%<br><br>1 wt%<br><br>1 wt% | Oringe juice: 18.95 & 28.92 ± 0.01 µg/L<br><br>W: 6.4 ± 0.01µg/L<br><br>Oringe juice: 0.029± 0.01µg/L |
| Ntim et al., (2015) | Nano-Silver | PP LDPE PES | Commercial<br><br>- baby bottles<br>- cutting boards<br>- food storage bags<br> -food storage containers | 3% AA (w/v) | 4 h at 100 ᵒC + repeated contact | ICP-MS | 0.88 µg/g<br>7.16 µg/g<br>36.0 µg/g<br>24.7 µg/g | Nano-silver: n.d.<br>6.60 µg/g<br>35.8 µg/g<br>24.1 µg/g |

| | | | (purchased through the US. FDA office in China). | | | | | |
|---|---|---|---|---|---|---|---|---|
| Artiaga et al., (2015) | Nano-Silver | PP LDPE | Commercial food containers | 3%AA(w/v) 10% E (v/v) 95% E (v/v) | 10 d at 20 $^o$C 10 d at 40 $^o$C 2 h at 70 $^o$C | ICP-MS | 28 µg/g | 3% AA: 0.001 ng/g (20 $^o$C), 0.01 ng/g(40 $^o$C), 0.01ng/g (70 $^o$C) 10% E: <0.0001 (20 $^o$C) 95% E: < 0.1 |
| EFSA (2014) | Nano Silica | LDPE | Films (thickness: 60 µm) | 3%AA (w/v) 95% E (v/v) IO | 24 h at 40 $^o$C (IO) | AF4-MALLS | 3 wt% | Silica particles not detectable at the detection limit of the analytical method used (0.3-0.6 µg/kg simultant). |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Farhoodi et al., (2014) | Nanoclay (Cloisite 20 A) | PET | Bottle | 3% AA(w/v) | 7-90 d at 25 °C and 45 °C | ICP-OES | 3 wt% | 3%AA: Al: 0.18 mg/kg (25 °C) and 0.34 (45 °C), Si: 6.0 mg/kg (25 °C) and 9.5 mg/kg (45 °C) |
| Bott et al., (2014a) | Carbon Black | LDPE PS | Injection moulded plaques (thickeness: 3 mm) | 3%AA (w/v) 95% E (v/v) IO | 2-10 d at 60 °C | AF4-MALLS | 2.5 wt% 5 wt% | Carbon black particles not detectable. |
| Bott et al., (2014b) | Titanium nitirde (TiN) | LDPE | Films | 3%AA (w/v) 95% E (v/v) IO | 10 d at 60 °C, but Iso-octane: 24 h at 40 °C | ICP-MS | 0.01 wt% 0.05 wt% 0.1 wt% | 3% AA: 0.24 µg/kg 95% E, IO: 0.09-0.11 µg/kg |
| Cushen et al., (2014) | Nano-Silver | PE | Films | Chicken breast | 1-3 d at 8 °C and 22 °C | ICP-MS | 0.5 wt% | 0.003-0.005 mg/dm$^2$ |

| | Nano-Copper | PE | Films | Chicken breast | 1-3 d at 8 °C and 22 °C | ICP-MS | 0.5 wt% | 0.024-0.049 mg/dm² |
|---|---|---|---|---|---|---|---|---|
| Jokar et al., (2014) | Nano-Silver | LDPE | Films (thickness: 0.5 mm) | W 3 % AA w/v 10 % E v/v Apple juice | 30 dat 4 °C | AAS | 0.1 wt%, 0.5 wt% and 5 wt% | 0.30-1.12 mg/kg 0.37-1.12 mg/kg 0.33-0.96 mg/kg 0.34-0.76 mg/kg |
| | | | | W 3 % AA w/v 10 % E v/v Apple juice | 30 d at 40 °C | | | 0.38-1.10 mg/kg 0.49-1.43 mg/kg 0.42-1.29 mg/kg 0.56-1.08 mg/kg |
| Lin et al., (2014) | Nano-Titanium dioxide | PE | Films | 3%AA (w/v) 50% E (v/v) | 1-8 h at 25 °C 70 °C, 100 °C | ICP-MS | 254.84 mg/kg | 3% AA: 1.4/6.3/12.1 µg/kg |

| | | | | | | | | 50% E: 0.5/0.6/2.1 µg/kg |
|---|---|---|---|---|---|---|---|---|
| von Goetz et al., (2013) | Nano-micro- sized Silver | PP PE | Commercial plastic container | 3%AA(w/v) W 10% E (v/v) OO | 1-10 d at 20 °C | sp-ICP-MS | 9.7-23 µg/g | 3% AA: 9.5 ng/cm$^2$ W and 10% E: 4.75 ng/cm$^2$ OO: n.d. (LOD: 1 ng/g oil) |
| Cushen et al., (2013) | Nano-Silver (size particle: 10 nm and 50 nm) | Plasticiz ed PVC (50 % w/w DEHA) | Commercial plastic bags | W 3% AA 10% E | 1-10 d at 20 °C | sp-ICP-MS | 37 µg/g | 3% AA: 0.5 ng/cm$^2$ All other: n.d. (≤0.5 ng/cm$^2$) |
| | | | Film (thickness: 42- | Chicken breast | 1-4 d at 5 °C and 20 °C | ICP-MS | Nominal values: 500 (0.5%) and | 0.01-0.04 mg/dm2 (0.5%), 0.30-0.37 |

| | | | | | | | 50 000 (5%) =3.7 and 38.7 mg/dm2 | mg/dm2 (5%), no effect of particle size or temperature (or inverse effect, no time dependency) |
|---|---|---|---|---|---|---|---|---|
| Echegoyen et al., (2013) | Nano-Silver | LDPE PP | Commercial bags(FresherLonger$^{TM}$ Platic Storage bags$^{®}$) | 3%AA (v/v) 50% E (v/v) | 10 d at 40 $^o$C (repeated contact 2 h at 70 $^o$C) | ICP-MS | $2x10^4$ ng/cm$^2$) | 3 % AA: 3.74 ng/cm$^2$/ $3.1x10^{-3}$ ng/cm$^2$  50% E: 1.66 ng/cm$^2$/<LOQ |
| | | | Commercial container(Kinetic Go Green Basic Nanosilver Food Storage | 3%AA (v/v) 50% E (v/v) | 10 d at 40 $^o$C (repeated contact 2 h at 70 $^o$C) | ICP-MS | $39x10^4$ ng/cm$^2$ | 3 % AA: 31.46 ng/cm$^2$/50.3 $x10^{-3}$ ng/cm$^2$  50 % E: 9.48 ng/cm$^2$/ <LOQ |

| | | | Continer®) | | | | | |
|---|---|---|---|---|---|---|---|---|
| Huang et al., (2011) | Nano-Silver | PE | Commercial bags (thickness: 0.07 mm) | W 4%AA (w/v) 95% E (v/v) hexane | 3-15 d at r.t.; 40 $^{o}$C and 50 $^{o}$C | AAS | 100 µg (Ag)/g plastic materials | All simulants similar results, 40 $^{o}$C, 50 $^{o}$C: ~3-4 µg/dm$^{-2}$ of bag 25 $^{o}$C: ~1 µg/dm-$^{2}$ of bag |
| Schmidt et al., (2011) | Nano sized- Laurate - modified Al-Mg layered double hydroxide | PLA | Films | 95% E (v/v) | 10 d at 40 $^{o}$C | ICP-MS | 5 wt% | Ethanolysis of polymer |
| Song et al., (2011) | Nano-Silver (size particle:7 | PE | Commercial food contact film | 3%AA (w/v) 95% E (v/v) | 1-9 h at 20 $^{o}$C, 40 $^{o}$C, 70 $^{o}$C | ICP-MS | 234 mg/kg | 3% AA: 1.7/3/5.6 % 95% E: 0.24/0.23/0.22 % |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| nm) | | (thickness: 0.055 mm, Anson Nnanotechnolog y Co. Ltd, Zhuhai, China) | | | | | | |
| Emamifar et al., (2011) | Nano-Zinc oxide | LDPE | Films | Orange juice | 112 d at 4 $^{o}$C | AAS | 0.25 wt% 1 wt% | 0.68 µg/L 0.54 µg/L |
| Emamifar et al., (2010) | Nano-Zinc oxide | LDPE | Films | Orange juice | 28 d at 4$^{o}$C | AAS | 0.25 wt% 1 wt% | 0.11 µg/L 0.16 µg/L |
| Schmidt et al., (2009) | Nanoclay (Cloisite$^{®}$ Na$^{+}$ and Cloisite$^{®}$ | PLA | Film | 95% E (v/v) | 10 d at 40 $^{o}$C | AF4-MALS-ICP-MS | 5 wt% | Clay particles: n.d. |

| | 30B) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |

Abbreviations:

W: WATER; OO: olive oil; AA: acetic acid; E: ethanol; IO: octane;

 n.d: no detected;

PE: polyethylene; PP: polypropylene; LDPE: Low density polyethylene; PET: Polyethyleneterephtatale; PVC: polyvinyl chloride;

EVA: ethylene vinyl acetate; PC: polycarbonate; PLA:polylactitic acid; PES: polyphenylene ether sulfone; PS: polystyrene.
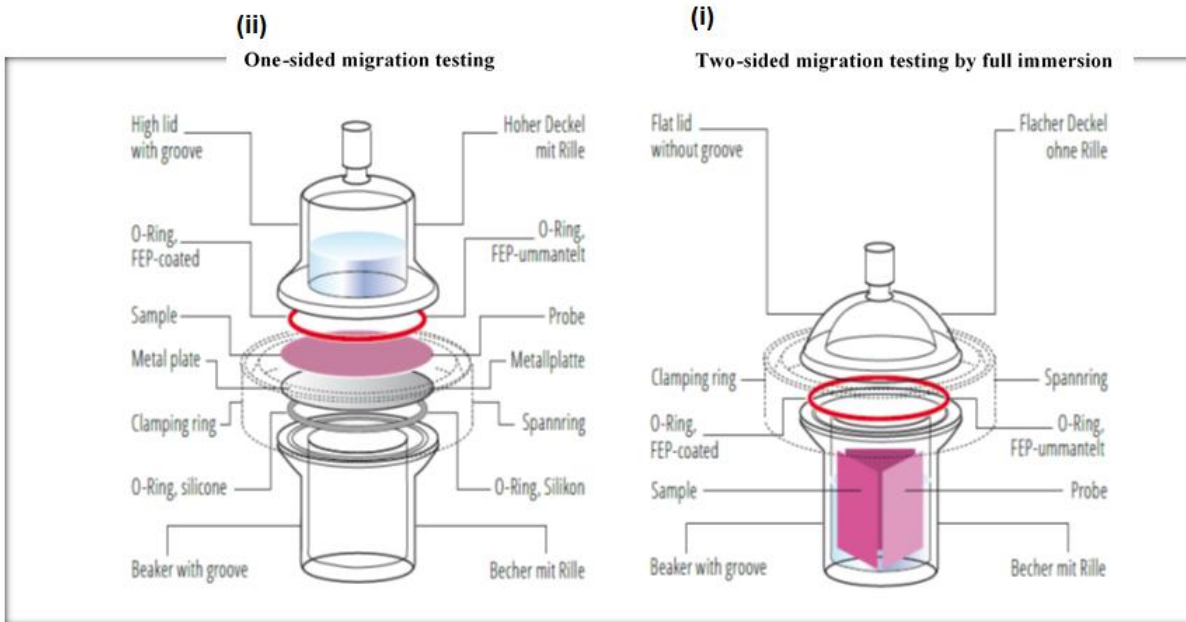
**Fig. 1**. Standardized cell (MigraCell®, FABES Forschungs-GmbH®, Germany) for overall/ specific migration of (i) *mono-* and (ii) *multi-layer* materials.
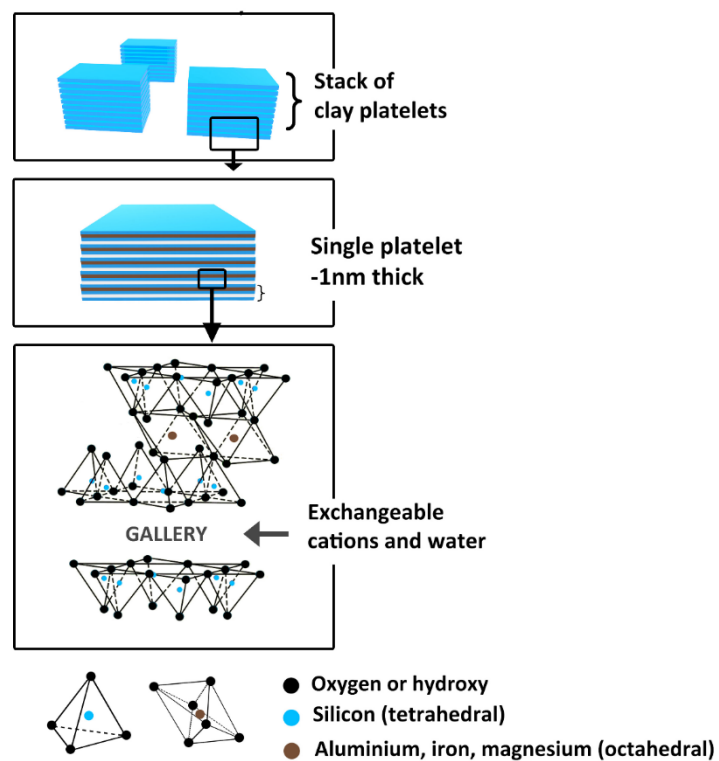
**Fig. 2.** Nano-clay chemical structure (Enescu et al., 2019).

**Supplemental**

**\*Declaration of Interest Statement**

Dear Editor Domingo,

I submit in your attention the manuscript entitle

"Recent advances and challenges on applications of nanotechnology in re-designing the traditional food

packaging as an active and intelligent one. A comprehensive review"

author: Daniela Enescu,  to be published in  "Food and Chemical Toxicology".

I mention that the above manuscript has not been published elsewhere.

Sincerely,

Ph. D. Daniela Enescu

daniela.enescu@inl.int

International Iberian Nanotechnology Laboratory (INL), Department Life Sciences, Research Unit: Nano for Food/Food Processing, Braga, Portugal

**\*Declaration of Interest Statement**

**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

*DEnescu*
24 May 2019