

Nanotechnologies in Food Packaging: an Exploratory Appraisal of Safety and Regulation

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Executive Summary

Food Standards Australia New Zealand (FSANZ) commissioned ToxConsult Pty Ltd to provide a literature review of the safety and regulation of nanotechnologies in food packaging. To achieve this target, a comprehensive literature search was undertaken in various scientific databases and agency websites; relevant references were sourced and reviewed. In addition, a patent search was conducted with the aim of identifying evidence for nanomaterials currently used in food packaging applications in Australia, New Zealand, United States, Europe, and Asia. It is recognised that not all such technologies may have been identified by the applied search techniques.

Current applications of nanomaterials in food packaging include:

- Enhancement of barrier properties through the incorporation of nano-fillers (e.g. nano-clay).
- 'Active' food packaging, with controlled release of active substances such as antibacterials to improve shelf-life of food (e.g. nanosilver).
- Improvement of physical characteristics to make the packaging more tensile, durable, or thermally stable (e.g. nano-titanium dioxide, titanium nitride).

Potential future applications include the use of 'smart' packaging (in the form of nanosensors, labels, etc), as well as polymer composites incorporating nanoencapsulated substances allowing consumers to modify food depending on their own nutritional needs or tastes.

The results of the patent search showed that although there is no direct evidence that nanomaterials are currently being used in food packaging applications in Australia and/or New Zealand, there is evidence they are being used overseas. These nanomaterials might be considered to be potentially in use in Australia and New Zealand if the associated products are imported. The two most common nanomaterials used in food packaging at present are likely to be nano-clays and nanosilver, based on the number of patents found and the types of products mentioned in other reviews. As a result of their likely widespread use, these two nanomaterials have been presented as case studies for exposure and safety assessment in this report.

Safety assessment of nanomaterials 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 nanomaterial constituents or the nanoparticles themselves from polymer nanocomposites into food or food simulants has been assessed by various authors using standard migration tests. These tests are European 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. The methods have not been validated for nanomaterials.

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 utilised 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. The results from migration experiments conducted with nano-clay, nanosilver, and other nanomaterial containing polymers were reviewed.

Nano-clay:

Clay (i.e. bentonite) is a naturally occurring substance with platelets whose thicknesses are in the nanoscale size range. Bentonite has a long history of permitted use as a food additive at levels up to 5% w/w in Europe and good manufacturing practice (GMP) levels in Australia; no evidence of adverse effects due to its use was found in the literature review conducted as part of this project. Although anecdotal evidence suggests it has been used as a food additive for decades (if not longer), definitive information for the extent and rate of its current or historical use as a food additive was not found. No evidence was found in this literature review to indicate that nano-clay is likely to cause adverse effects on health when used in food packaging.

Considering the probable extent of its use, there have been surprisingly few studies investigating the migration of nano-clay constituents into food simulants or foods. In some of the studies, migration of elemental components from nano-clay (particularly Si) into food and acidic food simulant has been detected from food packaging material, although overall migration in all cases (0-9.5 mg/kg) was significantly lower than the 60 mg/kg of foodstuff overall migration limit for Europe. Migration of aluminium from nano-clay was minimal (0-1 mg/kg food), and lower than the concentrations typically found in foods.

Only two studies examined migration of nano-clay particles *per se*, and in both their presence in food simulants was not detected. This indicates that the potential for consumer exposure and subsequent public health or safety issues, as a result of incorporation of nano-clay into polymer composites, is likely to be low. This is supported by *in vitro* and *in vivo* (90-day) toxicity experiments conducted with nano-clay polylactic acid composite migration simulant solutions, which have not found any adverse effects. Safety evaluations for nano-clay in food packaging are therefore likely to be driven by migration of elemental constituents, rather than by the 'nano-ness' of the material. However, this conclusion is tempered by the relatively few studies which have investigated the migration of nanoparticles *per se* from nano-clay, and the uncertainties in current analytical techniques for measuring nanoparticles in foods/simulants.

Nanosilver:

Silver is permitted for use as a food additive in Australia or as food colouring in Europe in confectionary, spirits and liqueurs to GMP. Colloidal silver and formulations containing silver salts were used historically for medical applications, but these uses have been largely discontinued. Since the 1990s, colloidal silver has been marketed as an alternative medicine, however its effectiveness for such uses has not been proven. After chronic medical or occupational exposure to silver, argyria (a permanent grey or blue grey discolouration of the skin and other organs) is the most common finding.

Unlike nano-clay, the antimicrobial function of nanosilver in food packaging materials means it is intended that silver ions be released to deter food spoilage. Thus a balance between what is considered too little to be effective and too much from a safety perspective needs to be achieved. A considerable number of migration studies were found for nanosilver containing polymer composites or coatings.

Overall the results from these studies suggest the production method of nanocomposites (e.g. incorporation or coating, surfactant modification), starting silver concentration, temperature, time and choice of contact medium are all factors which may have an effect on the extent of silver ion migration into food simulants. In general, the rate of migration increases when nanosilver is coated onto the food packaging material or surfactants are added, when the storage temperature and length of storage increases, and the acidity of the contact medium increases. There appears to be a specific time of storage, after which a steady state release of silver is achieved. This is supported also by a repeat contact migration experiment, which found silver migration decreased considerably (by an order of magnitude) after first contact.

Several studies have attempted to investigate whether nanoparticles *per se* migrate from nanosilver containing polymer composites into food simulant solutions, and mixed results have been obtained. Theoretical models predict migration of nanoparticles themselves from packaging to food would be detectable only when very small nanoparticles (i.e. ~1-3.5 nm) are embedded in polymer matrices which have low dynamic viscosities. The limitation in detectability of current measurement techniques, together with the lack of information provided on sample processing and handling prior to analysis makes it difficult to draw any concrete conclusions on whether silver ions or silver nanoparticles *per se* migrate into food simulants.

Until such a time analytical techniques are more refined and more information is available, safety assessment of nanosilver-containing food packaging materials will be limited to conventional considerations of ionic silver release into foods.

Nevertheless, there is some evidence to suggest that if silver nanoparticles do migrate into food/food simulants, they would most likely dissolve quickly into ionic silver. This stance was taken by the European Food Safety Authority in an assessment of zinc oxide nanoparticles in polyolefins, which led the agency to conclude that the substance does not migrate in nanoform and the resulting safety assessment was based on soluble ionic zinc. Furthermore, the toxicological effects of nanosilver observed in 28-90 day gavage studies with laboratory animals are qualitatively similar to those observed in dietary studies with silver salts, and in some instances less severe (ToxConsult 2015a). Though this is based on very limited information, this suggests any toxicity observed is unlikely to be due to the novel nano-ness of the material.

The majority of the migration studies found for nanosilver food packaging composites have shown levels of migration of ionic silver into foods and food simulants below the European specific migration limit (SML) of 0.05 mg Ag/kg food, suggesting low consumer exposure and subsequently low risk of adverse effects. However there are also several studies, in which migration exceeded this limit. This indicates that for new food packaging products containing nanosilver migration experiments should be conducted on a case-by-case basis.

Other nanomaterials:

In Europe, only 4 nanomaterials are currently authorised for use in food packaging applications:

- Titanium nitride nanoparticles in PET plastics up to 20 mg/kg (no migration of the nanoparticles into food is allowed).
- Carbon black (10-300 nm, aggregated to 100-1,200 nm in size), maximum level in polymer not to exceed 2.5% w/w. A specific migration limit is not set.
- Butadiene, ethyl acrylate, methyl acrylate copolymer cross-linked with divinylbenzene, in nanoform, in non-plasticised PVC up to 10% w/w (>20 nm, at least 95% by number >40 nm).
- Silanated silicon dioxide (SiO₂). Although this is not for nanoparticles *per se*, the European Food Safety Authority was recently informed that the substance had always been produced using synthetic amorphous silica in nanoform. A recently published safety assessment concluded, as no detectable migration of Si into food simulants was found, silanated SiO₂ does not raise a safety concern for the consumer in the currently authorised conditions of use.

The few regulatory safety assessments of nanoparticulates in food contact materials take a cautious approach in which no migration of nanoparticles is permissible. Since there are still limitations with measuring NPs *per se* in food/food simulants from migration experiments, this essentially means elemental constituent migration must be lower than the detection limit. This was the case in the European Food Safety Authority assessments for titanium nitride and silanated SiO₂.

For zinc oxide NPs in polyolefins, however, as discussed above, the European Food Safety Authority took a different approach. Although no direct evidence was available on the physical form of the released zinc in the migration experiments that were conducted, the agency concluded any zinc present in particulate form would be expected to dissolve immediately into ionic zinc on contact with acid foods or stomach acid. Therefore they focused their safety evaluation on soluble ionic zinc.

Few food packaging migration experiments for nanomaterials other than nano-clay and nanosilver were found in the literature. These either found no detectable migration of the nanomaterial or its constituents, or migration significantly less than the European permissible overall migration limit of 60 mg/kg. Although based on very limited data, this suggests that the potential for consumer exposure and subsequent public health or safety issues, as a result of incorporation of these nanomaterials (carbon black, TiN, TiO₂) in polymers studied is likely to be low.

Overall conclusion:

The data reviewed for this report indicate for most of the studied nanomaterials in food packaging, migration of intact nanoparticles into food simulants is negligible, implying consumer exposure to these materials is likely to be low. This suggests there is low potential for safety issues related to the 'nano-ness' of the materials incorporated into food packaging. If they were to migrate in nanoparticulate form, it would be anticipated at the resulting low concentration in food that many of the metal oxide nanoparticulates would likely dissolve into their ionic forms upon contact with acid foods or stomach acid. These conclusions are tempered by the relatively few studies which have investigated the migration of nanoparticles *per se* from food packaging materials and the uncertainties in current analytical techniques for measuring possible migrated nanoparticles in foods/simulants.

Summaries and conclusions for each type of nanotechnology food packaging are provided at the end of the relevant report section.

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Abbreviations

AA:	Atomic Absorption
ACCC:	Australian Competition and Consumer Commission
ADI:	Acceptable Daily Intake
AF4:	Asymmetric Flow Field-flow Fractionation
EU:	European Union
FSANZ:	Food Standards Australia New Zealand
GMP:	Good Manufacturing Practice
GRAS:	Generally Regarded as Safe
HDPE:	High Density Polyethylene
ICP-MS:	Inductively Coupled Plasma-Mass Spectrometry
JECFA:	Joint FAO/WHO Expert Committee on Food Additives
kg:	Kilogram
LDPE:	Low Density Polyethylene
MALS:	Multi-angle Light Scattering Detection
NICNAS:	National Industrial Chemicals Notification and Assessment Scheme
NP:	Nanoparticle
OML:	Overall Migration Limit
PET:	Polyethylene Terephthalate
PP:	Polypropylene
PS:	Polystyrene
PTWI:	Provisional Tolerable Weekly Intake
PVC:	Polyvinyl Chloride
RfD:	Reference Dose
SCF:	Scientific Committee for Food
SEM:	Scanning Electron Microscopy
SiO₂:	Silicon Dioxide
SML:	Specific Migration Limit
TDI:	Tolerable Daily Intake
TiN:	Titanium Nitride

1. Introduction

Food Standards Australia New Zealand (FSANZ) engaged ToxConsult Pty Ltd to provide a literature review of the safety and regulation of nanotechnologies in food packaging¹. Specifically, the aim of the review was to:

- Identify types of nanotechnologies currently used in food contact packaging with an aim to identify those that may result in migration of nanomaterials from the packaging into food.
- Where possible, identify publically available evidence that the nanotechnologies identified in the previous task are applied in Australia and/or New Zealand, either in domestically produced or imported products.
- Ascertain if there is reasonable scientific evidence that the application of nanotechnologies to food packaging materials may potentially pose a risk to public health and safety, due to the migration of nanomaterials into food and its subsequent ingestion.
- Include a brief synopsis of international regulations currently in place, or in development, which deal with the use of nanotechnologies in food contact packaging.
- Use case studies, based on data, to place the above tasks into context and assist to identify data gaps that may hinder formal risk assessment of a novel nanomaterial intended for food packaging.

Numerous applications for nanomaterials in food packaging have been proposed. Their purpose includes conveying antimicrobial and barrier properties to prevent food spoilage, enhancing film mechanical properties such as emulsification, foaming and water binding capacity, or enhancing other chemical-physical properties of polymers used in food packaging such as thermal stability and crystallinity (Aresta et al. 2013, Beltran et al. 2014).

Although many nanomaterials have been proposed for use in food packaging, this report focuses on those currently used in foods. The focus is also on food packaging *per se* (e.g. food containers, food wraps and films), rather than food contact materials (e.g. fridges, cutting boards, cutlery). Section 2 identifies the functions and types of nanomaterials proposed for use in food packaging, and Section 3 identifies those for which there is evidence of their use in Australia and/or New Zealand. The potential

¹ In Australia, the regulation of chemicals in articles for food use is shared by several Australian Government regulatory agencies; FSANZ and the state/territory food authorities for the food sold in packaging; the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) for the safety of the industrial chemicals used; and the Australian Competition and Consumer Commission (ACCC) for the safety of the packaging articles themselves.

In New Zealand, chemicals in food are also regulated by FSANZ, in conjunction with the New Zealand Ministry of Primary Industries. Chemicals in packaging are regulated by the New Zealand Environment Protection Authority and consumer products, by the New Zealand Ministry of Consumer Affairs.

for consumer exposure to nanomaterials in food packaging is discussed in Section 4, with specific reference to case studies for nano-clay and nanosilver. A brief overview of how nanomaterials in food packaging are being regulated internationally is provided in Appendix B. This report should be read in conjunction with the report on the *Potential Health Risks Associated with Nanotechnologies in Existing Food Additives* (ToxCR230215-RF2), which focuses on the safety of nanotechnologies in food. The latter report has been referenced as ToxConsult (2016a) in this document.

Nanoparticles in this report are defined as an engineered form of matter having at least one dimension in the nanometre scale (<100 nm). Similarly, nanomaterials are materials with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale.

2. Nanotechnologies used in food packaging

A study carried out by the 'Safety of Nanomaterials Interdisciplinary Research Centre' of the UK Food Standards Agency collated information on the current and projected processes, products and applications of nanotechnologies for food contact materials (Chaudhry et al. 2008). The information gathered indicated nanotechnology applications in the food sector are increasing worldwide, and many international food companies are exploring their potential applications. Among the nanotechnology applications for the food sector, nanotechnology-derived food contact materials make up the largest share of the current and short-term predicted market; a range of these are already available in some countries, and it is widely expected they will become increasingly available worldwide in the next few years (Chaudhry et al. 2008). In 2008 the global nano-enabled food and beverage packaging market was 4.13 billion US dollars and was projected to grow to 7.3 billion US dollars by 2014 (Duncan 2011, iRAP 2009).

2.1 Current uses

Among several thermoplastics, polyolefins are the most used plastics materials in the food packaging sector. Polypropylene (a type of polyolefin) films are often used because of their transparency, brilliance, low specific weight and chemical inertness. However, polypropylene (PP) (like other polyolefins and other polymers)² is also characterised by low barrier properties (i.e. an inherent

² Polymers most frequently used for food packaging include, but are not limited to, polyolefins such as polypropylene (PP) and various grades of polyethylene (HDPE, LDPE, etc), polyethylene terephthalate (PET), polystyrene (PS) and polyvinyl chloride (PVC). Different polymers have different strengths and weaknesses in relation to barrier properties. For example, PET provides a good barrier to oxygen, while HDPE fares much worse. On the other hand, HDPE offers a significantly better barrier against water vapour than PET (Duncan 2011).

permeability to gases and other small molecules), which results in poor protection of packaged foods. One of the methods to improve PP and other plastics' barrier deficiencies is to add a second component such as a polymer blend or multilayer, filler, etc (Avella et al. 2007, Duncan 2011, Fabra et al. 2013, Han et al. 2011, Manikantan and Varadharaju 2011, NanosafePACK 2012, Tang et al. 2008). Polymer-based nanocomposites are reported to achieve the same or better barrier properties than their conventional composite counterparts³ (Avella et al. 2005, 2007; Bott et al. 2014a, Mihindukulasuriya and Lim 2014). Such nanocomposites are reinforced with small quantities (typically up to 5% by weight) of nanoparticles⁴, which have very high aspect ratios ($L/h > 300$) (Chaudhry et al. 2008, FAO/WHO 2009, NanosafePACK 2012). They are incorporated in addition to the traditional fillers and additives.

Nanocomposites have also been researched for their use in so-called 'active' food packaging, which refers to the controlled release of active substances from the food packaging materials. 'Active' packaging is not a new concept; this type of packaging (not necessarily incorporating nanomaterials) has been on the market for decades. Examples include small pouches or sachets placed inside a sealed package, which act as a desiccant, corrosion inhibitor or oxygen scavenger. Nanocomposites which are being used or proposed for use in 'active' packaging include polymer composites with antimicrobial nanomaterials, e.g. silver, zinc oxide, magnesium oxide. 'Active' packaging is intended to enhance the condition of the packed food, extend shelf-life, or improve sensory properties while maintaining the freshness and quality of food (Chaudhry et al. 2008, Bott et al. 2014b, Dainelli et al. 2008, FoE 2008, Kuorwel et al. 2015). One of the most well-known examples of a nanocomposite currently used in 'active' food packaging is nanosilver in plastics which confers antimicrobial properties to improve food and beverage shelf-life (de Azeredo et al. 2013, Emamifar et al. 2010, Fortunati et al. 2013, Llorens et al. 2012, Valipour et al. 2013); nanosilver has been presented as a case study in this report (Section 4.3).

Apart from conferring barrier properties to extend the shelf life of food (e.g. through an antimicrobial function or an oxygen- or water vapour- permeability barrier), other nanocomposites confer various physical characteristics to make the packaging more tensile, durable, or thermally stable (Beltran et al.

³ Nanomaterials have large aspect ratios which, when incorporated as fillers into the walls of packaging, creates an obstacle for gas and moisture passing through packaging walls by increasing the path that the gas/moisture must travel (Hannon et al. 2015).

⁴ Nanomaterials are commonly immobilised within polymer packaging using two methods: formation of particles and/or polymer *in situ* (e.g. spin coating, casting) or attachment of particles and polymer in their final state. The method employed often determines the concentration and distribution of the nanomaterials within the polymer (Hannon et al. 2015).

2014, Duncan 2011, NanosafePACK 2012). Examples of other nanocomposites include UV absorbers (e.g. nano-titanium dioxide, iron oxides, silica, alumina) to prevent UV degradation of plastic polymers, titanium nitride (TiN) used to improve strength of packaging materials, nano-calcium carbonate-polymer composites, nano-chitosan-polymer composites, biodegradable nanoclay composites of starch and polylactic acid⁵, biodegradable cellulose nano-whiskers, and other gas-barrier coatings (e.g. nano-silica) (Reig et al. 2014, Sanchez-Garcia et al. 2010, Siracusa et al. 2008, Smolander and Chaudhry 2010). A well-known example of one of the first nanocomposites to be explored for use in food packaging to enhance barrier properties is nano-clay, which has been incorporated with nylons, polyolefins, copolymers, epoxy resins, polyurethane, polyethylene terephthalate, etc. Some materials are already commercially available, and used by beverage companies in certain countries. Nano-clay is presented in this report as a case study (Section 4.2). Formulation of nanobiocomposites combining nanosilver with nano-clay or other nanomaterials (e.g. titanium dioxide) to enhance both barrier and antimicrobial properties has also been studied for potential future application in food packaging (Busolo et al. 2010, Cozmuta et al. 2014). Other examples of nanomaterials potentially used in food packaging⁶ include alumina (e.g. wheel-shaped alumina platelets used as fillers for plastic materials), nano-precipitated calcium carbonate (to improve mechanical properties, heat resistance and printing quality of polyethylene), polyhedral oligomeric silsesquioxane (POSS) nanoclay (to improve barrier properties), zinc oxide calcium alginate nanofilms (used as a food preservative), and silica/polymer hybrids (to improve oxygen-diffusion barriers for plastics) (Smolander and Chaudhry 2010, Bajpai et al. 2012).

2.2 Potential future uses

Potential future applications of nanotechnology relevant to the area of food packaging include:

- Use of carbon nanotubes or carbon nanodots for conductive and reinforcement applications in nanocomposites (Chaudhry et al. 2008, Das Purkayastha et al. 2014, Sanchez-Garcia et al. 2010).
- Nanocellulose conferring barrier properties for use as an economical biodegradable food packaging material (Li et al. 2015).
- Materials for 'smart' food packaging, e.g. nanosensors, biosensors, labels (APVMA 2014, Chaudhry et al. 2008, Dainelli et al. 2008, Duncan 2011, Han et al. 2011, Munro et al. 2009).
The purpose of these embedded sensors is to signal the condition of food by detecting food

⁵ It is unknown whether these nanocomposites are currently in commercial use anywhere in the world or in Australia.

⁶ It is unknown whether these materials are currently in commercial use anywhere in the world or in Australia.

pathogens and triggering a colour change in the packaging to alert the consumer about contamination or spoilage. A potential example of this is BioSilicon™ from pSivida⁷ (Australia). This is a nanostructured silicon with nanopores for ‘smart’ packaging applications, e.g. to detect pathogens in food, for food tracing, food preservation and detection of variations of temperature in food storage.

- Polymer composites incorporating nanoencapsulated substances (e.g. enzymes, catalysts, oils, adhesives, polymers, inorganic nanoparticles, biological cells, flavour and colour enhancers, vitamins, etc), which would allow consumers to modify the food depending on their own nutritional needs or tastes, or act as an alternative antimicrobial application for improving shelf life of food (Chaudhry et al. 2008, Duncan 2011, Liang et al. 2012).

3. Evidence for use in Australia and/or New Zealand

Whilst there are a number of projective applications for nanomaterials in food packaging, there is currently little evidence for regulatory approvals being sought for these products⁸.

In order to gain an indication whether nanomaterials in food packaging are currently used in Australia and/or New Zealand, either in domestically produced or imported products, a patent search was conducted⁹. Details of the search methodology can be found in Appendix A. Since nanomaterials currently patented for use in the food packaging market overseas might be considered to be potentially in use in Australia and/or New Zealand, the search was conducted for patents in Australia, New Zealand, Europe, the United States and Asia. In total 59 relevant patents were found; most of these stem from the United States. No relevant patents were found to be registered in Australia and/or New Zealand. A summary of the patents found, and the function of the nanomaterial in food packaging is presented in Table 3.1 below. The two nanomaterials with the most patents were nano-clays (18) and nanosilver (14).

⁷ pSivida is a global company specialising in miniaturised, sustained-release drug delivery products technology, with a primary focus on ophthalmology. pSivida launched an Australian spinout company (pSiNutria Limited) in December 2005, to develop applications of their silicon technology in the food industry. An Australian patent search for pSivida or pSiNutria Limited produced no hits relevant to food packaging, which suggests these applications are still in the research and development stage and have not been commercially released.

⁸ Verbal communication with FSANZ, 14th April 2016.

⁹ It is noted the identification of nanotechnologies in current use in Australia is difficult to determine due to intellectual property issues and closely guarded propriety data. Not all such technologies may have been identified by the applied search techniques.

Table 3.1: Results of patent search

Type of nanomaterial ^a	Patent Location	Patented use in food packaging	# patents found
<i>Nanosilver</i>	USA	- Antimicrobial film - Catalyst for production of polymers used in food contact coatings - Antistatic-Antimicrobial	11
	Europe	- Antimicrobial - Laminate (food wrap) preservation	2
	Asia	- Antimicrobial	1 ^b
	Aust/NZ	Nil Found	0
Total			14
<i>Copper NPs</i>	USA	- Conductivity	1
	Europe	- Antimicrobial - Barrier properties in food wrap	3
	Asia	Nil Found	0
	Aust/NZ	Nil Found	0
Total			4
<i>Nano-clays</i>	USA	- Polymer nano composites to improve mechanical properties (e.g. toughness, ductility, strength) - Flexible barrier packaging - Moisture barrier layer - Flexible (oxygen) barrier packaging	18
	Europe	Nil Found	0
	Asia	Nil Found	0
	Aust/NZ	Nil Found	0
Total			18
<i>Graphite nanoplatelets</i>	USA	- Barrier and/or conductive properties	1
	Europe	Nil Found	0
	Asia	Nil Found	0
	Aust/NZ	Nil Found	0
Total			1
<i>Nano-Titanium Dioxide</i>	USA	- Flexible barrier packaging	1
	Europe	Nil Found	0
	Asia	Nil Found	0
	Aust/NZ	Nil Found	0
Total			1
<i>Nano- metal phosphonates</i>	USA	- Barrier properties	1
	Europe	Nil Found	0
	Asia	Nil Found	0
	Aust/NZ	Nil Found	0
Total			1

Type of nanomaterial ^a	Patent Location	Patented use in food packaging	# patents found
<i>Nano-silicon dioxide (SiO₂)</i>	USA	- Hydrophobic coating - 'Active' packaging for food preservation	5
	Europe	Nil Found	0
	Asia	Nil Found	0
	Aust/NZ	Nil Found	0
Total			5
<i>Zinc oxide (ZnO) NPs</i>	USA	- Enhanced barrier properties - UV filter - Antimicrobial	7
	Europe	Nil Found	0
	Asia	Nil Found	0
	Aust/NZ	Nil Found	0
Total			7
<i>Mixed metal oxide NPs (e.g. ZnO, Al₂O₃, TiO₂, SiO₂)</i>	USA	- Durability - Barrier - Antimicrobial	6
	Europe	- Antimicrobial	2
	Asia	Nil Found	0
	Aust/NZ	Nil Found	0
Total			8

NPs = nanoparticles.

^a Specific size ranges or further characteristics were not reported.

^b This is a patent application, not yet registered.

Chaudhry et al. (2008) conducted a literature search for nanomaterials or nanoparticles potentially in use for food packaging applications. As part of the search, the authors visited relevant company websites. Such a detailed search was not within the scope of the current report. Chaudhry et al. (2008) listed a number of examples of commercially available food packaging products containing antimicrobial nanomaterials or nano-clays. More recent reviews by Han et al. (2011), Reig et al. (2014), Hannon et al. (2015), and Friends of the Earth (FoE 2014) listed additional examples. They include some food packaging materials which may not be under FSANZ's remit.

They include:

- Nanosilver Food Containers and water bottles (from A-DO, Korea).
- Nanosilver salad bowl (from Changmin Chemicals, Korea).
- FresherLonger™ Miracle Food Storage and FresherLonger™ Plastic Storage Bags (from Sharper Image® USA), containing nanosilver.
- Fresh Box® food storage containers (from BlueMoonGoods™, USA), containing nanosilver.
- Fresh food containers (from Oso Fresh, USA), containing nanosilver (40-30 nm).
- Smartwist food storage with nanosilver (from Kinetic Go Green, USA).

- Nanosilver storage box (from Quan Zhou Hu Zeng Nano Technology[®] Co., Ltd, China).
- Nano Silver Baby Mug Cup and Baby Milk Bottle (from Baby Dream[®] Co., Ltd in Korea), containing nanosilver.
- Nanobox (from Hopack, Australia)¹⁰, a paper food box/container containing nanosilver.
- Agion[®] (from 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.
- Zeomic Sinanen (from Zeomic Co Ltd, Japan), packaging film containing silver zeolites.
- Nano Plastic Wrap (from SongSing Nano Technology Co., Ltd in Taiwan), containing nano-zinc oxide as a light catalyst to sterilise in indoor lighting.
- Imperm[®] (from 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 CO₂ from drinks and penetration of O₂ into bottles, thus keeping the beverage fresher and extending the shelf life by up to 6 months.
- NanoSeal[™] Barrier Coating and Bairicade XT[™] Barrier Coating (from NanoPack Inc, USA): described as a water based coating comprised of a masterbatch and a liquid dispersion of clay platelets ('nano' or particle size is not mentioned, however the company and product names suggest the presence of nano-clay). The coating is applied to traditional packaging films 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).
- Duretham[®] LDPU 601 (from 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 CO₂.
- Aegis[®] OX (from 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 O₂ scavengers and passive nano-clay particles to enhance barrier properties, retain CO₂ but keep O₂ out.
- Nanolok[™] (from InMat[®] Inc, USA), containing nano-clay for barrier properties.
- PET bottles, containing nano-titanium nitride (from Colormatrix, USA) to confer barrier properties.

¹⁰ FoE (2014) indicate the Nanobox food packaging is available for purchase in Australia. However the link for the reference to this information provided in FoE (2014) no longer works. An internet search for the product "Nanobox" only found a product statement (Hopack 2012) from what appears to have been a showcase for "gohospitality", Australia's Hospitality Directory. The product statement has the Hopack (an Australian company) logo in it, however an internet search for "Nanobox" on the Hopack website and a search in the gohospitality website came up with zero results. The product may not yet be commercially available, or if it is, it may not be sold by that name.

- Eco Plastic™ (from Plantic Technologies Ltd, Australia), a biodegradable polymer made of corn starch. Although Han et al. (2011) indicates the polymer is a nano-composite, the authors do not provide a reference for the information. No direct evidence and no mention of the word “nano” could be found in the product descriptions from Plantic Technologies, therefore it is uncertain whether these products in fact contain nanomaterials.

Conclusions:

From the results of a patent search, it is concluded that although there is no direct evidence that nanomaterials are currently being used in food packaging applications in Australia and/or New Zealand, there is evidence they are being used overseas. These nanomaterials might be considered to be potentially in use in Australia and New Zealand if the associated products are imported. The two most common nanomaterials used in food packaging at present are likely to be nano-clays and nanosilver, based on the number of patents found and the names of products mentioned in other reviews.

4. Is consumer exposure likely?

Theoretically, there may be potential consumer exposure to nanomaterials incorporated into food packaging if:

- they migrate into foodstuffs or drinks from the packaging¹¹, or
- if the nanocomposite polymers degrade and ‘dissolve’ into food or drinks.

It is assumed the latter type of exposure is unlikely to occur, as this is expected to compromise the integrity of the packaging to the extent that the consumer is unlikely to eat or drink the product within. This chapter therefore focuses on the potential migration of nanoparticles from food packaging made with nanocomposites, specifically focusing on nano-clays and nanosilver, the two most common nanomaterials likely to be currently used in food packaging applications (See Section 3).

Migration of nanoparticles from food packaging material into food may be affected by multiple factors including temperature, time, concentration gradient, material properties, position of the nanoparticles

¹¹ Migration can theoretically occur if nanoparticles desorb from the surface of the packaging material due to weak bonding at the surface (only really relevant for coatings), diffuse into foods as a result of a concentration gradient, or dissolve resulting in ions released into food (Noonan et al. 2014).

in the packaging material, interaction between the nanoparticle and the material, and the nature of the food (Hannon et al. 2015, Noonan et al. 2014).

A theoretical study by Simon et al. (2008) predicted migration of nanoparticles¹² *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 nanocomposites of silver with polyolefins, but not by nanosilver composites with polyethylene terephthalate (PET) and polystyrene (PS), nor by any surface-modified nano-clay embedded in polymer matrices (Simon et al. 2008). Other authors, after developing a particle volume (size) related migration model based on an existing model for migration of conventional plastic additives, came to similar conclusions (Bott et al. 2014a). The modelling from these authors suggested only the smallest spherical NPs up to 3.5nm in diameter may result in measurable migration, if present at high concentrations in a polymer. Larger particles have an exponential decrease in mobility in the polymer and therefore very limited, or no potential to migrate out of the polymer. The overall conclusion from this study was consumer exposure to NPs via migration from food contact plastics into foods is not expected (Bott et al. 2014a). However, Noonan et al. (2014) have emphasised that until experimental methods are sufficiently developed to generate reliable migration data for nanomaterials, such theoretical models as described above cannot be validated and are only of academic relevance (see also Section 4.1).

4.1 General considerations

Migration testing from polymers used for food packaging is typically conducted by the manufacturer or food packager. In these tests overall migration of all components from the packaging is normally evaluated to ensure compliance with a regulatory limit¹³. In addition, compliance may also be required for migration of certain individual monomers and/or additives, the limit is set according to specific hazard assessments for these components.

¹² The type of nanoparticle was not accounted for, rather the size of the NP and the properties of the polymer were used to generate equations for the migratability, diffusion rate and amount of migrating particles (Simon et al. 2008).

¹³ In the EU, the overall migration limit into food for all components from food-contact plastics is 10 mg/dm² of packaging. For a cubic packaging containing 1 kg of food, this results in a migration of 60 mg/kg of foodstuff (EC 2011, 2014, 2015). For small packaging where the surface to volume ratio is higher the resulting migration into food is higher. Specific migration limits (SMLs) are set according to the Acceptable Daily Intake (ADI) or the Tolerable Daily Intake (TDI) established by the Scientific Committee on Food. The limits are set on the assumption that every day throughout a person's lifetime, a 60 kg person eats 1kg of food packed in plastics containing the substance in the maximum permitted quantity.

Standard migration tests are conducted with food simulants under standard incubation time and temperature conditions which are chosen according to the type of food that will be packaged. These methods are used to measure the 'overall' or 'specific migration', which is compared to the respective limits¹⁴. The standard methods may be modified to cover specialist uses, for example plastics for use in microwave ovens. An overview of a standard migration test from two different jurisdictions is provided in Table 4.1. This overview is not intended to be an exhaustive review of available testing or measurement protocols. Rather it is intended to provide the reader an indication of what is involved in food packaging migration testing.

Table 4.1: Overview of standard migration tests for food packaging from two jurisdictions

Jurisdiction	Legislation (Reference)	Comments
Europe	Council Directive 82/711/EEC, Commission Directives 93/8/EEC, 97/48/EC; food simulants specified in Council Directive 85/572/EEC (EC 1982, 1985, 1993, 1997) Supported by European Standard BS EN 1186:2002 (BSi 2002)	- 4 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 (dry food does not need to be tested). - Times & temperature selected to correspond to worst foreseeable conditions of contact & to any labelling information on maximum temperature for use. - Where no labelling given, depending on food type(s), simulant(s) A and/or B and/or C shall be used for <i>4 hrs at 100°C</i> or for 4 hrs at reflux temperature and/or simulant D shall be used only for <i>2 hrs at 175°C</i> . - Where materials are labelled for use at room temperature or below, test shall be carried out at <i>40°C for 10 days</i> .
USA	Nonbinding recommendations (FDA 2007)	- 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. ^b

^a Simulant A = distilled water or water of equivalent quality (used for aqueous food, pH > 4.5); Simulant B = 3% w/v acetic acid (used for acidic foods, pH ≤ 4.5); Simulant C = 10% v/v ethanol, shall be adjusted to actual alcoholic strength of food if >10% (v/v) (used for alcoholic foods); Simulant D = rectified olive oil or other fatty food simulant (used for fatty foods). Alternatives to Simulant D include 95% ethanol in aqueous solution or isoctane.

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

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

- 121°C for 2 hrs (high temperature, heat sterilised), followed by the remainder of 10 days (i.e. 238 hours) at 40°C.
- Same as above but 100°C for 2 hrs instead of 121°C (boiling water sterilised).
- 66°C for 2 hrs, followed by 238 hrs at 40°C (pasteurised above 66°C).
- 40 °C for 10 days (room temperature filled & stored, i.e. no thermal treatment in container).
- 20 °C for 10 days (refrigerated storage, no thermal treatment in container).
- 20° C for 5 days (frozen storage, no thermal treatment in container).
- 100°C for 2 hrs (frozen or refrigerated storage, intended to be re-heated at time of use).

¹⁴ 'Overall migration limit' (OML) means the maximum permitted amount of non-volatile substances released from a material or article into food simulants (EC 2011). The 'specific migration limit' (SML) is defined as the maximum permitted amount of a given substance released from a material or article into food or food simulants. Standard migration tests are used to measure overall and specific migration.

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. However it is also permissible to demonstrate compliance with an overall migration limit by using a more severe test, i.e. a total immersion test, in which both inner and outer surfaces are in contact with the food simulant. Testing samples with representative specimens prepared by cutting sections from the plastic and completely immersing them in food simulant is also regarded as a more severe test, and therefore will give more conservative results (i.e. higher migration rates) than anticipated to occur in actual use (BSi 2002). The surface to volume ratio in a total immersion test is typically 1 dm² of food contact area to 100 mL of food simulant. 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 (BSi 2007). In the literature reviewed as part of this project, only one study was found which considered migration from food packaging after repeated use (von Goetz et al. 2013).

The literature search conducted as part of this project did not find studies which investigated the applicability of the food simulants for nanomaterial *per se* migration. Nevertheless, the standard simulants for assessing hydrophilic and hydrophobic materials have been used in migration studies with nanomaterial-containing polymers (e.g. Chaudhry et al. 2008, Bott et al. 2012, 2014a, b, c; Artiaga et al. 2015, Echegoyen and Nerin 2013, Farhoodi et al. 2014). In some of these studies, the suitability of the food simulant for the sensitive detection of metal derived from the nanomaterial (e.g. Ti or Ag) was verified. In a few investigations, migration has also been tested into actual foods (Avella et al. 2005, Chaudhry et al. 2008, Cushen et al. 2013, 2014a, Metak and Ajaal 2013). There are issues with this latter testing as the complex matrix of most foods may interfere with accurate measurements, and there seems to be no way or ready way of distinguishing between migrated particles or ions within the food (Cushen et al. 2013, 2014a; Kuorwel et al. 2015). In addition, it has been suggested use of specific low fat foods (e.g. turkey or chicken meat) in migration testing may result in underestimation of potential migration into other more fatty foods (Hannon et al. 2015). The majority of migration studies identified as part of this project have investigated nanomaterials incorporated within polymers, with only a few assessing the migration from food packaging coatings (Jokar and Rahman 2014, Smirnova et al. 2012, Nobile et al. 2004).

Most migration experiments with nanomaterial-containing food contact materials have determined migration by measuring the elemental component of the nanomaterial by standard analytical

techniques [e.g. atomic absorption (AA), inductively coupled plasma mass spectrometry (ICP-MS)¹⁵, etc], without determining whether the nanoparticle *per se* is present in the food simulant solution (e.g. Avella et al. 2005, Fortunati et al. 2013, Farhoodi et al. 2014). This is in part due to the limitations of available measurement techniques (Noonan et al. 2014). Scanning electron microscopy (SEM) of food simulant at the conclusion of migration testing has been used in some studies to determine the presence of nanoparticles, but is also limited in resolution in some respects (e.g. Artiaga et al. 2015, Noonan et al. 2014, Kuorwel et al. 2015). Most recently, asymmetric flow field-flow fractionation (AF4), at times in combination with SEM and/or ICP-MS, appears to be the technique of choice for detecting/measuring intact nanoparticles in the food simulant. AF4 is a fractionation method for separating macromolecules and particles in a suspension. Smaller particles are transported more rapidly along the sample channel than larger particles, resulting in the smaller particles eluting before the larger ones. One of the main advantages of the technique, when coupled with a detector such as ICP, is that it allows detection and quantitation of nanoparticles in relatively low concentrations. However, there are still issues to be resolved with this technique, they relate to membrane-nanoparticle interaction, behaviour of different nanoparticle types in the field-flow fractionation channel, the influence of dilution of suspensions during separation, the lack of suitable calibration strategies and improvement of recovery rates (Krystek et al. 2011). In addition it cannot rule out the formation of nanoparticles from ions during post-migration handling (Noonan et al. 2014, Tiede et al. 2008). Some of these aspects relating to nanoparticle characterisation in food matrices have been the subject of recent research (e.g. Lee 2013).

Another limitation for interpretation of the available migration studies is that they vary significantly in test sample preparation as well as the level of description provided for how these methods were carried out.

4.2 Nano-clay

Polymer nanocomposites incorporating nano-clay (i.e. clay nanoparticles) 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 (Chaudhry et al. 2008, de Abreu et al. 2010, Hannon et al. 2015, Lagaron et al. 2005, Kuorwel et al. 2015). Montmorillonite is a soft 2:1 layered phyllosilicate clay comprised of platelets separated by thin layers of water. The platelets have an average thickness of ~1nm and average

¹⁵ These methods often require acid digestion of samples prior to analysis. Therefore they provide information on total elemental concentrations, but provide no information on physical characteristics of migrated nanomaterials or even whether whole nanoparticles migrated in the first place (Noonan et al. 2014).

lateral dimensions ranging from a few tens of nm to several μm . Each platelet contains a layer of aluminium or magnesium hydroxide octahedral sandwiched between two layers of silicon oxide tetrahedral (Figure 4.2.1) (Duncan 2011, Hannon et al. 2015, Mauricio-Iglesias et al. 2010, Kuorwel et al. 2015).

Because it is a natural material, nano-clay is not considered to be a novel product *per se*. Bentonite has a long history of permitted use as a food additive and no evidence of adverse effects due to its use was found in the literature. In the European Union, bentonite is authorised as an additive for plastic materials and articles in contact with foods with no specific restrictions (EC 2011). The substance is also an approved food additive (E558) included in European Directive 95/2/EC (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. Anecdotal evidence suggests bentonite has been used as a food additive for decades (if not longer), and it is frequently marketed on the internet as an oral medicinal remedy for various intestinal ailments. However definitive information for the extent and rate of its current or historical use as a food additive was not found.

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) of 7 mg/kg bw for aluminium for all intake sources¹⁶, established previously by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 1988). Recently at its sixty-seventh meeting, JECFA re-evaluated 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)¹⁷. JECFA also noted that “*the PTWI*

¹⁶ The 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 bw aluminium (JECFA 2007).

¹⁷ The Committee concluded the available studies have many limitations and were not adequate for defining the dose-response relationship for aluminium, therefore the evaluation was based on combined evidence from several dietary exposure studies in animals. The lowest LOELs for aluminium in a range of different dietary studies in mice, rats and dogs were in the region of 50-75 mg/kg bw/day expressed as aluminium. The Committee applied an uncertainty factor of 100 to the lower end of this range (50 mg/kg/d) to allow for inter- and intraspecies differences. Although the Committee noted there were deficiencies in the database, notably the absence of NOELs in the majority of studies evaluated and the absence of long term studies on relevant toxicological endpoints, they indicated these deficiencies are counterbalanced by the probable lower bioavailability of the less soluble aluminium species present in food. Overall they considered an additional uncertainty factor of 3 appropriate to cater for these uncertainties, establishing a PTWI of 1 mg/kg bw applicable to all aluminium compounds in food, including additives (JECFA 2007).

is likely to be exceeded to a large extent by some population groups, particularly children, who regularly consume foods that included aluminium-containing additives”.

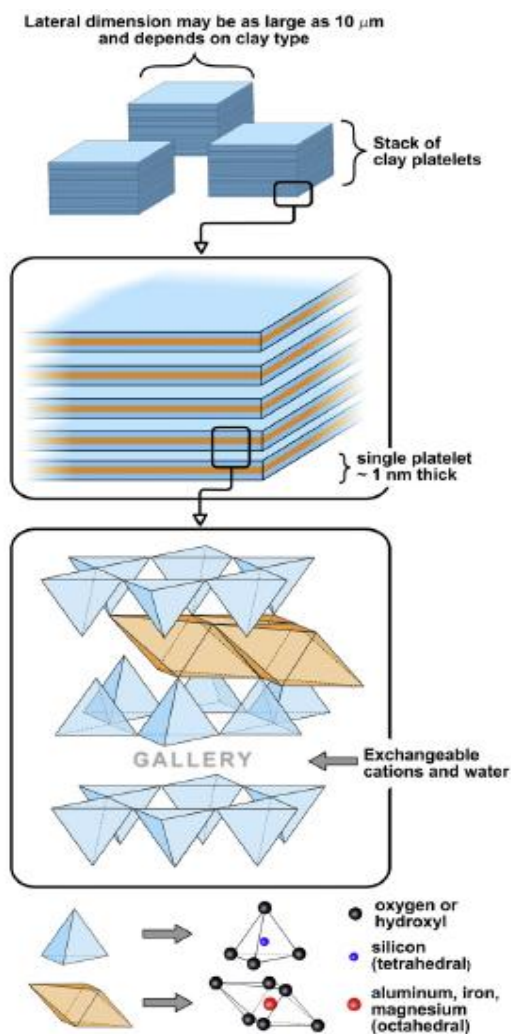


Figure 4.2.1: Structure of montmorillonite (phyllosilicate nano-clay).
Reproduced from Duncan 2011.

The natural nano-scaled layer structure of nano-clay restricts the permeation of gases, which has led to its use in food packaging for a variety of food and drinks, e.g. processed meats, cheese, confectionary, 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).

Thermoset or thermoplastic polymers used for making the nano-clay composites include polyamides, nylons, polyolefins, polystyrene, ethylene-vinylacetate, copolymer, epoxy resins, polyurethane, polyimides, and polyethyl-terephthalate (PET) (NanosafePACK 2012). Biodegradable nano-clay composites produced using other materials such as polylactic acid (PLA), polyhydroxyalkanoates (PHA), starch, cellulose, casein, and whey have also been the subject of recent research (Robinson and Morrison 2009, Peelman et al. 2013, Siracusa et al. 2008, Kuorwel et al. 2015, Smolander and Chaudhry 2010).

Several studies have investigated the migration of nano-clay constituents from nano-clay containing polymer nanocomposites into food. Each of the studies are described in the dot points below. An overall summary of the studies and conclusions are provided in Section 4.2.1.

- Avella et al. (2005) studied the migration of nano-clay from bags made of either potato starch, or a potato starch-polyester blend, and their respective composites with nano-clay into vegetable samples (lettuce and spinach) contained therein. The nanocomposite films were produced in house. Bags were heated at 40°C for 10 days, subsequently cooled, acclimatised (to 50% w/w humidity), and migration of Si, Fe and Mg determined by atomic absorption after hydrochloride acid digestion of the vegetables. The results indicated no consistent increase in the levels of Fe and Mg in the vegetables as a result of contact with the bags, but a slight increase in the amount of Si (the main component of nano-clay)¹⁸. While this study provides some information, it does not investigate the likely migration of the nanoparticles *per se* and does not investigate the potential enhanced migration of 'normal' chemical constituents of plastics (e.g. monomers, additives) brought about by the incorporation of nanomaterials into the plastic.
- Chaudhry et al. (2008) investigated the migration of nanoparticles from two nanocomposites used in food packaging, as well as control plastics (the same polymer type but not containing nanoparticles), into food simulants. The nanocomposites investigated were nano-clay-polyethylene terephthalate (PET) bottles (obtained from a commercial source in the UK) and nanosilver containing polypropylene (PP) containers (from a commercial firm in the USA). The results for nanosilver are provided in Section 4.3. The food simulants, exposure times and

¹⁸ Concentrations of Si in lettuce and spinach samples before contact with the bags were approximately 3 mg/kg, whereas after contact with potato starch film (no nano-clay) or potato starch film incorporating biodegradable polyester (no nano-clay) Si concentrations were 13 mg/kg. This concentration increased to 16-19 mg/kg after contact with the nano-clay containing composite film.

exposure temperatures were selected based on worst foreseeable conditions of use of the PP containers and PET bottles, in accordance with existing rules for migration testing¹⁹. After exposure to the relevant simulant, aliquots were analysed by liquid chromatography (LC)-UV and inductively coupled plasma- mass spectrometry (ICP-MS).

The authors found no detectable migration from the materials which had nano-clay sandwiched between PET layers²⁰, therefore they did not proceed with migration experiments in actual food for the PET bottles.

In both cases, the presence of nanomaterials did not affect the migration of non-nano components in the plastics. Indeed, other researchers have found the presence of nano-clay polymer nanocomposites slowed down the rate of migration of non-nano components from the polymer matrix into food simulants by up to six times²¹ (de Abreu et al. 2010). In the Chaudhry et al. (2008) experiments, since some migration was observed from the PP containers containing nanosilver (see Section 4.3), the study authors concluded migration of nanoparticles is likely to be dependent on the type and composition of the polymer.

The authors also made attempts to image nanoparticles in aqueous extracts prepared from the nano-clay PET bottles and nanosilver PP containers, as well as within the food contact matrices under wet conditions (Chaudhry et al. 2008). They used electron microscopy coupled with analysis by energy dispersive electron (EDX) analysis. The results did not show any

¹⁹ EU legislation (Directive 82/711/EC, as amended; Directive 85/572/EEC, as amended). Migration was tested in 3% acetic acid for 4 hours at 100°C.

²⁰ Antimony was found at virtually identical levels in both the nano-clay PET bottle and in the control PET samples and migration of antimony was also measured into acetic acid. Antimony migration was 33 µg/L from the nano-clay PET and 67µg/L from the control PET. These levels represent worst case as the polymer was exposed by total immersion, including cut edges. Taking into account the area of polymer exposed (2.5 dm²) and the volume of simulant used (100 mL) then these concentrations are equivalent to migration values of 1.3 and 2.7 µg/dm² for the nano-clay and control PET samples, respectively (Chaudhry et al. 2008). The presence of the nano-clay did not increase the extent of migration. In fact a lower migration level was observed. As a result of these findings, the authors did not consider migration testing into food (as opposed to food simulants) as necessary (Choudhry et al. 2008).

²¹ Polyamide films were manufactured in the laboratory containing 94.5% polyamide, 5% nano-clay, and 0.5% of a model migrant (triclosan or trans-1,4-diphenyl-1,3-butadiene, i.e. DPBD). Nanocomposite polyamide films were cut into pieces, and migration experiments carried out at various temperatures with diverse food simulants (3% acetic acid, water, isooctane, 10% ethanol, and 95% ethanol); concentrations of migrants were measured by HPLC and diffusion coefficients calculated for nano-clay containing polyamides and compared to non-nano containing controls. Migration was lower in nanocomposite polyamide film than in polyamide film, probably as a result of the barrier effect of nano-clay (de Abreu et al. 2010). The study did not investigate the migration of nanoparticles or clay minerals.

nanoparticles in the extracts, but this could have been due to limitations of the technology rather than the absence of nanoparticles (Chaudhry et al. 2008).

- Farhoodi et al. (2014) investigated the migration of aluminium and silicon (determined by ICP-OES) from PET/clay nanocomposite bottles (containing 3% wt nano-clay) manufactured in the laboratory into 3% acetic acid stored at 25 and 45°C for 90 days. Migration of both increased with storage time and temperature. Concentrations of aluminium after 90 days were 0.18 mg/kg at 25°C and 0.34 mg/kg at 45°C, whereas silicon concentrations were 6 and 9.5 mg/kg, respectively, with no aluminium or silicon detected in blank samples. No attempt was made to determine whether nanoparticles *per se* had migrated into the acidic simulant.
- In another study, polylactic acid (PLA) nanocomposites containing 4% modified nano-clay²² were prepared by extrusion and used in a standard migration experiment with distilled water as a food simulant (40°C for 10 days) (Maisanaba et al. 2014a). Controls consisted of PLA without nano-clay. At the conclusion of the migration experiments, simulant solutions were evaporated and weighed and compared with controls. In addition, simulant solutions were analysed for metal content (Al, Fe, Mg, Si, Ca) by ICP-MS or ICP-OES. Overall migration was found to be $0.1 \pm 0.2 \text{ mg/dm}^2$ (i.e. 0.6 mg/kg) in all samples, with no difference between controls and nano-clay containing composites. No statistical differences between elemental concentrations in simulant solutions were found, therefore migration of nano-clay in this experiment can be considered negligible. Cytotoxicity and mutagenicity tests conducted with simulant solutions at the conclusion of the migration experiments were negative. The migration extract from one of the nano-clay PLA composites was later used in a 90-day repeat oral toxicity study in Wistar rats, where distilled water or migration extract was provided to rats as their sole source of drinking water (Maisanaba et al. 2014b). No adverse effects on any of the parameters²³ investigated were observed.
- Mauricio-Iglesias et al. (2010) studied overall migration, and the migration of aluminium and silicon from wheat gluten/nano-clay films (5% nano-clay) into aqueous (water, 3% acetic acid,

²² Two modified nano-clays were produced with a cation-exchange method, which consists of displacement of sodium cations with the ammonium cations of quaternary ammonium salt hexadecyltrimethyl-ammonium bromide (HDTA) or a combination of HDTA and acetylcholine chloride (ACO).

²³ Parameters investigated included mortality, clinical signs, body weight, body weight gain, food and water/extract consumption, organ weights, somatic index of different organs, clinical chemistry; gross and histopathology of liver, kidneys, lung, spleen, brain, testes, gastrointestinal tract, and heart; IL-6 leakage in serum, oxidative stress biomarkers, or genetic expression.

15% ethanol), fatty (olive oil), and solid (Tenax²⁴ and agar gel) food simulants. Migration²⁵ was measured just after the high pressure, high temperature (HP/T) treatment²⁶ and after HP/T treatment plus 10 days storage at 40°C. Protein migration from wheat gluten was also assessed. The nanocomposites could not withstand the extreme HP/T conditions of sterilisation, only those mimicking pasteurisation. Aluminium was only found in the acidic simulant (1 mg/kg), whereas silicon was found in other simulants but concentrations were highest in 3% acetic acid (~3.2-4.5 mg/kg). The amount of silicon detected was higher after HP/T treatment, but HP/T treatment had no effect on overall or protein migration. As the ICP-OES analysis was outsourced to a contract laboratory, experimental conditions for the analysis (e.g. sample digestion parameters) were not provided. No attempt to determine presence of nanoparticles in simulant solutions was made.

- Schmidt et al. (2009) used a combination of AF4 with multi-angle light scattering detection (MALS) and ICP-MS analyses to determine migration of nano-clay particles from a biopolymer nanocomposite consisting of PLA and 5% Cloisite[®]30B (a derivatised montmorillonite clay) as a filler²⁷ into 95% ethanol. Migration experiments for the nanocomposite polymer and pure PLA films (no nano-clay) were undertaken at 40°C for 10 days. In addition, a food simulant blank and food simulant spiked with 2 mg Cloisite[®]30B were included. Food simulants were evaporated to dryness, resuspended in 20 ml food simulant and analysed by AF4-MALS-ICP-MS without any further sample preparation. Although particles ranging from 50-800 nm were detected in 95% ethanol food simulant, none of the characteristic clay minerals were detectable after acid digestion and analysis by ICP-MS, suggesting the particles detected were not of nano-clay origin. The authors could not determine the source of the particles.

²⁴ Tenax is modified poly(phenylene oxide). Tenax and agar gel were used to simulate contact with solid food.

²⁵ Overall migration in aqueous simulants was determined by drying the samples until a constant weight was reached. In olive oil, overall migration was determined as the difference in the weight of the film and further quantification of absorbed or stuck olive oil. In solid simulants, Tenax powder and agar gel were desorbed after 10 days storage with pentane, which was evaporated to dryness and dried residues compared with controls. Migration of Al and Si was measured by ICP-OES, either directly or after acid dissolution (for olive oil).

²⁶ High pressure/ high temperature treatment consisted of treatments simulating pasteurisation (800 MPa, 5 minutes, 20-40°C) and sterilisation (800 MPa, 5 minutes, 90-115°C).

²⁷ Nanocomposite PLA films were produced by drying PLA under vacuum at 40°C for 3 hours and the clay at 100°C for 3 hours. The nano-clay was added to PLA granules at a 5% w/w loading, mixed and produced by twin-screw extrusion.

4.2.1 Summary and Conclusions

Bentonite is a naturally occurring substance comprised of platelets whose thickness are in the nanoscale size range. Bentonite has a long history of permitted use as a food additive at levels up to 5% w/w in Europe and GMP in Australia; no evidence of adverse effects due to its use was found in the literature review conducted as part of this project. Although anecdotal evidence suggests it has been used as a food additive for decades (if not longer), definitive information for the extent and rate of its current or historical use as a food additive was not found.

No evidence was found in this literature review to indicate that nano-clay is likely to cause adverse effects on health when used in food packaging.

When compared to the vast number of migration studies found for nanosilver (see Section 4.3) and considering the probable extent of its use, there have been surprisingly few investigations on migration of nano-clay constituents into food simulants or foods. Only two studies investigated migration of nano-clay particles per se, and in both studies the presence of nano-clay in food simulants was not detected. In one study (Schmidt et al. 2009), although particles ranging from 50-800 nm were detected in 95% ethanol food simulant, none of the characteristic clay minerals were detectable after acid digestion and analysis by ICP-MS, suggesting the particles detected were not of nano-clay origin. The findings from the various migration experiments with nano-clay in food packaging are summarised in Table 4.2.1 below. In the table, the migration rates of elemental constituents have been converted to standardised units of mg/kg foodstuff in order to facilitate comparison²⁸.

Table 4.2.1: Summary of migration experiments conducted with nano-clay composites

Study	Packaging material			Food or Simulant	Migration (mg/kg food)	Were NPs detected?
	Type	Production method	Commercially sourced?			
Avella et al. 2005	Starch-polyester films	Casting ^a	No	Food (lettuce & spinach)	None (Fe & Mg) 3 - 6 (Si)	Not investigated
Chaudhry et al. 2008	PET bottles	-	Yes	Simulant (3% acetic acid, 10% ethanol, isooctane)	None (Sb & Al)	No

²⁸ Conversion from units of mg/dm² into mg/kg food assumed a contact area of 6 dm² per kg of food. This assumption is used by European authorities when assessing results from migration testing into foods or food simulants (EFSA 2004, 2005).

Study	Packaging material			Food or Simulant	Migration (mg/kg food)	Were NPs detected?
	Type	Production method	Commercially sourced?			
Farhoodi et al. 2014	PET bottles	Melting & blow moulding	No	Simulant (3% acetic acid)	0.18 – 0.34 (Al) 6 – 9.5 (Si)	Not investigated
Maisanaba et al. 2014a	PLA composites	Melting & extrusion	No	Simulant (distilled water)	None (overall migration by weight, Al, Fe, Mg, Si, Ca)	Not investigated
Mauricio-Iglesias et al. 2010	Wheat-gluten/nano-clay films	Mixing & heated pressing	No	Simulant (water, 3% acetic acid, 15% ethanol, olive oil, Tenax, agar gel)	0 - 1 (Al) 0 – 4.5 (Si)	Not investigated
Schmidt et al. 2009	PLA films	Mixing & extrusion	No	Simulant (95% ethanol)	None (Mg)	No ^b

- Information not provided; PET = polyethylene terephthalate.

^a This production method uses a mould, immersed in a tank of polymer and nanomaterial solution, so that the resulting nanocomposite is homogeneously distributed and incorporated into the polymer.

^b Although particles ranging from 50-800 nm were detected in 95% ethanol food simulant, none of the characteristic clay minerals were detectable after acid digestion and analysis by ICP-MS, suggesting the particles detected were not of nano-clay origin (Schmidt et al. 2009).

In some of the studies summarised in Table 4.2.1, migration of elemental components from nano-clay (particularly Si) into food and acidic food simulant has been detected from food packaging material, although overall migration in all cases (0-9.5 mg/kg food) was significantly lower than the 60 mg/kg of foodstuff overall migration limit for Europe (EC 2011, 2014, 2015). Migration of aluminium from nano-clay was minimal (0-1 mg/kg food), and lower than the concentrations typically found in foods (JECFA 2007)²⁹.

In both studies (Chaudhry et al. 2008, Schmidt et al. 2009) which have investigated the migration of nanoparticles per se, no evidence for nano-clay nanoparticles was found in food simulants. This indicates the potential for consumer exposure and subsequent public health or safety issues as a result of incorporation of nano-clay into polymer composites is likely to be low. This is supported by in vitro and in vivo (90-day) toxicity experiments conducted with nano-clay PLA composite migration simulant solutions after the migration study had been undertaken, which have not found any adverse effects. Safety evaluations for nano-clay in food packaging are therefore likely to be driven by migration of elemental constituents, rather than by the 'nano-ness' of the material. However, the

²⁹ JECFA (2007) indicated most foods contain aluminium at concentrations less than 5 mg/kg.

conclusion of negligible exposure is tempered by the relatively few studies which have investigated the migration of nanoparticles per se from nano-clay, and the uncertainties in current analytical techniques for measuring nanoparticles in foods/simulants (Section 4.1).

4.3 Nanosilver

In Australia and New Zealand, silver is permitted for use as a food additive in confectionary, spirits and liqueurs to GMP. It is also approved for use in food colouring³⁰ (E174) in the European Union, also to GMP (EC 1994). The Joint FAO/WHO Expert Committee on Food Additives evaluated silver in 1977, but did not set an acceptable daily intake (JECFA 1977). The US EPA derived an oral reference dose (RfD) (i.e. the dose that can be ingested daily for a lifetime without adverse effects) for silver in humans of 0.005mg/kg/d (i.e. 5 µg/kg/d) (US EPA IRIS 1996)³¹. This is similar to the daily dose which would be equivalent to the human NOAEL (10 g over a lifetime) for argyria from the WHO *Guidelines for Drinking Water Quality* (WHO 2011)³².

Colloidal silver (consisting of silver particles suspended in liquid) and formulations containing silver salts were used by physicians in the late 19th century, but their use was largely discontinued in the 1940s following the development of modern antibiotics (Fung et al. 1996). Since the 1990s, colloidal silver has been marketed as an alternative medicine, however its effectiveness for such uses has not been proven (Fung et al. 1996). After chronic medical or occupational exposure to silver, argyria (a permanent grey or blue grey discolouration of the skin and other organs) is the most common finding. As discussed in ToxConsult (2015a), this condition is considered medically benign but permanent.

Traditional animal toxicity studies, where silver salts were administered in drinking water or diet over a long period of time, have observed various effects including liver necrosis (in Vitamin E deficient animals), reduced growth (in copper and selenium deficient animals), cardiac enlargement (in copper and selenium deficient animals), decreased nucleic acid levels in the brain and liver, histopathologic

³⁰ Only allowed for external coating of confectionary, decoration of chocolates, and in liqueurs.

³¹ The RfD was based on the lowest dose (i.e. 1g of metallic Ag) resulting in argyria in one of 13 individuals following intravenous medical therapy over a 2 - 9.75 year period. This was converted to an oral dose of 25 g by accounting for oral absorption (i.e. 1 g ÷ 0.04 = 25 g), converting this to a lifetime dose [(25 g x 1000 mg/g) ÷ (70 kg body weight x 25,500 days) = 0.014 mg/kg/d] and applying an uncertainty factor of 3 for minimal effects in a subpopulation which has exhibited an increased propensity for the development of argyria (US EPA IRIS 1996). It is noted this RfD is old and was established well before many of the toxicological studies on nanosilver were available.

³² 10 g over a lifetime = 10,000 mg ÷ 70 years ÷ 365 days/year ÷ 70 kg body weight = 0.0056 mg/kg/d.

changes in liver and brain, and hypoactivity (JECFA 1977, Rungby and Danscher 1984, Jensen et al. 1974)³³. The doses at which these effects occurred were not provided, with the exception of a study in rabbits at which doses of 0.025-0.25 mg Ag/kg bw/d administered in drinking water (for 11 months) resulted in histopathological changes in the brain. These changes were not observed at lower doses (≤ 0.0023 mg/kg/d) (JECFA 1977). Although short or long term dietary investigations with nanosilver are not available, 28-90 day gavage dosing studies with nanosilver indicate No Observed Adverse Effect Levels (NOAELs) for traditional toxicological endpoints range from 0.5 - ~500 mg Ag/kg/d (ToxConsult 2016a). The effects are qualitatively similar to those observed with silver salts, and in some instances less severe.

Nanosilver is widely incorporated into a range of packaging and food contact materials³⁴, including polymer nanocomposites for 'active' packaging to improve the shelf-life of food or beverages through its antimicrobial properties³⁵. In contrast to nano-clay, which is primarily sandwiched between layers of polymer to improve barrier properties of food packaging and therefore is more unlikely to be released into food, silver from nanosilver must migrate to the food/packaging surface to some extent in order to be effective. Therefore a compromise must be made between the level of migration and antimicrobial activity (Hannon et al. 2015). Current understanding indicates the antimicrobial action of nanosilver is caused by the release of silver ions from the surface of particles (Bott et al. 2014b, Busolo et al. 2010, Cushen et al. 2013, de Azeredo 2013, Jokar and Rahman 2014, Wagner 2013). 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 Ag/kg food (EFSA 2004, 2005)³⁶.

³³ Adding Vitamin E or copper to the diets typically offset the silver-induced changes.

³⁴ Nanosilver is often incorporated into coatings on products including containers, mugs, dishes, cutlery, fridges, chopping boards, etc where the antimicrobial action occurs at the surface.

³⁵ 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* Typhmuriium, *Micrococcus luteus*, *Listeria monocytogenes*, and *Klebsiella pneumoniae* (Duncan 2011). Nanosilver is also effective against strains of these organisms that are resistance to potent chemical antimicrobials, as well as being toxic to fungi, and being inhibitory to at least two viruses (HIV and monkey pox) (Duncan 2011, Mohammed Fayaz et al. 2009, Fernandez et al. 2010, Hannon et al. 2015).

³⁶ 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*).

Several studies have investigated the migration of ionic silver (and silver nanoparticles) from nanosilver containing polymer nanocomposites into food simulants or food. Each of the studies has been described in subsequent dot points. A summary of the studies and conclusions are provided in Section 4.3.1.

- A description of the study conducted by Chaudhry et al. (2008) is provided in Section 4.2. The authors investigated the migration of nanoparticles from two nanocomposites used in food packaging, as well as control plastics (the same polymer type but not containing nanoparticles), into food simulants³⁷. The nanocomposites investigated were commercially sourced nano-clay-polyethylene terephthalate (PET) bottles and nanosilver containing polypropylene (PP) containers. The results for the nano-clay part of the experiment are provided in Section 4.2. After exposure to the simulant, aliquots were analysed by liquid chromatography (LC)-UV and inductively coupled plasma- mass spectrometry (ICP-MS).

The authors found no difference in the levels of silver detected in the acetic acid simulant exposed to the nanosilver PP compared to the control. However, since it has been reported that silver is not readily dissolved by organic acids (such as acetic acid) the authors indicate this result was not unexpected, and decided to proceed with additional migration testing into acidic foodstuffs for the PP containers (Choudhry et al. 2008). For these tests, worst case foreseeable foods (apple sauce, pizza, lasagne)³⁸ and contact conditions were selected³⁹ such that worst case migration could be ascertained.

Authors found very low level of migration of silver (less than the method limit of quantification⁴⁰) was detectable from the PP food containers containing nanosilver. The technique used to perform this analysis does not differentiate between nano- and non-nano trace elements⁴¹.

³⁷ To determine the migration of silver from the PP containers and metals associated with the nano-clay in PET bottles, the food simulant used was simulant 'B' (3% w/v aqueous acetic acid), as metal ions are known to migrate at the highest levels into acidic media. Simulant was exposed to the articles for 4 hours at 100°C.

³⁸ Apple sauce was expected to represent worst case, as it is an acidic food.

³⁹ The selected foodstuffs were apple sauce, pizza and lasagne both with a tomato sauce with reheating for 2.5 minutes followed by stirring/shaking and continued heating for a further 2.5 minutes. The food was allowed to cool, homogenised and analysed by ICP-MS.

⁴⁰ The limit of detection was 1 µg/kg silver. Silver was detected at levels less than the method limit of quantification (1 and 2 µg/kg silver) in two of the three apple sauce samples; no silver was detected in pizza; the third successive exposure to lasagne gave detectable levels of silver (1 µg/kg) but again this was less than the limit of quantification.

- The migration of silver from an in-house manufactured silver plasticised polyvinyl chloride (PVC) nanocomposite film⁴² into raw chicken breast under varying storage conditions was studied by Cushen et al. (2013). Chicken breasts were wrapped in the nanocomposite film or the negative control, vacuum packed and held for designated periods (1.1, 2, 3.1 or 4 days) at 5 or 20°C, after which packaging was removed and food extracts acid digested and analysed for silver content by ICP-MS. Test samples had higher levels of silver than control samples (mean 2.11 mg/kg vs. 0.053 mg/kg); migration of silver ranged from 0.01 – 0.46 mg/dm² (i.e. 0.15 – 1.4% of the starting nominal silver concentration in film). The quantity of silver found in the samples did not differ significantly with the size of the silver NPs used in the films, but increased with increasing starting concentration and storage time. In a similar experiment conducted with nanosilver containing polyethylene (PE) nanocomposites, migration of silver was 0.003 – 0.005 mg/dm² (Cushen et al. 2014a)⁴³. These studies are limited in that they did not investigate the likely migration of the NPs *per se* into food, or the state, appearance or size of the NPs in the PVC or PE nanocomposites.
- Researchers from the Fraunhofer Institute in Germany carried out migration studies using low density polyethylene (LDPE) films with different concentrations of incorporated nanosilver in contact with different EU-official aqueous and fatty food simulants (Bott et al. 2014b). The films were produced in the testing laboratory, and contained nominal silver concentrations of 0, 50, 150, and 250 mg/kg⁴⁴. The distribution and size of nanosilver in the polymer films with the lowest (50 mg/kg) and highest (250 mg/kg) concentrations were determined by transmission electron microscopy (TEM). This analysis showed the NPs were of different sizes (smallest about 10 nm) with most being spherical and 50 nm in size, and were distributed homogeneously⁴⁵ in the polymers; aggregates were also found approximately 100-270nm in

⁴¹ The food samples were acid-digested before ICP-MS analysis, therefore the test results for silver include any migration of solubilised silver plus any nano-particulate silver.

⁴² Films contained silver NPs with a diameter of 50 nm or 10 nm. However, the NP appearance and size in the PVC film was not ascertained by the authors of this study (Cushen et al. 2013).

⁴³ Assuming 6 dm² of contact per kg of food (as per assumptions used in Europe), this would equate to silver mass migrations of 0.06 – 2.76 mg/kg food from PVC film and 0.018 – 0.03 mg/kg food from PE.

⁴⁴ The measured concentrations of Ti after acid digestion were 0.1 ± 0.03, 48.7 ± 2.6, 185.2 ± 27.4, and 249.8 ± 5.7 mg/kg, respectively.

⁴⁵ Homogeneous distribution was also confirmed by the small variability between measured silver concentrations (by ICP-MS) in triplicate samples.

size. Asymmetric flow field-flow fractionation (AF4) was also used to characterise the stability of silver nanoparticles in a 3% acetic acid and ultrapure water solution. Silver content in LDPE films was determined by nitric acid digestion and analysis with inductively coupled plasma mass spectrometry (ICP-MS). The extent of migration of the NPs was also determined by measuring the silver content of the food simulants after drying and nitric acid digestion. Simulants used were 3% acetic acid, 10% ethanol, and 95% ethanol (by total immersion) at 60°C for 3, 6, 8, and 10 days, as well as isooctane at 40°C for 24 hours. Migration experiments were conducted according to European standards in triplicate (EN 13130-1 and EN 1186-3).

In the aqueous food simulants (10% ethanol and 3% acetic acid), Ag migration was measurable and dependent on the initial Ag concentration in the polymer⁴⁶. No detectable Ag migration was observed into the fatty food simulants (isooctane and 95% ethanol), although the method validation showed good recovery and the capability of low detection of silver in these simulants. In the AF4 experiments, injections of Ag nanoparticle dispersions (10 nm in diameter) which had been stored for 5 hours at room temperature in 3% acetic acid no longer produced a signal, indicating the Ag nanoparticles had likely completely dissolved in the migration experiments within this short time span (since silver was still measurable by ICP-MS even after 10 days at 60°C). The authors therefore deduced the detected Ag in the 3% acetic acid migration experiments was in ionic form, not in nanoparticulate form (Bott et al. 2014b). EFSA (2015) came to a similar conclusion in their assessment of zinc oxide nanoparticles in food packaging. They concluded the particles did not migrate in nanoform, therefore the safety evaluation was focused on soluble ionic zinc (Section 4.4.4). Bott et al. (2014b) indicated it seems impossible to measure migration of silver nanoparticles *per se* as an end parameter from nanosilver containing polyolefins.

- Artiaga et al. (2015), after conducting European standard migration experiments⁴⁷, used a combination of ICP-MS and AF4 techniques to determine the presence of silver nanoparticles in the standard food simulant solutions in which silver was detected. Scanning electron microscopy- Energy dispersive X-ray spectroscopy (SEM-EDX) was used to determine the

⁴⁶ Migration of total Ag after 10 days at 60°C was 2.4 µg/dm², 13.2 µg/dm², and 115.1 µg/dm² into 10% ethanol and 168.5 µg/dm², 444.8 µg/dm², and 1010.9 µg/dm² into 3% acetic acid for the 50, 150, and 250 mg/kg materials, respectively. Assuming 6 dm² of contact per kg of food (as per assumptions used in Europe), this would equate to silver mass migrations of 0.014 – 6 mg/kg food. No migration of Ag was found in controls.

⁴⁷ All four food simulants were used for the experiments conducted at 20°C for 10 days. These were distilled water, 3% acetic acid, 10% ethanol and 95% ethanol. Only the most efficient simulants (distilled water and 3% acetic acid) were used for the migration tests at 40°C for 10 days, and 70°C for 2 hours.

presence and morphology of nanosilver in the plastics studied before and after their exposure to food simulants, as well as in the extracts obtained after the migration assays. The tests were done with pieces of a commercial plastic bag of high density polyethylene (HDPE) manufactured in the USA (FresherLonger brand). SEM-EDX confirmed the presence of silver nanoparticles in the bags as aggregates (~1.59 μm) before and after the migration experiments. In the 20°C for 10 day exposures, detectable amounts of ionic silver were found in the distilled water and 3% acetic acid simulants. While ionic silver was found in distilled water only after 10 days of experimental exposure time, silver migration was observed into 3% acetic acid from the first day of bag exposure. Silver migration increased when the experiments were conducted at 40°C for 10 days. Under extreme conditions (70°C for 2 hours), similar concentrations of silver were obtained for acetic acid and distilled water (i.e. 17 ng/g, 0.06% of total silver content in bag) as in the 10 day experiment conducted at 40°C (Figure 4.3.1). This may indicate that the food simulant was not a significant factor for the extent of silver migration, i.e. after a sufficiently long exposure time, all releasable silver from the bag was effectively transferred to the food simulant solution.

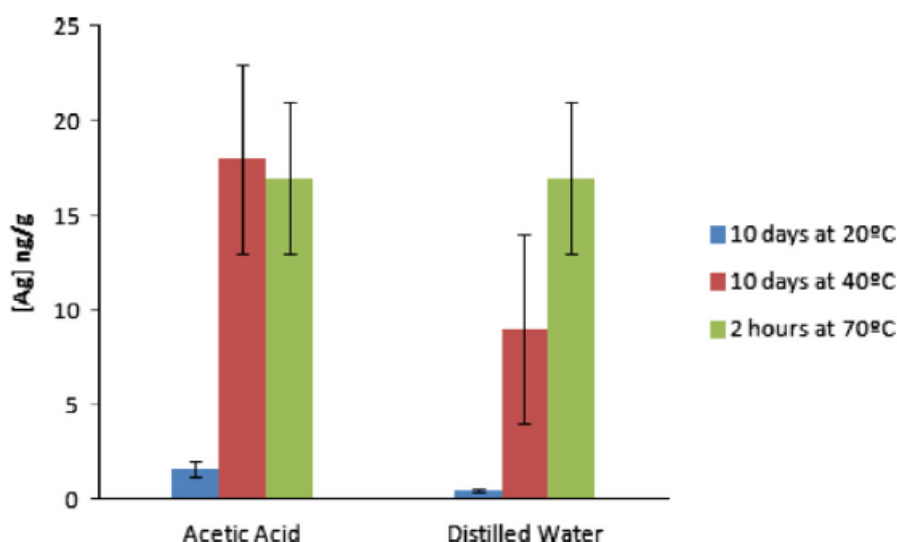


Figure 4.3.1: Mean concentration of Ag which migrated into 3% acetic acid and distilled water after extraction from HDPE bags containing AgNPs.

1 ng/g = 1 $\mu\text{g}/\text{kg}$

Source: Artiaga et al. 2015

In contrast to the findings of the Fraunhofer Institute researchers (Bott et al. 2014b), Artiaga et al. (2015) detected the presence of Ag nanoparticles (40 nm)⁴⁸ in the food simulant solutions (after treatment at 70°C for 2 hours) using AF4-ICP-MS. The authors estimated about 30% of the silver released from HDPE bags into the food simulants after 2 hours at 70°C was in nanoparticulate form.

- Cushen et al. (2014b) manufactured PE-based nanocomposites containing 0.1% or 0.5% of silver nanoparticles and other composites containing 0.5%, 1% or 2% of commercially available silver ion packaging material filler 'Agion™' (mean size: 3 µm). The filler also contains zinc, ammonium, zeolite A and potentially water. Standard immersion migration tests were carried out with these materials, as well as PE packaging films containing no fillers (negative controls), in distilled water and 3% acetic acid at 40°C for 10 days. Undigested food simulants were examined with transmission electron microscopy (TEM) and Hach Lange spectrometry (this technique only measures silver ions). Nitric acid digested simulants were analysed for total silver content by inductively coupled plasma atomic emission spectroscopy (ICP-AES). TEM imaging of food simulants which had been in contact with 0.5% w/w Agion™ in PE revealed particles with a mean diameter of 9.24 ± 2.51 nm (notably smaller than the Agion™ particles in solution⁴⁹). All the particles found were entrapped within what appeared to be organic matter. TEM imagery of food simulants which had undergone migration tests with PE containing silver NPs revealed particles of small diameter (14 – 66 nm). Silver levels in the simulants ranged from approximately 0.05 – 0.5 mg/L for the Ag nanoparticle PE composite, and 0.0003 – 0.05 mg/L for the Agion™ fill containing PE film. For the simulants in contact with Ag NP nanocomposites, the migrated silver levels were within the detection ranges of both quantification methods, and there was no significant difference between the two. This suggests that total silver and silver ion concentrations in the simulants were the same, indicating only silver ions migrated into food simulants, or silver particles that were present dissociated into ions readily in the simulants. This finding is similar to the conclusion by Bott et al. (2014b), i.e. any silver NPs which do migrate dissociate rapidly in food simulant to silver ions.

⁴⁸ For both simulants (distilled water and 3% acetic acid) analysed using AF4-ICP-MS, a peak was observed between 11 and 30 minutes (peak maximum at 20 min), which was assigned to silver nanoparticles of 40-60 nm in size with the help of an internal standard silver nanoparticle solution. When the distilled water extract was sonicated for 1.5 minutes with an ultrasonic probe prior to analysis (instead of 5 mins in an ultrasonic bath), the peak maximum shifted to 15 minutes, which approached the retention time of silver nanoparticles 40 nm in size. This suggests silver NPs are likely to aggregate in food simulants.

⁴⁹ Agion™ particles in solution had a mean length of 1507 ± 581 nm, and were a mixture of cubic and slightly cuboid shapes with rounded corners.

- The migration of silver and silver NPs from three commercially obtained nanosilver-containing plastic food containers⁵⁰ was investigated by Echevoyen and Nerin (2013). Two simulants (50% ethanol and 3% acetic acid) at two test conditions (40°C for 10 days and 70°C for 2 hours) were used for the migration experiments, and food containers were filled with an amount of simulant enough to cover the bottom surface. After sonication for 5 minutes, migration solutions were analysed for silver content by ICP-MS. Solutions were also drop cast onto silicon chips for analysis of nanoparticulate content by SEM-EDX. Silver migration was observed for all samples studied at 1.66 – 31.5 ng/cm², and values were highest in the acetic acid simulant. This is approximately equivalent to 0.001 – 0.019 mg/kg food⁵¹. Migration values were higher after heating for 2 minutes in a microwave oven. The extent of migration of silver was well below the European permissible SML of 0.05 mg/kg for silver. Analysis of all migration solutions by SEM-EDX showed the presence of silver NPs⁵², as well as other NPs (most likely sodium chloride and polymer particles).
- Fortunati et al. (2013) conducted standard migration tests (by total immersion) with pristine and surfactant modified cellulose polylactic acid (PLA)-based nano-biocomposites containing 1% silver NPs⁵³. Food simulants used were 10% ethanol (40°C for 10 days) and isooctane (20°C for 2 days); at the conclusion of the experiments, simulants were analysed for silver content⁵⁴ by ICP-MS. Silver migration ranged from 0.00079– 0.023 mg/kg in isooctane and 0.061 – 0.087 mg/kg in 10% ethanol for the surfactant modified cellulose nanocrystals. Silver migration from unmodified cellulose nanocrystals was considerably lower (0.00028 – 0.0012 mg/kg in isooctane; 0.0018 – 0.004 mg/kg in 10% ethanol), and well below the European SML of 0.05 mg/kg. The migration of nanoparticles themselves was not investigated.

⁵⁰ The containers, obtained in the USA, were: Kinetic Go Green Basic Nanosilver Food Storage container (0.32% silver), Oso Fresh Food Storage container (0.33% silver), and FresherLonger™ Plastic Storage bags (0.33% silver). The containers are made of polyolefins: the bags of LDPE and the two other containers of PP.

⁵¹ $1.66 - 31.5 \text{ ng/cm}^2 \times 100 \text{ cm}^2/\text{dm}^2 \times 10^{-6} \text{ mg/ng} = 0.000166 - 0.00315 \text{ mg/dm}^2$. If, as in Europe, it is assumed 6 dm² of product is potentially in contact with 1 kg of food, this equates to 0.001 – 0.019 mg/kg food.

⁵² For the Kinetic Go Green sample, individual particle sizes were 10-20 nm and aggregates 50-200 nm. In the Oso Fresh sample, NPs were 40-60 nm.

⁵³ Silver NPs ranged in primary size of 20 – 80 nm. TEM images of the nano-biocomposites showed some isolated particles as well as agglomerates.

⁵⁴ Before the analysis, isooctane extracts were evaporated until dryness and re-suspended in HNO₃ 1%. Ethanol extracts were analysed with no further treatment.

- The potential migration of silver and silver nanoparticles into standard food simulants⁵⁵ from commercially available nanosilver-containing polyethylene fresh food bags was studied by Huang et al. (2011). A single-surface exposure regime was used, where bags were filled with simulating solution and sealed for storage at 40 or 50°C for 3, 6, 9, 12, and 15 days. At the respective time frames, simulant solutions were dried, redissolved in water and nitric acid by ultrasonicator, then diluted and analysed for silver content by AAS. The researchers used SEM-EDX to determine the presence of nanoparticles in the bag and simulant solutions after an up- and down- concentration method⁵⁶. The analysis found the presence of individual spherical particles larger than the accepted definition of a nanomaterial (100-300 nm in size) in the bag, and 300 nm particles in the water simulant after storage for 15 days. It is unknown whether the preparation procedure for the food simulant solution prior to analysis could have had an impact on the results, especially since a negative control (i.e. bags not containing nanosilver) was not analysed. Huang et al. (2011) found that the amount of migrated silver increased with time and temperature, and in contrast to findings from other authors, there was virtually no difference between the extents of migration into different simulant solutions. After 15 days, silver migration rates ranged from 0.75 – 4 µg/dm², this is approximately equivalent to 0.0045 – 0.024 mg/kg food⁵⁷.
- One of the few studies that compared the migration of silver from nanosilver coatings on LDPE films⁵⁸ with melt blended nanocomposites (0.1, 0.5, or 5% nanosilver) was by Jokar and Rahman (2014). Total silver content in each dried nanocomposite was determined by AAS, and X-ray diffraction (XRD) was used to characterise the size of particles in the composites. Standard migration tests were carried out by immersion in food simulants (water, 3% acetic acid, 10% ethanol) and apple juice. Immersion liquid was changed after 1, 3, 5, 7, 10, 15, 20, 25 and 30 days' storage at 4 and 40°C, and silver ions measured by AAS. Migration of

⁵⁵ Simulants used were standard simulants according to Chinese standard GB/T 5009.60-2003. They were ultrapure water, 4% acetic acid, 95% ethanol and hexane. The 95% ethanol represents a worst case scenario.

⁵⁶ The bag was turned to ash at 600°C, and simulant solutions were concentrated. The respective ash and concentrated solutions were dispersed in alcohol solution and evaporated in droplets onto aluminium foil prior to analysis by EDX-SEM.

⁵⁷ Using the European assumption that 6 dm² of food packaging is in contact with 1 kg of food.

⁵⁸ Coatings were produced by the layer-by-layer deposition method, in which LDPE films were ultrasonically washed with acetone, functionalised in dilute aqueous solution of polyethylenimine for 10 minutes, and sequentially dipped into anionic silver colloid dispersions containing PEG-capped silver NPs or cationic chitosan for 10 minutes. Films were rinsed with deionised water and dried in a nitrogen stream after each immersion.

nanoparticles *per se* was not investigated. Measured silver concentrations in the nanocomposites were 0.11, 0.67, and 2.3% (melt blended) and 0.04, 0.47, and 1.6% (coated).

Silver particles were 18-32 nm and 22-30 nm in size in melt-blended and coated composites, respectively. As expected, silver migration into 3% acetic acid and apple juice were significantly higher than distilled water and 10% ethanol. Coated nanocomposites had a higher silver release after 30 days storage than melt-blended composites. As seen in other studies, starting silver concentration and increasing temperature were significant determinants of extent of silver ion migration. In worst case conditions (40°C), silver migration in melt-blended composites after 15 days was 0.4 – 0.9 mg/kg (distilled water), 0.5 – 1 mg/kg (3% acetic acid), 0.4 – 0.9 mg/kg (10% ethanol), and 0.6 – 0.9 mg/kg (apple juice). In coated nanocomposites, this was 0.6 – 1.1 mg/kg (distilled water), 1.1 – 1.4 mg/kg (3% acetic acid), 0.6 – 1.3 mg/kg (10% ethanol), and 0.7 – 1.1 mg/kg (apple juice).

- Another study also evaluated migration of silver ions from nanosilver coatings, this time on PE films⁵⁹ immersed in distilled water, acidified malt extract broth and apple juice at 44°C for 5 days (Nobile et al. 2004). Tests were run in both shaken and quiescent solutions. TEM imaging revealed Ag NPs deposited on the films about 90 nm in size. Silver ions were measured at the conclusion of the tests by nitric acid digestion and analysis by ICP-AES. Silver content in the solutions unexpectedly were highest in the distilled water simulant (1-1.9 mg/kg), with lower concentrations in the malt extract broth and apple juice (0.2-0.38 mg/kg). Concentrations were higher in the shaken solutions. The study did not investigate the presence of NPs *per se* in the food simulant/food solutions.
- Migration of silver from commercial nanosilver/nano-TiO₂ containers⁶⁰ into a wide range of food samples was investigated by Metak and Ajaal (2013). Food samples selected included fresh apples, white slice bread, fresh carrot, pre-packed soft cheese, modified atmosphere packaging milk powder and fresh orange juice. Samples were stored in nanosilver/nano-TiO₂ or control containers at 40°C for 7 or 10 days. After the exposure period, food samples were

⁵⁹ The authors produced the coating in polyethylene oxide on the surface of PE film by plasma-based vapour deposition (Nobile et al. 2004).

⁶⁰ Fresh Box containers sourced from Blue Moon Goods, USA.

processed⁶¹ and analysed for silver and titanium content by ICP-MS. SEM-EDX and X-ray diffraction analysis of the containers confirmed the presence of silver and TiO₂ nanoparticles 20-70 nm in size, with some aggregated (100 nm), incorporated into the polymer (but not coated onto the surface). Overall, insignificant levels of Ag and Ti were measured in the food samples after 7 and 10 days exposure to the containers. The highest level of silver was measured in orange juice ($5.7 \pm 0.02 \mu\text{g/L}$ vs. $0.16 \pm 0.01 \mu\text{g/L}$ in controls), with others ranging from approximately 2 – 5.2 $\mu\text{g/L}$. The concentration in orange juice is approximately equivalent to 0.0057 mg/kg food. The highest level of Ti was also found in orange juice ($2.5 \pm 0.03 \mu\text{g/L}$). No attempt was made to detect actual nanoparticles in foods.

- Panea et al. (2014) determined the migration of silver and zinc by ICP-MS from a mixed nanosilver/ZnO (5% and 10% w/w) LDPE blend into an aqueous food simulant after storage at 40°C for 10 days. Very limited experimental detail was provided, including which simulant was used, sample preparation method for analysis, and no information was provided on the physical characteristics of the nanoparticles once in the LDPE matrix. Silver migration was always below the detection limit (<0.001 mg/kg), and zinc was detected at $2.44 \pm 0.37 \text{ mg/kg}$.
- Song et al. (2011) investigated migration of silver from a commercial nanosilver-PE food packaging film⁶² into food simulants (3% acetic acid, 95% ethanol) stored at 20, 40, and 70°C for 1-9 hours. Migration solutions were evaporated to dryness, nitric acid digested and analysed for silver content by ICP-MS. The extent of migration was higher in the acetic acid simulant, increased with time (until reaching steady state at 6 hours), and increased with temperature (for the acetic acid simulant only). The authors expressed the extent of silver migration as a percentage of the original silver concentration in the film. Maximum migration ranged from 1.7-5.6% (i.e. 3.9 – 13 mg/kg) in acetic acid, and 0.22-0.24% (0.5 – 0.6 mg/kg) in ethanol, depending on the storage temperature. No attempt was made to determine whether nanoparticles *per se* migrated, and characterisation of the commercial film itself was limited.
- von Goetz et al. (2013) also investigated silver migration into food simulants (water, 3% acetic acid, 10% ethanol, olive oil) from two commercial nanosilver-containing food storage

⁶¹ Food samples were weighed, heated to dryness at 105°C for several hours, cooled for 40 minutes, carbonised using a Bunsen burner, before heating again to 550°C for several hours until ash was formed. Ash was dissolved in nitric acid solution, heated slowly and filtered prior to analysis by ICP-MS.

⁶² The film was purchased from Anson Nanotechnology Co, Ltd (China). It contained nanosilver of approximately 7 nm in diameter (according to the manufacturer). The initial concentration of Ag in the film was $234 \pm 4 \text{ mg/kg}$ (by ICP-MS).

containers⁶³. Migration experiments were carried out in the dark at 20°C for various storage times ranging from 1 hour to 10 days. After incubation, simulant solutions were sonicated and analysed by solution nebulisation ICP-MS (SN-ICP-MS) after adding yttrium (internal standard) and diluting with 2% nitric acid without further treatment. In order to distinguish between migrating ionic silver and nanoparticulate silver, samples with higher silver concentrations were generated with distilled water and analysed by SEM or TEM with energy dispersive X-ray spectroscopy and electron diffraction (ED)⁶⁴. An additional experiment was conducted to assess migration under multiple-use conditions in the food simulant showing the highest migration rates (3% acetic acid).

From this combination of techniques, the researchers observed silver migration (into simulants, to varying degrees) and concluded that most of the silver migration could be accounted for by silver ion migration. Nevertheless, they did report the presence of silver NPs in some of the migration solutions. The authors acknowledged that they were unable to determine whether the silver NPs migrated via the diffusion mechanism or whether they were released from the surface or formed post-dissolution during sample handling. The latter process may be indicated because the detected silver NPs in the simulant residuals were composed of AgCl and AgS, in agglomerated form. The authors estimated 12% of the mass of silver released from the food packaging container was in nanoparticulate form⁶⁵. The highest migration of silver was observed for the containers in acetic acid (9.5 ng/cm²), approximately equivalent to

⁶³ Two commercial plastic food containers labelled as containing “nano” or “micro” silver were investigated. These were Kinetic Go Green Nano Silver Basic PP containers and FresherLonger™ PE bags, both sourced in the USA. Silver content was measured by solution nebulisation ICP-MS (SN-ICP-MS) to be 9.7-11.9 mg/kg (for Kinetic Go Green) and 37.1 mg/kg for FresherLonger™.

⁶⁴ This method produced a constant peak height for the signal of ionic Ag in solution, and peak heights significantly above the ionic Ag peaks for Ag in particulate form.

⁶⁵ TEM/EDXS and TEM/ED detected the presence of metallic nanoparticulate silver in concentrated migration solutions (prepared using a larger polymer/food simulant ratio). Nanoparticle agglomerates with primary particle sizes of 20-100 nm were found by SEM. The analysis by SP-ICP-MS with microdroplet dispenser also showed silver in nanoparticulate form and was used to determine the ratio between ionic and particulate silver. In this analysis, constant signals of approximately 500 counts per second (cps) and peaks above 2000 cps were found. The constant signals at 500 cps were generated from single droplets at intensities that correspond to silver ions in solution, whereas the peak signals represent nanoparticles or nanoparticle agglomerates in solution. Based on the number of peaks for each silver form and the atomic mass of silver, the authors estimated that 12% of the mass of silver that is released from the food container is in nanoparticulate form. By comparison with a calibration point, the detected events were assigned to particles of 100-350 nm in size. However, the authors noted agglomerates such as those detected with SEM and TEM can cause peaks >2000 cps, so that the primary particle sizes may be smaller.

0.0057 mg/kg⁶⁶. For water and 10% ethanol, half as much migration was observed, whereas in olive oil none was found. Negligible migration (<0.5 ng/cm², i.e. <0.0003 mg/kg) was detected from plastic bags. Under conditions of repeated use, silver migration into food simulants decreased dramatically (up to one order of magnitude) after the first use. The authors compared the likely consumer exposure to silver from the assessed food packaging materials with background exposures, and concluded exposure from the packaging is negligible.

4.3.1 Summary and Conclusions

Silver is permitted for use as a food additive in Australia, or as food colouring in Europe, in confectionary, spirits and liqueurs to GMP. The US EPA derived an oral reference dose (RfD) for silver in humans of 0.005mg/kg/d (i.e. 5 µg/kg/d), which is similar to the human NOAEL for argyria derived by the World Health Organization.

Colloidal silver and formulations containing silver salts have been used historically for medical applications, but these uses have been largely discontinued. Since the 1990s, colloidal silver has been marketed as an alternative medicine, however its effectiveness has not been proven. After chronic medical or occupational over-exposure to silver, argyria (a permanent grey or blue grey discolouration of the skin and other organs) is the most common finding.

The antimicrobial function of nanosilver in food packaging materials requires silver ions be released to deter food spoilage. Thus a balance between what is considered too little to be effective and too much from a safety perspective needs to be achieved. A large number of migration studies were found for nanosilver containing polymer composites or coatings. The results are summarised in Table 4.3.1. In the table, the migration rates of elemental constituents have been converted to standardised units of mg/kg foodstuff in order to facilitate comparison⁶⁷.

⁶⁶ $9.5 \text{ ng/cm}^2 \times 100 \text{ cm}^2/\text{dm}^2 \times 10^{-6} \text{ mg/ng} = 0.00095 \text{ mg/dm}^2$. If, as in Europe, it is assumed 6 dm² of product is potentially in contact with 1 kg of food, this equates to 0.0057 mg/kg food.

⁶⁷ Conversion from units of mg/dm² into mg/kg food assumed a contact area of 6 dm² per kg of food. This assumption is used by European authorities when assessing results from migration testing into foods or food simulants (EFSA 2004, 2005).

Table 4.3.1: Summary of migration experiments conducted with nanosilver containing food packaging material

Study	Packaging material			Food or Simulant	Migration ^a (mg/kg food)	Were NPs detected?
	Type	Production method	Commercially sourced?			
Cushen et al. 2013	PVC films	Casting	No	Food (raw chicken breast)	0.06 – 2.76	Not investigated
Cushen et al. 2014a	PE composite	Mixing & extrusion ^b	No	Food (raw chicken breast)	0.018 – 0.03	Not investigated
Metak and Ajaal 2013	Nanosilver/nano-TiO ₂ polymer composite	-	Yes	Food (various)	0.002 – 0.0057	Not investigated
Chaudhry et al. 2008	PP container	-	Yes	Simulant (3% acetic acid) Food (apple sauce, pizza, lasagne)	None (Ag, Sn, Ba) <0.002 (Ag)	No
Jokar and Rahman 2014	LDPE films	Melt blended or Layer-by-layer deposition (i.e. coating)	No	Simulant (distilled water, 3% acetic acid, 10% ethanol) Food (apple juice)	Melt-blended: 0.4 – 1 Coated: 0.6 – 1.4	Not investigated
Nobile et al. 2004	PE films	Coating	No	Simulant (distilled water) Food (malt broth extract, apple juice)	1 – 1.9 (distilled water) 0.26 – 0.38 (malt broth extract) 0.2 – 0.25 (apple juice)	Not investigated
Bott et al. 2014b	LDPE films	Mixing & extrusion ^b	No	Simulant (3% acetic acid, 10% & 95% ethanol)	0.014 – 6 (aqueous simulants) None (fatty simulant)	No ^c
Artiaga et al. 2015	HDPE bag	-	Yes	Simulant (distilled water, 3% acetic acid, 10% & 95% ethanol)	0.017	Yes ^d (40 nm aggregates)

Study	Packaging material			Food or Simulant	Migration ^a (mg/kg food)	Were NPs detected?
	Type	Production method	Commercially sourced?			
Cushen et al. 2014b	PE films (made with AgNPs or Agion™ Ag ion filler)	Mixing & extrusion ^b	No	Simulant (distilled water, 3% acetic acid)	0.05 – 0.5 (Ag NPs) 0.0003 – 0.05 (Agion™)	Yes ^e
Echegoyen and Nerin 2013	LDPE bag 2 x PP containers	-	Yes	Simulant (3% acetic acid, 50% ethanol)	0.001 – 0.019	Yes
Fortunati et al. 2013	PLA composites (unmodified or surfactant-modified)	Casting	No	Simulant (10% ethanol, isooctane)	Unmodified: 0.0018 – 0.004 (ethanol) 0.00028 – 0.0012 (isooctane) Modified: 0.061 – 0.087 (ethanol) 0.00079 – 0.023 (isooctane)	Not investigated
Huang et al. 2011	PE bags	-	Yes	Simulant (pure water, 4% acetic acid, 95% ethanol, hexane)	0.0045 – 0.024	No ^f (300 nm in size)
Panea et al. 2014	LDPE film (Ag/ZnO NP)	Melting & injection moulding	No	Simulant (aqueous, no further details provided)	<0.001 (Ag) 2.44 (Zn)	Not investigated
Song et al. 2011	PE film	-	Yes	Simulant (3% acetic acid, 95% ethanol)	3.9 – 13 (acetic acid) 0.5 – 0.6 (ethanol)	Not investigated
von Goetz et al. 2013	PP containers, PE bags	-	Yes	Simulant (water, 3% acetic acid, 10% ethanol, olive oil)	PP containers: 0.0057 (acetic acid) ~0.0029 (water & ethanol) None (olive oil) PE bags: <0.0003	Yes (~12% of migrated Ag) ^g

- Information not provided; PVC = polyvinyl chloride; PP = polypropylene; PE = polyethylene; LDPE = Low Density Polyethylene; HDPE = High Density Polyethylene; PLA = polylactic acid.

^a Migration of silver unless otherwise specified.

- ^b This process is one which incorporates the nanomaterial into the polymer mix, and involves melting the polymer nanocomposite mix and forming it into a continuous profile.
- ^c Although the presence of NPs was not tested in the food simulant experiments, the authors tested the presence of NPs using AF4 methods and injections of Ag nanoparticle dispersions (10 nm in diameter) which had been stored for 5 hours at room temperature in 3% acetic acid. These dispersions no longer produced a signal after the 5 hour storage time, indicating the Ag nanoparticles had likely completely dissolved in the migration experiments within this short time span (since silver was still measurable by ICP-MS even after 10 days at 60°C). The authors therefore deduced the detected Ag in the 3% acetic acid migration experiments was in ionic form, not in nanoparticulate form.
- ^d The authors estimated about 30% of the silver released from HDPE bags into the food simulants after 2 hours at 70°C was in nanoparticulate form (Artiaga et al. 2015).
- ^e NPs were detected by TEM in food simulant solutions from both PE films. This indicates the larger particles in Agion™ fill likely dissociated into smaller particles in solution. Interestingly, there was no significant difference between the migrated silver levels of both quantification methods, one of which only measures silver ions (Hach Lange) and the other which measures silver ions and silver nanoparticles after acid digestion (ICP-AES). This suggests that total silver and silver ion concentrations in the simulants at the conclusion of migration testing were the same, indicating only silver ions migrated into food simulants, or silver particles that were present dissociated into ions readily in the simulants.
- ^f The commercially-obtained PE bags contained individual spherical particles larger than the accepted definition of a nanomaterial (100-300 nm in size); particles were found in all simulants after migration experiments were completed. In the water simulant, particles were 300 nm in size. It is unknown if the complicated up- and down-concentration procedure used to prepare the samples for SEM-EDX analysis could have affected the outcome.
- ^g The authors acknowledged that they were unable to determine whether the silver NPs migrated via the diffusion mechanism or whether they were released from the surface or formed post-dissolution during sample handling. The latter process may be indicated because the detected silver NPs in the simulant residuals were composed of AgCl and AgS, in agglomerated form. The authors estimated 12% of the mass of silver released from the food packaging container was in nanoparticulate form.

Overall the results from migration studies for nanosilver suggest the production method of nanocomposites (e.g. incorporation or coating, surfactant modification), the starting silver concentration, temperature, time and choice of contact media are all factors which may have an effect on the extent of silver ion migration from the nano-silver into food simulants. In general, the rate of silver ion migration increases when nanosilver is coated onto the food packaging material or surfactants are added, when the storage temperature and length of storage increases, and the acidity of the contact medium increases. There appears to be a specific time of storage, after which a steady state release of silver is achieved.

The limitation in detectability of current measurement techniques, together with the lack of information provided on sample processing and handling prior to analysis makes it difficult to draw any concrete conclusions on whether silver ions or silver nanoparticles per se migrate into food simulants⁶⁸.

Until such a time analytical techniques are more refined and more information is available, safety assessment of nanosilver-containing food packaging materials will be limited to conventional considerations of ionic silver release into foods. This is not unreasonable since the available weight of

⁶⁸ It is uncertain whether the detected particles are actually 'real', whether they are the result of migration of nanosilver, or whether they could have formed after ionic silver migrated into food simulants. It is also sometimes uncertain whether these NPs represent silver NPs at all.

evidence indicates the hazards associated with nano-silver are not nano-related but rather with the silver ion.

There is some evidence to suggest that if silver nanoparticles do migrate into food/food simulants, they would most likely dissolve quickly into ionic silver. Furthermore, the toxicological effects of nanosilver observed in 28-90 day gavage studies with laboratory animals are qualitatively similar to those observed in dietary studies with silver salts, and in some instances less severe (ToxConsult 2016a).

The majority of the migration studies found for nanosilver food packaging composites have shown levels of migration of ionic silver into foods and food simulants below the European SML of 0.05mg Ag/kg food, suggesting low risk of consumer exposure. However there are also several studies, in which migration exceeded this limit. This indicates that for new food packaging products containing nanosilver migration experiments should be conducted on a case-by-case basis.

4.4 Other nanomaterials

The European Commission has published a Union list of authorised substances for use in manufacturing polymer food contact materials (EC 2011). Since the original list was published, several updates have been released. The nanomaterials included in the list and therefore permitted for use in food packaging are (EC 2011, 2015):

- Titanium nitride nanoparticles are authorised for use as an additive or polymer production aid, specifically to be used in PET plastics 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 100-500nm 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 (Section 4.4.1). 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 2008, 2012).
- Carbon black with primary particles of 10-300 nm, which are aggregated to 100-1,200 nm in size, as long as the maximum level used in the polymer does not exceed 2.5% w/w. A specific migration limit is not set.
- Butadiene, ethyl acrylate, methyl acrylate copolymer cross-linked with divinylbenzene, in nanoform is authorised for use as an additive or polymer production aid, specifically in non-plasticised PVC up to 10% w/w in contact with all food types at room temperature or below

including long-term storage. When used together with the same copolymer in nanoform (which is not cross-linked), the 10% restriction applies to the sum of the substances. The diameter of particles shall be >20 nm, and for at least 95% by number it shall be >40 nm.

- Silanated⁶⁹ silicon dioxide (SiO₂). Although this entry is not for nanoparticles *per se*, EFSA (2014) was recently informed that the substance had always been produced using synthetic amorphous silica in nanoform.

Migration experiments have been conducted with these and other nanoparticles. Some of the studies found are summarised in subsequent sections.

4.4.1 Titanium nitride (TiN)

Titanium nitride (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 (3,000 °C) but at temperatures >500°C it may form titanium oxides in air. TiN is used in coatings for wear protection of tools or other objects and to improve thermal properties of plastics (DaNa2.0 undated).

The work on TiN nanoparticles was in part led by researchers from the Fraunhofer Institute in Germany (Bott et al. 2012, 2014a). TiN is used as a reheat additive in the production of PET bottles for its thermal stability. The researchers produced low density polyethylene (LDPE) films with three different concentrations of TiN in the polymer, as well as LDPE blanks without TiN, in-house. The nominal concentrations of TiN were 0, 100, 500 and 1000 mg/kg (i.e. nominal Ti 0, 77, 387, 774 mg/kg)⁷⁰. The distribution and size characteristics of the TiN nanoparticles in the polymer were visualised using transmission electron microscopy (TEM). This analysis showed the NPs with an average size of 20nm were distributed homogeneously in the polymers, but existed as aggregates approximately 100-500nm in size. Titanium content in LDPE films was determined by nitric acid digestion and analysis with inductively coupled plasma mass spectrometry (ICP-MS). The extent of migration of the NPs was also determined by measuring the titanium content of the food simulants after drying and nitric acid digestion. Simulants used were 3% acetic acid, 95% ethanol, and 0.2%

⁶⁹ Silanisation of silica refers to reacting silicon dioxide with methoxy or ethoxy molecules to form alkoxy silane molecules. When these are applied to surfaces it increases the hydrophobicity and generally reduces the adsorption of other molecules to the surface. Alkoxy silanes are also used to silanise glassware to reduce adherence of cells to the surface.

⁷⁰ The measured concentrations of Ti after acid digestion were 0, 80, 350, and 490 mg/kg, respectively.

surfactant solution⁷¹ (by total immersion) at 60°C for 10 days, as well as isooctane at 40°C for 24 hours. Migration experiments were conducted according to European standards in triplicate (EN 13130-1 and EN 1186-3). No measurable migration of Ti could be detected in any of the food simulants. The detection limits translate into food related migration values of 0.09-0.11 µg/kg food for 95% ethanol and isooctane and 0.24 µg/kg for 3% acetic acid (Bott et al. 2014a). The authors concluded migration of nano-TiN appears not to take place.

Since the conclusion is limited by the underlying analytical sensitivity, the authors developed a particle volume (size) related migration model based on an already existing model system for migration of conventional plastic additives and proposed this model to be applicable for predicting the migration of nanoscale materials in general (Bott et al. 2014a). Using carbon based spherical nanoparticles (with very low quasi-molecular weight) as a model particle (which would represent worst case), their results suggested:

- Only the smallest spherical NPs up to 3.5 nm in diameter may cause measurable migration, if present at high concentrations in a polymer.
- NPs in the 3-5 nm range (again only if present at high amounts in the polymer) could potentially migrate but at rates that would not be measurable even with highly sensitive methods.
- Larger particles have an exponential decrease in mobility in the polymer and therefore no potential to migrate out of the polymer.

This led the authors to the overall conclusion that consumer exposure to NPs via migration from food contact plastics into foods cannot be expected.

Based on these data, the European Food Safety Authority concluded the nanoparticulate substance would not give rise to consumer exposure via food and therefore was not of toxicological concern (EFSA 2008, 2012).

4.4.2 Carbon black

Carbon black consists of elemental carbon in the form of near spherical colloidal particles and coalesced particle aggregates/agglomerates, obtained by partial combustion or thermal decomposition of hydrocarbons. Therefore carbon black is considered an engineered nanomaterial.

⁷¹ The surfactant solution consisted of a mixture of surfactants, sourced from Postnova Analytics GmbH, Germany. It was chosen as an additional food simulant, since its composition stabilises TiN nanoparticles.

Different types of carbon black have a wide range of primary particle sizes, large surface areas per unit mass, low contents of ash and solvent-extractable materials and varying degrees of particle aggregation (IARC 2010). As a result of source material, production method, large surface area and surface characteristics, commercial carbon blacks typically contain varying quantities of adsorbed by-products/impurities, particularly aromatic compounds⁷² (IARC 2010). When considering the safety of carbon black in food packaging applications, it is also important to consider the safety of extractable impurities. The major use of carbon black is in rubber tyres to increase resistance to abrasion, tear, and flexing.

In food packaging applications, carbon black acts as a pigment, and is found in colour concentrates for plastics, epoxy-based enamels, paperboard, inks, can-end cement and sealants (Env Canada & Health Canada 2013). It is unknown what proportion of carbon black (if any) currently used for food packaging is likely to be nano-sized. The patent search conducted as part of this project did not identify any relevant patents for nano- carbon black in food packaging applications.

The potential migration of carbon black⁷³ incorporated in LDPE and polystyrene (PS) plaques at 25 g/kg (2.5%) and 50 g/kg (5% w/w) (manufactured in-house) into standard food simulants⁷⁴ was investigated by Bott et al. (2014c). The researchers used AF4 coupled to a multi-angle laser light-scattering (MALLS) detector to separate, characterise and quantify nanoparticle release. No carbon black peak was detectable (<12 µg/kg food) in the food simulant solutions, irrespective of carbon black concentration in the polymer and exposure time. The results were validated by spiking the migration solutions with a known amount of carbon black, after which it was recovered at the expected elution time. From both these results and the authors' theoretical considerations based on migration modelling, they concluded carbon black does not migrate into food once incorporated into a plastics food contact material (Bott et al. 2014c).

Carbon black (primary particles of 10-300 nm, aggregated to 100-1,200 nm in size) is approved for use in food packaging in Europe, as long as the maximum level used in the polymer does not exceed

⁷² The specific chemicals detected in carbon black extracts and their relative quantities vary widely from sample to sample. Extraction method, type and grade of carbon black and post-extraction treatments all appear to be factors that affect the type and quantity of impurities obtained, and substantial batch-to-batch variation is also common (IARC 2010).

⁷³ Carbon black in the polymers was homogeneously distributed, had a primary particle size of 16 nm, but aggregated to sizes 40 - >100nm.

⁷⁴ Migration tests were conducted by total immersion in 3% acetic acid and 95% ethanol at 60°C for 2, 4, 8, and 10 days according to EN standard 13130-1 and EN 1186-3, and in isooctane at 40°C for 24 hours. Whole plastic plates were cut in three 20 x 70 x 3 mm strips for a total area of 1 dm², cutting edges included.

2.5% w/w, and the level of extractable toluene and benzo(a)pyrene impurities are <0.1% and 0.25 mg/kg carbon black, respectively (EC 2011).

4.4.3 Silanated silicon dioxide (SiO₂)

Silanated silicon dioxide (SiO₂) 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 2011). Amorphous SiO₂ has been used as the food additive, E551, for decades. JECFA (1974) evaluated the safety of SiO₂ as a food additive in 1974 and assigned a “not limited” acceptance pending further studies. The “not limited” acceptance signifies no explicit indication of an upper limit of intake was assigned to the substance due to its very low toxicity. More recently, the European Food Safety Authority concluded exposures to SiO₂ 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). A recent review concluded synthetic amorphous SiO₂, such as that used as food additives, are not new nanomaterials with unknown properties, but are well-studied materials which have been in use for decades (Fruijtier-Pölloth 2012).

After EFSA was informed that silanated SiO₂ had always been produced using synthetic amorphous silica in nanoform, the agency reviewed the original assessment together with additional data provided by industry (EFSA 2014). Additional data included information on the basic SiO₂, the surface treated (i.e. silanated) SiO₂, and a migration study. The synthetic amorphous SiO₂ powder is prepared by a pyrogenic (fumed) process or a precipitation process, where the size range of primary particles produced is 5 – 40 nm or 2 – 60 nm, respectively. The basic SiO₂ is then surface modified with dichlorodimethylsilane or other silanes, which does not significantly change the size of the primary particles. Nevertheless, in the commercial products (the powder), TEM analysis showed the NPs are aggregated and agglomerated to 100 – 300 nm size or larger. No isolated primary particles were observed. Using AF4-MALS, the particle size distribution of the powder product was 160 – 600 nm, peaking at 300 nm. After formulation into LDPE at 0.5, 1 and 3% w/w, the size distribution in the plastic did not change. Standard migration testing of the film containing the highest level of 3% silanated SiO₂ (in 95% ethanol, 3% acetic acid and isooctane) under a variety of time and temperature conditions revealed no detectable migration (presumably of Si) at the detection limit of 0.3 – 0.6 µg/kg simulant. The details of the analytical method(s) used to determine migration potential were not provided in the EFSA report. EFSA (2014) considered the polymer studied and level of incorporation used representative or worst case of other applications. The Panel concluded silanated SiO₂ does not raise a safety concern for the consumer in the currently authorised conditions of use. Silanised SiO₂ nanoparticles in the stomach will be subject to acid hydrolysis.

4.4.4 Zinc oxide (ZnO)

In response to a request from the Ministry of Health, Welfare and Sport in the Netherlands, EFSA (2015) assessed the safety of zinc oxide NPs (uncoated and coated with 3-(methacryloxy)propyl trimethoxysilane) intended for use as transparent UV absorbers in polyolefins for food packaging. The substance is used as a powder in nanoform (average particle size ~44 nm). In the final polymer (LDPE) the NPs are still present but largely aggregated (~120 - 205 nm; 10-35% <100 nm).

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 (as zinc).

Standard migration tests were carried out with the nanocomposite LDPE films, containing the maximum use level of uncoated (2%) and coated (3%) zinc oxide. Food simulants used were 3% acetic acid, 10% ethanol, and 50% ethanol; exposures were for 10 days at 60°C. Zinc was measured in food simulant solutions by ICP-MS and ICP-AA. Migration of zinc into 3% acetic acid was up to 7.6mg/kg (for 2% uncoated ZnO) and 17.3 mg/kg (for 3% coated ZnO). Migration into ethanol was much lower, up to 80 µg/kg. A stress test on migration test samples, which were in contact with isooctane for 10 days at 20°C (to generate polymer swelling) resulted in the exposed simulant containing <0.1 µg/kg of zinc. EFSA (2015) indicate the zinc detected in the other simulants is likely ionic zinc as a result of solubilisation of zinc oxide. They note, however, no direct evidence is available on the physical form of the released zinc. Nevertheless, EFSA (2015) commented if the zinc was in particulate form, it would be expected to dissolve immediately into ionic zinc on contact with acid foods or stomach acid. The agency concluded the substances do not migrate in nanoform, therefore they focused their safety evaluation on soluble ionic zinc. They noted that although the migration data comply with the current SML for zinc (25 mg/kg), in combination with dietary exposure from other sources the upper limit intake of 25 mg/person/day⁷⁵ could be exceeded. EFSA (2015) recommended that the Commission reconsider the SML of 25 mg/kg for zinc, taking into account that consumers are exposed to zinc from sources other than food contact materials.

⁷⁵ This upper limit intake was recommended by the Scientific Committee on Food (SCF) in 2003 and subsequently confirmed by EFSA in 2006 and 2014 (EFSA 2015). It is based on a NOAEL of 50 mg/day for the absence of any adverse effects on a wide range of relevant indicators of copper status (as the critical endpoint) in three human studies. An uncertainty factor of 2 was applied owing to the small number of subjects included in these relatively short-term studies but acknowledging the rigidly controlled metabolic experimental conditions employed.

4.4.5 Titanium dioxide (TiO₂)

Titanium dioxide (TiO₂) is naturally occurring and poorly soluble. It has been used as a pigment for food colouring for decades. In Australia, it is approved for use in processed foods to GMP. It is also approved for use as a food colouring (E171) in Europe (EC 1994), by the Codex Alimentarius of the FAO/WHO, and in the US at concentrations up to 1% w/w without the need to include it on the ingredient label (FDA 2015). The nano-form of TiO₂ 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-TiO₂' in food is not new. The Joint WHO/FAO Expert Committee on Food Additives (JECFA 1969) evaluated TiO₂ and concluded the studies in several species, including humans, show neither significant absorption nor tissue storage following ingestion of titanium dioxide. They considered the establishment of an acceptable daily intake unnecessary due to the substance's inertness (JECFA 1969). The TiO₂ in these safety tests was not characterised.

Dietary studies with nano-TiO₂ are not available. The weight of evidence from gavage studies in rodents indicates nano-TiO₂ is also poorly absorbed from the gastrointestinal tract but nevertheless some investigations have observed increased tissue titanium concentrations and sometimes the presence of nano-TiO₂ particles in tissues. In these studies tissue effects have been observed which is inconsistent with the high exposure chronic studies evaluated by JECFA. The relevance of these gavage studies for assessing the safety of nano-TiO₂ in food is obscure. Not only is the route of administration different from human dietary exposure but the relationship of the TiO₂ tested to 'food grade' TiO₂ is unknown and many of the studies have methodological limitations, for example not conducted to GLP and limited relevance of some evaluated endpoints. ToxConsult (2015a) concluded there is insufficient, directly relevant information available to confidently support a contemporary risk assessment of nano-TiO₂ in food. It was also noted the long history of use of TiO₂ as a food additive has not given rise to reports of adverse effects.

In standard migration tests, Lin et al. (2014) investigated the migration of Ti from nano-TiO₂-PE food packaging films into food simulants (3% acetic acid, 50% ethanol) stored at 25, 70 and 100°C for 1, 2, 3, 4, 5, 6, 7, and 8 hours. After testing, the simulant solutions were evaporated to dryness, and nitric acid dissolved before analysis by ICP-MS. Laser particle size analysis (LPSA) was used to determine the particle size distribution of particles in the food simulants after migration testing. Initial concentrations of Ti in the composite films studied ranged from 248.88 ± 4.74 to 254.84 ± 2.95 mg/kg. SEM imaging of the films revealed average particle size of TiO₂ NPs was 30 nm, with some

aggregates observed of 100 nm. In the 3% acetic acid simulant stored for 8 hours at 100°C, particles (50-90 and 100-150 nm) were detected by LPSA. It is unknown if these were TiO₂ NPs.

Migration of Ti into food simulants increased with increasing concentration in the film, time (until reaching an equilibrium after 6 hours), temperature, and acidity of the food simulant. Maximum migration values for Ti ranged from 0.0014 – 0.012 mg/kg food for the 3% acetic acid, and 0.0005 – 0.001 mg/kg in 50% ethanol (Lin et al. 2014). These values are significantly lower than the EU overall migration limit of 60 mg/kg.

4.4.6 Summary and conclusions

The European Commission has published a Union list of authorised substances for use in manufacturing polymer food contact materials. Only 4 nanomaterials are currently authorised for use:

- *Titanium nitride nanoparticles in PET plastics up to 20 mg/kg (no migration of the nanoparticles into food is allowed).*
- *Carbon black (10-300 nm, aggregated to 100-1,200 nm in size), maximum level in polymer not to exceed 2.5% w/w. A specific migration limit is not set.*
- *Butadiene, ethyl acrylate, methyl acrylate copolymer cross-linked with divinylbenzene, in nanoform, in non-plasticised PVC up to 10% w/w (>20 nm, at least 95% by number >40 nm).*
- *Silanated silicon dioxide (SiO₂). Although this entry is not for nanoparticles per se, EFSA (2014) was recently informed that the substance had always been produced using synthetic amorphous silica in nanoform.*

The few regulatory safety assessments of nanoparticulate materials in food contact materials (including food packaging) take a cautious approach in which no migration of nanoparticles is permissible. Since there are still limitations with measuring NPs per se in food/food simulants from migration experiments, this essentially means elemental constituent migration must be lower than the detection limit. This was the case in the EFSA assessments for titanium nitride and silanated SiO₂ (EFSA 2008, 2012, 2014).

For zinc oxide NPs in polyolefins, however, EFSA (2015) took a different approach. Although no direct evidence was available on the physical form of the released zinc in the migration experiments that were conducted, the agency concluded any zinc present in particulate form would be expected to dissolve immediately into ionic zinc on contact with acid foods or stomach acid. The agency

concluded the substances do not migrate in nanoform, therefore they focused their safety evaluation on soluble ionic zinc. This conclusion is also likely to be appropriate for nanosilver (see Section 4.3).

Few food packaging migration experiments for nanomaterials other than nano-clay and nanosilver were found in the literature. Experiments with carbon black (in LDPE and PS plaques) and TiN (in LDPE films) found no detectable migration of the nanomaterial or its constituents, whereas migration of Ti into food simulants in a study with nano-TiO₂-PE food packaging films was significantly less than the European permissible overall migration limit of 60 mg/kg. Although based on very limited data, this suggests that the potential for consumer exposure and any subsequent public health or safety issues as a result of incorporation of these nanomaterials in polymers studied is likely to be low.

5. Overall conclusions

From the results of a patent search conducted for nanomaterials in food packaging, it can be concluded that although there is no direct evidence that nanomaterials are currently being used in food packaging applications in Australia and/or New Zealand, there is evidence they are being used overseas. These nanomaterials might be considered to be potentially in use in Australian and New Zealand if the associated products are imported. The two most common nanomaterials used in food packaging at present are likely to be nano-clays and nanosilver, based on the number of patents found. It is noted the identification of nanotechnologies in current use in Australia is difficult to determine due to intellectual property issues. Not all such technologies may therefore have been identified by the applied search techniques.

Nano-clay:

Bentonite is a naturally occurring substance with platelets whose thickness are in the nanoscale size range. Bentonite has a long history of permitted use as a food additive at levels up to 5% w/w in Europe and GMP in Australia; no evidence of adverse effects due to its use was found in the literature review conducted as part of this project. Although anecdotal evidence suggests it has been used as a food additive for decades (if not longer), definitive information for the extent and rate of its current or historical use as a food additive was not found. No evidence was found in this literature review to indicate that nano-clay is likely to cause adverse effects on health when used in food packaging.

Considering the probable extent of its use, there have been surprisingly few studies investigating the migration of nano-clay constituents into food simulants or foods. Only two studies investigated migration of nano-clay particles *per se*, and in both their presence in food simulants was not detected.

In some of the studies, migration of elemental components from nano-clay (particularly Si) into food and acidic food simulant has been detected from food packaging material, although overall migration in all cases (0-9.5 mg/kg) was significantly lower than the 60 mg/kg of foodstuff overall migration limit for Europe (EC 2011, 2014, 2015). Migration of aluminium from nano-clay was minimal (0-1 mg/kg food), and lower than the concentrations typically found in foods.

This indicates that the potential for consumer exposure and subsequent public health or safety issues as a result of incorporation of nano-clay into polymer composites is likely to be low. This is supported by *in vitro* and *in vivo* (90-day) toxicity experiments conducted with nano-clay PLA composite migration simulant solutions after the migration testing was carried out, which have not found any adverse effects. Safety evaluations for nano-clay in food packaging are therefore likely to be driven by migration of elemental constituents, rather than by the 'nano-ness' of the material. However, the conclusion is tempered by the relatively few studies which have investigated the migration of nanoparticles *per se* from nano-clay, and the uncertainties in current analytical techniques for measuring nanoparticles in foods/simulants.

Nanosilver:

Silver is permitted for use as a food additive in Australia or as food colouring in Europe in confectionary, spirits and liqueurs to GMP. Colloidal silver and formulations containing silver salts were used historically for medical applications, but these uses have been largely discontinued. Since the 1990s, colloidal silver has been marketed as an alternative medicine, however its effectiveness for such uses has not been proven. After chronic medical or occupational exposure to silver, argyria (a permanent grey or blue grey discolouration of the skin and other organs) is the most common finding.

Unlike nano-clay, the antimicrobial function of nanosilver in food packaging materials means it is intended that silver ions be released to deter food spoilage. Thus a balance between what is considered too little to be effective and too much from a safety perspective needs to be achieved. A large number of migration studies were found for nanosilver containing polymer composites or coatings.

Overall the results from migration studies for nanosilver suggest the production method of nanocomposites (e.g. incorporation or coating, surfactant modification), starting silver concentration, temperature, time and choice of contact media are all factors which may have an effect on the extent of silver ion migration into food simulants. In general, the rate of migration increases when nanosilver is coated onto the food packaging material or surfactants are added, when the storage temperature and length of storage increases, and the acidity of the contact medium increases. There appears to be a specific time of storage, after which a steady state release of silver is achieved.

Several studies have attempted to investigate whether nanoparticles *per se* migrated into food simulant solutions, and mixed results have been obtained. Theoretical models predict migration of nanoparticles *per se* from packaging to food would be detectable only when very small nanoparticles (i.e. ~1-3.5 nm) are embedded in polymer matrices which have low dynamic viscosities. The limitation in detectability of current measurement techniques, together with the lack of information provided on sample processing and handling prior to analysis makes it difficult to draw any concrete conclusions on whether silver ions or silver nanoparticles *per se* migrate into food simulants.

Until such a time analytical techniques are more refined and more information is available, safety assessment of nanosilver-containing food packaging materials will be limited to conventional considerations of ionic silver release into foods.

Nevertheless, there is some evidence to suggest that if silver nanoparticles do migrate into food/food simulants, they would most likely dissolve quickly into ionic silver. Furthermore, the toxicological effects of nanosilver observed in 28-90 day gavage studies with laboratory animals are qualitatively similar to those observed in dietary studies with silver salts, and in some instances less severe (ToxConsult 2016a). Though this is based on very limited information, this suggests any toxicity observed is unlikely to be due to the novel 'nano-ness' of the material.

The majority of the migration studies found for nanosilver food packaging composites have shown levels of migration of ionic silver into foods and food simulants below the European SML of 0.05mg Ag/kg food, suggesting low risk of consumer exposure and subsequently low risk of adverse effects. However there are also several studies, in which migration exceeded this limit. This indicates that for new food packaging products containing nanosilver, it is still necessary to conduct migration experiments on a case-by-case basis.

Other nanomaterials:

The European Commission has published a Union list of authorised substances for use in manufacturing polymer food contact materials. Only 4 nanomaterials are currently authorised for use:

- Titanium nitride nanoparticles in PET plastics up to 20 mg/kg (no migration of the nanoparticles into food is allowed).
- Carbon black (10-300 nm, aggregated to 100-1,200 nm in size), maximum level in polymer not to exceed 2.5% w/w. A specific migration limit is not set.
- Butadiene, ethyl acrylate, methyl acrylate copolymer cross-linked with divinylbenzene, in nanoform, in non-plasticised PVC up to 10% w/w (>20 nm, at least 95% by number >40 nm).
- Silanated silicon dioxide (SiO₂). Although this entry is not for nanoparticles *per se*, EFSA (2014) was recently informed that the substance had always been produced using synthetic amorphous silica in nanoform.

The few regulatory safety assessments of nanoparticulate materials in food contact materials take a cautious approach in which no migration of nanoparticles is permissible. Since there are still limitations with measuring NPs *per se* in food/food simulants from migration experiments, this essentially means elemental constituent migration must be lower than the detection limit. This was the case in the EFSA assessments for titanium nitride and silanated SiO₂.

For zinc oxide NPs in polyolefins, however, EFSA (2015) took a different approach. Although no direct evidence was available on the physical form of the released zinc in the migration experiments that were conducted, the agency concluded any zinc present in particulate form would be expected to dissolve immediately into ionic zinc on contact with acid foods or stomach acid. This is in line with the suggested findings from the migration experiments with nanosilver. The agency concluded the substances do not migrate in nanoform, therefore they focused their safety evaluation on soluble ionic zinc.

Few food packaging migration experiments for nanomaterials other than nano-clay and nanosilver were found in the literature. These either found no detectable migration of the nanomaterial or its constituents, or migration significantly less than the European permissible overall migration limit of 60 mg/kg. Although based on very limited data, this suggests that the potential for consumer exposure and subsequent public health or safety issues as a result of incorporation of these nanomaterials (carbon black, TiN, TiO₂) in polymers studied is likely to be low.

Overall conclusion:

The data reviewed for this report indicate for most of the studied nanomaterials in food packaging, migration of intact nanoparticles into food simulants was negligible, implying consumer exposure to these materials is likely to be low. This suggests there is low potential for safety issues related to the 'nano-ness' of the materials incorporated into food packaging. If they did migrate in nanoparticulate form, it would be anticipated many of the metal oxide nanoparticulates would likely dissolve into their ionic forms upon contact with acid foods or stomach acid. These conclusions are tempered by the relatively few studies which have investigated the migration of nanoparticles *per se* from food packaging materials and the uncertainties in current analytical techniques for measuring nanoparticles in foods/simulants.

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Appendix A: Patent search strategy

Websites searched:

- Australia: <http://www.ipaustralia.gov.au/get-the-right-ip/patents/search-for-a-patent/>.
- New Zealand: <http://www.iponz.govt.nz/cms/patents>.
- USA: <http://patft.uspto.gov/netahtml/PTO/search-bool.html>.
- Europe: <https://www.epo.org/searching.html>.
- Worldwide (includes Europe and Asia): <https://patentscope.wipo.int/search/en/search.jsf>.
- Worldwide (Google):
https://www.google.com.au/?tbm=pts&gws_rd=cr,ssl&ei=j4pAVbqeMsPRmwWAz4CIBq.

Search terms:

Search terms evolved throughout the project and with each website. More specific search terms were used for the worldwide patent searches, as the general terms resulted in too many hits. The general search terms were:

- nanomaterial food packaging
- nanoparticle food packaging
- nanomaterial food
- nanoparticle food
- nanomaterial AND food
- nanoparticle AND food

Appendix B: Regulatory Aspects

Summary:

- Regulatory authorities have taken different approaches to managing the commercialisation of nanomaterial-containing food packaging, whether it is in the form of a guidance document, specific regulation or amendment to existing regulations (Hannon et al. 2015).
 - Although Australia and New Zealand have a general requirement that food packaging be safe, there are no specific requirements or guidance documentations for nanomaterials in food packaging as of yet. However, Food Standards Australia New Zealand (FSANZ) is currently undertaking a review of the legislation regarding chemical migration into food packaging. Although the review does not include consideration of nanomaterials in food packaging *per se*, any amendments made to existing legislation may also be useful in helping to manage risks from nanomaterials.
 - Regions such as USA, EU and Canada have made amendments to current food packaging legislation and/or have provided guidance documents for nanomaterials. As with Australia and New Zealand, they ultimately require food packaging to be safe. They also require nanomaterial-containing food packaging materials be assessed on a case-by-case basis until more information is known.
 - For countries such as Brazil, Argentina, China, Japan and Mexico there has been limited regulation relating to nanomaterials. For most of the countries (except Japan and Mexico, which do not formally regulate food contact materials), current regulation requires the level of food additives in food (which includes contaminants that may inadvertently become a part of food)⁷⁶ to be safe. None of these jurisdictions have established regulations specific to nanomaterials (Magnuson et al. 2013).

⁷⁶ Under the broad sense of this definition, nanomaterials in food packaging would be considered as food additives.

Jurisdiction	Regulatory strategy for nanomaterials	Reference
<p>Australia & New Zealand (Food Standards Australia New Zealand, state/territory food authorities, National Industrial Chemicals Notification and Assessment Scheme, Australian Competition and Consumer Commission)</p>	<ul style="list-style-type: none"> - Currently managed using existing food safety standards (<i>Australia New Zealand Food Standards Code</i>). - In Australia, the regulation of chemicals in articles for food use is shared by several Australian Government regulatory agencies; FSANZ and the state/territory food authorities for the food sold in packaging; NICNAS for the safety of the industrial chemicals used; and the ACCC for the safety of the packaging articles themselves. - In New Zealand, chemicals in food are also regulated by FSANZ, in conjunction with the New Zealand Ministry of Primary Industries. Chemicals in packaging are regulated by the New Zealand Environment Protection Authority and consumer products, by the New Zealand Ministry of Consumer Affairs. - Although there is a general requirement in the regulation in the Code which covers articles and materials in contact with food (Standard 1.4.3) stating food packaging must be safe, there are no specific requirements (e.g. migration limits) for substances in food packaging. However, the maximum limits (MLs) in food must be adhered to and contaminant levels must be kept as low as reasonably achievable if no limit exists. - FSANZ is currently reviewing the current legislation in terms of chemical migration from food packaging. - The amended Application Handbook now requires information on particle size be provided where it is important to achieving the technological function or may relate to a difference in toxicity. 	<p>FSANZ 2013, 2014 Fletcher & Bartholomaeus 2011</p>

Jurisdiction	Regulatory strategy for nanomaterials	Reference
<p>European Union (European Commission, Parliament and Council)</p> <p>- Commission advised by European Food Safety Authority (EFSA), which provides guidance on risk assessment of NMs in food & food contact substances and conducts risk assessments at request of Commission.</p>	<ul style="list-style-type: none"> - Managed using existing regulation, as well as amended legislation. (e.g. EC No 1935/2004, 1895/2005; Commission Directive 2002/72/EC; Commission Regulation No. 975/2009, 10/2011). - Any material or article intended to come into contact directly or indirectly with food must be sufficiently inert to preclude substances from being transferred to the food in quantities large enough to endanger human health, or to bring about an unacceptable change in the composition of the food or a deterioration in its organoleptic properties. - Regulation distinguishes between new 'active' and 'intelligent' food contact materials and articles and materials and articles that have been traditionally used to release their natural ingredients into specific types of food (e.g. wooden barrels), although 'nano' is not specifically mentioned. - Nevertheless, it is stated that new technology (e.g. nanoparticles) should be assessed on <u>a case-by-case basis</u> until more information is known. - Union List contains a number of nanomaterials which are approved for food packaging use (see Section 4.4). 	<p>Choudhry et al. 2008, Hannon et al. 2015</p> <p>EC 2004, 2005, 2009, 2011</p>

Jurisdiction	Regulatory strategy for nanomaterials	Reference
United States (Food and Drug Administration)	<ul style="list-style-type: none"> - Regulated under existing legislation (<i>Federal Food, Drug, and Cosmetic Act</i>) (i.e. product-focused regulation). - Review of new products before they are commercialised (i.e. pre-market approval). Premarket review includes attention to whether use of nanomaterials suggests need for additional data on safety or effectiveness. - Under this system, products are evaluated on a <u>case-by-case basis</u>, except if prior sanctioned or generally recognised as safe (GRAS)⁷⁷. - FDA guidance documents indicate for a food contact material to be made commercially available the product manufacturer must provide a safety assessment which includes studies on humans and animals evaluating its safety under the worst case conditions of use. It is stressed that the reduction of any substance to nanoscale is considered a significant deviation from conventional manufacturing processes and consequently merits particular examination. 	FDA 2011, 2014a, b
Canada (Health Canada, Environment Canada)	<ul style="list-style-type: none"> - Regulated under existing <i>Food and Drug Regulations (Canadian Environmental Protection Act 1999)</i>. - Assessment on a <u>case-by-case basis</u>. - Health Canada has a policy statement describing their working definition of a nanomaterial, providing general guidance on the type of information that may be required for a safety assessment of a nanomaterial or nanomaterial-containing product. 	Health Canada 2011, 2014
Argentina (Ministry of Health and the National Administration of Drugs, Foods and Medical Technology)	<ul style="list-style-type: none"> - Managed under existing regulations (<i>Grupo Mercado Comun – GMC 26/03</i>) - The approval process for new substances used in food packaging is the same as for direct food additives (i.e. all non-listed food additives can be submitted for evaluation). 	Magnuson et al 2013

⁷⁷ For example, one listed prior sanction is for TiO₂ used in the manufacture of paper and paperboard products for food packaging, where under normal conditions of use, the substance would not reasonably be expected to migrate to food. It is unclear whether this prior sanction would apply to nanoscale TiO₂ when intended for that use. In this case the “no migration” restriction could be a matter of needing proof. In addition, SiO₂ is GRAS as a substance migrating to food from paper and paperboard food packaging, but whether a nanoscale version of the compound used for that application would be covered by that regulation is debatable (FDA 2011).

Jurisdiction	Regulatory strategy for nanomaterials	Reference
Brazil (Ministry of Health)	<ul style="list-style-type: none"> - Managed under existing regulations (<i>Grupo Mercado Comun – GMC 26/03</i>) - The approval process for new substances used in food packaging is the same as for direct food additives (i.e. all non-listed food additives can be submitted for evaluation). 	Magnuson et al 2013
China (Ministry of Health)	<ul style="list-style-type: none"> - Managed under existing regulations (<i>Food Safety Law of the People’s Republic of China</i>) - Pre-market application should demonstrate technological need & safety for the additive, and shall meet certain conditions with respect to migration. Specific reference to ‘nano’ not made. 	Magnuson et al 2013
Japan (Ministry of Health, Labour and Welfare)	<ul style="list-style-type: none"> - No regulations available for food contact materials. - In 1973, the Japan Hygienic Olefin and Styrene Plastics Association (JHOSPA) established the industry’s voluntary standards made up of a positive list describing raw materials that can be used safely for food packaging. - No authoritative guidance document or approval process for a new substance (including nanomaterials) in food packaging found. 	Magnuson et al 2013
Mexico (Ministry of Health)	<ul style="list-style-type: none"> - Managed under existing regulations (<i>The Regulation on Sanitary Control of Products and Services</i>) - No specific process available for approval of a new packaging material (including nanomaterials in packaging materials). However, most major customers require legal confirmation that a specific compound is allowed, so they are often encouraged to consult with the authorities. 	Magnuson et al 2013
International (Codex Alimentarius)	<ul style="list-style-type: none"> - Recommendations, not binding legislation. - Expert Meeting (FAO/WHO) concluded current risk assessment approaches used in Codex are suitable for ENMs in food. 	FAO/WHO 2009

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