Final Report

Project A03063

Assessment of Current and Projected Applications of Nanotechnology for Food Contact Materials in Relation to Consumer Safety and Regulatory Implications

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Executive Summary **3**
1.0 Introduction **5**
1.1 Objectives 6
1.2 Methodology 6
2.0 Current status and future trends in nanotechnology applications for FCMs **7**
2.1 Market drivers for nanotechnology applications 7
2.2 Current Nanotechnology Applications to Food Contact Materials 9
2.2.1 Nanoparticle Reinforced Materials 9
2.2.2 Coatings Incorporating Nanoparticles 11
2.2.3 Biodegradable Polymer Nanocomposites 12
2.3 Products and Applications at R&D Stage 13
2.3.1 Polymer Nanocomposites Incorporating Carbon Nanotubes and Nanofibres 13
2.3.2 ‘Smart’ Packaging Incorporating Nanosensors and Labels 14
2.3.3 Polymer Composites Incorporating Nanoencapsulated Substances 15
2.3.4 Dirt Repellent Coatings at Nanoscale 15
3.0 Potential Hazards and Consumer Safety Issues **15**
3.1 Migration of Nanoparticles from FCMs 16
3.1.1. Approach 17
3.1.2. Samples 18
3.1.3. Analytical screening 19
3.1.4. Migration into food simulants 24
3.1.5 Migration into food 27
3.1.6. Conclusions 28
4.0 Adequacy of relevant regulations and implications of nanotechnology-derived FCMs **29**
4.1 Regulatory Aspects Relating to Products and Applications of Nanotechnology for FCMs 29
4.1.1 Regulation (EC) 1935/2004 30
4.1.2 Potential regulatory gaps in relation to Regulation (EC) 1935/2004 32
4.2 Legislation relating to specific materials and articles in contact with food 32
4.2.1 Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with foodstuffs 33
4.2.2. Commission Regulation (EC) No 1895/2005 on the restriction of use of certain epoxy derivatives in materials and articles intended to come into contact with food. 33
4.2.3 The Plastic Materials and Articles in Contact with Food (England) Regulations 2008 33
4.2.4 Commission Directive 2005/31/EC amending Council Directive 84/500/EEC 34
4.2.5 Potential regulatory inadequacies 34
4.3 General legislation relating to materials and articles in contact with food 35
4.3.1 EC Food Law Regulation 178/2002 35
4.3.2 Commission Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs 35
4.3.3 Potential regulatory inadequacies in relation to general food law 35
4.4 Summary of Regulatory Uncertainties 36
4.5 The Precautionary Principle in Relation to Nanotechnology Derived FCMs 37
4.6 The EFSA Opinion on Nanotechnology Applications 37
5.0 Dissemination of Findings **39**
6.0 Conclusions and Gaps in Knowledge Requiring Further R&D **39**
7.0 References **41**
Table 1: List of relevant patent applications 44
Annex-A: Results of Nanomaterial Migration Testing 48
Annex-B: CSL/FSA Workshop on Applications and Implications of Nanotechnology for FCMs 87
Annex-C: Publications and Presentations Resulting from the Study 91
List of Abbreviations: 92

Executive Summary

1. This project was aimed at assessing the current and projected applications of nanotechology for food contact materials (FCMs) in relation to implications for consumer safety and regulatory controls.
2. The study has been carried out by SnIRC (Safety of nanomaterials Interdisciplinary Research Centre), led for this project by Central Science Laboratory.
3. As part of the study, extensive searches were carried out to collate information on the current and projected processes, products, and applications of nanotechnologies for FCMs. The information gathered indicates that nanotechnology applications in the food sector are on the increase worldwide, and many large international food companies are exploring the potential of nanotechnologies for applications in the food sector.
4. Among the nanotechnology applications for the food sector, nanotechnology-derived FCMs make up the largest share of the current and short-term predicted market. A range of nanotechnology-derived FCMs is already available in some countries, and it is widely expected that they will be increasingly available on the UK/ EU market in the next few years.
5. The rapid proliferation of nanotechnology into consumer products has also raised a number of technological, health and safety and social issues. There is a growing body of scientific evidence, which indicates that some free nanoparticles may cause harm to biological systems because of their ability to penetrate cellular barriers, and induce oxyradical generation that may cause oxidative damage to the cell. However, toxicological studies in this regard are sparse, and are mainly in relation to inhalation of free nanoparticles. Thus, the nature and extent of risks to consumer health from ingestion of engineered free nanoparticles via food and drinks are currently unknown.
6. The likelihood of consumer exposure from the use of nanotechnology derived FCMs is intrinsically linked to the migration of nanoparticles into food and drinks. Such migration data for nanotechnology-derived FCMs had not been available, which posed a major barrier to assessment of the risks to an average consumer. Pioneering research was, therefore, carried out as part of this project to test migration of nanoparticles from two typical FCMs made of nanomaterial-polymer composites.
7. Results of the migration study showed that there was no detectable migration from the polymer composite that had nanoclay embedded between PET layers. A very low level of migration of silver (less than the method limit of quantification) was detectable in the case of food containers made of polypropylene-nanosilver composite. In either case, the presence of nanoparticles did not affect migration of non-nano components. Whilst this study has generated some reassurance in the safety of nanotechnology-derived FCMs, it has also shown that migration of nanoparticles is likely to be dependent on the type and composition of the polymer. There is thus a need for testing other types of nanotechnology-derived polymer composites to build a broader picture in regard to the potential migration of nanoparticles from different FCMs.
8. The study has highlighted a number of major knowledge gaps that require further research; for example, to understand the behaviour, fate and toxicology of nanoparticles, their interactions with other food components in the gastrointestinal

tract, effects on the function of gut epithelium and other cells, and on the natural gut microflora.

9. Assessment of the current regulatory frameworks for this study has identified a few uncertainties in relation to nanotechnology-derived FCMs that may be addressed through clarifications and/ or appropriate adjustments.
10. Following completion of the study, a workshop was organised as part of the project to discuss state-of-the-art, and the key issues emerging from the applications of nanotechnologies for FCMs. The workshop was attended by representatives from scientific community, industry, consumer forums, and regulatory agencies. A number of UK/ EU experts presented their studies and views at the workshop, highlighting the enormous benefits that nanotechnology applications would bring to the food sector, and any potential risks to the consumer health and the environment.
11. Parts of the findings of this study were presented at national and international forums. A review article has been published in a peer-reviewed journal, and a book is currently being edited by the participants of this project.
12. In brief, the study has collated the available information in regard to current and projected applications of nanotechnologies for FCMs, highlighted potential risks to the consumer, identified the main gaps in current knowledge and regulatory frameworks, disseminated the findings to key stakeholders, and suggested priority areas for further research.

1.0 Introduction

Food packaging is intended to maintain quality of foods during storage and transportation, and to extend shelf life by controlling the permeation of moisture, gases (oxygen, carbon dioxide), and volatile components (e.g. flavours). The Food and Beverage industry is always seeking new technologies to improve quality, shelf life, safety and traceability of their products. The advent of nanotechnology, that involves manufacture and use of materials in the size range of up to 100 nanometres in one or more dimensions, has opened up new opportunities for the development of new packaging materials with improved properties for use as food contact materials (FCMs). It is therefore not surprising that one of the fastest moving sectors to embrace nanotechnology and realise the potential benefits is the food and beverage industry. A number of the world's largest food companies have been reported to be actively exploring the potential of nanotechnologies for use in food or food packaging[1,2].

Nanotechnology has already opened up a way for a multibillion dollar global industry in recent years. The market impact of nanotechnology is widely expected to reach 1 trillion US$ by 2015, with around 2 million workers (Roco and Bainbridge, 2001). Whilst the majority of manufacturing and use of nano-scale materials occurs in the United States, the European Union (EU), with its 30% global share of this sector, is not lagging far behind. Within the European Union, the UK accounts for nearly a third of the sector (Chaudhry *et al*., 2005; Aitken *et al*., 2006). A variety of consumer products that contain nanomaterials are already available in the UK and the EU. Examples of these range from self-cleaning glass, anti-microbial wound dressing, paints and coatings, fuel catalysts and cosmetics[3].

In terms of specific applications for FCMs, nanotechnology processes and products have been used to develop polymer composites with improved mechanical properties, durability, temperature/ moisture/ pH stability; and barrier properties against moisture, volatiles and gases. The technology is also being explored for the development of 'Intelligent' and 'Smart' food packaging that can monitor the condition of the food during storage and transportation, and 'Active' packaging intended to change the condition of the packed food, to extend shelf-life, or improve sensory properties while maintaining the freshness and quality of the food.

Whilst nanotechnology offers a lot of opportunities for innovation in the food sector, its rapid proliferation into a wide range of consumer products has also raised a number of safety, environmental, ethical, policy and regulatory issues. The main concerns stem from the lack of knowledge over the potential effects and impacts of nano-sized materials on human health and the environment. This is because physicochemical and biological properties of materials at nano-size can be substantially different from their conventional forms, and therefore their effects and impacts may not be accurately predicted from the existing knowledge on micro- or macro- scale equivalents. Such lack of information also makes it more likely that nanotechnology applications for the food sector will raise public concerns over their safety to the consumer.

In this context, Food Standards Agency funded the project A03063 to study the current and projected applications of nanotechnology for FCMs, and to identify any implications with regard to consumer safety and regulatory controls.

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[1] Cientifica 'Nanotechnologies in the Food Industry', published August 2006; www.cientifica.com/www/details.php?id=47

[2] 'Quietly, nanotechnology joins the food chain': The Sunday Telegraph, 22 October 2006, page 5.

[3] The Woodrow Wilson Nanotechnology Consumer Products Inventory www.nanotechproject.org/consumerproducts

1.1 Objectives

This study has been carried out with the following main aims:

1. Project scoping meeting with FSA to discuss the scope of work to ensure that all aspects of interest are included
2. Review of nanotechnology related processes, products and applications in relation to FCMs;
3. Identification of the key issues emerging from the use of nanomaterials in FCMs in relation to consumer safety and regulatory controls, and any gaps in knowledge;
4. Experimental work to determine the potential migration of nano and other (non-nano) components from FCMs;
5. Organisation of a workshop to inform regulators, industry, academia and other stakeholders about the findings of the project, and to discuss the main issues, gaps in knowledge, and needs for further research.
6. Development of conclusions, recommendations for future research.

1.2 Methodology

As part of this study, extensive searches for relevant information were carried out. The main sources of information accessed were:

- Published literature, relevant company websites, and patent databases for nanomaterials or nanoparticles for potential uses for food packaging, and information relevant to consumer safety and regulatory implications;
- The international inventory of nanotechnology consumer products developed by the Woodrow Wilson Institute (www.nanotechproject.org/consumerproducts) for information on the available consumer products packaged in a nanotechnology derived FCM.
- The Defra/ CSL database of nanomaterials manufactured and used in the UK: (http://nanotech.csl.gov.uk/) for information on relevant materials/ applications.
- Other sources of information included the first EFSA advice published on a nanotechnology derived coating for FCM[4]. Key reports, such as from the Institute of Nanotechnology (Joseph and Morrison 2006), the Institute of Food Technologists (Weiss et al. 2006) and a market analysis report from Cientifica (2006). Up-to-date information was also gathered at the first European International Workshop "*Nano and Micro Technologies in the Food and HealthFood Industries'* (October 2006, Amsterdam); the "*Nanotoxicology Conference"*, (April 2007, Venice, Italy); the "*2007 CSL/JISFAN Joint Symposium on Food Safety and Nutrition – Nanotechnology in Foods and Cosmetics"* (June 2007, Greenbelt MD, USA); and the *Food Contact Plastics* Conferences (June 2006 and 2007, Brussels).

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[4] The EFSA Journal (2007) 452-454: 1-10

2.0 Current status and future trends in nanotechnology applications for FCMs

The information gathered for this study indicates that there has been an increasing trend in recent years in R&D into the development of packaging materials based on nanomaterial-polymer composites. The incorporation of certain nanomaterials into polymers has been reported to improve mechanical and/or barrier properties, which is of great interest for applications in food packaging. This is because there has always been a need for improved packaging materials to control certain degradation processes in foods (e.g. rancidity of fats and oils, degradation due to microbial growth) through the control of diffusion of oxygen. Similarly, carbonated soft drinks can be stored for longer periods by controlling the permeation of carbon dioxide.

Unlike some conventional fillers and additives, a very low level of nanoparticles is generally sufficient to improve the properties of packaging materials without any significant changes in density, transparency, and processing characteristics (Lei et al., 2006). The addition of certain nanoparticles into shaped objects (e.g. bottles, containers), and other forms of packaging (e.g. films) can render them light, fire resistant, and stronger in terms of mechanical and thermal performance, and may also make them less permeable to gases. For example, improvement in properties of certain nanomaterial-polymer composites has been reported with regard to durability due to increased strength (Wang et al., 2003); temperature resistance (Alexandra & Dubois, 2000, Kotsilkova et al., 2001); flame resistance (Ray et al., 2002); barrier properties (Alexandra and Dubois 2000, Xu et al., 2006); optical properties (Wan et al., 2003); processability due to lower viscosity (Schartel et al., 2005); and recycling properties (McGlashan & Halley, 2003; Park et al., 2002; Park et al., 2003).

2.1 Market drivers for nanotechnology applications

It is clear from a number of reports, reviews, patent applications, and company products that the nanotechnologies have started to make an impact on different aspects of the food and associated industries (Chen et al., 2006). The nanofood (including nanocomposites for packaging) sector is currently led by the USA, followed by Japan and China; but Asian countries (led by China) are expected to be the biggest market for nano products for food industry by 2010 (Helmut Kaiser Consultancy report)[5].

Estimates of the current global market size and number of companies involved in the nanofood sector are varied because of the scarcity of exact information due to commercial and environmental sensitivities. Such sensitivities have led major food corporations, who had been, until a few years ago, at the forefront of food nanotechnology R&D, to disassociate themselves from publicity in this field. Some Non Governmental Organisations, such as the ETC Group, have called for a moratorium on the use of nanotechnologies in food, until they are proven to be safe to consumers[6]. Recently the Soil Association has proposed a ban on products or ingredients produced using nanotechnology, including for the agriculture and food sectors[7]. A report on the survey of public opinion, due to be published soon by the

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[5] Helmut Kaiser Consultancy. 2004. Study: nanotechnology in food and food processing industry worldwide 2003–2006–2010–2015 www.hkc22.com/Nanofood.html - accessed 30 July 2007

[6] ETC Group Report. 2004. "Down on the farm: the impact of nano-scale technologies on food agriculture". www.etcgroup.org/upload/publication/80/01/etc_dotfarm2004.pdf. Accessed 16 February 2007.

[7] www.soilassociation.org/web/sa/saweb.nsf/d39dda83e1f3c019802570ad005b4516/ebd8481eeee81872802570b5005e192d!OpenDocument - accessed 25 February 2008

Federal Institute of Risk Assessment (BfR), is expected to conclude that consumer opinion in the EU is not favourable to the use of nanotechnologies in food[8]. Such sensitivities have made the task of information gathering in this area very difficult. Furthermore, a lot of the available information in 'grey' literature is aimed at projecting the 'magic' of nanotechnologies when applied to food or food packaging, rather than 'real' products and applications that are available now or in a few years time. This study has, therefore, sifted the information objectively to separate the facts from fiction, and considered those products and applications that are identifiable, whilst at the same time also kept a track of the 'anticipated' ones.

It has been suggested that the number of companies undertaking research or using nanotechnology for food or food packaging applications may be between 200 (IFST, 2006) and 400 (Cientifica, 2006)[9]. These include some major international food and beverage firms. It is also widely anticipated that the number of companies applying nanotechnologies to food and food packaging will increase dramatically in the near future. For example, the Cientifica report (2006) concluded that the majority of the existing applications of nanotechnology in the food sector are for food packaging materials, and the most promising future applications are likely to be for active packaging.

The predictions for the future growth of the nanofood market are also varied. The report by Helmut Kaiser Consultancy has estimated the overall nanofood market (including packaging) would have grown to US$7 billion in 2006, and will reach US$20.4 billion by 2010. The report by the consulting firm Cientifica has estimated the food applications of nanotechnologies in 2006 to be around $410m (including food packaging $210m). According to the report, the current applications of nanotechnology are mainly for food packaging (improved barrier properties etc), with some applications for delivery systems for nutraceuticals. The report has estimated that by 2012 the overall market value will have reached $5.8 billion (including food packaging $2930m). Thus, nanotechnology-derived innovative food packaging forms the largest share of the current and short-term predicted market of nanofood applications. These and other reports also clearly indicate that nanotechnology applications for the food sector are likely to grow rapidly in the coming years.

The current study has indicated that there are several available applications of nanotechnologies in food packaging in a number of countries outside the EU, mainly in the USA, Australia, New Zealand, Korea, China and Taiwan. The use of barrier layered PET bottles in Germany has been reported[10]. However, it is not clear whether they incorporate nanoparticle based barrier layers. Considering the rapid developments in this field, and the global setup of international food companies, it is not unreasonable to anticipate that nanotechnology-derived food packaging will start appearing on the UK/ EU markets in the next few years. Further surveillance will, therefore, be needed to monitor the ingress of nanotechnology-derived FCMs in the UK/ EU.

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[8] www.dairyreporter.com/news/ng.asp?n=82217-bfr-nanotechnology-consumer-acceptance - accessed 25 February 2008

[9] Cientifica Report. 2006. "Nanotechnologies in the Food Industry" published August 2006. Available: www.cientifica.com/www/details.php?id=47. Accessed 24 October 2006.

2.2 Current Nanotechnology Applications to Food Contact Materials

This study has identified the following broad categories of nanotechnology applications for FCMs:

- Improved FCMs incorporating nanomaterials for better packaging properties (flexibility, durability, temperature/ moisture stability)
- 'Active' FCMs that incorporate nanoparticles with antimicrobial or oxygen scavenging properties
- 'Intelligent' and 'Smart' food packaging; e.g. that incorporate nanosensors to monitor the condition of the food
- Biodegradable polymer-nanomaterial composites

2.2.1 Nanoparticle Reinforced Materials

These are polymers reinforced with nanoparticles to provide a composite material with enhanced properties. Also termed as 'nanocomposites', these are reinforced with small quantities (typically up to 5% by weight) of nanoparticles, which have high aspect ratios (L/h>300). Such reinforcement can radically modify the properties and performance of a polymer. The composites developed so far include a variety of thermoplastic, thermoset and elastomer polymers, and biodegradable polymers such as potato starch and polylactic acid (Garland, 2004). Nanocomposite films have also been developed for improving mechanical and barrier properties of plastic films or polymers against permeation of gases and moisture to increases the shelf life of packaged food products (Garland, 2004; Joseph and Morrison, 2006). Barrier plastic coatings using embedded nanocrystals have also been developed for use on the inner plastic lining of cans. This technology is also used in plastic beer bottles to increase the shelf life by preventing the migration of oxygen (Garland, 2004; Food Packaging using Nanotechnology Methods, 2004).

2.2.1.1 Polymer Nanocomposites Incorporating Clay Nanoparticles

These are among the first nanocomposites to emerge on the market as improved materials for packaging (including food packaging). The nanoclay mineral used in these nanocomposites is montmorillonite (also termed as bentonite), which is a natural clay commonly obtained from volcanic ash/ rocks. Nanoclay has a natural nano-scaled layer structure that restricts the permeation of gases. Substantial improvements in gas barrier properties of polymer composites containing nanoclay have been claimed (Ke and Yongping, 2005). This has led to the development of nanoclay-polymer composites for potential use in a variety of food packaging applications, such as processed meats, cheese, confectionery, cereals, boil-in-the-bag foods, as well as in extrusion-coating applications for fruit juices and dairy products, or co-extrusion processes for the manufacture of bottles for beer and carbonated drinks (Akbari et al., 2006).

Nanoclay-polymer composites are made from a thermoset or thermoplastic polymer. The polymers used for nanocomposites are polyamides (PA), nylons, polyolefins, polystyrene (PS), ethylene-vinylacetate (EVA) copolymer, epoxy resins, polyurethane, polyimides and polyethyleneterephthalate (PET). Other additives used in polymer nanocomposites include Polyhedral Oligomeric Silsesquioxane (POSS). The POSS-Nanoclay is a relatively new hybrid based on the silsesquioxane-cage structures, one of the smallest forms of silica also known as molecular silica, the physical forms of which can be liquid, wax or crystalline solid (Garland, 2004).

Examples of the available nanoclay-polymer composites with Nylon (6) include: Imperm®, Duretham® LDPU 601 and Aegis® OX.

- Imperm® (from Nanocor Inc) is used in multi-layer PET bottles and sheets for food and beverage packaging[10]. It works by minimising both the loss of CO_2 from the drink and the ingress of O_2 into the bottle thus keeping beverages fresher and extending the shelf-life by up to six months.
- Duretham® LDPU 601 (from Bayer AG) is a transparent plastic film with nylon/ PA6 which is enriched with silicate particles. It is a hybrid system and its primary purpose is to prevent the packaging contents from drying out and protect them from moisture and CO_2. It is used in multilayer packaging and comes in 2 grades; moderate barrier improvement (25% reduction in O_2 transmission rate) and high barrier improvement (50% reduction) with only slight changes to the residual PA specific properties. Duretham KU 2-2601 uses Nanocor's clay to produce a film with increased barrier properties in the plastic, enhanced gloss and stiffness. The application is used where conventional PA is too permeable and EVOH coatings too expensive e.g. paperboard juice containers.
- Aegis® OX (from Honeywell) polymerized nanocomposite film is an oxygen-scavenging barrier resin formulated for use in co-injection PET bottle applications e.g. beer, fruit juice and soft drinks. The resins are a blend of active and passive nylon using O_2 scavengers and passive nanoclay particles to enhance the barrier properties, retain CO_2 and keep O_2 out.

Known applications of nanoclay-based multilayered film packaging include bottles for beer and carbonated drinks, and thermoformed containers[10]. Miller Brewing Co. USA and Hite Brewery Co. South Korea are reported to be using the technology in their beer bottles[10,11].

2.2.1.2 Polymer Nanocomposites Incorporating Metal (oxide) Nanoparticles

Polymer nanocomposites developed for 'Active' packaging are based on the antimicrobial action of metal or metal oxide nanoparticles. Other improvements in polymer performance through incorporation of metal(oxide) nanoparticles include abrasion resistance, UV absorption, and strength. Some nanomaterials have been used to develop active packaging that can absorb oxygen and therefore keep food fresh. Other applications include UV absorbers (e.g. nano-titanium dioxide) to prevent UV-degradation in plastics such as PS, PE, PVC. The commercially important nanoparticulate materials in this respect are metals such as silver (Ag), gold (Au), zinc oxide (ZnO), and metal oxides such as silica (SiO_2), titanium dioxide (TiO_2), alumina (Al_2O_3) and iron oxides (Fe_3O_4, Fe_2O_3). Polymer composites incorporating titanium nitride (TiN) have also been developed for rigidity and strength of the packaging objects.

Utilising the antimicrobial action of nanosilver, a number of 'active' FCMs have been developed that are claimed to preserve the food within the materials longer by inhibiting the growth of microorganisms. Antimicrobial properties of other nanoparticles, such as zinc oxide and magnesium oxide, have recently been discovered at University of Leeds (Zhang et

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[10] Plastic Technology www.plastictechnology.com/articles/200508fa1.html - Accessed 30 July 2007.

[11] Big Idea Investor: www.bigideainvestor.com/index.cfm?D=603

al., 2007). It is hoped that nano forms of zinc oxide and magnesium oxide may provide a more affordable and safe food packaging solutions in the future.

Examples of available FCMs incorporating antimicrobial nanomaterials

Product	*Company*	*Brief description*	*Nanoparticles*
Nano Silver Food Containers	A-DO (Korea)	Containing antibacterial nanosilver claimed to prevent bacterial growth in food	Nano-silver
FresherLonger™ Miracle Food Storage and FresherLonger™ Plastic Storage Bags	Sharper Image® (USA)	Plastic containers and plastic bags incorporating nanosilver, claimed to keep foods (fruits, vegetables, herbs, breads, cheeses, soups, sauces and meats) fresher 3-4 times longer than conventional plastic storage containers	Nano-silver
Nano Plastic Wrap	SongSing Nano Technology Co., Ltd. (Taiwan)	Nano ZnO light catalyst, which can sterilize completely in indoor lighting, anti UV and reflects IR	Nano-zinc oxide
Nano Silver Baby Mug Cup and Baby Milk Bottle	Baby Dream® Co. Ltd. (Korea)	Silver nano poly system, claimed to prevent 99.9% of germs, and a deodorizing function that keeps freshness	Nano-silver
Domestic fridge	Samsung	Silver nanoparticles incorporated into the inner surface of fridges to prevent microbial growth to maintain a clean and hygienic environment in the fridge.	Nano-silver

2.2.2 Coatings Incorporating Nanoparticles

Coatings incorporating nanoparticles have been used to develop antimicrobial, scratch-resistant, anti-reflective, or corrosion-resistant surfaces (Garland, 2004). These coatings contain nanoparticulate form of a metal or a metal oxide, or a film resin containing nano-particles. Nano-coatings may also be applied in a multi-layer deposition process, which is referred to as layer-by-layer (LBL) or electrostatic self-assembly (ESA) coating.

Examples of food contact materials with nano-coating

Product	*Company*	*Brief description*	*Nanoparticles*
Antibacterial Kitchenware, Antibacterial Tableware and Antibacterial Pet products	Nano Care Technology, Ltd. (China)	Antibacterial ware with nano silver coating	Nano-silver

Nano Silver Cutting Board	A-DO (Korea)	Containing antibacterial nanosilver claimed to keep the cutting board clean and hygienic	Nano-silver
Nano Silver Teapot Nano Silver Spray	SongSing Nano Technology Co., Ltd. (Taiwan)	Nanosilver for disinfecting and water-clearing Antimicrobial nanosilver spray for sterilising action	Nano-silver

2.2.2.1 Nano-structured Barrier Coatings

These are hybrid organic-inorganic nanocomposite coatings of hybrid precursors and sol-gel systems (e.g. propyltrimethoxysilane- PTMO and Bayresit), and are being developed for active packaging applications, e.g. foil treatment (Garland, 2004; Paulussen and Vangeneugden, 2006). The coatings are produced through atmospheric plasma technology using dielectric barrier discharges.

Other high-barrier nanocoatings consist of numerous nanodispersed platelets per micron of coating thickness to increase barrier properties of PET, which enhances the oxygen barrier ensuring longer shelf life of food and drink products (Garland, 2004). The coatings have been reported to be very efficient at keeping out oxygen and retaining carbon dioxide and can rival traditional active packaging technologies such as oxygen scavengers. Examples include InMat Nanocoatings, which is an aqueous-based nanocomposite barrier coating (possibly non-elastomeric) providing an oxygen barrier with 1-2 micron coating, and is said to be cost effective relative to EVOH multilayer films and PVDC coated films used in the packaging industry. Another example is silica-polymer hybrids manufactured by sol-gel process, which can also improve oxygen-diffusion barriers for plastics (such as PET) in the packaging industry.

2.2.2.2 Antimicrobial Nano Emulsions

Nanoemulsions have been developed for use in the decontamination of food packaging equipment and in packaging of food. An example of an available product is *Nano-in Natural Environmental Cleaning Agent* from Nano-Infinity Nanotech Co., Ltd. (Taiwan)**.** This nano micelle based product contains natural glycerine, and is claimed to completely remove pesticide residues on fruits and vegetables, as well as the oil/dirt on cutlery.

2.2.3 Biodegradable Polymer Nanocomposites

This is an emerging area of R&D with potential application of nanotechnology to improve properties of biodegradable polymers. For example, starch based polymers have poor moisture barrier properties due to their hydrophilic nature, and inferior mechanical properties compared to plastic films. The incorporation of nanoclay in starch polymers has been reported to improve moisture barrier and mechanical properties (Alexandra & Dubois, 2000).

Similarly, poly lactic acid (PLA) is a biodegradable thermoplastic polymer that has a high mechanical strength, but low thermal stability and low water vapour and gas barrier properties compared to plastic polymers. The incorporation of 5% (w/w) of montmorillonite or microcrystalline cellulose into PLA has been reported to improve tensile modulus and yield strength in the case of montmorillonite, but only marginal improvement in yield strength in the case of microcrystalline cellulose. A reduction in the oxygen permeability has also been reported in the case of nanoclay-PLA nanocomposite, but not in the case of microcrystalline cellulose-PLA composite (Akbari et al., 2006).

2.3 Products and Applications at R&D Stage

In terms of R&D activities, it has been estimated that over 200 companies worldwide are conducting R&D into the use of nanotechnology in engineering, processing, packaging or delivering food and nutritional supplements[5,12]. Whilst a few nanotechnology based FCMs are already commercially available, over 150 applications of nanotechnology in food and food packaging are reported to be at different stages of development (Cientifica, 2006).

The prominent institutions carrying out R&D into different aspects of nanofood include:

- Denmark's Center for Advanced Food Studies (LMC) is an alliance of Danish institutions working in food sciences. The Center is aiming to manufacture nanomaterials with functional properties, and nanosensors and nanofluidic technologies for applications in food sciences.
- Wageningen Bionanotechnology Center at Wageningen University (The Netherlands) is focusing on the applications of nanotechnology in the food industry.
- NanoteK Consortium, USA: In 2000, Kraft Foods launched the NanoteK Consortium of research groups from 15 universities, 3 national labs and 3 start-up companies to explore the applications of nanotechnologies to make interactive foods. The consortium planned to develop smart foods that release nutrients in response to deficiencies detected by nanosensors.
- Nanofood Consortium. A cluster of scientists from Northern European food industries to nurture applications of nanotechnology in the food industry, to develop healthy and safe foods, to develop sensors for toxic compounds or bacteria in food products; anti-bacterial surfaces for food production machines; thinner, stronger and cheaper packaging for food; and healthier nutritional food compositions.
- Leatherhead Food International formed a new working group 'NanoWatch' in 2007 to investigate the use of nanotechnology in the food and drink industry, with particular emphasis on ingredients and hydrocolloids.

The search of patent databases for this study found a total of 464 patent entries with regard to applications of nanotechnology in food or FCMs. Out of these, the 52 patents relevant to food contact applications were selected for further examination (Table 1). The main nanotechnology R&D activities relevant to the area of FCMs have been listed below.

2.3.1 Polymer Nanocomposites Incorporating Carbon Nanotubes and Nanofibres

Carbon nanotubes (CNT) are a particular form of fullerenes (Iijima (1991). They are similar in structure to carbon bucky balls (C_{60}), but are elongated to form tubular structures, 1-2 nm in diameter. They can be produced with very large aspect ratios and can be more than 1 mm in

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[12] Institute of Food Science and Technology (IFST) Trust Fund. 2006. Nanotechnology information statement www.ifst.org/uploadedfiles/cms/store/attachments/nanotechnology.pdf

length. In their simplest form, nanotubes comprise a single layer of carbon atoms arranged in a cylinder. These are known as single-wall carbon nanotubes (SWCNTs). They can also be formed as multiple concentric tubes (multi-wall carbon nanotubes, MWNTs) with diameters up to 20 nm, and length greater than 1 mm. CNTs have great tensile strength and are considered to be 100 times stronger than steel, whilst being only one sixth of its weight, making them potentially the strongest, smallest fibre known (www.thomas.swan.co.uk). They also exhibit high conductivity, high surface area, unique electronic properties, and potentially high molecular adsorption capacity (Maynard *et al;* 2004). Carbon nanofibers are hollow, solid carbon fibres, usually no longer than a few microns. They consist of a mix of various forms of carbon from layers of graphite stacked at various angles to amorphous carbon. Potential applications in packaging could include conductive and reinforcement applications in composites (e.g. Pyrograf-III from Applied Sciences Inc.), and processing nanotubes to functionalise and disperse plastics and coatings.

Because of the enormous strength they can provide to polymers, SWCNTs are being studied for use as a reinforcing agent for intercalation matrices in polymer composites for a variety of packaging applications. CNT reinforced composites have been developed with different matrix resins such as PA, polyesters, polycarbonates & blends, PS, polyphenylene sulphide (PPS), PEI and polyether ether ketone (PEEK). There is also a possibility in future for CNT nanocomposites with polyolefins. However, to date, there is not a known example where CNTs or nanofibres have been incorporated in a food contact material.

Carbon nanofibres have a similar structure to asbestos fibres and there are concerns that these may cause respiratory problems when inhaled in large amounts over long periods. There are also reports that indicate the potential for carbon nanotubes to cause harmful effects in living organisms, and therefore there may be a risk to human health through contact with skin, ingestion or inhalation of commercial products (Lam et al., 2004, Shvedova et al., 2005, Jia et al., 2005).

2.3.2 ‘Smart’ Packaging Incorporating Nanosensors and Labels

Materials for ‘Smart’ packaging are being developed that incorporate a variety of nanosensors, biosensors, labels or the so called ‘Electronic Tongue’ technology being developed at the University of Texas (USA) that is made up of sensor arrays to signal the condition of the food (Garland, 2004; Food Packaging using Nanotechnology Methods, 2004). When developed, these embedded sensors will be able to detect food pathogens and trigger a colour change in the packaging to alert the consumer about contamination or spoilage. Also another application under development is smart packaging, which would optimise shelf life, repair small holes and tears, and also respond to environmental conditions. Examples include Nano Bioswitch/ ‘Release-on-Command’ (Food Packaging using Nanotechnology Methods, 2004) that will provide a basis for intelligent preservative packaging technology that will release a preservative if a food begins to spoil.

Another application under development is Nano Bioluminescence Detection Spray (Garland, 2004; Joseph and Morrison, 2006). These nanosensors contain a luminescent protein of small molecular weight modified to bind to target microbial surfaces such as *Salmonella* and *E. coli*. When bound, the protein emits a visible glow thus allowing easy detection of contaminated foods and beverages. R&D into designing such spray techniques include, for example, spraying on ocean freight containerised shipping of perishable goods; defence and security applications in the event of terrorist attacks on food supplies (bioterrorism) etc. DNA based biochips are also under development (Garland, 2004) that will enable detection of pathogens and presence of different harmful bacteria in meat or fish, or fungi affecting fruit.

A further example is BioSilicon™ from pSivida Australia that is nanostructured silicon with nano-pores for potential applications in food packaging. The potential pSiNutria products being developed include; products to detect pathogens in food, for food tracing, for food preservation, and products to detect variations of temperature in food storage. These products may include ingestible BioSilicon™, which will dissolve into silicic acid in the body or silicon used in modified atmosphere packaging[13].

There is also research under the 'Good Food Project' that has developed a portable, micro-array based hand-held nano-sensor for identification of pathogens, chemical contaminants and toxins in food (Garland, 2004; Joseph and Morrison, 2006; Food Packaging using Nanotechnology Methods, 2004). Other micro-sensors under development will identify pesticides on fruit and vegetables and monitor environmental conditions at the farm. Nanoscale-sensing devices are also under development that, when attached to food products and packaging, will enable the food or food ingredients to be traced back to the source of origin. Radio Frequency Identification Display (RFID) incorporated in smart labels is expected to enable more quick and accurate distribution of products with a limited shelf-life. Also under development are RFID incorporating polymeric transistors that use nanoscale organic thin-film technology. The RFID systems will be designed to operate automatically, and will provide exception reports for anomalies like temperature, short-life span products, etc (Garland, 2004).

2.3.3 Polymer Composites Incorporating Nanoencapsulated Substances

These are polymers that incorporate nano-sized different substances in encapsulated form. They are being developed as part of interactive nanofoods, which would allow consumers to modify the food depending on their own nutritional needs or tastes. Their potential use in anti-bacterial or scented packaging is also being explored. The substances that could potentially be added to nanocapsules include enzymes, catalysts, oils, adhesives, polymers, other inorganic nanoparticles, latex particles, biological cells, flavour and colour enhancers, or nutritional compounds such as vitamins (Garland, 2004).

2.3.4 Dirt Repellent Coatings at Nanoscale

These coatings have been developed by researchers at University of Bonn (Germany) with potential applications in packaging. The dirt repellency is thought to be due to a 'Lotus Effect' (referring to the phenomenon that water beads and runs off the surface of lotus leaves dues to nanoscale wax pyramids which coat the leaves). The projected uses include self-cleaning surfaces that can help prevent growth of microorganisms and ensure food safety, such as in abattoirs and meat processing plants (Garland, 2004; Chaudhry Q., Nanomaterials for Food Contact Plastics. Paper presented at Food Contact Plastics 2006 Conference).

3.0 Potential Hazards and Consumer Safety Issues

In the UK, the median adult intake of fine particles via inhalation has been estimated to be in the region of 10^{12} - 10^{13} microparticles/individual per day (Tran et al 2005). Microparticles may enter the gut mainly through ingestion of food and drinks. However, very little is known about the dietary toxicity of microparticles in the gut, and even less about the toxicity of

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[13] http://www.psivida.com/application/other.asp

nanoparticles (Tran et al, 2005). The main consumer safety implications from a nanotechnology-derived food packaging would be intrinsically linked to the physicochemical properties of nanoparticles, and the likelihood and level of exposure from migration into food and drinks from FCMs or active surfaces. It is known that materials manufactured at nano-scale may have substantially different physicochemical, and biological properties from their conventional forms. This is because conventional physicochemical rules may not be fully applicable at the nanometer size scale. Depending on the size and physicochemical nature of the material, quantum effects may have a much greater influence on the properties of a nanomaterial compared to larger equivalents. Also, on a weight per weight basis, nanomaterials have enormously large surface areas compared to conventional equivalent materials.

Very few studies have been carried out so far into the toxicology of nanoparticles, and their potential harmful effects in biological systems are therefore largely unknown. There is concern that exposure to some of the engineered free nanoparticles may pose unforeseen health or environmental hazards. Such concerns have arisen from a growing body of scientific evidence which indicates that free nanoparticles can cross cellular barriers, and that exposure to some engineered nanoparticles may lead to increased production of oxyradicals and consequently oxidative damage to the cell (Oberdörster, 2000; 2004; Donaldson et al., 2002; 2004, Tran et al., 2005).

Despite claims over the antimicrobial effects of a number of engineered nanoparticles, such as nanosilver, there is currently no published research on the effect that food/ drinks, contaminated due to migration of nanoparticles from packaging, may have on the gastrointestinal tract or on the natural gut microflora. Also, whilst there is some evidence on the potential of certain nanoparticles to cause harm, the likelihood and extent of the consumer exposure due to migration from FCMs into food and drinks is currently unknown. It has also not been known whether the incorporation of nanomaterials into plastic polymers would lead to a greater migration of non-nano components.

Other potential sources of food contamination with nanoparticles may arise from indirect routes; such as through contact of foodstuffs with:

- devices such as catalytic devices used in food processing; such as the frying-oil refining catalytic nanoceramic device - OilFresh™ 1000 by OilFresh® Corporation (USA) - claimed to prolong freshness of the oil used for deep frying;
- self cleaning surfaces, dirt-repellent coatings, active coatings;
- nanosensors and labels incorporated in packaging for tagging and monitoring of food items.

3.1 Migration of Nanoparticles from FCMs

A major unknown that has limited our ability to assess the potential risks from nanotechnology derived FCMs to an average consumer is the likelihood and extent of migration of nano (and non-nano) components of nanocomposites. The need for such testing has been highlighted by a number of researchers (e.g. Lagaron et al., 2005), but there is only one published study by Avella et al. (2005) that has experimentally determined the extent of migration of minerals from biodegradable starch/ nanoclay nanocomposite films, developed for use in food packaging. Briefly, the experimental work involved putting vegetable samples (lettuce and spinach) into bags made of either potato starch, or potato starch-polyester blend, and their respective composites with nano-clay. The bags were heated at 40^{O}C for 10 days, cooled, acclimatised, and migration of minerals determined by atomic absorption method after digestion of the vegetables. The results of the tests indicated an insignificant trend in the levels of Fe and Mg in the vegetables, but a consistent increase in the amount of Si (the main component of nano-clay). The concentrations of Si detected in the vegetables were 16-19 ppm in the case of nanoclay composites of potato starch, and potato starch-polyester bend, compared to 13 ppm for the same polymers without nanoclay, and around 3 ppm in neat vegetables. Whilst this study provides a small piece of the information, it only relates to a biodegradable material, and does not indicate what will be the likely migration of nano (and non-nano) components of plastic polymers more commonly used in making food and drink containers, such as PET, PE, PP.

In view of the scarcity of information on potential migration of nanoparticles from FCMs, experimental work was carried out as part of this study on two nano-polymer composites that are used for food packaging. The main questions on the potential migration of nanoparticles from FCMs have three focal points:

- migration of the nanoparticles themselves into foodstuffs
- migration of any impurities or decomposition products or interaction products formed when the nonmaterial is incorporated into plastics
- enhanced migration levels of ‘normal’ chemical constituents of plastics, such as monomers and additives, brought about by the incorporation of nanomaterials into the plastic

The experimental work was, therefore, designed to establish the migration of nano (and non-nano) components of two representative nanocomposite materials to inform assessment of exposure risks to an average consumer.

3.1.1. Approach

Analytical screening methodologies were applied to the two nanocomposites as well as control plastics (the same polymer type but not containing nanoparticles). The techniques used were:

1. headspace gas chromatography-mass spectrometry (GC-MS) to detect very volatile substances
2. GC-MS to detect semi-volatile substances
3. liquid chromatography (LC)-time of flight (TOF)-MS to detect polar and non-volatile substances
4. inductively coupled plasma (ICP)-MS to detect inorganics.

The rationale of the study was to compare the substances detected in the nanocomposite materials with the similar control plastics to identify any potential migrants in the nanotechnology-derived materials. In this way not only can the nanomaterials (as particulates or solubilised constituents) be identified but also any impurities of the nanomaterials, degradation products, or reaction products formed between nanomaterials and monomers, or any additives present in the host plastic.

3.1.2. Samples

Nanoclay-polyethylene terephthalate (PET) bottles (Figure 1) were obtained from a commercial source in the UK. These typically consist of a modified clay incorporated into a polyamide or a metaxylelene apidamide polymer which is sandwiched between two layers of PET (Fig 2).

Figure 1. Nano-silver polypropylene containers and nano-clay polyethylene terephthalate bottles

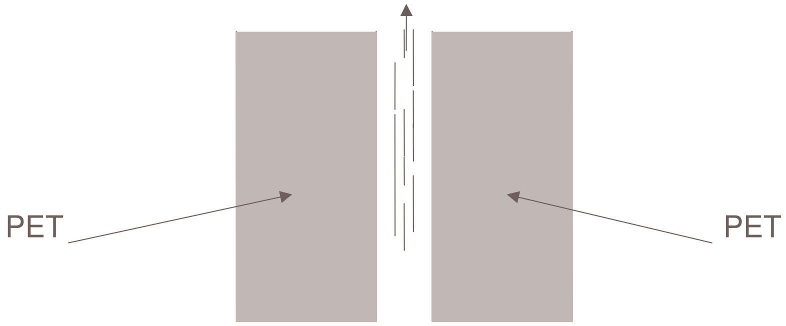

Fig. 2: Polyamide or metaxylelene apidamide polymer containing the nano-clay

Prior to incorporation into the polymer the nano-clay was likely to be modified to render it compatible, by surface modification using 12-amindodecanoic acid. The 12-amindodecanoic acid allows the clay to link into the polymer chains in the polymerisation process. In this way the clay has strong links in the nylon or metaxylelene apidamide polymer. This, along with the fact that the clay-containing polymer is sandwiched between layers of PET, means that this 12-amindodecanoic acid, to which a specific migration limit of 0.05 mg/kg has been assigned, has a limited migration potential. By dispersing the clay in the polymer matrix the nano-clay particles are separated into individual platelets and are exfoliated - i.e. they have a continuous plastic phase between them and they present a barrier to the passage of gaseous molecules. It has also been reported that ‘recycling studies show 93% of the barrier layer can be removed using a standard recycling protocol’ although no evidence was provided.

Nano-silver containing polypropylene (PP) containers were purchased through the Internet from a commercial firm in the USA (Fig.2). Information available on the internet describes the nano-silver PP containers as ‘infused with naturally anti-bacterial silver nano-particles’. No information was provided on the level of nano-silver added nor if the silver was applied mainly at the surface or incorporated uniformly throughout the PP.

Corresponding non-nanoparticle containing materials and articles were obtained from a large supermarket in the north of England.

3.1.3. Analytical screening

3.1.3.1 Sample preparation

3.1.3.1.1 Sample preparation for headspace GC-MS

The samples were cut into small pieces and the pieces were mixed. 0.5 g sub-samples of each polymer were transferred to 10 ml headspace vials and the vials were securely capped. Additional samples were prepared containing 0.5 g of test sample and 10 µl of a 1 mg/ml solution of fluorobenzene in a 10 ml headspace vial. Triplicate samples were prepared.

3.1.3.1.2 Sample preparation for GC-MS

The samples were cut into small pieces and the pieces were mixed. 1 g sub-samples, to which 100 µl of a 1 mg/ml solution of d_{10}-benzophenone was added and allowed to infuse into the polymer, were extracted with isooctane or 95% aqueous ethanol (10 ml) for 24 hours at room temperature. A portion of the extract (0.5 ml) was removed and transferred to a vial for analysis. The remainder was concentrated to a volume of 1 ml and this concentrated extract was transferred to a vial for analysis.

3.1.3.1.3 Sample preparation LC-TOF-MS

The samples were cut into small pieces and the pieces were mixed. 1 g sub-samples, to which 100 µl of a 1 mg/ml solution of d_{10}-benzophenone was added and allowed to infuse into the polymer, were extracted with isooctane or 95% aqueous ethanol (10 ml) for 24 hours at room temperature. Further sub-samples (10 g) were refluxed in water (100 ml) for 24 hours. For isooctane and 95% aqueous ethanol the extraction solvent was transferred to a clean vial and was removed by evaporation under a gentle stream of nitrogen. The residue was reconstituted in acetonitrile and transferred to a vial for analysis. For samples refluxed in water a portion of the water was transferred to a clean vial and analysed directly.

3.1.3.1.4 Sample preparation ICP-MS

Portions (0.05 g) of the polymer were digested in a nitric and hydrochloric acid mixture using quartz high pressure closed vessels and microwave heating prior to quantification by ICP-MS. Reagent blanks, a reagent blank spiked with a known amount of each analyte, and a certified reference material, were analysed with the test samples to estimate recovery and accuracy.

3.1.3.2 Analysis

3.1.3.2.*1* *Analysis by headspace GC-MS*

The samples were incubated for 30 minutes at 100°C. The resulting volatiles were analysed using an Agilent 6980 gas chromatograph (Agilent, Palo Alto, CA, USA) coupled with an Agilent 5973inert mass selective detector by splitless injection of 1 ml of the headspace gas onto an DB-VRX capillary column (30 m x 0.25 mm i.d. x 1.2 µm film thickness; J & W Scientific, Folson, Ca, USA). Following injection, the oven was held at 40°C for 1 minute and then raised at 10°C/minute to 320°C. The injector was held at 250°C. Helium (1 ml/min constant flow) was employed as the carrier gas. The MS was operated in electron impact mode with scanned monitoring between 40 - 450 amu.

3.1.3.2.*2* *Analysis by GC-MS*

The ethanol and isooctane extracts were analysed by GC-MS using an Agilent 6980 gas chromatograph (Agilent, Palo Alto, CA, USA) coupled with an Agilent 5973inert mass selective detector. Splitless injection of 1 µl of extract was carried out into a DB-5MS capillary column (30 m x 250 µm i.d., 0.25 µm film thickness; J & W Scientific, Folson, Ca, USA). Following injection the oven was held at 60°C for 5 minutes and then raised at 10°C/minute to 320°C. The injector was held at 250°C. Helium (1 ml/min constant flow) was employed as the carrier gas. The MS was operated in electron impact mode with scanned monitoring between 40 - 450 amu.

3.1.3.2.*3* *Analysis by LC-TOF-MS*

The extracts were analysed by LC-TOF-MS using an Agilent 6210 LC/MS TOF (Agilent, Santa Clara, California, USA) consisting of a 1200 Series LC and a time of flight mass spectrometer. Two separate LC-MS methods were used in order to increase the coverage of compounds that could be detected in this way. In both cases separation used an Agilent ZORBAX Eclipse XDB-C18 (100 x 2.1 mm, 3.5 µm) column. For positive mode electrospray the mobile phase consisted of 0.1% aqueous acetic acid (channel A) and acetonitrile (channel B). For negative mode electrospray the mobile phase was 5 mM ammonium formate at pH 5.5 (channel A) and 0.1% 5 mM ammonium formate at pH 5.5 in acetonitrile (channel B). The mobile phase gradient for both positive and negative mode electrospray was the same: a starting mixture of 80% A and 20% B that changed to 50% B over 25 minutes. This was held for 20 minutes and then went to 100% B at 60 minutes. This was held for a further 10 minutes before returning to 20% B over 10 minutes. The flow rate was 0.2 ml/min with an injection volume of 5 µl.

TOF-MS analysis was carried out in positive and negative mode electrospray with nebuliser pressure 45 psi, capillary 4000 V, gas temperature 325°C, drying gas flow at 10 l/min, skimmer 60 V, fragmentor 150 V and octopole RF voltage 250 V. The mass range measured was 100 – 1100 m/z. The TOF-MS data produced was processed using Agilent Molecular Feature Editor (MFE) software with signal to noise threshold 50, minimum relative volume 5% and a mass accuracy of 10 ppm. The elements used in the Molecular Formula Database along with the potential number of atoms (in brackets) were C (1-100), H (0-200), O (0-20), N (0-20), P (0-10), S (0-5), Cl (0-5), Si (0-10), Na (0-5) and K (0-1).

3.1.3.2.*4* *Analysis by ICP-MS*

All solutions were measured by flow injection using an Elan6000 ICP-MS, operating with a nebuliser flow rate 1.3-1.5 l/min argon, R.F. power (for the ICP) 1250 Watts. Peak jump acquisition. The instrument was optimised to achieve < 3% oxide formation (based on CeO) and < 1% double charged species (based on Ba2+).

3.1.3.3 Analytical screening results and discussion

Following headspace GC-MS and liquid GC-MS analysis, the resulting chromatograms were studied to identify any potential migrants indicated by the chromatography. Chromatograms were integrated and peaks of interest compared to the d_{10}-benzophenone internal standard used. The concentrations of these substances in the polymers were estimated by comparing the peak area of each substance with that of the internal standard. Peak identification was achieved by comparison of the mass spectrum of each potential migrant with NIST library spectra.

The TOF-MS data produced was processed using Agilent Molecular Feature Editor and Masshunter software. These packages examine the data, extract chromatographic peaks and produce mass spectra for those peaks. Some of the chromatographic peaks seen in the total ion chromatograms (TIC) obtained from the analysis of the extracts are very small, but are elucidated nevertheless, highlighting the power of TOF-MS. Many of the peaks are not visible from the TICs but are extracted from the raw data by the data processing software and their mass spectra were generated. Two user-prepared databases were searched. A database of potential structures was prepared as a part of another FSA funded project (A03054, An investigation into the reaction and breakdown products from starting substances used to produce food contact plastics, 2007). These structures were produced based on known additives and predicted reaction/breakdown products. A database of PET oligomers was also prepared. This contained over one thousand potential PET oligomers of varying lengths, both linear and cyclic, which were theoretically possible from a number of common poly-functional alcohols and poly-functional carboxylic acid starting materials. These databases were used to assign the identities of the chromatographic peaks that were detected. Searches of scientific literature and the Internet were also carried out to propose possible identities for the substances detected in the extracts that could not be assigned from the knowledge of additives and/or reaction/breakdown products. Quantification of the substances detected could not be estimated as the internal standard used (d_{10}-benzophenone) was suited for the GC-MS analysis but not seen in the TIC of the sample extracts. The substances detected using each of the analytical techniques are described below.

3.1.3.3.*1* *Headspace GC-MS analysis*

Nano-silver containers and controls – The chromatograms obtained from the analysis of the headspace gas above the nano-silver containing polypropylene container and the 'control' polypropylene container are shown in Figures 3 and 4 respectively. Many alkanes, straight chain and branched, were detected in the headspace above the two samples. The combined estimated concentrations of these substances are shown in Table 1. Other substances were also detected in the headspace above the samples. The best library matches obtained and their estimated concentrations (per kg of plastic) are shown in Tables 2 and 3. Similar substances were detected in all cases. The differences in the profiles of the two sets of materials are more likely to be due to any differences in the polypropylene grades used rather than the presence of the nano-particles.

Nano-clay bottles and controls – The chromatograms obtained from the analysis of the headspace gas above the nano-clay bottles and the 'control' PET bottles are shown in Figures 5 and 6 respectively. No peaks were detected in the headspace of the nano-clay bottle and only three substances were detected in the headspace of the 'control' PET bottle, ethylbenzene and two xylene isomers. The estimated concentrations (in kg of plastic) are shown in Table 4.

3.1.3.3.2 *Solvent extraction followed by GC-MS analysis*

Nano-silver containers and controls – The chromatograms obtained from the analysis of the isooctane extracts of the nano-silver containing polypropylene container and the 'control' polypropylene container are shown in Figures 7 and 8 and those obtained from the analysis of the corresponding ethanolic extracts are shown in Figures 9 and 10. The majority of the peaks in the chromatograms obtained from the analysis of the isooctane extracts can be assigned to straight chain and branched alkanes as expected for a polyolefin. The combined estimated concentrations of these substances (per kg of plastic) are shown in Table 5. The other substances detected in the isooctane extracts and their estimated concentrations calculated per kg of plastic are shown in Table 6. Polypropylene polymers are non-polar and therefore only little interaction occurs between the polypropylene and the polar ethanol extraction solvent. Therefore the number of extractable substances was much less for this solvent (Tables 7 and 8). As well as little interaction between the polymer and extraction solvent the non-polar alkanes (the main constituents of the isooctane extracts) are less soluble in the polar ethanolic solvent. As for the headspace GC-MS data, the differences in the profiles of the two sets of materials are more likely to be due to differences in the polypropylene rather than a result of the inclusion of the nano-additives.

Nano-clay bottles and controls – The chromatograms obtained from the analysis of the isooctane extracts of the nano-clay bottles and the 'control' PET bottle are shown in Figures 11 and 12 and those obtained from the analysis of the corresponding ethanolic extracts are shown in Figures 13 and 14. Two substances were identified in the isooctane extracts of the nano-clay bottles, nonane and squalene. The estimated concentrations of these are shown in Table 10. Both of these substances were also detected in the isooctane extracts of the 'control' PET bottle (Table 11), with nonane also being detected in the ethanolic extracts. As expected, due to the more polar nature of the PET polymers, a greater number of peaks were detected in the ethanolic extracts of the nano-clay bottles and controls than in the isooctane extracts. The proposed identities and estimated concentrations for these substances are shown in Tables 11 and 12. Of the peaks detected nonane, nonanal, di-(2-ethylhexyl) phthalate and eicosane were detected in both the nano-clay bottles and the 'control' PET bottles. The best library matches for the substances that were detected in the nano-clay bottles only were 1-hexadecene, octadecanal and 4-octadecyl-morpholine. These identities have not been confirmed. The ions expected for 12-aminododecanoic acid, believed to be used to modify the nano-clay to make it compatible with the nylon or metaxylelene apidamide polymer, were specifically searched for in the chromatograms but were not detected. This is not unexpected as this substance is incorporated into the polymer structure and it is 'protected' from the extraction solvent by layers of PET.

3.1.3.3.*3* *Solvent extraction followed by LC-TOF-MS analysis*

As LC-TOF-MS is amenable to aqueous sample compositions, as well as extraction into isooctane and 95% aqueous ethanol, the samples were refluxed in water and analysed in order to increase the coverage of compounds that could be detected. For the same reasons, and because the analysis was for unknown compounds with unknown ionisation potentials, LC-TOF-MS was run in both positive and negative electrospray modes. Procedural blanks were also analysed for each solvent system. All sample chromatograms were compared to the chromatograms for the appropriate solvent blank and any peaks present in both were discounted as procedural contamination.

Nano-silver containers and controls – The results obtained from the analysis of the ethanolic, isooctane and water extracts of the nano-silver containing polypropylene container, the 'control' polypropylene container and the procedural blank sample in both positive and negative mode electrospray ionisation are summarised in Table 14. All the LC-TOF-MS data including mass spectra and predicted molecular formula statistics are given in Appendix 1. Only 2 compounds were exclusively detected in the nano-silver container. One was assigned the proposed identity diethyleneglycol monodecyl ether, a surfactant, and the other, with a mass 636.2990, could not be identified. The range of possible molecular formulae are given in Appendix 1. Other compounds detected in the nano-silver container and also in the polypropylene control container were assigned as dicarboxy-1,10-diphenoxydecane, dibutyl phthalate, dibutyl sebacate, an erucamide related compound, tris(2,4-ditert-butylphenyl)phosphate, an oxidation product of tris(2,4-ditert-butylphenyl)phosphite, and a compound with mass 404.3122, assigned a molecular formula of $C_{22}H_{44}O_6$, for which an identity could not be proposed. All other compounds were found only in the polypropylene control container. No compounds were detected in the water extracts.

Nano-clay bottles and controls – The results obtained from the analysis of the ethanolic, isooctane and water extracts of the nano-clay bottles, the 'control' PET bottle and the procedural blank sample in both positive and negative mode electrospray ionisation are summarised in Table 15. All the LC-TOF-MS data including mass spectra and predicted molecular formula statistics are given in Appendix 2. Fewer compounds were detected in these extracts than with the nano-silver containers. Five compounds were detected in the nano-clay bottles and not the controls. Two compounds were assigned as 3-sulfopropyl dimethyl-3-methacrylamidopropyl ammonium salt and N-(2,6-dimethylphenyl)-2-oxo-1-pyrrolidine acetamide. Identities for the other three, with masses 492.2732, 555.2639 and 369.2048, could not be proposed. Only one other compound (mass 508.1300) was detected, which was present in both water extracts of the nano-clay bottle and the PET control bottle, but again an identity could not be proposed. As for the analysis of the extracts by GC-MS, 12-aminododecanoic acid was specifically searched for in the chromatograms. Again, it was not detected.

3.1.3.3.*4* *Acid digestion followed by ICP-MS analysis*

Nano-silver containers and controls – The concentrations of the trace elements detected in the nano-silver containing PP and the control are shown in Table 16. Due to the relatively high limit of detection for silver in this semi-quantitative analysis another specific analysis of the digest was performed for this element. The results of this analysis are given in Table 17. The average concentration of silver, the active antimicrobial nano-ingredient, was 28 mg/kg. The technique used to perform both the semi-quantitative and specific analysis does not differentiate between nano and non-nano trace elements. Tin and barium were also detected at elevated levels in the nano-silver PP containers (Table 16). These elements may be present as impurities in the silver or it may be that they are ingredients that are intentionally incorporated into the silver to leach out and so expose a larger silver area to give controlled release. Although other trace elements were detected in the digests their concentrations were either less than the limit of quantification or the levels detected in the nano-silver containing PP was not significantly greater (> 3 times higher) than the levels in the control PP.

Nano-clay bottles and controls – The concentrations of the trace elements detected in the nano-silver containing PP and the control are shown in Table 18. From Table 18 the empirical formula of the major elements detected (taking the abundance and adjusting for atomic weight) is $Na_{0.6}\,Ca_{0.8}\,Al_{5.9}\,Mg_{1.0}\,Ti_{0.7}\,Cu_{0.1}$ .The nano-clay incorporated into the PET bottle is understood to be a montmorillonite a natural alumino-silicate clay obtained from volcanic ash / rocks. Chemically, Montmorillonites are sodium calcium aluminium magnesium silicate hydroxide $(Na,Ca)_{0.33}(Al,Mg)_2(Si_4O_{10})(OH)_2{\cdot}nH_2O$. Potassium, iron, and other cations are common substitutes and the exact ratio of cations varies with source. The elements found in the bottles are consistent with this composition although the aluminium level is elevated and this may indicate that another additive is present too. It was not possible to measure silicon due to interferences (from nitrogen) during measurement and non-quantitative digestion. Many other trace elements were also detected in the nano-clay PET that were not in the control. As the montmorillonite is a natural clay their presence is not unexpected. These may be considered as part of the nano-clay itself rather than as impurities within it. Both the nano- and the control PET bottles contained antimony which is a typical catalyst used in the PET polymerisation process.

Excluding antimony, the PET contained about 500 mg/kg of other elements (Table 18). Considering the elemental composition of a typical Montmorillonite (see above) and the fact that neither silicon nor oxygen results are available from the ICP-MS, the level of clay used in the PET bottles can be estimated to be about 2000 mg/kg averaged over the three-layer structure. Clearly the concentration in the sandwiched polyamide or metaxylelene apidamide layer will be higher by a factor depending on the relative thickness of the 3 layers.

3.1.4. Migration into food simulants

3.1.4.1 Selection of substances to measure

Nano-silver containers and controls – From the analytical screening studies (Section 4) only silver was detected at high levels exclusively in the nanomaterials. Therefore this substance was selected for migration studies. As mentioned previously the differences in the GC-MS chromatograms obtained from the analysis of the nano-silver PP containers and the controls were expected to be due to difference in the PP itself rather than the nano-additive and therefore no impurities could be proposed for migration testing. It was considered important to establish whether or not the presence of the nanoparticles in the PP had any effect on the migration of other additives present in the formulation. Of these dibutyl sebacate and tris(2,4-ditert-butylphenyl)phosphite were both detected in the nano-silver and control PP and therefore these two substances were selected to investigate this.

Nano-clay bottles and controls – From the analytical screening studies several trace elements were detected exclusively in the nano-clay containing PET. Therefore the same trace element scan performed on the acid digests was the selected approach here. This allows the migration of the nano-clay (aluminium) itself as well as it’s impurities to be determined as well as investigating the effect of the nano-clay on the extent of the migration of the antimony catalyst to be determined. Very few potential migrants were detected by GC-MS and those that were detected were present at too low levels to offer any potential for measurable migration taking into account the food contact area and mass of simulant required to fill the bottles. Several substances were detected by LC-TOF-MS in the nano-clay PET bottles however their identities could not be confirmed and the levels present were low (although not quantified only small peaks were observed in the TIC) therefore no additional migration tests were proposed.

3.1.4.2 Selection of food simulants and test conditions

The rules for migration testing are enshrined in existing EU legislation (Directive 82/711/EC, as amended) in which the use of food simulants to mimic migration into foods is described along with appropriate test conditions. Which simulant(s) should be used for a given foodstuff is given in Directive 85/572/EEC, as amended. Using this legislation the food simulants, exposure times and exposure temperatures have been selected based on the worst foreseeable conditions of use of the PP containers and the PET bottles.

Nano-silver containers and controls – The PP containers may be used for long term ambient storage of dry non-perishable foodstuffs or for chilled or frozen storage of aqueous, acidic or fatty foods. They may also be used to reheat these foodstuffs. Legislation states that for articles that may be used at a variety of temperatures and for varying times, or their conditions of use are not known, simulants(s) A and/or B and/or C should be used for 4 hours at 100°C or for 4 hours at reflux temperature and/or simulant D shall be used only for 2 hour at 175°C. As metal ions are known to migrate at the highest levels into acidic media then only simulant B (3% (w/v) aqueous acetic acid) was selected as the test media to determine the migration of silver. The migration of dibutyl sebacate and tris(2,4-ditert-butylphenyl)phosphite was determined into simulants B and C (10% aqueous ethanol) as specified in the project proposal. As the containers are intended for repeat use then tests should be carried out three times on a single sample and using fresh simulant each time.

Nano-clay bottles and controls – The PET bottles are intended for contact with alcoholic or acidic beverages for long-term storage at ambient temperature. Corresponding test conditions are 10 days at 40°C. However as the aim was to demonstrate the worst case then test conditions of 4 hours at reflux temperature were selected.

3.1.4.3 Exposure to food simulants

Due to limited material being available all migration tests were carried out by total immersion on representative portions of the selected articles.

Nano-silver and control PP, migration of dibutyl sebacate and tris(2,4-ditert-butylphenyl)phosphate – The samples were cut into small pieces and the pieces were mixed. 20 g sub-samples (equivalent to 2 dm^2) were exposed to 50 ml of simulant (either 3% acetic acid or 10% ethanol) for 4 hours at 100°C. A reduced volume of simulant, compared to the conventional 1 dm^2 to 100 ml polymer ratio, was used to provide more concentrated migration test solutions to increase the chances that any migration that occurs would be measurable. Three successive exposures were carried out using fresh simulant.

Nano-silver and control PP, migration of metal ions – 10 g sub-samples (equivalent to 1 dm^2) were also exposed to 100 ml of simulant (3% acetic acid) for 4 hours at 100°C. It has been reported that silver is not readily taken-up by organic acids such as acetic acid therefore only a single exposure was performed as if migration of the nano-silver present on the surface of the PP did occur then it was expected that it would do so at the highest levels during the first exposure. If silver or other metal ions had been detected (Section 5.5) then further exposures would have been performed.

Nano-clay and control PET, migration of metal ions – The samples were cut into small pieces and the pieces were mixed. 10 g sub-samples (equivalent to 2.5 dm^2) were exposed to 100 ml of simulant (3% acetic acid) for 4 hours at 100°C.

3.1.4.4 Analysis

3.1.4.4.1 Analysis of dibutyl sebacate and tris(2,4-ditert-butylphenyl)phosphite

The extracts were analysed by LC-UV using an Agilent 1100 LC-UV system (Agilent, Santa Clara, California, USA). For both analytes separation was facilitated using a Waters (Hertfordshire, UK) XBridge C18 (150 x 4.6 mm, 5 µm) column. For dibutyl sebacate analysis the mobile phase consisted of water (channel A) and methanol (channel B) with a gradient starting at 80% B, held for 5 minutes and then changed to 100% B over 10 minutes. A post run equilibration time of 5 minutes was used. The flow rate was 1.0 ml/min with an injection volume of 25 µl. UV absorbance detection was carried out at 220 nm with reference at 360 nm. For tris(2,4-ditert-butylphenyl)phosphite analysis the isocratic mobile phase was 95% aqueous ethanol for 10 minutes. The flow rate was 0.6 ml/min with an injection volume of 25 µl. UV absorbance detection was carried out at 230 nm with reference at 360 nm.

3.1.4.4.2 Trace element analysis

Aliquots (2.5 ml) of the exposed acetic acid were diluted with dilute nitric acid (2.5 ml) prior to quantification by ICP-MS. Reagent blanks and selected test samples spiked with a known amount of each analyte were analysed with the test samples for recovery estimate purposes. ICP-MS analysis was performed as described in Section 4.2.4.

3.1.4.4.3 Results and discussion

Nano-silver and control PP – There was no measurable migration of dibutyl sebacate or tris(2,4-ditert-butylphenyl)phosphite into the two simulants tested. Detection limits were equivalent to 0.625 and 0.018 mg/dm^2 respectively. The detection limit for dibutyl sebacate was rather high because this substance does not have a strong chromophore for LC-UV analysis. It is concluded that the presence of the nano-silver does not result in a measurable increase in migration of either substance.

There was no difference in the levels of silver detected in the acetic acid exposed to the nano-silver PP compared to the control. It would be expected that nano-silver would be atomised and detected in the plasma if this ion was present. The same was also true for the tin and barium which were also detected at in the acid digests of the nano-silver PP. As mentioned previously it has been reported that silver is not readily dissolved by organic acids such as acetic acid so this was not unexpected. As in all migration testing the most important parameter to measure is the actual concentration of a given substance in a foodstuff. Although the silver ions are not readily extracted into acetic acid this may not be the case for an acidic foodstuff and therefore migration tests into foods were also carried out (Section 6).

Nano-clay and control PET – The results of the acid digestion of the nano-clay and control PET samples identified high levels of several trace elements in the nano-clay containing polymer. This was not the case for the exposed acetic acid simulant. The levels of these trace elements detected in the exposed simulant were of the same order of magnitude irrespective of the material. PET exhibits low diffusivity and the nano-clay containing polymer layer of this multi-layer plastic is sandwiched between two layers of PET. When subjected to acid digestion the strong acids attack the external layers to such an extent that the nano-clay containing layer is also attacked. This is not the case with the acetic acid which only interacts weakly with the sample. Despite the small nano-scale size of the clay it appears that the PET layers do act as a barrier to the migration of the elements.

Antimony was found at virtually identical levels (Table 18) in both the nano-clay PET bottle and in the control PET samples and the migration of antimony was measured into acetic acid. Antimony migration was 33 µg/L from the nano-clay PET and 67 µg/L from the control PET. These levels represent a worst case as the polymer was exposed by total immersion, including cut-edges. Taking into account the area of polymer exposed (2.5 dm^2) and the volume of simulant used (100 ml) then these concentrations are equivalent to migration values of 1.3 and 2.7 $\mu g/dm^2$ for the nano-clay and control PET samples respectively. Therefore the presence of the nano-clay did not increase the extent of the migration of this catalyst. In fact a lower migration level was observed. As a result of these findings migration testing into food was not considered to be necessary.

3.1.5 Migration into food

3.1.5.1 Exposure

As mentioned above it has been reported that silver is not readily dissolved by organic acids such as acetic acid. Therefore migration of this element into foods was determined. Worst foreseeable foods and contact conditions were selected (microwave reheating of acidic foods) such that the worst case migration could be ascertained. 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. Triplicate articles were exposed to each foodstuff and for the apple sauce (expected to be the worst case) the articles were exposed to three fresh portions of food as these are repeat use articles. For the pizza and lasagne only one of the three articles was exposed three times.

3.1.5.2 ICP-MS analysis

Aliquots (0.5 - 1 g) of foodstuff plus a certified reference material were digested in a nitric and hydrochloric acid mixture using quartz high pressure closed vessels and microwave heating prior to quantification by ICP-MS. Reagent blanks and a reagent blank spiked with a known amount of each analyte were analysed with the test samples for recovery estimate purposes. ICP-MS analysis was performed as described in Section 4.2.4.

3.1.5.3 Results

Using a method of analysis with a limit of detection of 1 µg/kg silver was detected albeit at levels less than the limit of quantification (1 and 2 µg/kg) in two of the three apple sauce samples on the first exposure. No silver was detected in the pizza. The third successive exposure to the lasagne gave detectable levels of silver (1 µg/kg) but again this was less than the limit of quantification. A replicate analysis of this sample gave a higher result which draws into question the significance of the migration concentration. Either way only low or non-detectable migration was observed. This suggests that even though the silver is present on the food contact surface, where it is required to fulfil its antimicrobial function the migration levels are low.

Since the food samples were acid-digested before ICP-MS analysis the test results for silver include any migration of solubilsed silver plus any nano-particulate silver as such. The 1 µg/kg detection limit can be expressed in terms of the number of nanoparticles. Taking for the purposes of exposition a particle size of 50 x 50 x 50 nm (giving each a volume of 1.25E-16 cm^3) and an effective density the same as bulk silver (say 10 g/cm^3, giving each a mass of 1.25E-15 g) then the LoD equates to (1E-6/1.25E-15) equals 8E8 particles per kg of food.

3.1.6. Electron Microscopy

The results of the migration study showed that whilst the currently available methods are adequate for detection and quantification of nanoparticles migrating from FCMs, they are not yet sensitive enough to enable determination of the size and shape of nanoparticles, especially when they are in a very low concentration or are in a matrix.

For this study, attempts were made to image nanoparticles in aqueous extracts prepared from the two study FCMs (nanoclay-PET bottles, nanosilver-PP containers), using electron microscopy coupled with analysis by energy dispersive electron (EDX) analysis. The results did not show any nanoparticles in the extracts, but this could be due to limitations of the technology rather than the absence of nanoparticles. Attempts were also made to image nanoparticles within FCM matrices under water/wet conditions but this also did not prove successful, probably because materials are subjected to very high charging effects under the microscope, and the carbon backbone of FCMs is not electron dense enough to allow proper imaging. Thus there are a number of technical limitations to the currently available imaging techniques, and a great deal of experimental work remains to be done to overcome these difficulties and limitations that have been highlighted in a recently published review by Tiede et al. (2008).

3.1.7. Conclusions

Screening ICP-MS analysis of nanoparticle containing plastic food contact articles facilitated the detection of the nano-particles themselves. There was no measurable migration of the nano-silver or of the trace elements found to make up the nano-clay into food simulants. As silver is not readily dissolved by organic acids such as the recognised acidic simulant (3% aqueous acetic acid) then migration into foods was also investigated. Low levels (< 2 µg/kg) of silver were detected in apple sauce microwave heated in the nano-silver containing PP articles but these concentrations are less than the method limit of quantification. No impurities or reaction/breakdown products were identified in the extracts high enough to be measurable in a migration experiment. The extent of the migration of additives included in the PP formulation (and also present in the control PP) was not influenced by the presence of the nano-silver. The extent of the migration of the antimony catalyst included in the PET formulation (and also present in the control PET) was not influenced by the presence of the nano-clay.

Therefore for the two materials studied migration of the nano-particles was low or not detectable. For the nano-clay containing PET the nano-clay layer is understood to be sandwiched within layers of PET. Any migration would require the nano-clay to first diffuse through the PET and then into the food simulant. This was not the case under the conditions investigated. What has not been investigated here is whether or not the same results (i.e. no measurable migration) would be obtained if the nano-clay was mixed into the food contact surface of the PET as it may be if these bottles entered the PET recycling chain.

In either of the FCMs tested, the presence of nanoparticles did not affect migration of non-nano components. Whilst this study has generated some reassurance in the safety of nanotechnology-derived FCMs, it has also shown that migration of nanoparticles is likely to be dependent on the type and composition of the polymer. There is thus a need for testing a few more types of nanotechnology-derived polymer composites to build a broader picture in regard to the potential nanoparticle migration from FCMs. More details of the results of migration study are given in Annex-A.

See Annex A

4.0 Adequacy of relevant regulations and implications of nanotechnology-derived FCMs

The Food Standards Agency (FSA) has published a (draft) review that considers the implications arising from nanotechnology applications in food for regulatory and risk assessment frameworks (Draft report of FSA regulatory review, 2006). Another regulatory gap study by Chaudhry et al. (2006) has assessed the existing EU regulatory frameworks in relation to a wider range of known and projected products and applications of nanotechnologies (including food and food packaging).

It emerges from these reviews that most nanotechnology applications in food would come under some sort of approval process. The FSA (draft) review further concludes that the existing models for risk assessment should be applicable to nanomaterials, but there are major gaps in information on hazard and exposure.

4.1 Regulatory Aspects Relating to Products and Applications of Nanotechnology for FCMs

The main regulatory controls governing the composition, properties and use of food contact materials or articles in the UK stem from Regulation (EC) 1935/2004. Other EU legislation that impacts appreciably on food contact materials includes Commission Directive 2002/72/EC, followed by a number of Regulations and Directives that deal other aspects of the control of food contact materials. These Regulations and Directives, along with perceived regulatory gaps relating to nanomaterials, are dealt with separately below.

4.1.1 Regulation (EC) 1935/2004

The main statutory instrument controlling the use of food contact materials in the EU is *Regulation (EC) No.1935/2004 on materials and articles intended to come into contact with food*. This framework Regulation aims to ensure the effective functioning of the internal market in relation to the placing on the market of materials and articles that are intended to be brought into contact with food, or can reasonably be expected to be brought into contact with food, or that might reasonably be expected to transfer their constituents to food, whilst providing the basis for securing a high level of protection of human health and the interests of consumers.

Regulation (EC) 1935/2004 concerns all materials which are intended directly or indirectly to come into contact with foodstuffs, including all types of packaging, bottles (plastic and glass), cutlery, domestic appliances (e.g. coffee makers) and even adhesives and inks for printing on labels or directly onto packaging.

The Regulation applies, *inter alia*, to the use of:

- ‘active food contact materials and articles’, where this refers to materials and articles that are intended to extend the shelf-life or to maintain or improve the condition of packaged food. Such materials are designed to deliberately incorporate components that release or absorb substances into or from the packaged food or the environment surrounding the food; and
- ‘intelligent food contact materials and articles’, where this refers to materials and articles which monitor the condition of packaged food or the environment surrounding the food.

The principle underlying this Regulation is that 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. The Regulation recognizes that active and intelligent food contact materials are not inert by design, unlike traditional food contact materials, and therefore addresses the main requirements for their use. The Regulation distinguishes between new active food contact materials and articles and materials and articles that have been traditionally used to release their natural ingredients into specific types of food during the process of their manufacture, such as wooden barrels. Active food contact materials and articles may change the composition or the organoleptic properties of the food only if the changes comply with the Community provisions applicable to food, such as the provisions of Directive 89/107/EEC[14] on food additives. The Regulation does not apply to covering or coating materials forming part of the food and possibly being consumed, but does encompass covering or coating materials which do not form part of the food and are not intended to be consumed (e.g. those that cover cheese rinds).

Of the sixteen provisions in Regulation (EC) 1935/2004, Article 3 is of most interest since it states the general requirement that all food contact materials and articles must be safe. Other provisions of particular interest in this report, apart from the general requirement already discussed, relate to:

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[14] Council Directive 89/107/EEC of 21 December 1988 on the approximation of the laws of the Member States concerning food additives authorised for use in foodstuffs intended for human consumption (OJ L 40, 11.2.1989, p. 27). Directive as last amended by Regulation (EC) No 1882/2003.

- Active and intelligent systems;
- Labelling of active and intelligent food contact materials and articles;
- Traceability of materials and articles intended to come into contact with food;
- Declaration of compliance;
- A description of the procedure for the authorization of substances;
- Safeguard, inspection and control measures;
- Specific measures.

In summary those provisions require that:

Active and intelligent systems - active and intelligent food contact materials and articles should not change the composition or the organoleptic properties of food or give information about the condition of the food that could mislead consumers. For example, they should not release or absorb substances such as aldehydes or amines in order to mask an incipient spoilage of the food. Similarly, such materials and articles which produce colour changes to the food that give the wrong information concerning the condition of the food are forbidden. Any change to the food must comply with EC provisions applicable to food.

Labelling – adequate labelling or other such information should support users in the safe and appropriate use of active materials and articles in compliance with food legislation. In the case of active materials and articles, information must be provided on the permitted use or uses and other relevant information such as the name and quantity of the substances released by the active component. This is to enable food business operators who use these materials and articles to comply with any other relevant Community or national provisions applicable to food and food labelling.

Traceability - the traceability of materials and articles must be ensured at all stages in order to facilitate control, the recall of defective products, consumer information and the attribution of responsibility. To fulfil this requirement business operators must have appropriate systems and procedures in place to trace products, and the information must be made available to the competent authorities on demand.

Declaration of compliance - materials and articles covered by specific measures (see ‘Specific measures’ below) shall be accompanied by a written declaration stating that they comply with the rules applicable to them, and appropriate documentation must be available to demonstrate such compliance.

Safeguard, inspection and control measures – if, as a result of new information or a reassessment of existing information, a Member State has grounds for concluding that the use of a material or article endangers human health, it may temporarily suspend or restrict the sale or use of that material or article within its territory. The Commission will examine these grounds and take appropriate measures. In addition Member States are required to carry out official controls in order to enforce compliance with the Regulation.

Specific measures – as well as setting out the main requirements for food contact materials, the Regulation also stipulates that a positive list of authorised substances may be established (excluding all other substances) when drawing up specific regulations for each type of material, and it will be necessary for substances to undergo a safety assessment before they are placed on a positive list. Such groups of materials and articles include, for example, ion-exchange resins, plastics, printing inks and metals and alloys, and measures may include: a list of substances authorised for use in manufacturing, purity standards for such substances, specific limits on the migration of certain constituents into or on to food, an overall limit on the migration of constituents into or on to food and provisions aimed at protecting human health against hazards arising from oral contact with materials and articles.

In England The *Materials and Articles in Contact with Food Regulations (England) 2005* provide for the enforcement of Regulation EC 1935/2004. They apply to all materials and articles within the scope of EC 1935/2004 and contain the specific requirements for materials other than plastics.

4.1.2 Potential regulatory gaps in relation to Regulation (EC) 1935/2004

Regulation (EC) 1935/2004 is inclusive in that it deals with any food contact material or article that may transfer its constituents into food with deleterious results, rather than dealing with specific components/ materials or types of component/ material. As such it is broad enough to encompass the migration of 'nanocomponents' into food from food contact materials and articles. However it only precludes the use of such materials or articles where they are transferred 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, where they might mislead the consumer. This implies, therefore, that the transfer rate and the properties of the substance have to be known. The 2004 Royal Society report recommends that chemicals in the form of nanoparticles be treated as new substances under the existing Notification of New Substances (NONS) regulations and the Regulation concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Thus in order for Regulation (EC) 1935/2004 to be effective in terms of any potential risk from nanocomponents, pro-active testing of such components has to be carried out in order to identify their potential hazard and determine any dose response. The legislation places the onus squarely on the shoulders of manufacturers to ensure the safety of their products. Thus the gap is not necessarily a regulatory one, but potentially one of compliance by manufacturers if they have not carried out proper risk assessment based on data for migration, toxicity and intake. Given this, it should not be necessary to regulate separately for nanosubstances.

Another potential regulatory gap concerning Regulation (EC) 1935/2004 is that the regulatory conditions it imposes are applicable only under 'normal or foreseeable conditions of use'. It is possible, therefore, that food contact materials may be used outside their normal scope of operations. For example, they may be subjected to extremes of temperature that would not normally be envisaged, or come into contact with certain food types (e.g. those with high fat content) for which they were not designed. This may lead to a change in the properties of nanomaterials resulting in, for example, increased migration rates and/or increased bio-availability of potentially harmful substances. It is, therefore, necessary that manufacturers ensure their customers are aware of the technical limitations of the product.

4.2 Legislation relating to specific materials and articles in contact with food

A number of materials and articles, including plastics, epoxy derivatives, regenerated cellulose film and ceramic articles, are subject to specific regulations. The regulations covering these materials and articles, and any potential regulatory gaps, are set out below:

4.2.1 Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with foodstuffs

Directive 2002/72/EC (as amended) applies to materials and articles that consist exclusively of plastic and are intended to come into contact with foodstuffs. It specifies maximum migration limits to foodstuffs and includes a positive list of monomers and other starting substances that may be used in the manufacture of plastic materials and articles. Additives that are incorporated into plastics to achieve a technical effect in the finished product are currently subject to an open list system, which stipulates the restrictions and/or specifications on their use. However, a closed list system is being introduced. Directive 2002/72/EC (as amended) does not apply to materials and articles composed of several layers in which one or more layers do not consist exclusively of plastics, even if the layer intended to come into direct contact with the food consists exclusively of plastics. In Great Britain, these rules are implemented by *The Plastic Materials and Articles in Contact with Food (England) (No 2) Regulations 2006* (see below).

4.2.2. Commission Regulation (EC) No 1895/2005 on the restriction of use of certain epoxy derivatives in materials and articles intended to come into contact with food.

Regulation (EC) No. 1895/2005 is directly applicable throughout the EU and permits the use of 2,2-bis(4-hydroxyphenyl) propane bis(2,3-epoxypropyl) ether (BADGE) in all food contact plastics, adhesives and coatings provided that any migration is within specific limits (measured in mg/kg of food or food simulant, or mg/dm^2 in the case of containers, films, etc.). The Regulation re-affirms the ban on the use of BFDGE (bis(4-hydroxyphenyl)methane, bis (2,3-epoxypropyl)ethers and NOGE (novolac glycidyl ethers) and, in the case of materials and articles containing BADGE and its derivatives, products must be accompanied by a written declaration of compliance at the marketing stage. Compliance must be documented and the documentation must be made available to the competent authorities on demand. In Great Britain, these rules are implemented by *The Plastic Materials and Articles in Contact with Food (England) (No.2) Regulations 2006* (outlined below).

4.2.3 The Plastic Materials and Articles in Contact with Food (England) Regulations 2008

Applicable in England, *The Plastic Materials and Articles in Contact with Food (England) Regulations 2008* implement a number of EU Directives (including *Directive 2002/72/EC* - see above, *Council Directive 82/711/EEC laying down the basic rules necessary for testing migration of the constituents of plastic materials and articles intended to come into contact with foodstuffs*, as amended, and *Council Directive 85/572/EEC laying down the list of simulants to be used for testing migration of constituents of plastic materials and articles intended to come into contact with foodstuffs*). The aim of the Regulations is to simplify the rules on food contact plastics by revoking a suite of older Regulations and consolidating them into one set of comprehensive Regulations on food contact plastics. Amongst other things the Regulations prohibit specific activities in relation to plastics that fail to meet appropriate required standards, prohibit the use of monomers and additives in the manufacture of plastics other than in accordance with specified conditions, stipulate required standards relating to the capability of a monomer or an additive to confer its constituents to food and specify the overall migration limits from plastic materials or articles to food. The regulations also provide for the enforcement of *Commission Regulation (EC) No 1895/2005 on the restriction of use of certain* *epoxy derivatives in materials and articles intended to come into contact with food*. The devolved administrations, Scotland, Wales and Northern Ireland have made separate but parallel legislation.

4.2.4 Commission Directive 2005/31/EC amending Council Directive 84/500/EEC

Commission Directive 2005/31/EC amending Council Directive 84/500/EEC as regards a declaration of compliance and performance criteria of the analytical method for ceramic articles intended to come into contact with foodstuffs. Directive 2005/31/EC (amending Council Directive 84/500/EEC which sets a positive list of authorised substances), requires that manufacturers of ceramic food contact materials provide a written declaration of compliance confirming that the article meets the relevant requirements with regard to lead and cadmium migration limits. The Directive also specifies the method of analysis for lead and cadmium, the migration limits of which are laid down in Directive 84/500/EEC *on the approximation of the laws of the Member States relating to ceramic articles intended to come into contact with foodstuffs*. Documentation on the results of the chemical analysis must be made available to the competent national authorities on request. In England *The Ceramic Articles in Contact with Food (England) Regulations 2006* transpose Council Directive 84/500/EEC, as amended by Directive 2005/31/EC.

4.2.5 Potential regulatory inadequacies

The provisions in the legislation for plastic materials and articles in contact with food make no distinction between macro- and nano-scale components, thus the recommendation by the Royal Society (2004) that nanomaterials should be treated as new chemicals (or new substances) has not yet been implemented in the legislation. Substances such as monomers and starting substances that must be included in a positive list have restrictions imposed on their migration into foodstuffs, which will also be applicable in terms of nanomaterials. However such safe maximum migration limits have been determined for macro-components and may not apply in the case of their nano-equivalent substance, due to possible differences in their physical or biological properties. Additives to the polymer in order to achieve a technical effect are currently subject to an open list system, and although this is due to change in the future the Commission has not set a date for this. In the case of substances not included in the lists in the legislation (i.e. those that directly influence the formation of the polymer, colorants and solvents), they are subject to the requirements of the general Regulation (EC) 1935/2004 described above.

A potential regulatory gap concerning Commission Regulation (EC) No 1895/2005 on the restriction of use of certain epoxy derivatives is that it does not apply to storage tanks having a capacity greater than 10,000 litres or to certain pipelines connected with them. It follows that if nanocomponents were to diffuse from large capacity tanks into food the dilution factor would be considerable due to the small surface area to volume ratio, hence any risk is likely to be minimal. When it comes to pipelines, which will have a relatively large surface area to volume ratio, the same may not be true and the migration of nanoparticles may be significantly greater.

Directive 2002/72/EC (as amended) relating to plastics does not apply to materials and articles composed of several layers in which one or more layers do not consist exclusively of plastics, even if the layer intended to come into direct contact with the food consists exclusively of plastics. In the context of nanocomponents it is possible to foresee situations where multi-layered materials or articles that do not wholly consist of plastics, but do contain nanocomponents, come in contact with food. Such materials or articles would not be covered by Directive 2002/72/EC, although they would still come under the auspices of Regulation (EC) 1935/2004.

4.3 General legislation relating to materials and articles in contact with food

EC legislation includes a number of Regulations and Directives concerned with general food safety and consumer health protection. Most of these are specific to food, although some are wide-ranging enough to encompass food contact materials. General legislation which may impinge on materials and articles in contact with food is set out below:

4.3.1 EC Food Law Regulation 178/2002

EC Food Law Regulation 178/2002 *laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety*, provides the basis for the assurance of protection of human health and consumers' interest in relation to food. It lays down the general principles and requirements of food law and establishes procedures in matters relevant to food safety, including common principles and responsibilities. Its main tenet is that food must not be placed on the market if it is unsafe i.e. if it is harmful to health and/or unfit for consumption. In determining whether any food is unsafe, consideration is given to, *inter alia*, the likely immediate or delayed effect on health and the cumulative toxic effects. The Regulation applies to all stages of production, processing and distribution of food and feed. In the UK *The Food Safety Act 1990* (as amended) and the *General Food Regulations 2004* align domestic legislation with the general principles and requirements of Regulation 178/2002.

4.3.2 Commission Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs

The intention of Commission Regulation 466/2001 is to provide consumers with an increased measure of protection by setting maximum levels for a number of specified contaminants in relation to certain specified categories of food. Substances that are restricted in terms of quantity present in food include heavy metals (lead, cadmium and mercury) environmental chemical contaminants (dioxins, nitrate) and mycotoxins (aflatoxins and patulin). In England *The Contaminants in Food (England) Regulations 2005* make provision for the execution and enforcement of Commission Regulation (EC) 466/2001.

4.3.3 Potential regulatory inadequacies in relation to general food law

Due to the inclusive nature of EU Food Law Regulation 178/2002, which requires all food placed on the market to be safe, the general safety articles embodied therein will, by implication, impact on food contact materials, including those containing nano-materials. Specifically, food would not be eligible for marketing if it contained substances harmful to health derived from food contact materials. Despite this, however, a degree of uncertainty remains over who is ultimately responsible for ensuring that the final food is safe – is it the food contact materials manufacturer or the food processor? Regulation 178/2002 does not make this clear. Another potential gap exists in Regulation 178/2002 due to the fact that primary production, preparation and consumption for private domestic use are exempted when it comes to matters of food safety. In actuality it is highly unlikely that food contact materials containing nanocomponents will come into contact with food in a private domestic situation, unless such materials have already been approved under Regulation (EC) 1935/2004.

In terms of Regulation (EC) 466/2001, it is unlikely that nanomaterials used in the production of food contact materials would include those specified in the Regulation (i.e. heavy metals, environmental contaminants and mycotoxins). Even if these materials were employed, it is unlikely that they would be present in quantities above the threshold limits, which are set in the order of mg/kg.

4.4 Summary of Regulatory Uncertainties

It is clear from the assessment of the relevant EU and national legislation that most food contact materials that utilize nanotechnology will be subject to some form of assessment process before being permitted for use. However, an ongoing problem concerning nanotechnology is the general lack of knowledge relating to the potential health risks of nanomaterials, due to their distinctly different properties compared to macromaterials. This conclusion is in agreement with the FSA (draft) review (Draft report of FSA regulatory review, 2006). Current legislation pertaining to food contact materials does not differentiate between substances produced routinely by ‘standard’ manufacturing methods and those that may be developed by nanotechnology (although there is some differentiation in the food additives area, for example minimum particle size in the case of microcrystalline cellulose).

Currently there is no scope in the European legal framework for food contact materials and articles for the development of specific measures to deal with ‘nanocomposites’ as separate class of material. However, Regulation (EC) no.1936 (2004) does contain provision for substances used in materials covered by specific measures to be listed. This could, of course, include nano-substances. In a joint statement on nanomaterial toxicology issues the independent Committees on Toxicity, on Mutagenicity and on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COT, COM, COC)[15] the Committees stated that there was no need to develop a new approach to risk assessment of nanomaterials but there was a clear need to provide hazard identification data on the widest possible range of nanomaterials. In the absence of such data the committees stated that it was not possible to derive conclusions about the spectrum of toxicological effects that might be associated with nanomaterials.

A working document is currently being discussed with the European Commission and other Member States that may become a proposal for rules on substances and materials that are problematic and not dealt with elsewhere in the legislation (including that relating to FCMs). Until such legislation is completed and adopted the products of nanotechnology will continue to be dealt with by a combination of general EU food law and more specific controls on particular materials and articles. Whether or not this is satisfactory in terms of risk to human health depends on the degree of difference between the nanomaterials and their macro-equivalents. Specific legislation dealing with nanocomponents in food contact materials is only likely to be made if there is sound scientific evidence to show that nanomaterials present a higher risk than macro-materials. Until an adequate assessment is carried out on the risks posed by nano-scale components it is not possible to say whether such materials should be treated differently from their macro-scale counterparts, and the fundamental question of whether regulatory gaps exist remains largely academic.

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[15] http://www.food.gov.uk/multimedia/pdfs/cotstatements2005nanomats.pdf

4.5 The Precautionary Principle in Relation to Nanotechnology Derived FCMs

In the absence of detailed toxicological data, but in view of the potential of nanoparticles to cause harm, it is also appropriate to consider applying the precautionary principle (PP) to applications of nanotechnology in food/ food packaging. The PP is a well-accepted tenet of international law, and is an attempt to legally codify the maxim "better safe than sorry". Originally applied in the EU in terms of environmental protection, it has since been accepted that its scope is much wider, and can be applied to the protection of human health. In 2000, the EC adopted a Communication on the use of the PP (Communication from the Commission on the precautionary principle', 2000), which provides a reasoned and structured framework for action in the face of scientific uncertainty or absence of scientific consensus. The Communication gives grounds for assigning responsibility for producing the scientific evidence necessary for a comprehensive risk assessment. Recourse to the PP presupposes that potentially harmful effects deriving from a product or process have been identified, and that existing scientific evaluation does not allow the risk to be determined with sufficient certainty.

The PP is also incorporated into EU food law in Article 7 of Regulation 178/2002, which states that where, following an assessment of available information, the possibility of harmful effects on health is identified but scientific uncertainty persists, provisional risk management measures necessary to ensure the high level of health protection chosen in the Community may be adopted, pending further scientific information for a more comprehensive risk assessment. However, despite the preliminary evidence, which suggests that certain engineered nanoparticles have the potential to cause harm to human health, it is not clear at present whether there is enough scientific basis to invoke the PP in all applications of nanotechnology for food contact materials. A 2006 IFST report[16] has recommended that nanoparticles be treated as new, potentially harmful materials, until testing proves their safety (The Institute of Food Science and Technology Report). More research is needed to provide a better understanding of the level of risk, but it would be prudent to consider application of the PP in certain high-risk applications. These would include where there is evidence of migration of free engineered nanoparticles into food/ drinks from an FCM, and where such foods/ drinks are likely to be consumed in relatively large quantities, or by a large proportion of the population, or by a particularly vunerable group of the population such as infants and young children.

4.6 The EFSA Opinion on Nanotechnology Applications

The European Food Safety Authority (EFSA) has recently issued their first opinion on a nanomaterial applied as a coating on a food contact material. The material in question is silicon dioxide coating (SiOx) formed from the monomers hexamethyldisiloxane and hexamethyldisilazane that are used to make a silicon dioxide coating *in situ* on the inner surface of PET articles. The coating is intended to provide gas barrier properties and the maximum thickness is 100 nm. The final articles are intended for all types of food, including hot fill and pasteurization at temperatures up to 95°C, and long-term storage at room temperature.

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[16] www.ifst.org/uploadedfiles/cms/store/ATTACHMENTS/ResponseFSA_NanotechnologiesT405app.pdf

According to the EFSA document the data provided with the submission included:

Physical / Chemical data: identity, physical and chemical properties, hydrolysis studies, use and authorization, analysis for migrateable oligomers or other reaction products, determination of residual content.

Toxicity data: gene mutation in bacteria (2 assays), *in vitro* mammalian cell gene mutation test, chromosome aberration tests (2 assays), alkaline elution assay, sister chromatid exchange assay, *in vivo* mammalian bone marrow chromosome aberration test, and cytotoxicity test in mouse lymphoma cells

The evaluation by EFSA considered that hexamethyldisilazane is not stable in moist air or in aqueous media and is converted rapidly into hexamethyldisiloxane. The residual content of hexamethyldisiloxane in PET bottles treated with both monomers to give a 43 nm barrier coating was 0.9 microgram per 6 dm^2. No other constituents with a molecular mass below 1000 g/mol were detected, using LC-MS. Overall migration was not determined, but given the very low thickness of the coating this information is not required

The EFSA opinion showed that, based on the data provided, the substance is classified as SCF_List: 3[17], with restrictions to 0.05 mg/ kg of food (measured as hexamethyldisiloxane), and only to be used as a surface treatment agent on PET. An added remark for the Commission says ‘only a method of analysis for the determination of the residual content of hexamethyldisiloxane on the treated surface is provided’. The document also did not identify any requirement for more data or information. Other (non-nano) components considered were Bis(2,6-diisopropylphenyl)carbodiimide, 89120 (stearic acid, butyl ester) and 70480 (palmitic acid, butyl ester).

An important aspect of this first EFSA opinion on a nanomaterial in an FCM is the statement ‘*overall migration was not determined but given the very low thickness of the coating this information is not required*’. As this may have bearing for future assessments on other nanomaterial based coatings on FCMs, we will consider this opinion, along with the information generated by our migration studies, to draw conclusions in relation to consumer safety and regulatory implications of the nanotechnology-derived coatings on FCMs.

Another FCM containing nano-sized titanium nitride (TiN) is currently under consideration by EFSA (personal communication). Whilst EFSA opinion is awaited, we understand that the nanomaterial is not applied as a coating, but is dispersed in the polymer matrix. It will therefore be interesting to see if migration data is asked for in this case, as the amount of nanoparticles in the polymer matrix will be relatively much greater than in a nano-coating.

An EFSA Working Group on nanotechnology is currently collating scientific information and evidence to submit their opinion on nanotechnology applications for food/ packaging. The outcome of the discussions, and the opinion that the Working Group submits to the European

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[17] List 3 Substances for which an ADI or a TDI could not be established, but where the present use could be accepted. Some of these substances are self-limiting because they are organo-leptic or are volatile and therefore unlikely to be present in the finished product. For other substances with very low migration, a TDI has not been set, but the maximum level to be used in any packaging material or a specific limit of migration is stated. This is because the available toxicological data would give a TDI, which allows that a specific limit of migration or a composition limit could be fixed at levels very much higher than the maximum likely intakes arising from present uses of the additive.

Depending on the available toxicological studies a restriction of migration into food of 0.05 mg/kg of food (3 mutagenicity studies only) or 5 mg/kg of food (3 mutagenicity studies plus 90-day oral toxicity study and data to demonstrate the absence of potential for bio-accumulation in man) may be allocated.

Commission (around September 2008), may provide further views on the issues related to nanotechnology-derived FCMs.

5.0 Dissemination of Findings

Following completion of the study, a workshop was organised as part of the project to discuss state-of-the-art, and the key issues emerging from the applications of nanotechnologies for FCMs. The workshop was attended by representatives from scientific community, industry, consumer forums, and regulatory agencies. A number of UK/ EU experts presented their studies and views at the workshop, highlighting the enormous benefits that nanotechnology applications would bring to the food sector, and any potential risks to the consumer health and the environment.

See Annex B

Parts of the findings of this study were presented at national and international forums. A review article has been published in a peer-reviewed journal, and a book is currently being edited by the participants of this project.

See Annex C

6.0 Conclusions and Gaps in Knowledge Requiring Further R&D

This study has shown that nanotechnologies offer a wide range of opportunities for innovation and benefits to the food packaging sector. The use of nanotechnologies to develop ‘improved’, ‘intelligent’ and ‘smart’ packaging materials by incorporating nanomaterials, nano-sensors, and nano-labels is rapidly becoming a commercial reality. Nanotechnology applications for FCMs form the largest share of the current, and short-term predicted market for nanotechnology applications in the food sector. Several nanotechnology-derived FCMs are already available in some countries. At present, the majority of these FCMs are only available outside the UK/ EU. However, as such applications are predicted to grow rapidly, it is widely expected that they will emerge on the UK/ EU markets in the next few years.

The study has also highlighted that the information is currently very sparse in relation to toxicology of nanoparticles, which can provide a basis for assessment of risks to consumer health. Much of the currently available information is specific to inhalation toxicology of a narrow range of engineered nanoparticles (mainly carbon materials), and hence not directly applicable food packaging.

The likelihood of consumer exposure from the use of nanotechnology-derived FCMs is also intrinsically linked to the migration of nanoparticles into food and drinks. Such migration data for nanotechnology-derived FCMs had not been available, which posed a major barrier to assessment of any risks to the consumer. The limited but pioneering research, carried out as part of this project, showed that there was no detectable migration from a polymer composite that had nanoclay embedded between PET layers. A very low level of migration of silver (< 2 µg/kg – which is less than the method limit of quantification) was detected in the case of food containers made of polypropylene-nanosilver composite. In either case, the presence of nanoparticles did not affect migration of non-nano components. The small study also showed that migration of nanoparticles is likely to be dependent on the type and composition of the polymer. There is thus a need for testing more types of nanotechnology-derived polymer composites to build a broader picture in regard to the potential nanoparticle migration from FCMs.

This study has also highlighted a number of knowledge gaps that require further research; for example, in relation to the behaviour, fate and toxicology of nanoparticles, their interactions with other food components in the gastrointestinal tract, absorption through the gut, effects on the gut epithelium and other cells in the body, and on the natural microflora in the gut. In view of these knowledge gaps, a few possible priorities for further research have been given below:

- Assessment of the potential contamination of food/ drinks due to migration of nanomaterials from FCMs and active surfaces, using different material types and application scenarios;
- Physicochemical properties of manufactured nanoparticles (anticipated to be incorporated in FCMs) to establish whether any nanoparticles leaching out of FCMs would bind to other food components, agglomerate, or remain available as free particles in the gastrointestinal (GI) tract;
- Potential effects of different nanoparticles on the function of the GI tract epithelium and function of other cells and tissues, and on the natural gut microflora;
- An exposure based assessment of risks to the average consumer from consumption of food/ drinks packaged in nanotechnology-derived FCMs;

Because of significant changes in material properties, and processing technologies used, any nanotechnology-derived FCMs will require approval on a case-to-case basis. It is clear that, in the absence of a nano-specific regulation (anywhere in the world), the products and applications of nanotechnology will be regulated under the existing frameworks. It is, therefore, important that any uncertainties and ambiguities in the regulatory frameworks are addressed in the light of novel properties and risks of nanomaterials to enable the regulatory decision-making processes.

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Table 1: List of relevant patent applications

Patent Assignee	Patent reference	Title/ Brief description
Atofina (FR)	W004012998* February 2004	'Composition for food packaging based on vinyl aromatic lean containing mineral platy filler in the form of nanoparticles.'
Borealis Technology (Finland)	W004063267* July 2004	'Article comprising stretched polymer composition with nanofillers: Polymer article (e.g. film for food packaging), comprises polymer composition containing polyolefin matrix and nanofiller dispersed in the matrix.'
Cellresin Technologies (USA)	TJS20030129403* July 2003	'Barrier material with nanosize metal particles as coating of plastic diaper or for food-contact packaging materials, comprises particles of zinc or similar reacting metal or metal alloy, dispersed in matrix material'
Lu Bingkun (CN)	CN1298902* 2001-06-13	'Process for preparing antibacterial plastics for food or beverage containers using nano-scale antibacterial powder'
Pengcheng Vocational University (CN)	CN1408746* April 2003	'Antibiotic fresh preserving plastic film and its producing method'
Shanxi Coal Chemistry Institute, Chinese Academy Of Sciences (CN)	CN1454939* November 2003	'The preparation method of nano titanium dioxide granule whose surface is coated with aluminum oxide. The grain diameter of the preparednano titanium dioxide is 10-100 nm, its surface is coated with aluminum oxide membrane …Nano titanium dioxide coated with aluminum dioxide has good dispersion property, can implement single granule dispersion, can be used as excellent UV-ray screening agent, and can be used in the fields of paint, rubber, fiber, coating material, sun protection products, printing ink & food package, etc.'
Tetra Laval Holdings & Finance S.A. (Switzerland)	US6117541* September 2000	'Polyolefin material integrated with nanophase particles: Packaging laminate, used in a container for fluid foods e.g. milk 01 juice - comprising a layer of polyolefin interspersed with nanometer size clay particles for gas barrier properties'
Wolffwalsrode AG, (DE)	DE19937117* February 2001	'Film, useful for the packaging of food stuffs, contains at least one copolyamide layer comprising 10-2000 ppm dispersed nano-scale nucleating particles'
Anthony, Michael M. (11899 NW. 31st St., Coral Springs, FL, US)	US5946930 September 1999	'Self-cooling beverage and food container using fullerene nanotubes' … a container such as a metal or plastic can .. a refrigerant receptacle is provided … the receptacle is filled with a sold or powdered form of carbon molecules of Fullerene nanotubes….
Fuji Photo Film Co., Ltd. (Minami-Ashigara-shi, JP)	US20060099232 May 2006	'Active oxygen eliminator and production method thereof' Active oxygen eliminator useful in e.g. foods, drinks and cosmetics, comprises composite particles produced by bonding platinum group element to dendritic molecules and/or having platinum group element included in dendritic molecules …… An active oxygen eliminator comprises: composite particles produced by bonding a platinum group element to dendritic molecules and/or having the platinum group element included in the dendritic molecules'.
E. I. Du Pont De Nemours and Company (Wilmington, DE, US)	US7265176 September 2007	'Composition comprising nanoparticle TiO2 and ethylene copolymer' Ultraviolet barrier composition for use to protect products susceptible to ultraviolet, e.g. foodstuffs, medicines, medical devices and cosmetics, comprises nano-titanium dioxide, and polymer
Toyo Seikan Kaisha, Ltd. (3-1, Uchisaiwai-Cho 1-Chome Chiyoda-Ku, Tokyo, JP)	EP0436725 April 1995	'method of producing antibacterial polymer and its use' Polyester film having excellent antibacterial property, which has coating layer of silver nanoparticles

Alcan Packaging Capsules, 7 Place du Chancellier Adenauer, 75218 Paris Cedex 16, FR	EP1765688 March 2007	'Dichtung aus mehrschichtigem material fur dichtmittel, insbesondere dichtungskapsel - 'seals made of a multi-layered material for sealing means, particularly a sealing capsule' Multi-layer seal e.g. for wine bottle cap has one oxygen-barrier layer made from or coated with a non-organic material
Mitsubishi Chem Corp	JP2005307190 November 2005	Thermoplastic resin composition for molded product, contains thermoplastic resin, and phosphorus-containing nanoparticles having ultraviolet ray absorptive properties, or ultraviolet ray absorptive particles and dispersant
Mekle, Walter, 10707 Berlin, DE	DE202005007199 April 2006	'Kratzfester Lack fur Scheiben und Glaser aller Art' Scratch-resistant lacquer, useful for panes and glasses and to coat drinking- glass or bottle, comprises a particle with special hardness
Cole, Carol L., Manassas Park, VA, US	US20060008738 January 2006	Formed body and methods thereof Formed body in the shape of a useful article formed from material, comprises a number of nanoparticles dispersed in the material
Cole, Carol L., Manassas Park, VA, US	US20060008737 January 2006	'Method of using a formed body' Slowing degradation of polymeric container used to hold consumable product(s), e.g. medicine, food stuffs e.g. meats, vegetables, food products, and dairy products, by forming polymeric container from composition comprising nanoparticles
A. Schulman Plastics, Pedro Colomalaan 25, 2880 Bornem, BE	EP1609816 December 2005	'Masterbatch-Zusammensetzung, enthaltend nanoskaliges Zinkoxid zur Herstellung von transparenten Polyolefinfolien mit UV-Barriere - Masterbatch Composition containing nano scalar zinc oxide for the production of transparent polyolefin films with UV-barrier properties' Polymer composition useful as a masterbatch in preparing polyolefin film for packaging material comprises polyolefin and nano scalar zinc oxide having specific particle size
Lanxess Deut GmBH	CA2508492 November 2005	Extrusion coating with polyamide, e.g. for production of packaging for food or cosmetics, involves high-speed mono- or co-extrusion coating with nanoparticle-modified polyamide at high coating speeds
(Except Us) ETC Products Gmbh, Ulrichsberger Strasse 17, 94469 Deggendorf, DE	WO2005115151 December 2005	'Funktionelle sol-gel-beschichtungsmittel - functional sol-gel coating agents' Antimicrobial or decorative sol-gel coatings for medical or domestic use (e.g. in contact with food) contain nanoparticulate metals as antimicrobial agents or nanoparticulate materials as decorative components
Eastman Kodak Company, US (East)	US20050226967 October 2005	Article for inhibiting microbial growth Packaging material for wrapping foodstuff, comprises metal-ion sequestering agent capable of removing designated metal ions from surfaces of foodstuffs and from liquid extrudates of foodstuffs
Bringley, Joseph F.(US), Patton, David L., (US), Wien, Richard W., (US), Lerat, Yannick J.F. (FR)	US20050226966 October 2005	Article for inhibiting microbial growth Packaging material useful for wrapping foodstuffs and for inhibiting the growth of microorganisms in foodstuffs comprises metal-ion sequestering agent
Eastman Kodak Company, US	US20050224423 October 2005	Container for inhibiting microbial growth in liquid nutrients Fluid container useful for inhibiting the growth of microbes in liquid nutrient in a container comprises an interior surface having a metal-ion sequestering agent and an antimicrobial agent
Colormatrix Euro Ltd	AU2005227732 October 2005	Composition useful for improving the reheat characteristics of a polymeric material comprises an inorganic material

Induflex Robert Morgan, Alte Dorfstrasse 39 B, 27337 Blender, DE	EP1686855 August 2006	'Verwendungen eines schichtmaterials - Applications of a coating material' Use of an antimicrobial coating comprising a biocide-containing layer covered with a transport control layer to coat packaging materials
Schott Ag, 55122 Mainz, DE	DE10345625 April 2005	Herstellungsverfahren eines Glaspulvers oder Glasproduktes mit antimikrobieller Wirkung Making glass powder with time-release antimicrobial action, for use in widely-diverse consumables, employs purely inorganic solutions and nanoparticle suspensions in sol-gel process
Krones AG	AU2003255336 March 2003	'Coated hollow body' Hollow article having on its external surface a barrier layer and a protective layer, useful particularly as bottles for foods and drinks, where the protective layer increases scratch resistance
Merck Patent GmBH; DE	CN1826389 August 2006	Nanoparticulate UV protectants used in cosmetic, sunscreen, dermatological or other protective uses (e.g. as textile or packaging coatings) comprise a metal oxide with a silicon dioxide coating
Chen J; CN	CN1539874 October 2004	Antibacterial, keeping foodstuff fresh, uv-resistant plastic fabricated from Nano material
Cryovac Inc	AU2004202397 December 2004	PVdC film with nanocomposite tie layer Film for packaging of object, e.g. food product such as ground meat products, includes first layer of vinylidene chloride polymer, second layer of dispersion of tie polymer and nanoparticles, and third layer of thermoplastic polymer
Dsm Ip Assets B.V., Het Overloon 1, 6411 Te Heerlen, NL	EP1479738 November 2004	Hydrophobe Beschichtungen enthaltend reaktive Nanopartikel Hydrophobic coatings comprising reactive nano-particles Revetement hydrophobe comprenant des nanoparticules reactives Coating composition useful in e.g. food industry comprises reactive inorganic nano-particles comprising reactive group and apolar group
Dsm Ip Assets Bv; NL	CN1820058 August 2006	Coating composition for hydrophobic coating used for food industry, decorative material, automobile industry and display industry, comprises reactive nano-particles having reactive organic groups and apolar groups on their surface
BSH Bosch Und Siemens Hausgerate GmBH	DE 10314513 October 2004	Katalysatorsystem zum Geruchsabbau Catalyst system for boiling, frying, backing and grilling equipment, especially oven or hot-plate, has odor catalyst and coating of binder containing porous particles without solid or liquid second phase in pores
Eisenwerke Fried. Wilh. Duker Gmbh & Co. Kgaa, 97753 Karlstadt, DE	DE 202004006472 September 2004	Element zum Transport und/oder zur Lagerung von Lebensmitteln Antimicrobial surface in contact with water and food during transport and storage includes silver
Joyce, Thomas W., Kalamazoo, Mi, US	US20040161594 August 2004	Nanoparticle barrier-coated substrate and method for making the same Preparation of barrier coating on substrate e.g. cellulosic substrate useful in e.g. food packaging involves application of coating solution comprising pigment nanoparticles, styrene-butadiene latex and water on substrate followed by drying
Daewoo Electronics Co Ltd; KR	CN1517652 August 2004	Refrigerator for use in preserving food in fresh state, comprises finish material containing antibacterial nanosilver particles
Daewoo Electronics Co Ltd; KR	CN1517651 August 2004	Refrigerator used to preserve food in fresh state for extended period of time, has inner case having inner sheet containing nanosilver particles and defining inner space of refrigerator, and outer sheet formed on inner sheet

Atofina SA; FR	FR2849442 July 2004	Styrene resin articles, especially films or containers, resistant to oils and expanding agents, obtained from resin composition containing nanoparticulate, plate-form mineral filler
ITN Nanovation GmBH	AU2002333181 March 2004	'Colloidal system with ceramic nanoparticles' Colloidal system of ceramic nanoparticles used to make e.g. ultra-fine ceramic membranes for food industry and medical applications, is maintained by energetic equilibrium
Atofina	AU2003269039 February 2004	'Composition for food packaging based on vinyl aromatic resin containing a mineral platy filler in the form of nanoparticles' Use for the production of food packaging of a composition containing styrene resin and flake-like mineral filler, e.g. montmorillonite, dispersed in the form of nano-particles
Krones AG, 93073 Neutraubling, DE	DE10231856 February 2004	'Beschichteter Hohlkorper' Coated hollow container, particularly for drinks or foodstuffs, has a protective layer which includes nanoparticles to increase scratch resistance
Rhodianyl	AU2003224214 October 2005	Thermoplastic material with high barrier properties Gas and liquid barrier material, for use in food packaging and storage and transport of liquid fuels, comprises a matrix of thermoplastic polymer and a nanoparticulate compound based on zirconium and/or titanium phosphate
Ems-Chem AG	CN1300224	Production of polyamide nanocomposites from base polymer and organically modified silicate layers useful for packaging semi-finished products, foodstuffs, toothpaste, cosmetics, drinks, paints, lacquers, and plasters
Commonwealth Sci & Ind Res Org (CSIR), Bottle Magic Australia Pty Ltd	AU2002248982 November 2002	Coating composition for containers for storing light sensitive products, comprises carrier dispersed with pigment having ultraviolet light absorber nanoparticles, or ultraviolet and visible light absorber nanoparticles
Valspar Corp; US	AU200220024 May 2002	Composition useful as barrier layer in packaging products, contains blend of specific amount of polyethylene terephthalate material and polyamide material
UCB SA; BE	AU200220699 May 2002	Planar self-supporting sheet for packaging article, e.g. food, has coating with nano-particles, and that is free of polymeric binder, synthetic hydrophilic resin and/or resin with hydrophilic/hydrophobic character
Plasco Plasma Coating GmBH Ehrich; DE	AU200150387 November 2001	Production of plastics articles containing nanoparticles, useful for packaging, e.g. film, bottle, tube or canister, involves pulverizing plastics film with metal(loid) oxide and/or nitride or amorphous carbon coating and melting
Wolff Walsrode AG; DE	AU199961993 May 2000	Multilayer film for food packaging applications has an outer layer of polyamide containing dispersed nano-scale filler particles and at least one other polyamide layer without such particles
Eastman Chem Co	BR199911256 March 2001	High gas barrier polyester composition useful in making articles e.g. bottles and sheets
Eastman Chem Co	BR199911262 March 2001	Polyester composition with improved gas barrier properties useful in making articles e.g. bottles
Tetra Laval Holdings & Finance SA	AU199879789 January 1999	Packaging laminate, used in a container for fluid foods e.g. milk or juice - comprising a layer of polyolefin interspersed with nanometer size clay particles for gas barrier properties
Wolff Walsrode AG	CA2209671 January 1998	Polyamide mixture containing nano-particles - used in production of packaging film with good oxygen barrier properties and transparency after sterilisation

* Source Cientifica (2006)

Annex-A: Results of Nanomaterial Migration Testing

Table 1. Alkane concentrations detected by headspace GC-MS analysis

Sample description	Number of alkanes detected (resolved peaks in chromatogram)	Combined estimated concentration (mg/kg)
Nano-silver containing polypropylene container	50	44
‘Control’ polypropylene container	60	103

Table 2. Estimated concentrations and proposed identities of the substances detected by headspace GC-MS in the nano-silver containing polypropylene containers

Retention time (minutes)	Concentration (mg/kg)	Best library match
12.74	0.14	4-Methyl-1-heptene
13.39	0.47	Toluene
13.88	0.10	Hexamethyl cyclotrisiloxane
14.07	0.03	*trans*-2-Methyl-3-octene
14.83	0.17	2,4-Dimethyl-1-heptene
15.95	0.07	Xylene
20.72	0.32	Decamethyl cyclopentasiloxane
23.6	0.30	3,5-Dimethyl benzaldehyde

Table 3. Estimated concentrations and proposed identities of the substances detected by headspace GC-MS in the 'control' polypropylene containers

Retention time (minutes)	Concentration (mg/kg)	Best library match
6.72	0.09	Isopropanol
7.26	4.44	2-Methyl-2-propanol
7.75	0.11	2-Methoxy-1-propene
7.97	0.06	4-Methyl-1-pentene
8.63	2.44	2-Methyl-1-pentene
9.28	0.69	2-Ethoxy-2-methyl-propane
9.63	1.73	2,2-Dimethyloxethane
9.76	0.41	Amelyene hydrate
10.02	0.11	2,4-Dimethyl-1-pentene
13.4	0.56	Toluene
14.83	9.79	2,4-Dimethyl-1-heptene
15.79	0.16	No good match (ions = 55, 67, 82, 99, 128)
17.28	0.18	No good match (ions = 43, 58, 69, 113)
17.53	1.47	No good match (ions = 59, 75, 91, 105, 133, 176)
17.76	0.09	No good match (ions = 43, 57, 71,87, 129)
17.85	5.05	No good match (ions = 43, 57, 105, 133, 176)
18.06	1.44	Tetrahydro-2,2,5,5-tetramethylfuran
19.09	0.17	3-Undecene
19.24	0.21	4-Undecene
20.06	1.31	7-Methyl-2-undecene
20.13	1.38	7-Methyl-2-undecene
23.61	0.39	3,4-Dimethyl benzaldehyde

Table 4. Estimated concentrations and proposed identities of the substances detected by headspace GC-MS in the 'control' PET bottle

Retention time (minutes)	**Concentration (mg/kg)**	**Best library match**
15.56	0.05	Ethylbenzene
15.84	0.15	o-Xylene
16.47	0.08	p-Xylene

Table 5. Alkane concentrations in the isooctane extracts of the nano-silver and control PP containers

Sample description	**Number of alkanes detected (resolved peaks in chromatogram)**	**Combined estimated concentration (mg/kg)**
Nano-silver containing polypropylene container	84	280
'Control' polypropylene container	95	313

Table 6. Estimated concentrations and proposed identities of the substances detected in the isooctane extracts of the nano-silver containing polypropylene containers

Retention time (minutes)	Concentration (mg/kg)	Best library match
6.28	0.34	Unspecified alkene
7.85	0.38	2,4-Dimethylbenzaldehyde
9.27	0.36	Heptyl butanoate
10.42	8.15	2,6-Di-t-butyl phenol
12.68	2.96	3-Hexadecanol
12.86	1.72	Isopropyl tetradecanoate
13.42	6.90	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione *
14.49	6.60	No good match (ions - 81, 99, 211, 239, 256)
17.09	12.98	Di-(2-ethylhexyl) phthalate
18.48	6.69	Squalene

* This quinone structure has been reported elsewhere to form in the GC injection port (it is an oxidation of 2,6-di-t-butyl-phenol which is also detected

Table 7. Estimated concentrations and proposed identities of the substances detected in the isooctane extracts of the 'control' polypropylene containers

Retention time (minutes)	Concentration (mg/kg)	Best library match
6.324	1.86	Unspecified alkene
8.54	3.04	Unspecified alkene
8.621	4.48	Unspecified alkene
10.419	2.87	2,6-Di-t-butyl phenol
11.66	1.65	1,1'-(1,3-Propoanediyl)bis-benzene
11.803	0.80	DIPN isomers
13.577	1.42	3,5-Bis(1,1-dimethylethyl)-4-hydroxy-benzenepropoanoic acid methyl ester
13.743	1.19	Dibutyl phthalate
14.128	0.71	No good match (ions = 57, 69, 111, 177, 205, 220)
16.772	3.36	No good match (ions = 101, 117, 130, 239, 315, 371)
17.091	11.0	Di-(2-ethylhexyl) phthalate
18.48	13.01	Squalene
22.36	55.96	Tris(2,4-di-tert-butylphenyl)phosphite

Table 8. Estimated concentrations and proposed identities of the substances detected in the ethanol extracts of the nano-silver containing polypropylene containers

Retention time (minutes)	Concentration (mg/kg)	Best library match
12.80	1.27	Isopropyl tetradecanoate
17.09	1.11	Di-(2-ethylhexyl) phthalate
18.48	3.29	Squalene

Table 9. Estimated concentrations and proposed identities of the substances detected in the ethanol extracts of the 'control' polypropylene containers

Retention time (minutes)	Concentration (mg/kg)	Best library match
17.10	1.05	Di-(2-ethylhexyl) phthalate
18.48	2.39	Squalene

Table 10. Estimated concentrations and proposed identities of the substances detected in the isooctane extracts of the nano-clay bottle

Retention time (minutes)	**Concentration (mg/kg)**	**Best library match**
4.71	86.5	Nonane
18.48	3.15	Squalene

Table 11. Estimated concentrations and proposed identities of the substances detected in the isooctane extracts of the ‘control’ PET bottle

Retention time (minutes)	**Concentration (mg/kg)**	**Best library match**
4.71	114	Nonane
18.48	5.13	Squalene

Table 12. Estimated concentrations and proposed identities of the substances detected in the ethanol extracts of the nano-clay bottle

Retention time (minutes)	**Concentration (mg/kg)**	**Best library match**
4.72	85.2	Nonane
7.07	0.67	Nonanal
13.07	2.36	1-Hexadecene
14.63	1.29	Octadecanal
17.49	4.63	4-Octadecyl-morpholine
17.57	4.68	Di-(2-ethylhexyl) phthalate
19.41	1.16	Eicosane

Table 13. Estimated concentrations and proposed identities of the substances detected in the ethanol extracts of the ‘control’ PET bottle

Retention time (minutes)	**Concentration (mg/kg)**	**Best library match**
4.72	388	Nonane
7.07	10.6	Nonanal
17.57	79.3	Di-(2-ethylhexyl) phthalate
19.41	40.3	Eicosane

Table 14. LC-TOF-MS data for the nano-silver containers and controls

Retention time (min)	**Mass**	**Proposed molecular formula**	**Proposed identity**	**Present in**					
				Nano-silver PP container			**Control PP container**		
				Ethanol	**Isooctane**	**Water**	**Ethanol**	**Isooctane**	**Water**
28.0	414.2047	C24H30O6	Dicarboxy-1,10-diphenoxydecane	ESI+			ESI+	ESI+	
50.4 and 51.9	278.1523	C16H22O4	Dibutyl phthalate		ESI+			ESI+	
57.6	274.2519	C16H34O3	Diethyleneglycol monodecyl ether	ESI+					
59.0	262.2301	C18H30O	Tri-tert butyl phenol					ESI+	
62.3	330.2773	C19H38O4	Methyl-9,10-dihydroxystearate					ESI+	
62.5	281.2844	C19H37O	None proposed					ESI+	
63.1	636.2990	Appendix 1	None proposed		ESI+				
63.8	314.2457	C18H34O4	Dibutyl sebacate	ESI+			ESI+		
64.1	351.3501	C23H45NO	Erucamide + CH_2	ESI+			ESI+		
64.4	225.1733	C13H23NO2	5,5-dimethyl-3(n-pentylamino)cyclohex-2-en-1-one					ESI+	
65.0	662.4464	C42H63O4P	Oxidised tris(2,4-ditert-butylphenyl)phosphite	ESI+			ESI+		
65.7	367.3817	C24H49NO	N,N-Dioctyl-octanamide				ESI+		
65.9	358.3087	C21H42O4	Glycerol monostearate					ESI+	
66.1	309.3035	C20H39NO	Erucamide – CH_2CH_2					ESI+	
66.2	404.3122	C22H44O6	None proposed		ESI-			ESI-	

Table 15. LC-TOF-MS data for the nano-clay bottles and controls

Retention time (min)	Mass	Proposed molecular formula	Proposed identity	Present in					
				Nano-clay bottle			PET control bottle		
				Ethanol	**Isooctane**	**Water**	**Ethanol**	**Isooctane**	**Water**
1.4	292.1464	C12H24N2O4S	3-Sulfopropyl dimethyl-3-methacrylamidopropyl ammonium salt	ESI-					
2.0	246.1364	C14H18N2O2	N-(2,6-Dimethylphenyl)-2-oxo-1-pyrrolidine acetamide	ESI+		ESI+			
4.7	492.2732	C27H40O8	None proposed	ESI+		ESI+			
5.3	555.2639	Appendix 2	None proposed	ESI-					
10.1	369.2048	Appendix 2	None proposed	ESI+		ESI+			
65.2	508.1300	Appendix 2	None proposed			ESI-			ESI-

Table 16. Semi-quantitative trace element concentrations detected in the acid digested nano-silver PP and in the PP control

		Concentration (µg/kg)			
	LoD (µg/kg)	**PP Box A**	**PP Box B**	**Nano Box A**	**Nano Box B**
Li	200	<LoD	<LoD	<LoD	<LoD
Be	1000	<LoD	<LoD	<LoD	<LoD
B	20000	<LoD	<LoD	<LoD	<LoD
Na	10000	<LoD	<LoD	<LoD	<LoD
Mg	2000	(6000)	8000	17000	16000
Al	10000	(40000)	(40000)	(40000)	(40000)
P	50000	<LoD	<LoD	<LoD	<LoD
K	100000	<LoD	<LoD	<LoD	<LoD
Ca	10000	<LoD	<LoD	<LoD	<LoD
Sc	500	<LoD	(500)	<LoD	<LoD
Ti	500	(1100)	(1100)	1800	1800
V	200	<LoD	<LoD	<LoD	<LoD
Cr	1000	<LoD	<LoD	<LoD	<LoD
Mn	1000	<LoD	<LoD	<LoD	<LoD
Fe	40000	<LoD	<LoD	<LoD	<LoD
Co	100	<LoD	<LoD	<LoD	<LoD
Ni	8000	<LoD	<LoD	<LoD	<LoD
Cu	500	<LoD	<LoD	<LoD	<LoD
Zn	1000	4000	<LoD	(1000)	(2000)
Ga	100	<LoD	<LoD	<LoD	<LoD
Ge	100	<LoD	<LoD	<LoD	<LoD
As	300	<LoD	<LoD	<LoD	<LoD
Se	10000	<LoD	<LoD	<LoD	<LoD
Rb	50	<LoD	<LoD	<LoD	<LoD
Sr	20	(40)	(50)	(60)	(50)
Y	10	<LoD	<LoD	<LoD	<LoD
Zr	100	<LoD	<LoD	<LoD	<LoD
Nb	30	<LoD	<LoD	<LoD	<LoD
Mo	100	<LoD	<LoD	<LoD	<LoD
Ru	20	<LoD	<LoD	<LoD	<LoD
Pd	20	<LoD	<LoD	<LoD	<LoD

Table 16 continued. Semi-quantitative trace element concentrations detected in the acid digested nano-silver PP and in the PP control

		Concentration (µg/kg)			
	LoD (µg/kg)	**PP Box A**	**PP Box B**	**Nano Box A**	**Nano Box B**
Ag	10000	<LoD	<LoD	(10000)	(30000)
Cd	20	<LoD	<LoD	<LoD	<LoD
Sn	20	140	500	700	1700
Sb	200	<LoD	<LoD	<LoD	<LoD
Te	100	(300)	<LoD	<LoD	<LoD
Cs	10	<LoD	<LoD	<LoD	<LoD
Ba	50	<LoD	(60)	1400	900
La	10	<LoD	<LoD	<LoD	<LoD
Ce	20	<LoD	<LoD	<LoD	<LoD
Pr	10	<LoD	<LoD	<LoD	<LoD
Nd	10	<LoD	<LoD	<LoD	<LoD
Sm	10	<LoD	<LoD	<LoD	<LoD
Eu	10	<LoD	<LoD	<LoD	<LoD
Gd	10	<LoD	<LoD	<LoD	<LoD
Tb	10	<LoD	<LoD	<LoD	<LoD
Dy	10	<LoD	<LoD	<LoD	<LoD
Ho	10	<LoD	<LoD	<LoD	<LoD
Er	10	<LoD	<LoD	<LoD	<LoD
Tm	10	<LoD	<LoD	<LoD	<LoD
Yb	10	<LoD	<LoD	<LoD	<LoD
Lu	10	<LoD	<LoD	<LoD	<LoD
Hf	50	<LoD	<LoD	<LoD	<LoD
Ta	50	<LoD	<LoD	<LoD	<LoD
W	50	<LoD	<LoD	<LoD	<LoD
Re	10	<LoD	<LoD	<LoD	<LoD
Ir	100	<LoD	<LoD	<LoD	<LoD
Pt	20	<LoD	<LoD	<LoD	<LoD
Au	1000	<LoD	<LoD	<LoD	<LoD
Hg	200	<LoD	<LoD	<LoD	<LoD
Tl	10	<LoD	<LoD	<LoD	<LoD

Table 16 continued. Semi-quantitative trace element concentrations detected in the acid digested nano-silver PP and in the PP control

		Concentration (µg/kg)			
	LoD (µg/kg)	**PP Box A**	**PP Box B**	**Nano Box A**	**Nano Box B**
Pb	50	<LoD	<LoD	<LoD	<LoD
Th	100	<LoD	<LoD	<LoD	<LoD
U	20	<LoD	<LoD	<LoD	<LoD

LoD = limit of detection

Concentrations given in brackets are less than the limit of quantification

Table 17. Measured silver concentrations in the acid digests of the nano-silver and control PP containers

	Concentration (µg/kg)			
	PP Box A	**PP Box B**	**Nano Box A**	**Nano Box A**
Ag	< 100	<100	27000	29000

Table 18. Semi-quantitative trace element concentrations detected in the acid digested nano-clay PET and in the PET control

		Concentration (µg/kg)			
	LoD (µg/kg)	**PET Bottle A**	**PET Bottle B**	**Nano Bottle A**	**Nano Bottle B**
Li	200	<LoD	<LoD	<LoD	<LoD
Be	1000	<LoD	<LoD	<LoD	<LoD
B	20000	<LoD	<LoD	<LoD	<LoD
Na	10000	<LoD	<LoD	(26000)	(27000)
Mg	2000	<LoD	<LoD	43000	46000
Al	10000	<LoD	<LoD	300000	300000
P	50000	<LoD	<LoD	<LoD	<LoD
K	100000	<LoD	<LoD	<LoD	<LoD
Ca	10000	<LoD	<LoD	60000	60000
Sc	500	<LoD	<LoD	(700)	(800)
Ti	500	<LoD	<LoD	59000	62000
V	200	<LoD	<LoD	<LoD	<LoD
Cr	1000	<LoD	<LoD	<LoD	<LoD
Mn	1000	<LoD	<LoD	<LoD	<LoD
Fe	40000	<LoD	<LoD	(40000)	<LoD
Co	100	<LoD	<LoD	700	800
Ni	8000	<LoD	<LoD	<LoD	<LoD
Cu	500	<LoD	<LoD	15000	16000
Zn	1000	<LoD	<LoD	(3000)	10000
Ga	100	<LoD	<LoD	<LoD	<LoD
Ge	100	<LoD	<LoD	<LoD	<LoD
As	300	<LoD	<LoD	<LoD	<LoD
Se	10000	<LoD	<LoD	<LoD	<LoD
Rb	50	<LoD	<LoD	<LoD	<LoD
Sr	20	<LoD	<LoD	200	180
Y	10	<LoD	<LoD	80	80
Zr	100	<LoD	<LoD	(300)	(300)
Nb	30	<LoD	<LoD	(40)	(40)
Mo	100	<LoD	<LoD	<LoD	<LoD
Ru	20	<LoD	<LoD	<LoD	<LoD
Pd	20	<LoD	<LoD	<LoD	<LoD

Table 18 continued. Semi-quantitative trace element concentrations detected in the acid digested nano-clay PET and in the PET control

		Concentration (µg/kg)			
	LoD (µg/kg)	**PET Bottle A**	**PET Bottle B**	**Nano Bottle A**	**Nano Bottle B**
Ag	10000	<LoD	<LoD	<LoD	<LoD
Cd	20	<LoD	<LoD	<LoD	<LoD
Sn	20	70	<LoD	(60)	<LoD
Sb	200	260000	240000	230000	236000
Te	100	<LoD	<LoD	<LoD	<LoD
Cs	10	<LoD	<LoD	<LoD	<LoD
Ba	50	<LoD	<LoD	(70)	(70)
La	10	(30)	(20)	200	180
Ce	20	<LoD	<LoD	380	400
Pr	10	<LoD	<LoD	(30)	40
Nd	10	<LoD	<LoD	130	140
Sm	10	<LoD	<LoD	(10)	(30)
Eu	10	<LoD	<LoD	<LoD	<LoD
Gd	10	<LoD	<LoD	(20)	(30)
Tb	10	<LoD	<LoD	<LoD	<LoD
Dy	10	<LoD	<LoD	(10)	(20)
Ho	10	<LoD	<LoD	<LoD	<LoD
Er	10	<LoD	<LoD	<LoD	<LoD
Tm	10	<LoD	<LoD	<LoD	<LoD
Yb	10	<LoD	<LoD	<LoD	<LoD
Lu	10	<LoD	<LoD	<LoD	<LoD
Hf	50	<LoD	<LoD	<LoD	<LoD
Ta	50	<LoD	<LoD	<LoD	<LoD
W	50	<LoD	<LoD	<LoD	<LoD
Re	10	<LoD	<LoD	<LoD	<LoD
Ir	100	<LoD	<LoD	<LoD	<LoD
Pt	20	<LoD	<LoD	<LoD	<LoD
Au	1000	<LoD	<LoD	<LoD	<LoD
Hg	200	<LoD	<LoD	<LoD	<LoD
Tl	10	<LoD	<LoD	<LoD	<LoD

Table 18 continued. Semi-quantitative trace element concentrations detected in the acid digested nano-clay PET and in the PET control

		Concentration (µg/kg)			
	LoD (µg/kg)	**PET Bottle A**	**PET Bottle B**	**Nano Bottle A**	**Nano Bottle B**
Pb	50	<LoD	<LoD	(110)	(150)
Th	100	<LoD	<LoD	(100)	(100)
U	20	<LoD	<LoD	130	130

LoD = limit of detection

Concentrations given in brackets are less than the limit of quantification

Figure 3. Headspace GC-MS chromatogram obtained from the analysis of the nano-silver containing polypropylene container

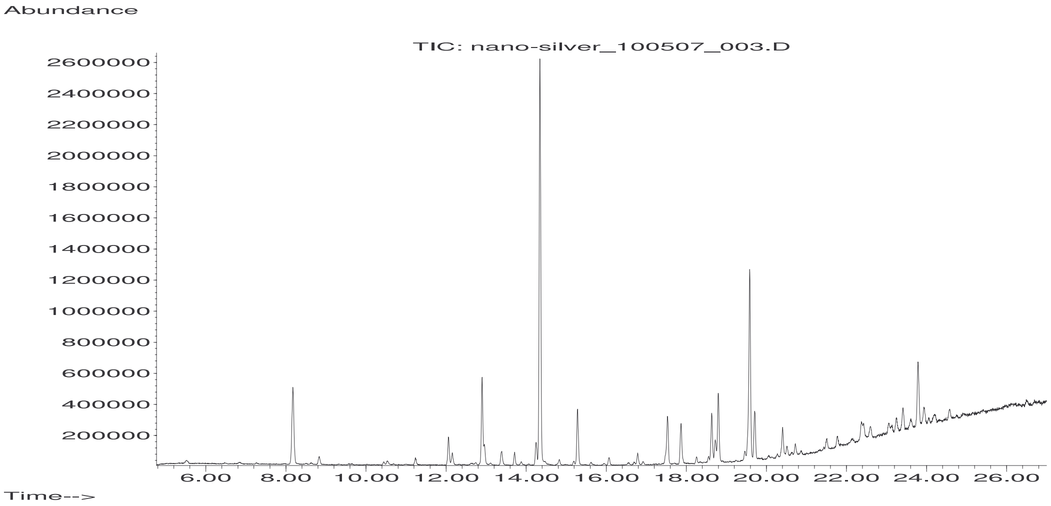


Figure 4. Headspace GC-MS chromatogram obtained from the analysis of the ‘control’ polypropylene container

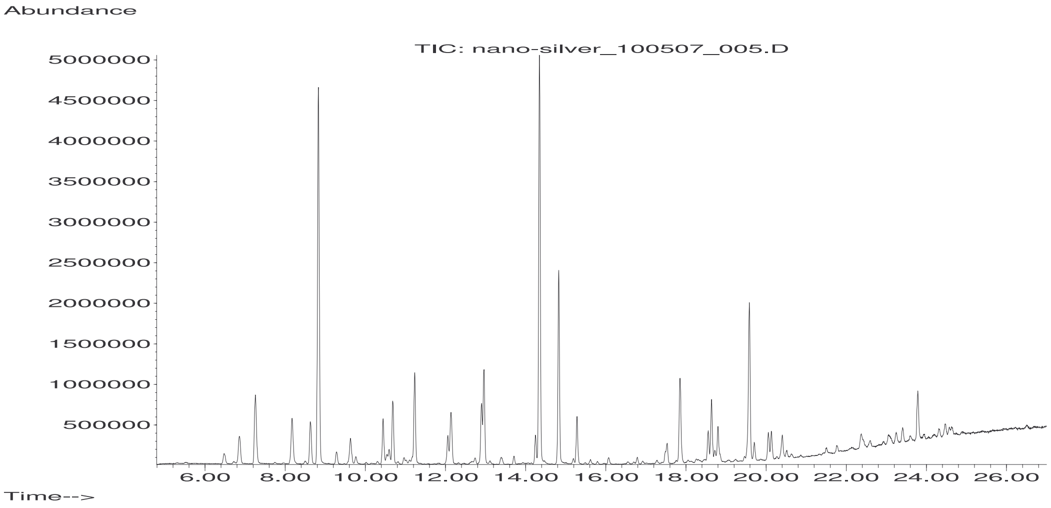


Figure 5. Headspace GC-MS chromatogram obtained from the analysis of the nano-clay bottle

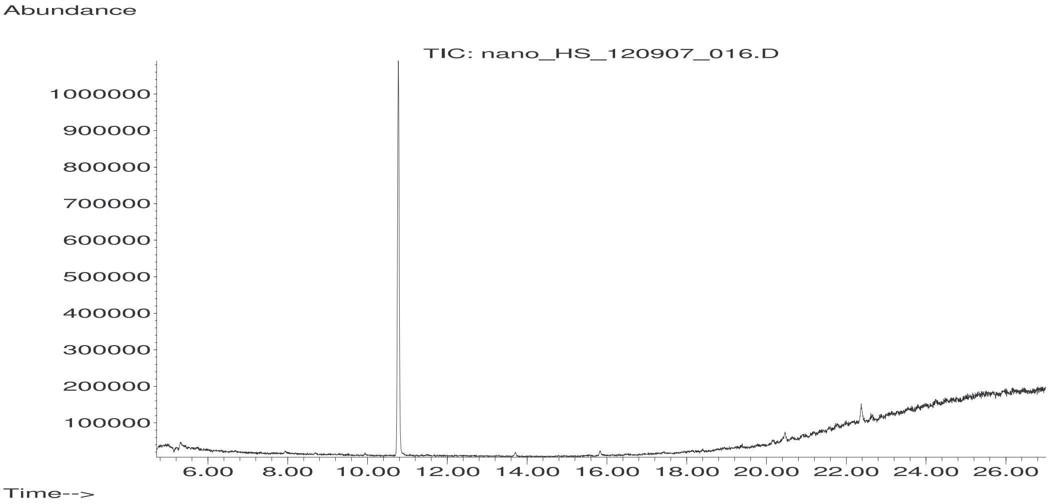


Figure 6. Headspace GC-MS chromatogram obtained from the analysis of the ‘control’ PET bottle

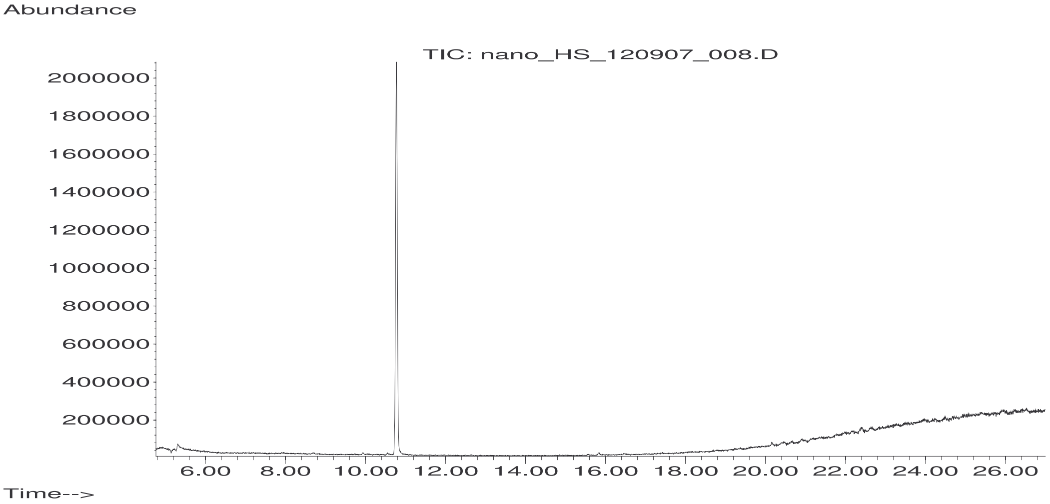


Figure 7. GC-MS chromatogram obtained from the analysis of the isooctane extract of the nano-silver containing polypropylene container

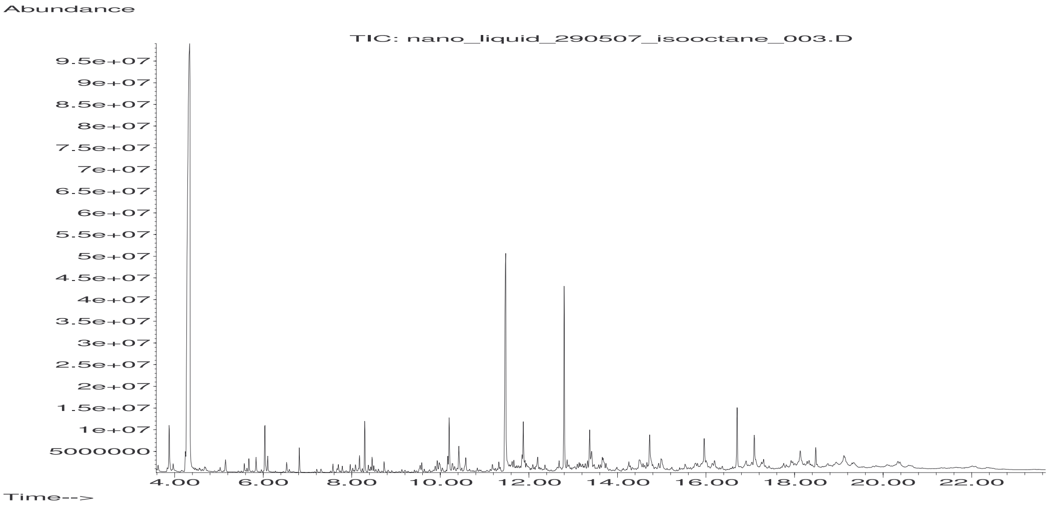


Figure 8. GC-MS chromatogram obtained from the analysis of the isooctane extract of the ‘control’ polypropylene container

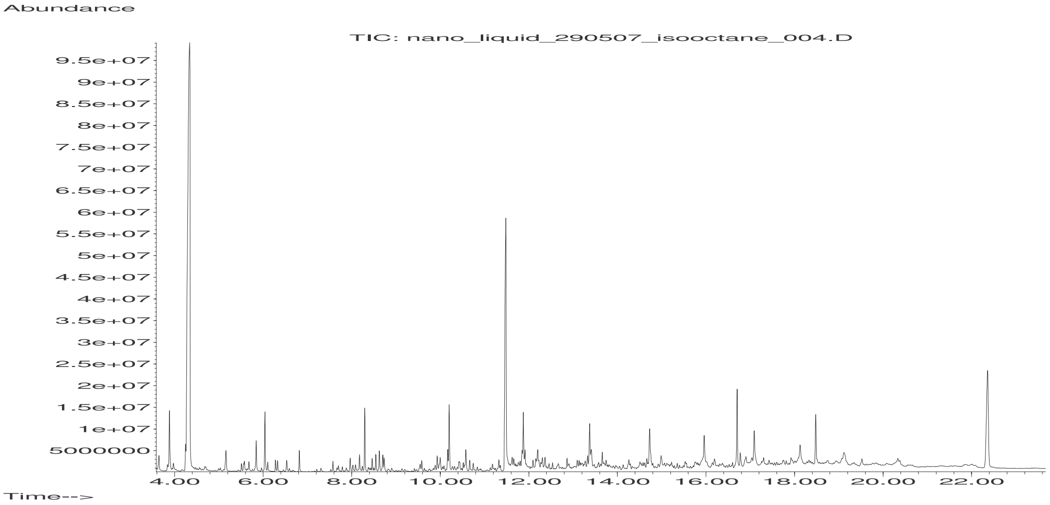


Figure 9. GC-MS chromatogram obtained from the analysis of the concentrated ethanolic extract of the nano-silver containing polypropylene container

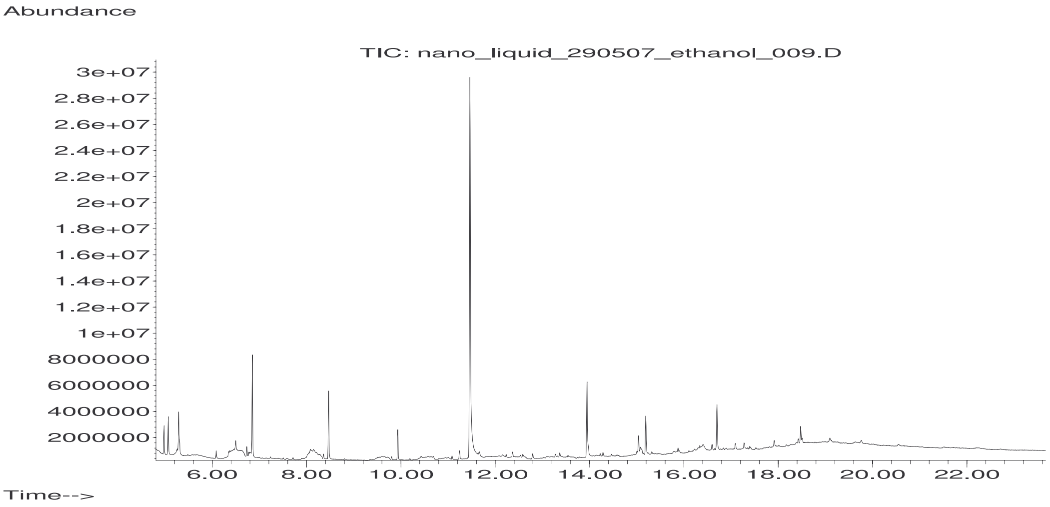


Figure 10. GC-MS chromatogram obtained from the analysis of the concentrated ethanolic extract of the ‘control’ polypropylene container

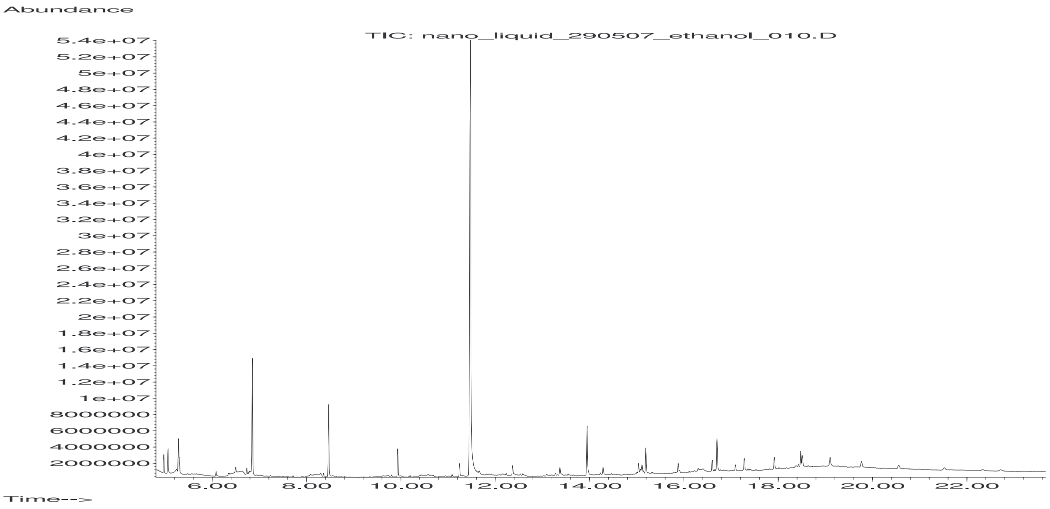


Figure 11. GC-MS chromatogram obtained from the analysis of the isooctane extract of the nano-clay bottle

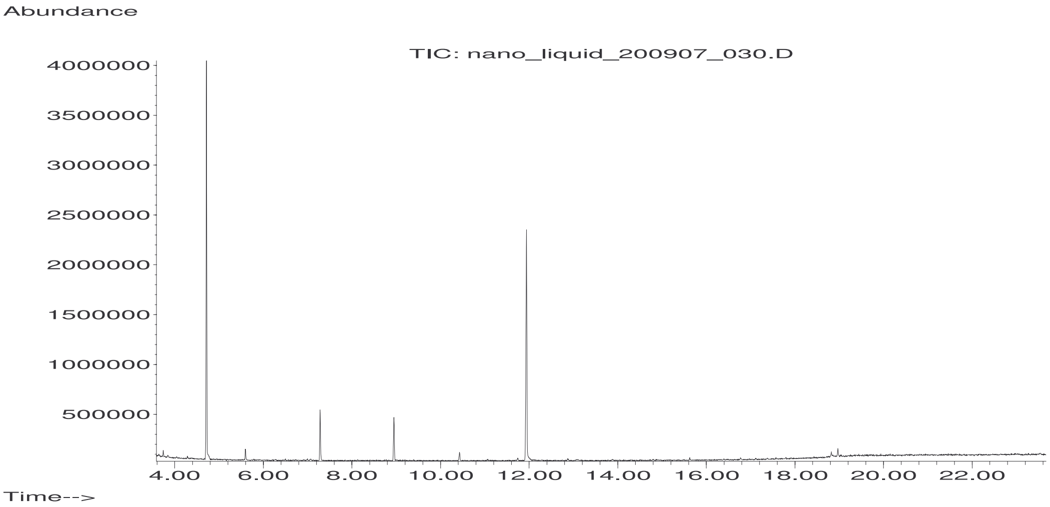


Figure 12. GC-MS chromatogram obtained from the analysis of the isooctane extract of the ‘control’ PET bottle

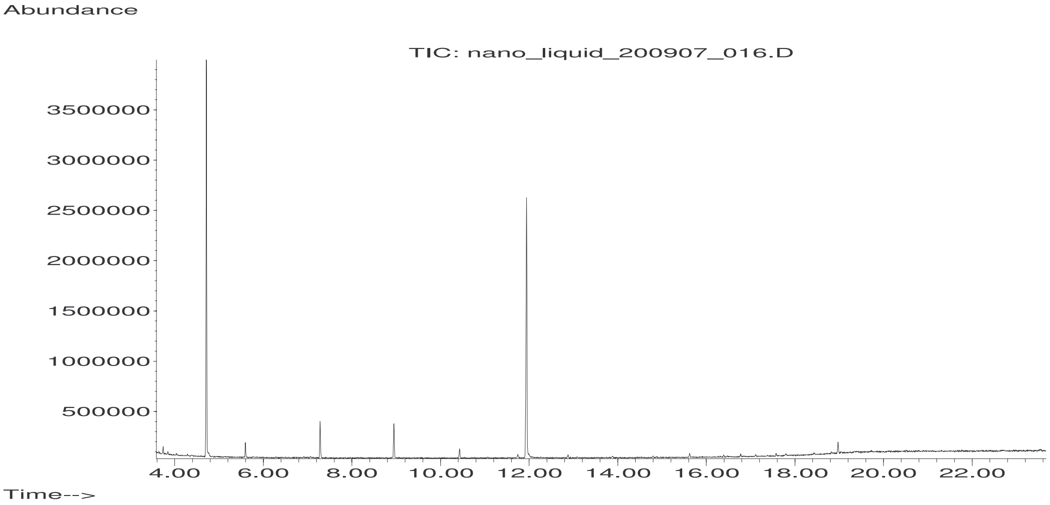


Figure 13. GC-MS chromatogram obtained from the analysis of the concentrated ethanolic extract of the nano-clay bottle

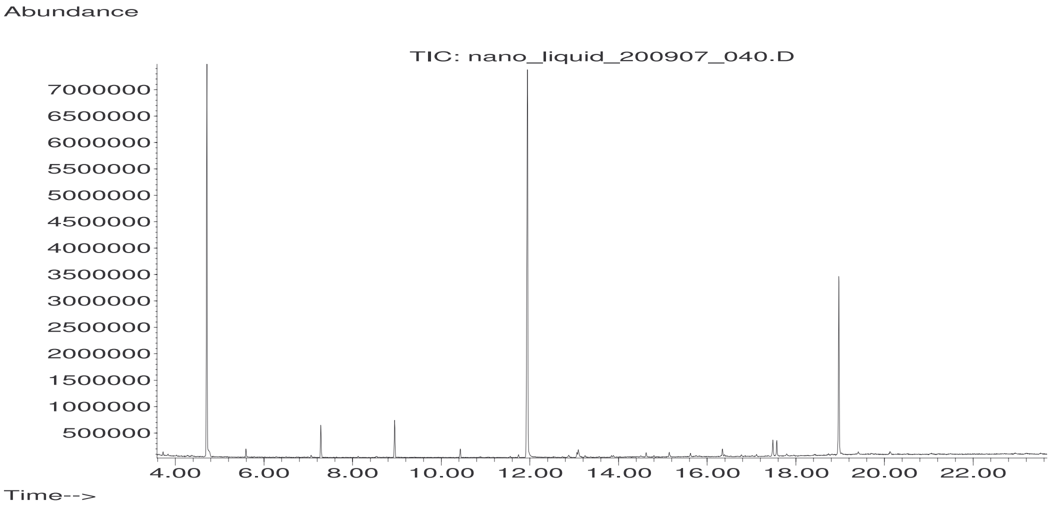
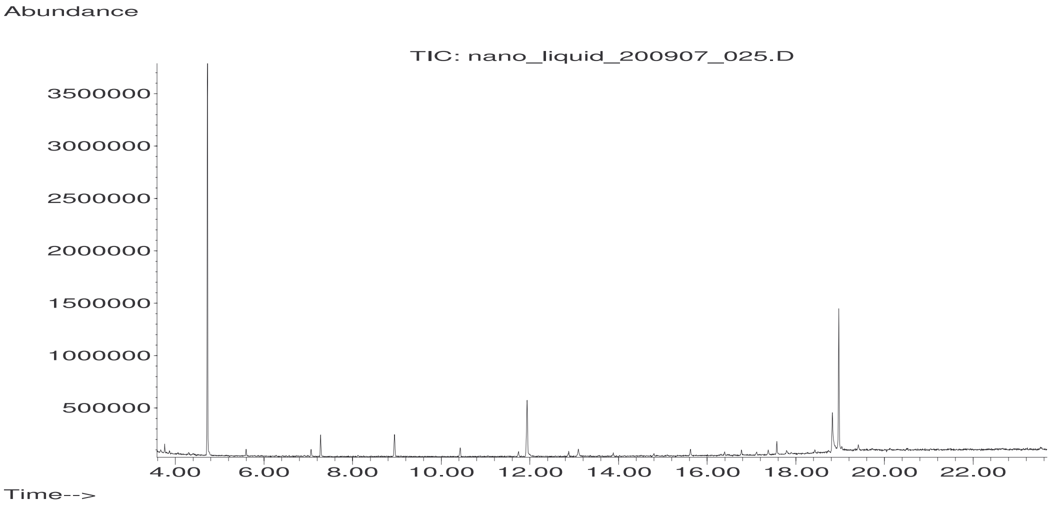
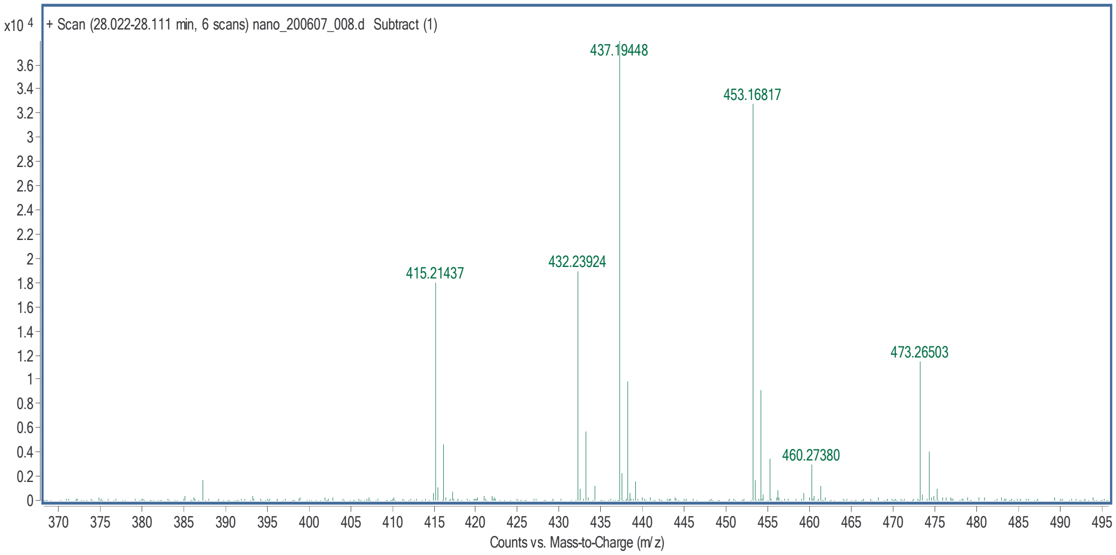


Figure 14. GC-MS chromatogram obtained from the analysis of the concentrated ethanolic extract of the 'control' PET bottle



Appendix 1 – LC-TOF-MS data for the nano-silver control PP containers

Peak at 28.0 min with mass 414.2047



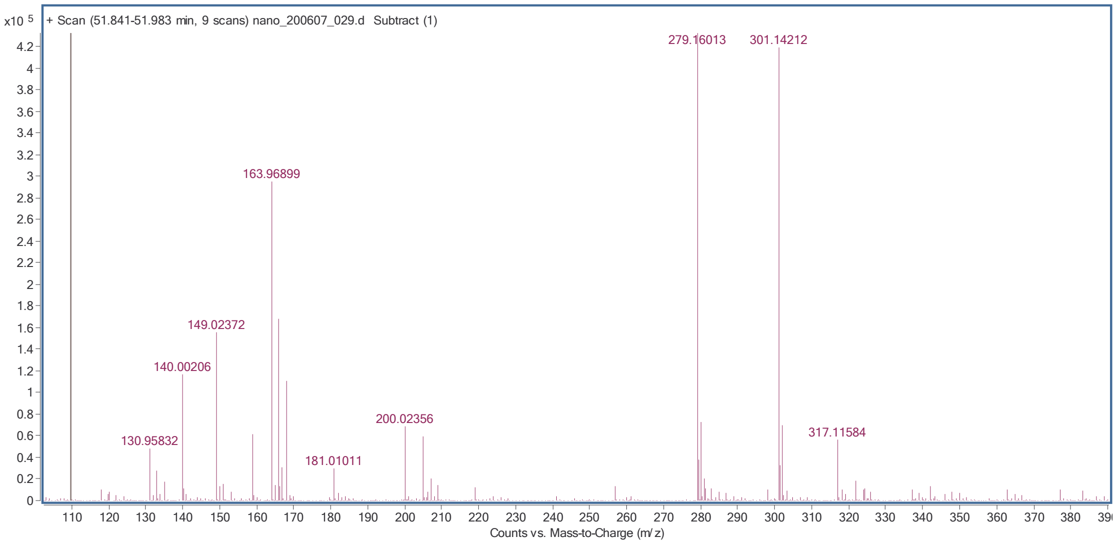
Ions present (above 5% of maximum intensity)

Ion	Mass
M+H	415.2144
M+NH4	432.2392
M+Na	437.1945
M+K	453.1692
No sensible suggestions with mass error < 5 ppm	460.2739
No sensible suggestions with mass error < 5 ppm	473.2650

Predicted formula

	Formula	Calculated m/z	Error (ppm)	Isotope Match (%)
1	C24H30O6	414.2042	0.2	99.8

Peak at 50.4 and 51.9 min with mass 278.1523



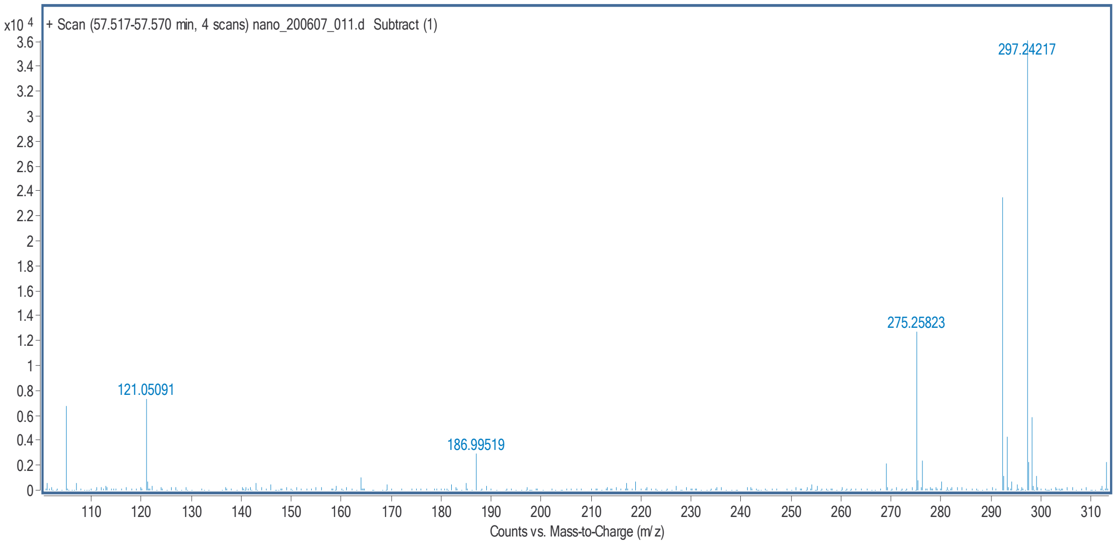
Ions present (above 5% of maximum intensity)

Ion	**Mass**
No sensible suggestions with mass error < 5 ppm	130.9593
No sensible suggestions with mass error < 5 ppm	140.0021
No sensible suggestions with mass error < 5 ppm	149.0237
No sensible suggestions with mass error < 5 ppm	163.9700
No sensible suggestions with mass error < 5 ppm	191.0101
No sensible suggestions with mass error < 5 ppm	200.0236
M+H	279.1601
M+Na	301.1421
M+K	317.1159

Predicted formula

	Formula	**Calculated m/z**	**Error (ppm)**	**Isotope Match (%)**
1	$C_{16}H_{22}O_4$	278.1518	1.8	99.9

Peak at 57.6 min with mass 274.2519



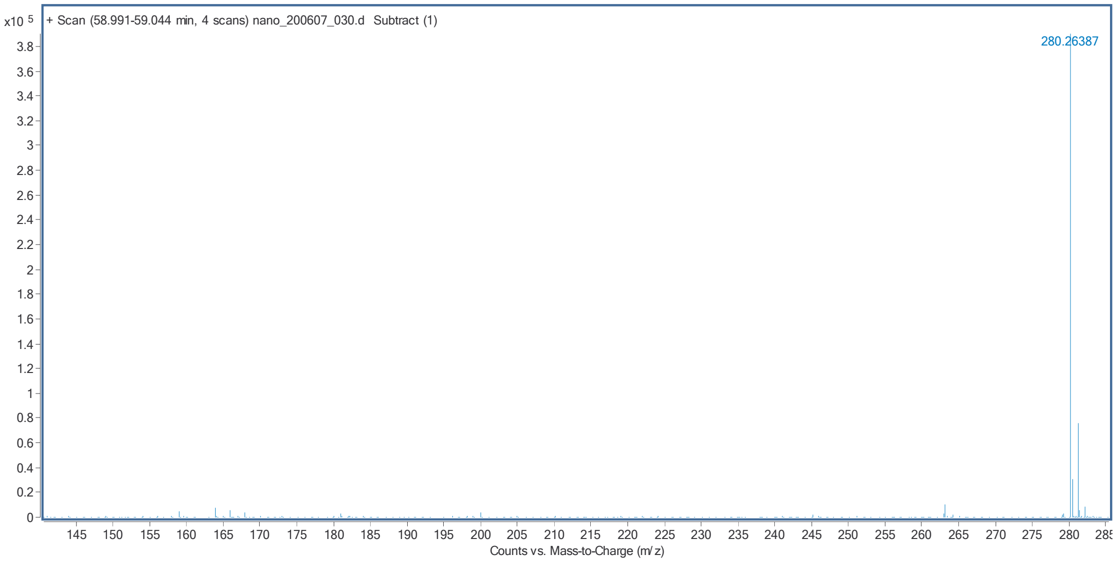
Ions present (above 5% of maximum intensity)

Ion	**Mass**
No sensible suggestions with mass error < 5 ppm	121.0509
No sensible suggestions with mass error < 5 ppm	196.9952
M+H	275.2592
M+NH4	292.2849
M+Na	297.2422

Predicted formulae

	Formula	**Calculated m/z**	**Error (ppm)**	**Isotope Match (%)**
1	$C_{16}H_{34}O_3$	274.2507	4.0	99.9

Peak at 59.0 min with mass 262.2301



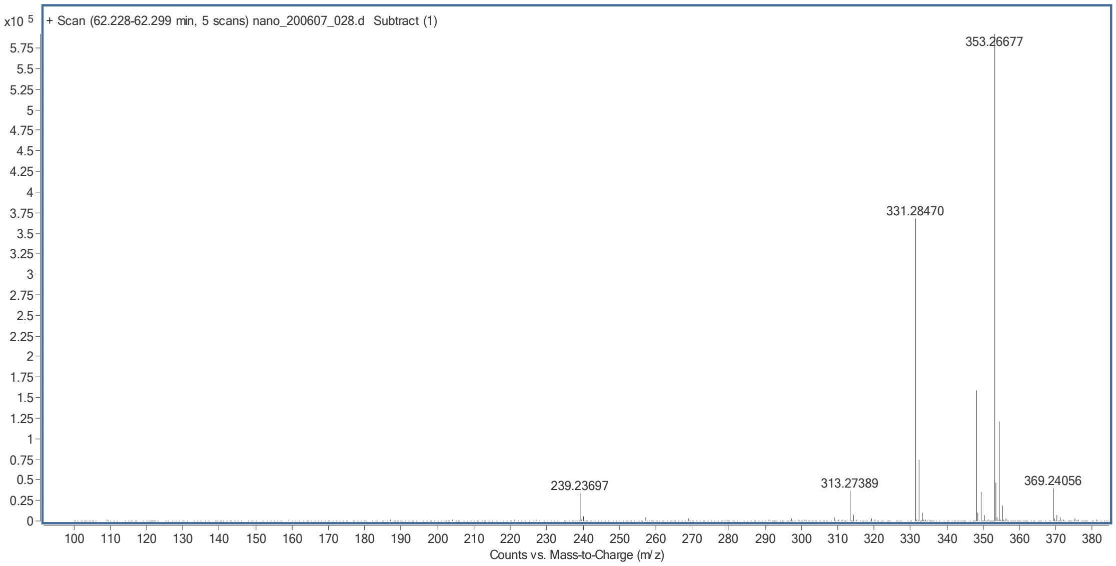
Ions present (above 5% of maximum intensity)

Ion	**Mass**
M+H	263.2356
M+NH4	290.2640

Predicted formula

	Formula	**Calculated m/z**	**Error (ppm)**	**Isotope Match (%)**
1	$C_{18}H_{30}O$	262.2296	1.7	99.8

Peak at 62.3 min with mass 330.2773



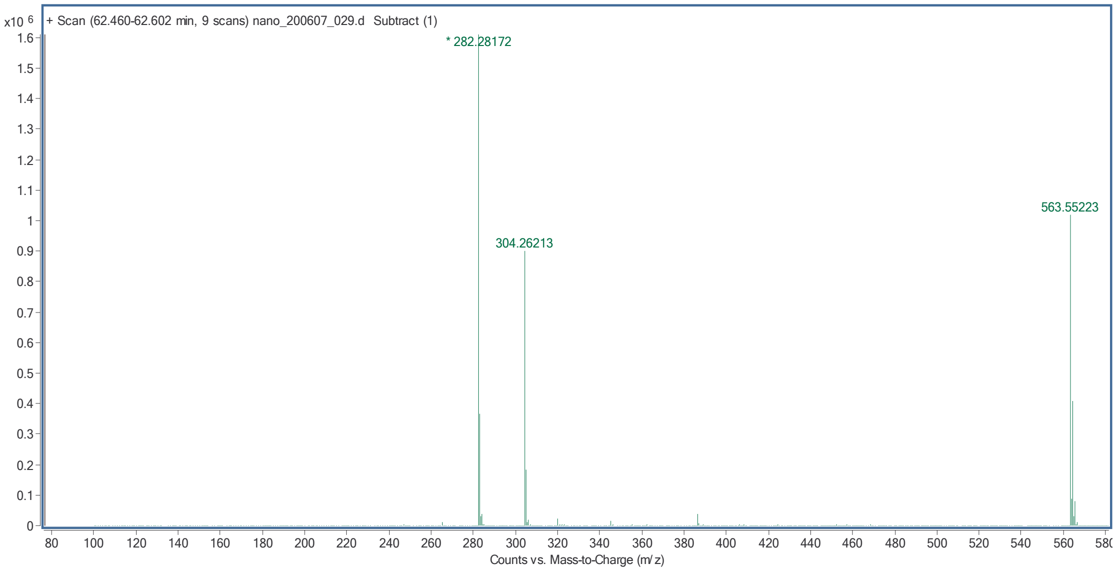
Ions present (above 5% of maximum intensity)

Ion	**Mass**
No sensible suggestions with mass error < 5 ppm	239.2370
M-H_2O	313.2739
M+H	331.2947
M+NH4	348.3100
M+Na	353.2668
M+K	369.2406

Predicted formula

	Formula	**Calculated m/z**	**Error (ppm)**	**Isotope Match (%)**
1	C19H38O4	330.2770	0.88	99.9

Peak at 62.5 min with mass 281.2844



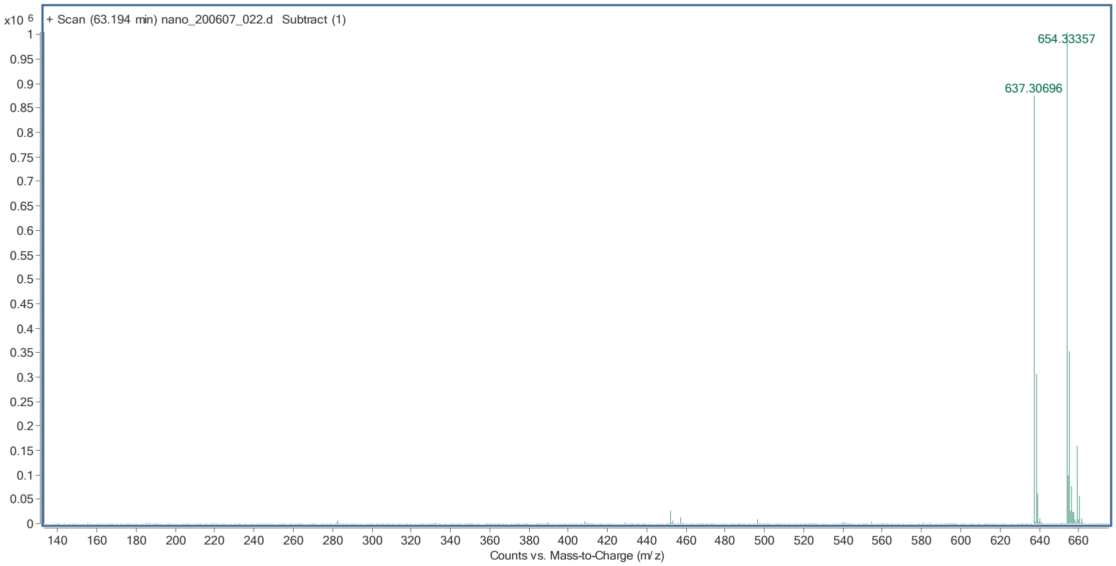
Ions present (above 5% of maximum intensity)

Ion	**Mass**
M+H	282.2917
M+Na	304.2621
2M+H	563.5522

Predicted formula

	Formula	**Calculated m/z**	**Error (ppm)**	**Isotope Match (%)**
1	$C_{19}H_{37}O$	281.2844	0.010	99.8

Peak at 63.1 min with mass 636.2990



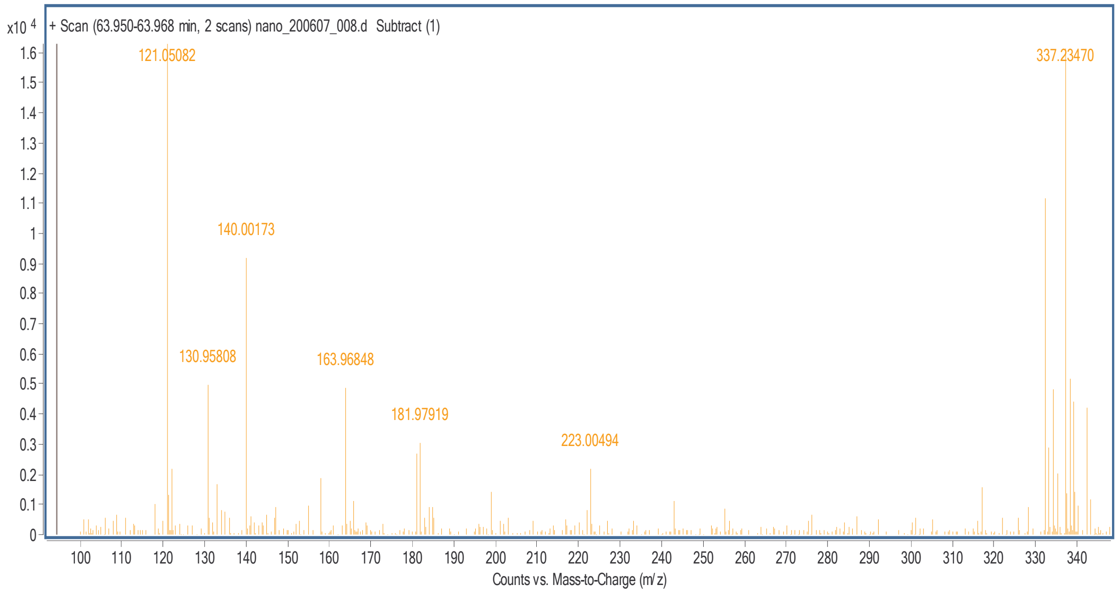
Ions present (above 5% of maximum intensity)

Ion	Mass
M+H	637.3070
M+NH4	654.3336

Possible formulae

	Formula	Calculated m/z	Error (ppm)	Isotope Match (%)
1	C35H48N4OS3	636.2990	-0.043	94.4
2	C34H54O3P2S2	636.2989	0.052	92.2
3	C32H54N3P4S	636.2988	0.15	93.4
4	C38H43N3O4P	636.2991	-0.18	95.6
5	C31H59NPS5	636.2988	0.19	70.5
6	C28H50N3O9S2	636.2988	0.23	64.5
7	C24H53N4O9PS2	636.2991	-0.25	63.5
8	C42H40N2O4	636.2988	0.30	65.6
9	C28H57N4P5S	636.2992	-0.33	63.6
10	C30H57NO3P3S2	636.2992	-0.43	65.7

Peak at 63.8 min with mass 314.2457



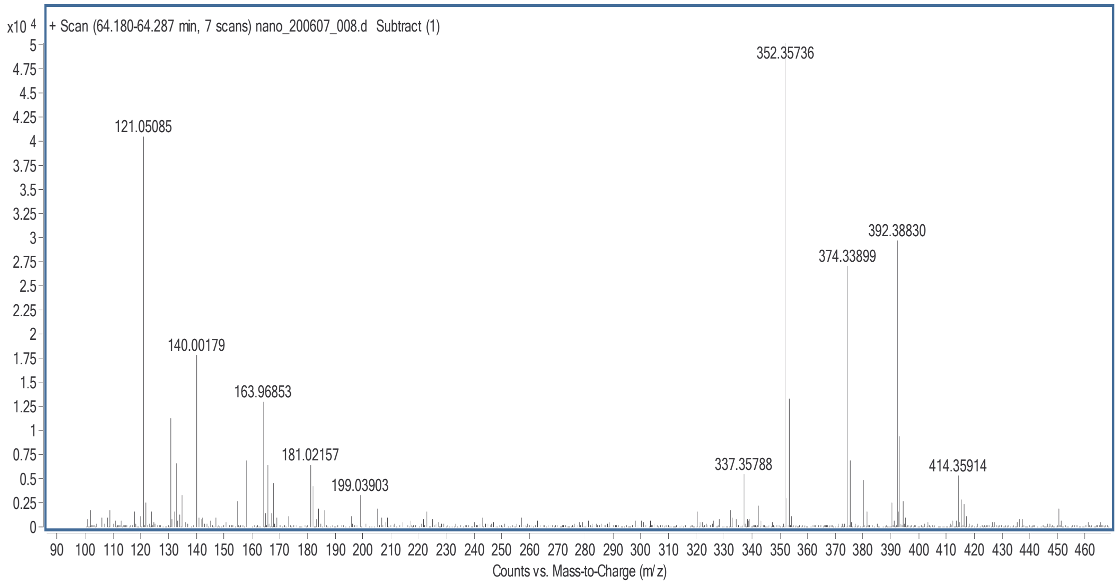
Ions present (above 5% of maximum intensity)

Ion	**Mass**
No sensible suggestions with mass error < 5 ppm	121.0509
No sensible suggestions with mass error < 5 ppm	130.9591
No sensible suggestions with mass error < 5 ppm	140.0017
No sensible suggestions with mass error < 5 ppm	169.9695
No sensible suggestions with mass error < 5 ppm	191.9792
No sensible suggestions with mass error < 5 ppm	223.0049
M+NH4	332.2815
M+Na	337.2347

Predicted formula

	Formula	**Calculated m/z**	**Error (ppm)**	**Isotope Match (%)**
1	C18H34O4	314.2451	1.7	99.8

Peak at 64.1 min with mass 351.3501



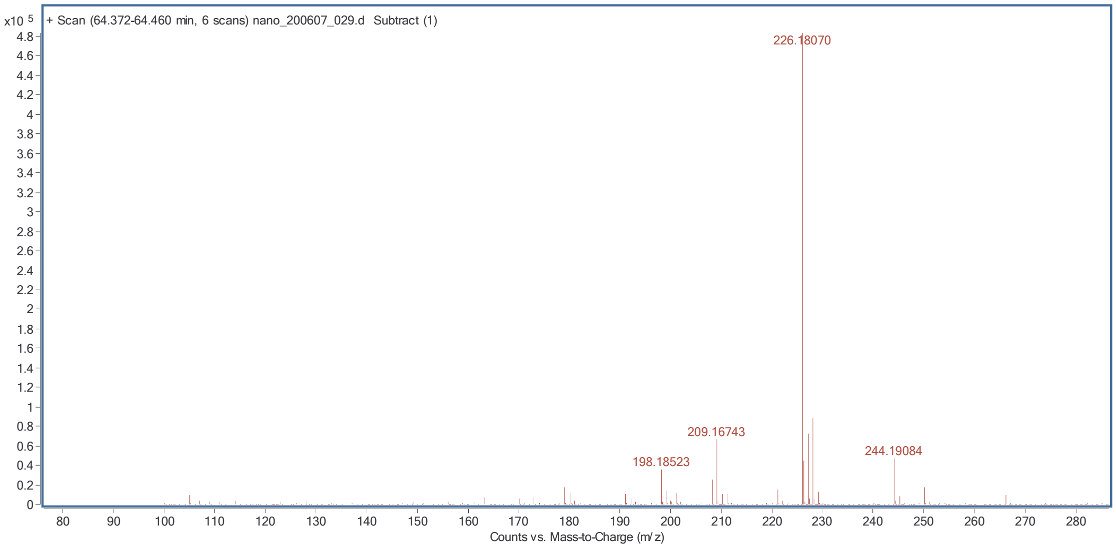
Ions present (above 5% of maximum intensity)

Ion	Mass
No sensible suggestions with mass error < 5 ppm	121.0510
No sensible suggestions with mass error < 5 ppm	140.0018
No sensible suggestions with mass error < 5 ppm	163.9695
No sensible suggestions with mass error < 5 ppm	191.0216
No sensible suggestions with mass error < 5 ppm	199.0390
Contamination from coeluting peak	337.3580
M+H	352.3574
M+Na	374.3400
No sensible suggestions with mass error < 5 ppm	392.3993
No sensible suggestions with mass error < 5 ppm	414.3591

Predicted formula

	Formula	Calculated m/z	Error (ppm)	Isotope Match (%)
1	C23H45NO	351.3501	0.010	99.9

Peak at 64.4 min with mass 225.1733



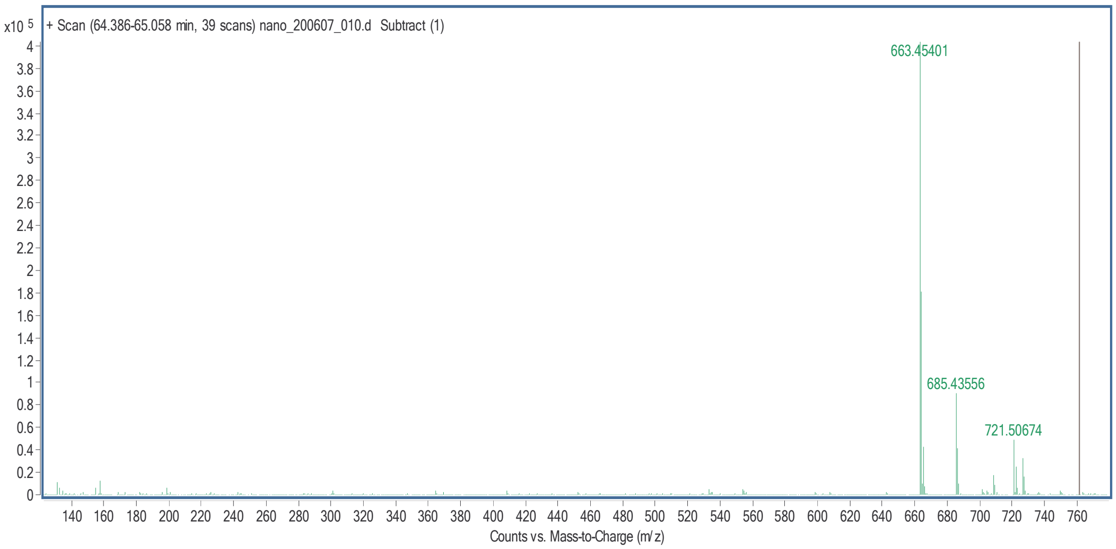
Ions present (above 5% of maximum intensity)

Ion	**Mass**
No sensible suggestions with mass error < 5 ppm	199.1952
No sensible suggestions with mass error < 5 ppm	209.1674
M+H	226.1907
No sensible suggestions with mass error < 5 ppm	244.1909

Predicted formula

	Formula	**Calculated m/z**	**Error (ppm)**	**Isotope Match (%)**
1	$C_{13}H_{23}NO_2$	225.1728	1.8	99.8

Peak at 65.0 min with mass 662.4464



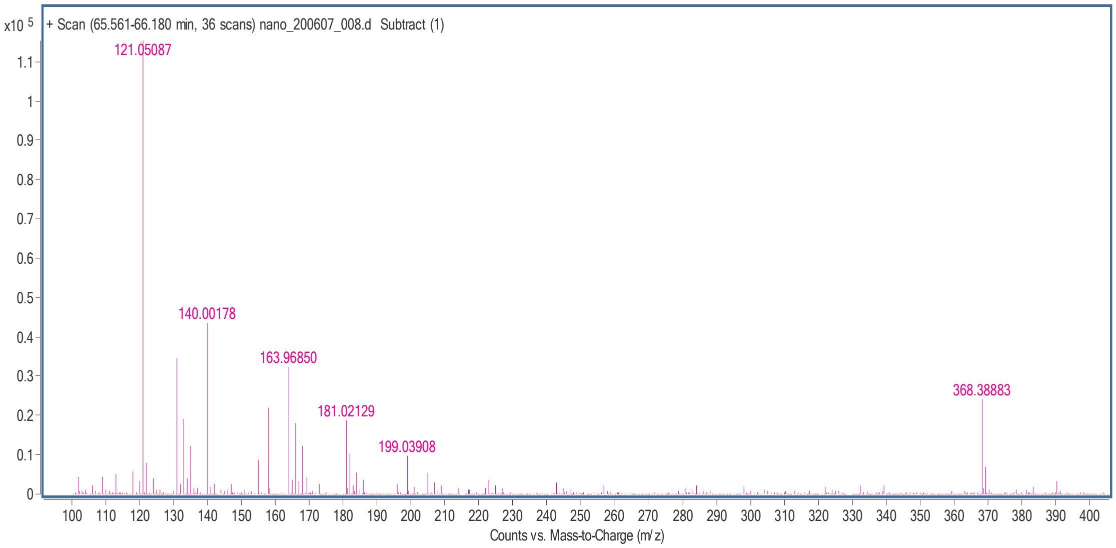
Ions present (above 5% of maximum intensity)

Ion	**Mass**
M+H	663.4540
M+Na	685.4356
No sensible suggestions with mass error < 5 ppm	721.5067

Predicted formula

	Formula	**Calculated m/z**	**Error (ppm)**	**Isotope Match (%)**
1	C42H63O4P	662.4458	0.8	99.8

Peak at 65.7 min with mass 367.3817



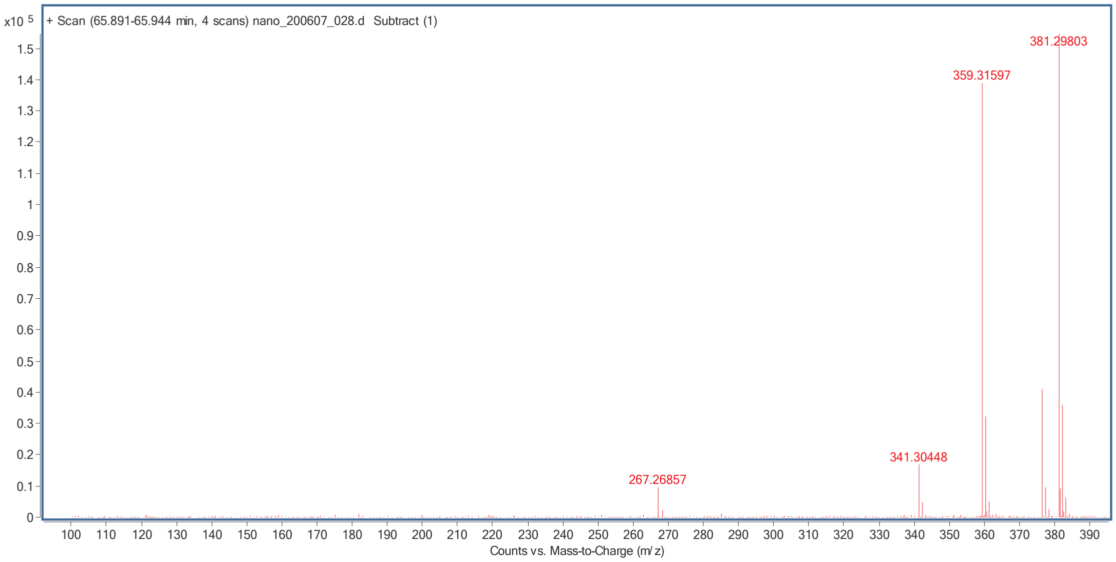
Ions present (above 5% of maximum intensity)

Ion	Mass
No sensible suggestions with mass error < 5 ppm	121.0510
No sensible suggestions with mass error < 5 ppm	140.0018
No sensible suggestions with mass error < 5 ppm	163.9695
No sensible suggestions with mass error < 5 ppm	191.0213
No sensible suggestions with mass error < 5 ppm	199.0391
M+H	368.3888

Predicted formula

	Formula	Calculated m/z	Error (ppm)	Isotope Match (%)
1	$C_{24}H_{49}NO$	367.3814	-0.040	99.4

Peak at 65.9 min with mass 358.3087



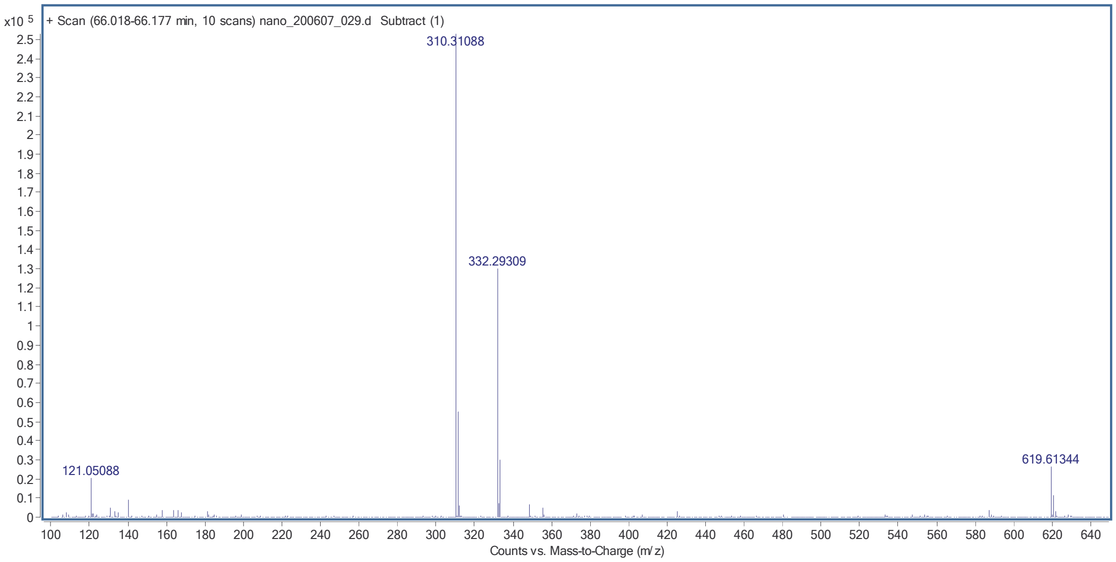
Ions present (above 5% of maximum intensity)

Ion	Mass
No sensible suggestions with mass error < 5 ppm	267.2696
M-OH	341.3045
M+H	359.3160
M+Na	381.2990

Predicted formula

	Formula	Calculated m/z	Error (ppm)	Isotope Match (%)
1	C21H42O4	358.3083	1.1	99.9

Peak at 66.1 min with mass 309.3035



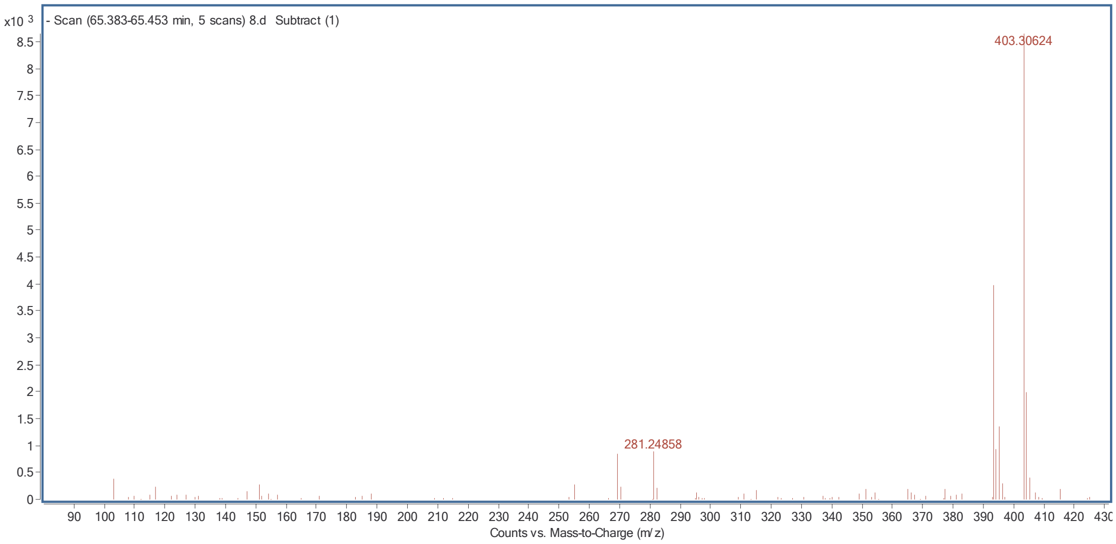
Ions present (above 5% of maximum intensity)

Ion	**Mass**
No sensible suggestions with mass error < 5 ppm	121.0510
M+H	310.3110
M+Na	332.2931
2M+H	619.6134

Predicted formula

	Formula	**Calculated m/z**	**Error (ppm)**	**Isotope Match (%)**
1	$C_{20}H_{39}NO$	309.3031	1.0	99.8

Peak at 66.2 min with mass 404.3122



Ions present (above 5% of maximum intensity)

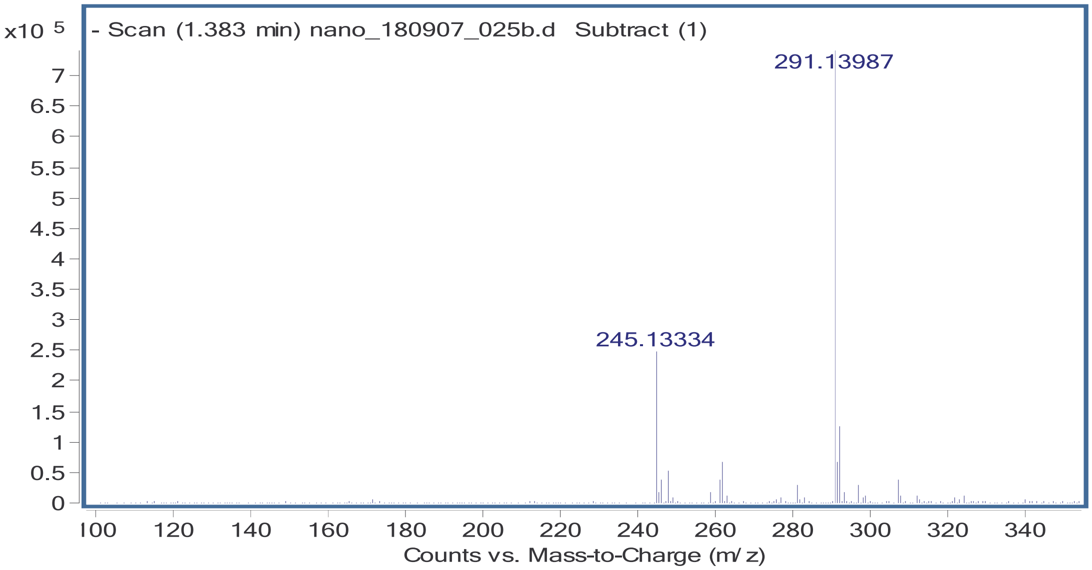
Ion	**Mass**
No sensible suggestions with mass error < 5 ppm	291.2496
No sensible suggestions with mass error < 5 ppm	393.2780
M-H	403.3062

Predicted formulae

	Formula	**Calculated m/z**	**Error (ppm)**	**Isotope Match (%)**
1	$C_{22}H_{44}O_6$	404.3137	-3.9	99.5

Appendix 2 – LC-TOF-MS data for nano-clay and control PET bottles

Peak at 1.4 min with mass 292.1464



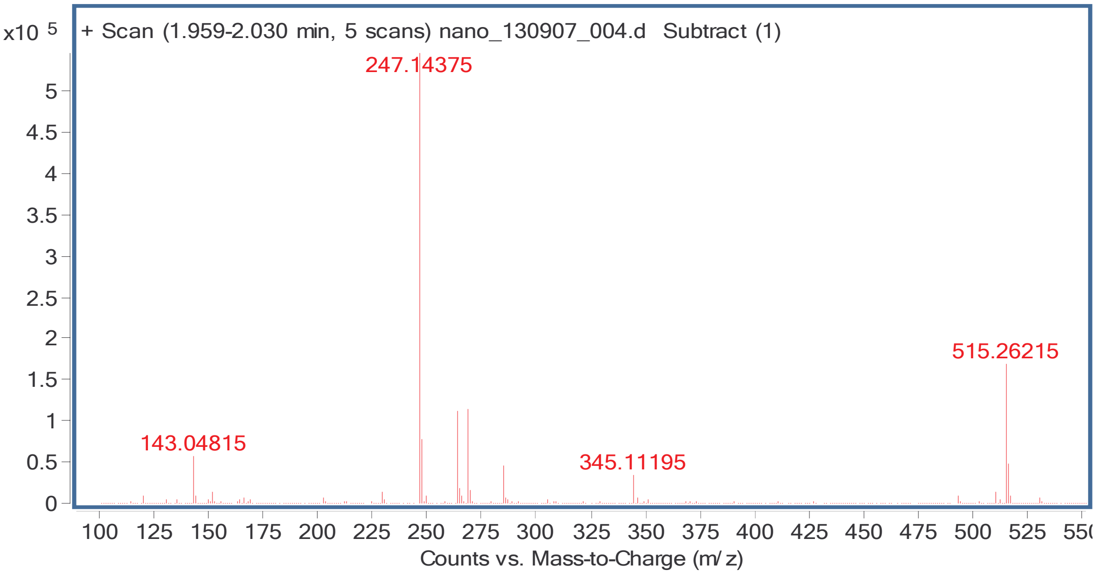
Ions present (above 5% of maximum intensity)

Ion	Mass
No sensible suggestions with mass error < 5 ppm	245.1333
M-H	291.1399

Predicted formula

	Formula	Calculated m/z	Error (ppm)	Isotope Match (%)
1	$C_{12}H_{24}N_2O_4S$	292.1457	-5.0	100

Peak at 2.0 min with mass 246.1364



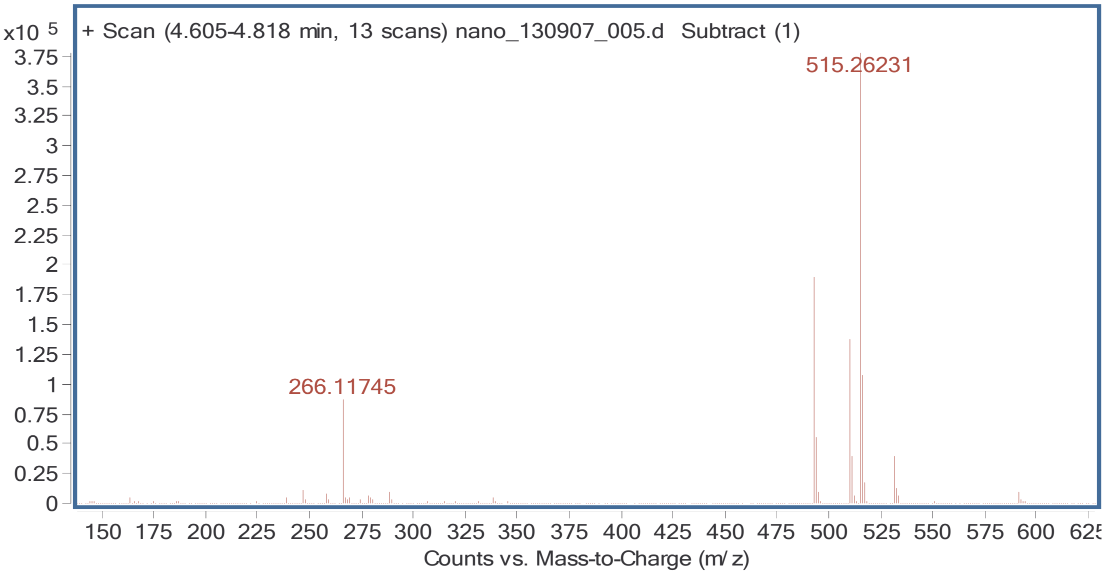
Ions present (above 5% of maximum intensity)

Ion	Mass
No sensible suggestions with mass error < 5 ppm	143.0482
M+H	247.1438
M+NH4	264.1699
M+Na	269.1258
No sensible suggestions with mass error < 5 ppm	345.1120
2M+Na	515.2622

Predicted formula

	Formula	Calculated m/z	Error (ppm)	Isotope Match (%)
1	C14H18N2O2	246.1368	-0.11	99.9

Peak at 4.7 min with mass 492.2732



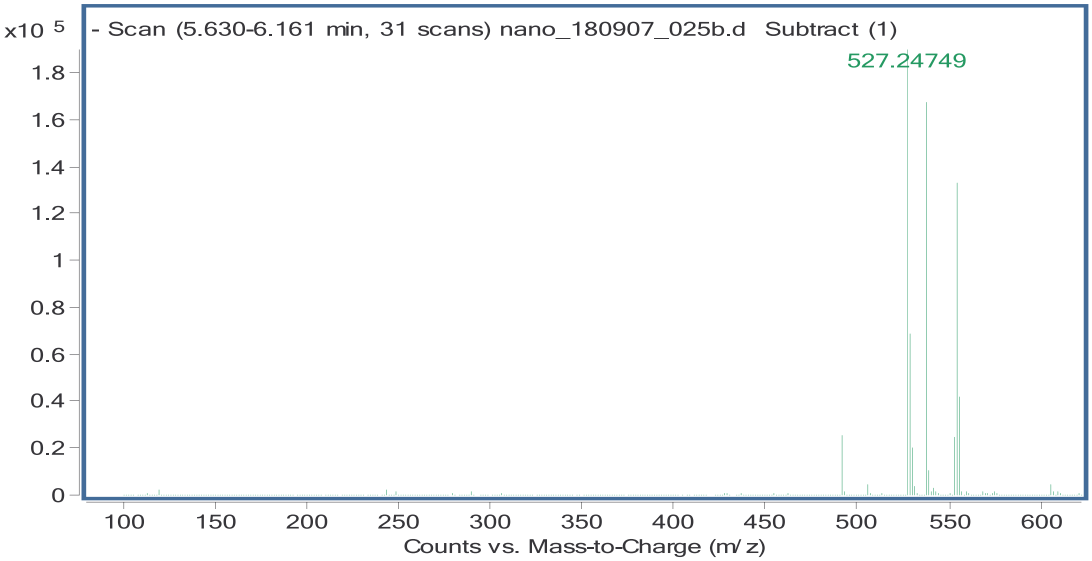
Ions present (above 5% of maximum intensity)

Ion	Mass
$C_{14}H_{18}O_5$	266.1175
M+H	493.2817
M+NH4	510.3068
M+Na	515.2623
M+K	531.2382

Predicted formula

	Formula	Calculated m/z	Error (ppm)	Isotope Match (%)
1	$C_{27}H_{40}O_8$	492.2723	-0.038	98.9

Peak at 5.3 min with mass 555.2639



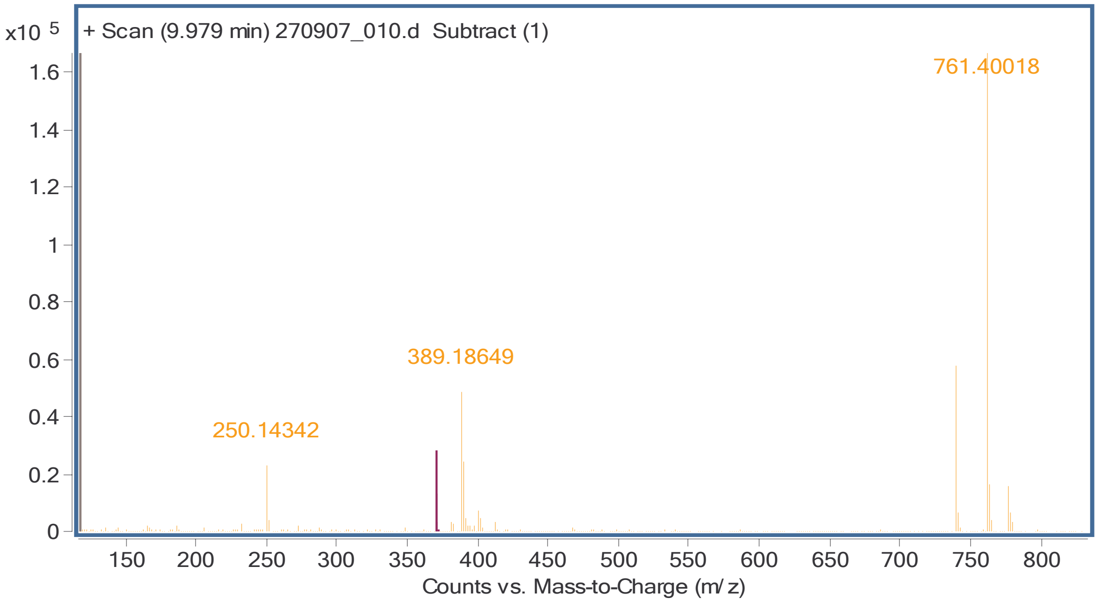
Ions present (above 5% of maximum intensity)

Ion	Mass
No sensible suggestions with mass error < 5 ppm	491.2710
No sensible suggestions with mass error < 5 ppm	527.2475
M-OH	537.2762
M-H	554.2667

Possible formulae

	Formula	Calculated m/z	Error (ppm)	Isotope Match (%)
1	$C_{27}H_{42}N_2O_8S$	555.2667	0.11	95.0
2	$C_{34}H_{40}N_3S_2$	555.2669	0.23	94.0
3	$C_{20}H_{44}NO_{16}$	555.2665	0.44	93.5

Peak at 10.1 min with mass 369.2048



Ions present (above 5% of maximum intensity)

Ion	Mass
C14H19NO3	250.1434
M+2H^{2+}	370.2130
No sensible suggestions with mass error < 5 ppm	389.1865
M+H	739.4171
M+Na	761.4002
M+K	777.3755

Possible formulae

	Formula	Calculated m/z	Error (ppm)	Isotope Match (%)
1	C38H62N2O10S	738.4125	1.4	98.1
2	C46H58O8	738.4132	2.3	96.7
3	C47H54N4O4	738.4145	4.1	86.5

Annex-B: CSL/FSA Workshop on Applications and Implications of Nanotechnology for FCMs

A workshop to discuss findings of this project, state-of-the-art, and the issues emerging from the use of nanotechnologies for FCMs was held at CSL Sand Hutton on 27 November 2007. The workshop was attended by around 50 representatives from scientific community, industry, consumer forums, and regulatory agencies. Presentation at the workshop highlighted the enormous benefits that nanotechnology applications are likely to bring to the food sector, as well as the potential risks that they might pose to consumer's health.

Workshop Presentations

- Dr. Laurence Castle (CSL) introduced the project objectives, background and aims of the workshop. Dr. Castle highlighted that it was not the aim of the workshop to provide a final word on the benefits or risks of nanotechnologies, but to bring important stakeholders together to discuss state-of-the-knowledge and to identify the way forward in which new developments can be realised in manner that safe to the consumer and the environment.
- Dr. Sandy Lawrie (FSA) presented the Food Standards Agency's perspective on nanotechnology applications for FCMs. Dr. Lawrie detailed government's strategy to assess the potential risks from nanotechnologies, and the Agency's efforts in supporting research into the safety of nanotechnology-derived foods and FCMs. He also outlined the relevance and adequacy of current regulatory frameworks in controlling the potential risks from products and applications of nanotechnology.
- Dr. Qasim Chaudhry (CSL) presented a detailed overview of the current and projected nanotechnology derived novel and improved FCMs, as well as certain issues in relation to consumer safety and regulatory controls that might emerge from the use of nanotechnology for FCMs. Dr. Chaudhry mentioned the increasing worldwide trend in nanotechnology applications for the food sector. Whilst a variety of nanotechnology-derived food, healthfood, and FCM is already available in some countries, it is widely anticipated that they will be increasingly available in the EU in the coming years, and FCMs are likely to make the largest share of the market.
- Dr. Lang Tran (Institute of Occupational Medicine) discussed state-of-the-knowledge with regard to toxicokinetics of nanoparticles in living organisms, and presented results of some of the unpublished studies on migration of nanoparticles into different organs and tissues following oral ingestion. Dr. Tran also highlighted the potential hazards to consumer health that need to be considered in relation to nanotechnology applications in food and FCMs.
- Dr. Emma Bradley (CSL) presented results of the pioneering tests carried out on migration of nanoparticles in the two types of nanotechnology-derived FCMs tested at CSL. The results showed that there was no detectable migration in the case where a nanoclay-polymer composite was embedded between two PET layers, and a very low level of migration of nanosilver from food containers made of polypropylene-nanosilver composite. In either case, the presence of nanoparticles in polymers did not affect migration of non-nano components from the two composites tested. Dr. Bradley also highlighted the need for further work into migration testing on other types of nanotechnology-derived FCMs to enable building a broader picture with regard to potential safety to consumers.
- Dr. Hans Bouwmeester (RIKILT) presented a detailed assessment of the potential risks to consumers from nanotechnology derived foods and FCMs. Dr. Bouwmeester stressed the need for further research into safety of nanotechnology-derived food and FCMs, and outlined the requirements for specific data to enable adequate risk assessment.

- Dr. Anna Gergely (Meyer Browne LLP) described the relevant EC legislation with regard to controlling the risks from nanotechnology-derived FCMs. In the absence of any nano-specific regulations, Dr. Gergely highlighted that provisions under the existing FCM legislation, and related legislation, such as the EU's new chemicals policy (REACH), and the General Product Safety Directive, should cover the risks from nanotechnology-derived food and FCMs.

Discussion Groups

The workshop attendees were split into three Discussions Groups in the afternoon session. Group-1 led by Dr. Qasim Chaudhry discussed the prospects for nanotechnology applications in the EU's food packaging industry, as well as market needs and gaps, commercial opportunities, and technological barriers. Briefly, the discussions concluded that:

- Nanotechnology-derived barrier products to extend shelf life of food products may be seen by consumers to mainly benefit the industry, and not the consumer. They may, however, be seen as a benefit to the consumer if there was a high-quality product at the end of the existing shelf life.
- Nanotechnology-derived antimicrobial packaging may be seen by the consumer as a camouflage for poor hygiene. However, active packaging releasing antimicrobials/ preservatives into food may be seen differently. Similarly, nano-coatings may be more acceptable especially when they are on the outside of food packaging.
- Radio Frequency Identification Display (RFID) based nano-sensors may be helpful in product recalls/ tracing products during transit, but may also lead to issues with regard to consumer privacy. Smart labels may be helpful in product quality assurance e.g. tempering of packs that have been packaged under modified atmosphere. Current high costs of smart labels (and high costs of implementing them into existing systems) is an obvious issue. Their use may thus be limited to high value products at least in the short term.
- With regard to adoption of nanotechnology-derived FCMs, the view from industry was that it was better to wait until safety issues were addressed, knowledge gaps filled and costs came down. There was certainly an interest if more robust but lightweight packaging was available.
- Questions were also raised over who would (or should) fund R&D. The view from industry was that they were not in favour of having to fund the basic R&D into innovation as well as the safety aspects of the new technology.

Group-2 led by Dr. Lang Tran discussed research priorities to identify and mitigate risks, risk communication, assessment of hazards, exposure, risk to consumers, possible industry code of best practice, and the issue of consumer education and communication of benefits and risks. Briefly, the discussions concluded that:

- Reference materials are required for instrumental and methodological calibration and to identify differences in nanoparticle behaviour. There is a need for determining the behaviour and fate of nano-particulates in real exposure situations, and for this elemental analysis alone will not be sufficient. However, in regard to availability of characterisation data on reference materials, industry IPR (intellectual property right) is an obvious issue and ways are needed to enable data sharing whilst maintaining confidentiality.
- Robust methodologies will be required to detect manufactured nanoparticles at low concentrations in food and drinks to enable life-cycle analysis. The manufactured nanoparticles present in (or leaching into) food/ drinks will need to be characterised for physicochemical properties such as particles number, size, surface charge etc.

- A major unknown in relation to predicting nanoparticle behaviour and effects in the gastrointestinal tract is the interactions with different food matrices, digestive enzymes, and the gut natural microflora.
- A full life cycle analysis for each nanotechnology-derived FCM will be needed (packaging, human exposure, environmental exposure) to enable appropriate exposure assessment.
- Biomarkers for nanoparticle exposure (eg oxidative activity) are needed to enable bioassays.
- In relation to human toxicity, the assessment of risk requires understanding of dose – nanoparticle loading in multiple organs.
- Ethical issues must be considered when carrying out safety assessments, i.e. reduction in the use of test animals, and perhaps more emphasis should be on validated *in vitro* methods that can be extrapolated to humans.
- There is also a need for prioritising nanoparticles for testing, as well as assessing the cocktail effects of different nanoparticles together.
- In relation to industry code of practice, there is need for a consensus from all parties/ stakeholders, and such schemes should be initiated by the industry.
- With regard to education and communication - grouping all nanoparticles together could give consumer the wrong impression. Despite the fact that "nano" is already being used as a selling point by some companies, distinction needs to be made between relatively safe nanoparticles and those that may pose greater risks to consumers. In this regard, lessons may be learnt from other issues – e.g. plasticiser migration or GM issues.
- The consumer should be made aware of the risks as well as benefits, and there should be transparency and openness on the part of industry and regulators. There is also a need to listen to consumer concerns – e.g. communicating what is being done to mitigate risks.
- In terms of duty of care, the potential impacts of nanoparticles on the environment need to be monitored – e.g. on microorganisms in wastewater treatment plants and landfill sites, and guidelines should be provided on safe disposal of wastes from incinerator plants and manufacturing facilities.

Group-3 led by Dr. Anna Gergely discussed the complex issue of specific regulatory activity on nanotechnologies in some depth, focusing on the practicalities of whether further regulation is necessary and if so what form it would take and how it would be implemented. The discussions included aspects of regulatory compliance for nanotechnology-derived FCMs, policy/ regulatory barriers in the way of new developments, and the need for new or amended regulation versus industry self-regulation schemes.

- The general conclusion of the group was that the existing EU regulatory framework for FCMs sufficiently covers the requirement to ensure that any FCM is safe, including innovative nano-based products. It is therefore fine for the current time. There are currently major gaps in knowledge and understanding of the majority of engineered nano-materials, but not in regulation.
- The group agreed that given the current knowledge gaps it would not be suitable to try to develop nano-specific regulation at present. There is no agreed definition of what nanotechnologies are and therefore it would not be possible to agree on the parameters that would need to be measured and controlled by specific legislation. For example, it was noted, that even within a single nanomaterial (e.g. gold) the particles may behaviour differently depending on their size within the nanoscale.

- The group further agreed that FCM manufacturers who currently wish to innovate in the field of nanotechnologies should ensure their activities meet the requirements of Article 3 of the FCM Framework Directive 1935/2004. This would need to be assessed on a case-by-case basis.
- Regarding the development of any future nano-specific legislation, the group advised that this would need to be correct, proportionate and, importantly for both manufacturers and regulators, enforceable.
- On the matter of self-regulation, it was noted that the creation of a code of conduct should not be ruled out at this stage. To date industry uptake of voluntary reporting schemes has been low but this may have more to do with the approach used than the exercise itself.
- Potential difficulties were noted with the self-regulation route. As soon as there is the first major issue, it is likely that there will be call for mandatory regulation. Difficulties with how to establish workable legislation would then arise again. The group queried whether the debate needed to be so polarised and if a middle ground between mandatory regulation and self-regulation on nanotechnologies could be found.

Annex-C: Publications and Presentations Resulting from the Study

Publications

- Chaudhry, Q; Scotter, M; Blackburn, J; Ross, B; Boxall, A; Castle, L; Watkins, R; Aitken, R (2008) Applications and implications of nanotechnologies for the food sector. *Food Additives and Contaminants 25(3): 241-258.*
- Chaudhry, Q., Castle, L. and Watkins, R. (Editors) The Outlook For Nanotechnologies in Food, Royal Society of Chemistry (In preparation - to be published later on in 2008)

Presentations

- Chaudhry, Q. (2006) Nanomaterials for Food Contact Plastics, Presentation at the *Food Contact Plastics Conference 2006*, June 2006, Brussels.
- Chaudhry Q. (2007) Potential Consumer Safety and Regulatory Issues in Relation to Products and Applications of Nanotechnology for the Food Sector, Presentation at the *Nanotoxicology Conference*, April 2007, Venice, Italy.
- Chaudhry, Q. (2007) Assessment of current and projected applications of nanotechnology for food contact materials in relation to consumer safety and regulatory implications, Presentation at the meeting of Working Party on Materials and Articles in Contact with Food or Drink, May 2007, London.
- Chaudhry, Q. (2007) Nanotechnology Derived Food Contact Plastics, Presentation at the *Food Contact Plastics Conference 2007*, June 2007, Brussels.
- Chaudhry, Q. (2007) Nanotechnology Applications for the Food Sector and Implications for Consumer Safety and Regulatory Controls, Presentation at the *2007 CSL/JISFAN Joint Symposium on Food Safety and Nutrition – Nanotechnology in Foods and Cosmetics*, June 2007, Greenbelt MD, USA.
- Chaudhry, Q. (2007) Consumer safety and regulatory issues in relation to the use of nanotechnologies in the food sector, IV International Symposium EU-Russia Co-operation in Biotechnology, Agriculture, Forestry and Food, August 30 – September 02, 2007, Suzdal, Russia.
- Chaudhry, Q. Applications and implications of nanotechnologies for the food sector, Presentation at the 2nd International Food and Nutrition Congress, October 2007, Istanbul Turkey.
- Chaudhry, Q. (2007) Consumer Safety and Regulatory Issues in Relation to Nanotechnology Applications for the Food Sector, High Level Conference on Nanotechnologies on 20-21 November 2007, Braga Portugal.
- Chaudhry, Q. (2008) Prospects of nanotechnologies for the EU’s food sector, and current knowledge gaps. EARTO Workshop on nanotechnology in food and medicine, 30 January 2008, Brussels.

List of Abbreviations:

FCM:	Food Contact Material
SnIRC:	Safety of nanomaterial Interdisciplinary Research Centre (www.snirc.org)
CSL:	Central Science Laboratory, York
FSA:	Food Standards Agency
Defra:	Department for Environment, Food and Rural Affairs
Elisa:	Enzyme Linked Immunosorbent Assay
R&D:	Research and Development
ETC:	The ETC Group (www.etcgroup.org)
BfR:	The German Federal Institute for Risk Assessment (www.bfr.bund.de)
PET:	Polyethyleneterephthalate
PA:	Polyamides
PS:	Polystyrene
EVA:	Ethylene-vinylacetate
PVH:	Polyvinyl methyl ether Homopolymer
POSS:	Polyhedral Oligomeric Silsesquioxane
EvOH:	Ethylene-Vinyl Alcohol Copolymer
UV:	Ultra Violet
PVC:	Poly Vinyl Acetate
PE:	Polyethylene
PTMO:	Propyltrimethoxysilane
PLA:	Poly Lactic Acid
CNT:	Carbon Nanotubes
SWCNT:	Single-walled Carbon Nanotubes
MWCNT:	Multi-walled Carbon Nanotubes
PPS:	Polyphenylene Sulphide
PEEK:	Polyether Ether Ketone
RFID:	Radio Frequency Identification Display
UK:	United Kingdom
EU:	European Union
EC:	European Commission
US/ USA:	United States of America
COT,COM,COC:	The independent Committees on Toxicity, on Mutagenicity and on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment
PP:	Polypropylene
IFST:	The Institute of Food Science and Technology (www.ifst.org)

SiOx:	Silicon oxides
LC-TOF-MS:	Liquid Chromatography-Time-of-flight-Mass Spectrometry
ICP-MS:	Inductively Coupled Plasma - Mass Spectrometry
IOM:	Institute of Occupational Medicine (www.iom-world.org/)
RIKILT:	The RIKILT-Institute of Food Safety, the Natherlands (www.rikilt.wur.nl/UK/)
REACH:	Registration, Evaluation, Authorisation of Chemicals
IPR:	Intellectual Property Right
GM:	Genetically Modified